Academic Team Project Integration Report

King County Orca Proviso

Wastewater Effluent Discharge Assessment – Impact to Marine Organisms

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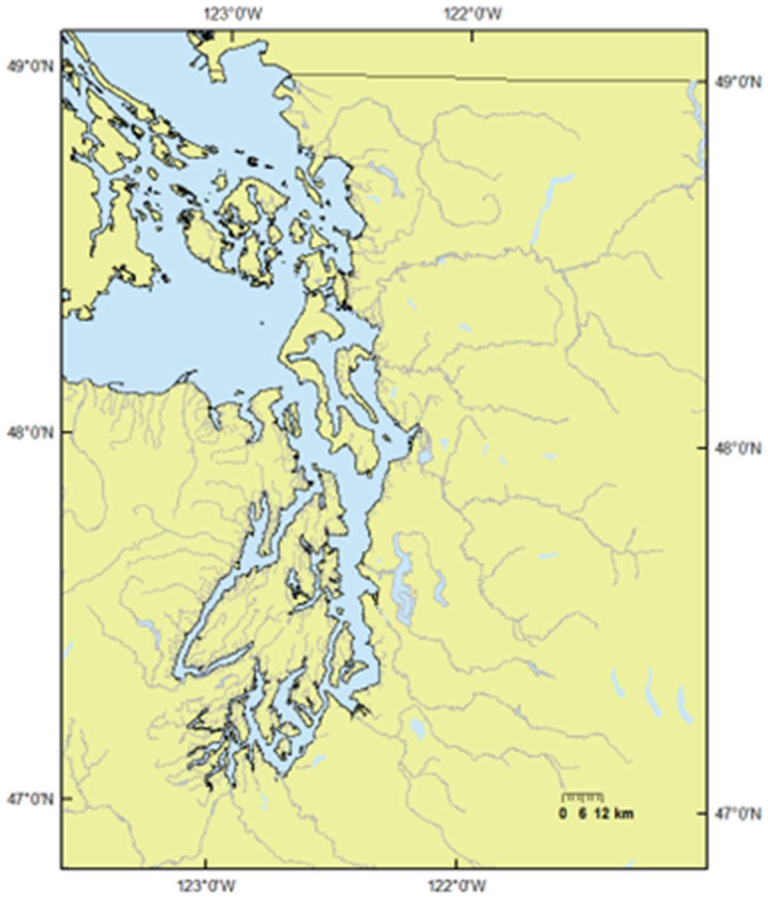

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Table of Contents

EXECUTIVE SUMMARY

Residential, commercial, and industrial water uses result in contamination of municipal water with myriad chemicals. Treatment of wastewater by municipal facilities reduces the amount and concentration of contaminants discharged to surface waters but the residual contaminants are a concern for the health of organisms in aquatic ecosystems. In Washington State, King County provides wastewater treatment service to over 1.9 million people. Three wastewater treatment plants (WWTPs) provide the majority of treatment; West Point in Seattle, South Plant in Renton, and Brightwater near Woodinville, with effluents released directly into Puget Sound. Chinook salmon (*Oncorhynchus tshawytscha*) in Puget Sound are listed as threatened under the U.S. Endangered Species Act, despite many restoration efforts. Chinook are exposed to wastewater effluent (WWE) when they migrate as juveniles into Puget Sound from the tributaries in which they were spawned, and again when they return from ocean migration as adults prior to migrating upstream to spawn. A portion of the population resides in Puget Sound until spawning. In addition to supporting a recreational and commercial fishery, Chinook are a critical prey for endangered southern resident killer whales (*Orcinus orca*). Among factors including degraded physical habitat, inadequate water quality is one factor contributing to the inability to restore Puget Sound Chinook to historical population levels.

To better understand the contribution of WWTPs to degraded water quality in Puget Sound, we conducted a focused chemical characterization of WWE from each of the three major WWTPs operated by King County; one sample from each WWTP during wet weather (high flow, spring 2021) and one during dry weather (low flow, summer 2021). Wet weather sampling of WWE included the added contribution from stormwater. Characterization focused on legacy organic compounds and chemicals of emerging concern. Concurrent with dry weather sampling, we analyzed water samples collected from Puget Sound near the outfalls for South Plant and West Point to gain a better understanding of conditions to which juvenile Chinook are exposed while residing in Puget Sound. Finally, concurrent with the dry weather/low flow sampling, we conducted a laboratory study exposing juvenile Chinook salmon to dilutions of WWE from South Plant in freshwater for 10 days. Tissue chemistry and biochemical analyses supported an exploration of potential impacts to Chinook salmon health from exposure to WWE.

Of more than 400 chemicals analyzed, targeted analytical chemistry detected 121 chemicals in wastewater. Additional non-targeted screening using high resolution mass spectrometry (HRMS) identified >250 chemicals. Among legacy compounds, WWE from King County WWTPs was determined to be an ongoing pathway of PCBs (polychlorinated biphenyls) to Puget Sound, whereas inputs of PBDEs (polybrominated diphenyl ethers) may be reduced from the prior decade. Chemicals were screened by comparing measured concentrations to concentrations associated with effects from toxicity databases. This approach identified nine of the targeted analytes as priority compounds for future monitoring. An additional 30 compounds were identified by HRMS for future monitoring. An analysis of chemical complexity using HRMS highlighted the chemical disparity of WWE from Brightwater with that from South Plant and West Point, reflecting the advanced treatment methods used at Brightwater compared with the older technologies at South Plant and West Point. The highest chemical similarity among all samples was for low flow WWE from South Plant and West Point, reflecting their similar treatment methods and inputs during low flow periods.

In the laboratory study, juvenile Chinook exposed to WWE showed evidence of endocrine disruption and alterations in the stress response, brain function, and metabolism. Brain function and total plasma protein were affected at low exposure concentrations, whereas other endpoints exhibited a dose-response relationship with measurable differences from control evident only at the higher concentrations. However, some of the endpoints (e.g., endocrine disruption) are expected to show more pronounced effects with longer exposure durations than in the laboratory study. Higher exposure concentrations in the laboratory study may therefore be indicative of effects resulting from chronic exposures, which occur in Puget Sound. Alterations in stress response and metabolism in the laboratory study could not be ascribed to any one contaminant or contaminant class, but estrogenic hormones in WWE were sufficient to cause the observed endocrine disruption, and selective serotonin reuptake inhibitors were implicated in the altered brain function.

Metabolomics analysis showed that WWE altered numerous endogenous biochemical pathways important for energy generation and utilization, lipid metabolism and biosynthesis, amino acid metabolism, growth, and oxidative stress. Pathway analysis implicated pharmaceuticals that act as antibiotics, antidepressants, antihistamines, analgesics and statins even at the lowest WWE concentrations tested (0.1% and 0.4%), although other chemicals present in WWE may have contributed.

Additional pharmaceuticals were predicted to cause harm based on a fish plasma model of bioaccumulation from tissue and water chemistry in exposed juvenile Chinook. As with metabolomics, impacts were in many cases predicted at environmentally relevant concentrations of WWE. Impacts to juvenile Chinook observed and predicted for this study are hypothesized to contribute to reduced availability as prey for SRKWs. Additionally, exposure to several classes of contaminants based on bioaccumulation modeling for Chinook likely contribute to health impairments in SRKW.

1 INTRODUCTION

This report presents the results and findings from an investigation performed under contract number 6113841 from King County to Washington State University (WSU), with subcontracts to the National Oceanic and Atmospheric Administration (NOAA) and the University of Washington (UW) Tacoma. The broad goals of the investigation were to improve our understanding of the occurrences of chemicals in the Puget Sound associated with wastewater treatment plant discharges, and the potential for impacts of those chemicals on key species. These goals were achieved through a limited monitoring program of estuary water and wastewater treatment plant effluent, and a focused exposure study of juvenile Chinook salmon exposed to varying concentrations of wastewater effluent from a King County facility. Results of individual components of the study have been described in individual deliverables (see Section 1.2); results are summarized and synthesized in this final report.

1.1 Project Background

1.1.1 Wastewater treatment plant effluent

Wastewater treatment plants (WWTPs) are known pathways to receiving waters for a variety of pharmaceuticals, personal care products, industrial compounds, metals, and legacy compounds. Additional pathways include stormwater runoff and air deposition, for example. Several of these chemicals affect fish at very low concentrations (Fairchild et al. 1999; Daughton and Brooks 2011; Schultz et al. 2011; Saaristo et al. 2017; Meador et al. 2017); however, few data exist on toxic responses for most of these poorly studied chemicals, especially as mixtures.

A high percentage of the chemicals in WWTP effluent are considered contaminants of emerging concern (CECs) that constitute a wide range of chemicals for which there is limited data on occurrence, environmental fate, and toxicity. Represented in this class of environmental contaminants are pharmaceuticals and personal care products (PPCPs) and a number of industrial compounds such as polybrominated diphenyl ethers (PBDEs), per-and polyfluoroalkyl substances (PFAS), alkylphenols, bisphenol A, phthalates, and current-use pesticides. Many of these compounds are present in our rivers, estuaries, and coastal areas due in part to direct discharge of WWTP effluent to these water bodies.

1.1.2 Study Area Description

The study area is central Puget Sound; the focus is on effluent released into estuarine waters from King County South Plant, West Point and Brightwater WWTPs (Table 1, Figure 1). Wastewater effluent (WWE) from South Plant and West Point is treated with secondary processes plus a chlorine disinfection step. Briefly, primary treatment removes particulates containing organic chemicals and metals, and secondary treatment removes additional organics via biodegradation by naturally occurring bacteria in aeration tanks, followed by particulate removal via clarifiers. Immediately prior to discharge, King County WWE is disinfected with sodium hypochlorite. At Brightwater, WWE undergoes the same process but uses a membrane bioreactor instead of clarifier in the secondary treatment process.

Table 1. Latitudinal and longitudinal coordinates of estuarine water outfall locations

Location	Outfall 1	Outfall 2	Outfall 3
Latitude/Longitude coordinates (d, m, s)	47° 39' 40" N 122° 26' 47.0" W	47° 36' 10" N 122° 25' 44.4" W	47° 46' 41" N 122° 24' 48" W
Identifying name:	West Point	South Plant	Brightwater

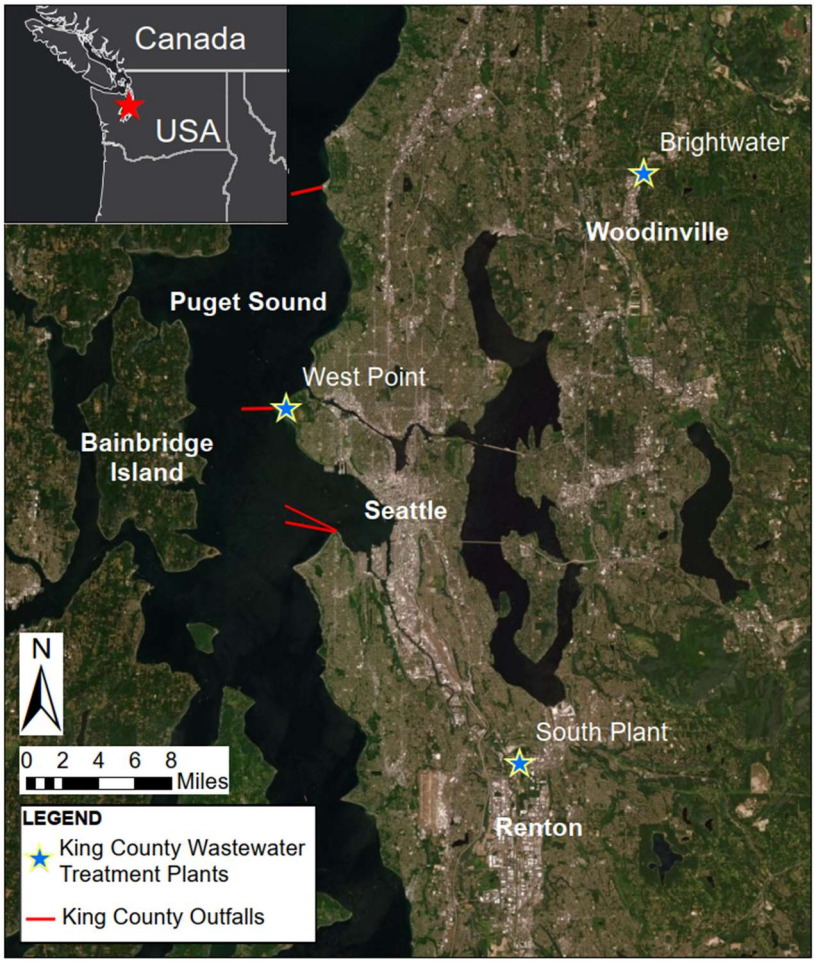

Figure 1. King County Wastewater Treatment Plants and their outfalls in Puget Sound.

1.1.3 Goals and Objectives

The goal of this project was to evaluate the occurrence and potential biological effects of CECs and other organic contaminants from King County WWTP effluents entering Puget Sound to:

- Provide baseline information on source inputs (i.e., environmental concentrations) of these chemicals in effluent and surface waters nearby outfalls and reduce the uncertainty assessing their biological impacts in the Puget Sound.
- Use quantifiable biological indicators of toxic effects to characterize differential responses between controls and effluent.
- Determine bioaccumulation potential for effluent related contaminants that can be used to evaluate potential risk.

1.1.4 Project Task Overviews

There were four primary tasks in this project, as described below.

Task 1. Chemical characterization of wastewater effluents.
We collected a single 24-hour time weighted composite sample of effluent from South Plant, West Point, and Brightwater WWTP effluent during a high-flow event and, several months later, during a low-flow event. For each event we characterized chemical occurrence using two different analytical approaches. For the first event, samples were analyzed for a large suite of targeted contaminants, focusing on those that may affect the health of Chinook salmon or SRKWs. The results are summarized in Appendix A. For the second event, effluent samples were analyzed with high resolution mass spectrometry (HRMS) instrumentation to support non-target screening approaches to understand the breadth of constituents in wastewater effluent.

Loading estimates for selected contaminant groups were developed from the chemical analytical results as well as flow information provided by King County for each WWTP.

The characterization included concentrations and mass loading estimations for selected chemicals or chemical groups, and an evaluation of their potential to cause adverse effects in juvenile Chinook. Comparison among WWTPs highlighted regional differences in contaminant concentrations, as noted in our previous study for Puget Sound (Meador et al. 2016).

Task 2. Chemical characterization of estuarine waters near WWTP outfalls.
We sampled estuarine waters near wastewater outfalls in Puget Sound to characterize the potential impacts of effluent in receiving waters. Estuarine samples were collected during one event in coordination with the WWTP low-flow effluent sampling (see Task 1). All samples were analyzed with both targeted (Appendix A) and non-targeted methods for chemical characterization. The estuarine sampling was performed with the support of personnel from King County Environmental Laboratory (KCEL) Field Service staff, and utilized the King County research vessel, the SoundGuardian.

Task 3. Laboratory exposures of juvenile Chinook salmon.
We conducted a 10-day exposure experiment consisting of a dilution series of whole effluent to juvenile Chinook salmon. This work was performed at the Aquatic Toxicology Lab at the Puyallup Research and Extension Center of WSU (WSU-PREC) in Puyallup, WA. We assessed a variety of molecular, cellular, and physiological endpoints to compare treatment effects among exposed and control fish. These included vitellogenin production (exposure to estrogenic compounds indicating effects to the endocrine system), brain and gill NKA activity (exposure to SSRI inhibitors and pesticides indicating effects to brain function and osmoregulation), and cortisol and glucose (exposure to pharmaceuticals and other compounds

indicating impairment of the stress axis). We also analyzed plasma for a large suite of blood chemistry parameters (enzymes, ions, lipids, etc.) and livers for indicators of metabolic changes. Metabolomic analysis examined more than 300 metabolites that were used to assess important physiological changes caused by effluent exposure.

Task 4. Bioaccumulation modelling and impacts assessment.
Using data derived from the chemical characterization assessment and the laboratory study, we modelled bioaccumulation of contaminants associated with wastewater effluent and provided predictions of potential adverse effects to biota, especially juvenile Chinook, with the fish plasma model.

1.2 Description of Deliverables

Thirteen prior deliverables were completed for this project. All deliverables were submitted to King County personnel for review prior to finalization; final versions were prepared based on consideration of all comments received. Final versions of each deliverable are available in the project files. The deliverables are listed below.

- Deliverable 1. Sample and Analysis Plan (SAP) - Entire Study
  Broad overview of the study design and individual tasks to be completed.
- Deliverable 2. SAP for Effluent and Estuarine Waters Chemistry
  Modified version of the original SAP with more detailed descriptions of methodologies for effluent and estuarine sampling.
- Deliverable 2.1.1. SAP for High Flow Sampling
  Detailed descriptions of methodologies for sampling effluent from the high flow event.
- Deliverable 2.1.2. High Flow Sampling Report and Appendix
  Detailed methodology of the high flow sampling after it was conducted. This included detailed day-of protocols, WWTP flow data, and any changes to the anticipated methodology.
- Deliverable 2.1.3. and 2.2.3. Chemistry and Data Quality Report
  Water chemistry data from the high flow, low flow, and estuarine sampling and tissue and water chemistry from the laboratory exposure study. The report focused on QAQC and data quality. This did not include summary results or other findings or implications of chemical occurrences.
- Deliverable 2.2.1. SAP for Low Flow and Estuarine Waters Sampling
  Detailed descriptions of methodologies for low flow effluent and estuarine sampling.
- Deliverable 2.2.2. Low Flow Sampling Report and Appendix
  Detailed methodology of the low flow sampling after it was conducted. This included detailed day-of protocols, WWTP flow data, and any changes to the anticipated methodology.
- Deliverable 2.3.1. SAP for Laboratory Exposure
  Detailed descriptions of the sampling plan for the laboratory exposure.
- Deliverable 2.3.2. Laboratory Exposure Report and Appendices
- Detailed methodology of the laboratory exposure after it was conducted. This included detailed day-f protocols, sampling charts, raw data collected during exposure, and any changes to the methodology.
- Deliverable 2.3.3. Laboratory Exposure Report
  Methodologies and results of analyses and assays from the laboratory exposure.
- Deliverable 2.4.1. Bioaccumulation Modeling Methods Plan

Detailed methodology of the bioaccumulation modeling.

- Deliverable 2.4.2. Bioaccumulation Modeling Report
  Results and discussion of the bioaccumulation modeling.

1.3 Background and Context of Investigation

This section provides background on juvenile Chinook salmon, a vulnerable life stage of an important prey species for Southern Resident Killer Whale, and some common measures and tools that are used to evaluate the impacts of exposures to anthropogenic contaminants.

1.3.1 Juvenile Chinook Salmon

Chinook salmon (*Oncorhynchus tshawytscha*) are important to the Pacific Northwest culture and economy and are a vital food resource for critically endangered southern resident killer whales (*Orcinus orca*) (Ford & Ellis 2006; Hanson et al. 2021). Chinook populations have declined drastically (approximately 60% since 1984) across the Pacific Northwestern U.S. in recent decades, and one source of stress is chemical pollution from WWE (Ecology & King County 2011). The juvenile life stage for salmonids is typically a bottleneck for survival. Smolting – the physiological changes observed during the outmigration of juvenile salmon to saltwater – is a challenging life phase that requires additional energy expenditure. Outmigrating salmon undergo many physiological changes, including rapid growth and development, making them more susceptible to the damaging effects of toxic contaminant exposure (Meado 2014; McCormic 2012). Juvenile salmon must be physically fit enough, grow to a competitive size, and gain enough nutrition and lipid content to survive migration and their first winter in marine waters (Zabel et al. 2004; Biro et al. 2004; Burrows 1969; Spromberg & Meador 2005).

It is estimated that one-third of the Chinook salmon migrating through Puget Sound in Washington State are exposed to toxicants at high enough concentrations to impair their health (O'Neill et al. 2015). Juvenile Chinook are particularly vulnerable to pollution in Puget Sound because they spend more time in estuaries compared to other salmonids (Quinn 2005). Additionally, Chinook may accumulate higher concentrations of CECs than other salmonids and fish species due to feeding at higher trophic levels and high rates of gill ventilation. Multiple aspects of Chinook physiology can be altered by exposure to contaminants, which can cause deleterious individual or population-level effects.

1.3.2 Bioaccumulation modeling and fish plasma model

1.3.2.1 Bioaccumulation modeling

In many cases, it is important to translate aqueous exposure concentrations to tissue concentrations (whole-body and plasma) for a more complete understanding of potential toxic effects. Several studies and reviews have examined the utility of using tissue concentrations as the dose metric, which provides an improved understanding of the relationship between exposure and toxicity (Escher and Hermens 2004, Meador et al. 2011). Tissue concentrations can then be used to predict potential adverse concentrations in salmonids based on existing literature. For this task, we applied bioaccumulation models for CECs and legacy compounds as described by several authors. For legacy compounds such as PCBs, we will use well established equilibrium partitioning models such as those highlighted in Meador (2006) and Meador et al. (2017). These models provide relatively accurate predictions of tissue concentrations for many compounds that can be used to evaluate toxicity, which may be compiled from

literature values. For some of the ionizable pharmaceuticals, more recent and updated methods accounting for variability in partitioning due to aqueous pH were employed as described by several authors.

We also used bioaccumulation modeling to evaluate the differences between observed and predicted water and tissue concentrations during the laboratory study as well as to predict water and tissue concentrations for the offshore marine waters of central Puget Sound based on effluent concentrations. In many cases, observed tissue concentrations are a result of aqueous concentrations occurring below their analytical limit of detection. Conversely, observed water concentrations may result in tissue concentrations that are below the limit of detection for tissue. Even though the predicted concentrations may be relatively low, some are potentially capable of eliciting toxic effects. Importantly, when many of these "below detection limit" compounds with the same or similar mechanism of action are added together, their summed concentrations may result in levels that may be potentially toxic. This is especially critical with wastewater effluent as there are likely a large number of poorly characterized compounds, which are generally not included in routine chemical analysis, but can be biologically important.

1.3.2.2 Fish Plasma Model for Predicting Adverse Effects

There are several ways to evaluate the potential toxicity of CECs, including bioassay results, alteration of biochemical and physiological pathways, biomarker responses, and the fish plasma model (FPM). Various endpoints can be used such as mortality, inhibited growth or behavior, altered gene transcripts, or altered physiological metabolites. For most CECs, including pharmaceuticals, such data for fish are limited.

The FPM, as described by Huggett et al. (2003) and explored by several researchers, was developed to assess the potential for adverse effects in fish. It is based on comparing observed or predicted plasma concentrations of pharmaceuticals and personal care products (PPCPs) in fish, to the effects levels that are characterized for humans. Comparing therapeutic levels in human plasma and fish plasma provides a basis to determine the relative risk to fish based on the similarity of those levels.

A key assumption of the FPM is the degree to which drug targets are conserved between fish species and humans, but also the hypothesis that human pharmaceuticals will interact with such targets to cause a similar, target-mediated pharmacological response as observed in humans. In this regard, the evolutionary conservation of a number of structurally and functionally conserved protein targets of drugs has been demonstrated in zebrafish (*Danio rerio*) (Gunnarsson et al. 2008) and other aquatic species (Brown et al. 2014). Gunnarsson et al. (2008) examined 1,318 human drug targets and found that 86% were conserved in zebrafish. Margiotta-Casaluci et al. (2014) found similarities in response for fish and humans at similar plasma concentrations for fluoxetine, which validates the cross-species extrapolation approach for that response pathway. More importantly, many pharmaceuticals can induce effects in fish that are unexpected. For example, metformin, a medicine for diabetes, caused reproductive effects in fish at environmental levels (40 ppb), which was an unexpected endocrine disruptor effect (Niemuth and Klaper 2015). In general, there is a paucity of data comparing fish and human responses in terms of magnitude and dose to pharmaceuticals; however, assuming similarity in response based on the above three studies is a valid although somewhat uncertain assumption until more data are available.

Another noteworthy assumption for the fish plasma model is that human therapeutic effect concentrations are generally considered as adverse physiological levels for fish, which is likely the case for many drugs that can alter behavior, metabolism, endocrine systems, and other physiological functions. In most cases, humans take these pharmaceuticals to correct an unhealthy condition. These effects may be beneficial for humans but can be deleterious for fish that rely on normal lipid metabolism, behavioral cues, and hormone levels to successfully complete their life cycle. Fish have highly tuned physiological systems that control a number of higher-level functions and often deviations from homeostasis result in adverse effects (Beyers et al. 1999).

1.3.3 Endocrine Disruption

A large number of xenobiotics in WWE are known to be estrogenic to fish, including, but not limited to, oral contraceptives (Aherne & Briggs 1989; Balaguer et al. 2017), some organochlorine pesticides (Bitman & Cecil 1970), bisphenols (Mihaich et al. 2012; U.S. EPA 2005a), polychlorinated biphenyls (Kloas et al. 2000), alkylphenols (Jobling et al. 1996; White et al. 1994), polycyclic aromatic hydrocarbons (PAHs) (Thomas et al. 2009), perfluorinated compounds (PFCs) (Balaguer et al. 2017), β-sitosterol (MacLatchy & Van Der Kraak 1995), tributyltin (Lagadic et al. 2017), and surfactants (Sumpter & Jobling 1995, Jobling et al. 1995). Exposure to these compounds can lead to the production of vitellogenin, an established biomarker of exposure to endocrine disrupting compounds (EDCs; Sumpter & Jobling 1995). Vitellogenin is a protein produced in the liver that is used by growing oocytes in the ovaries to produce egg yolk. Vitellogenin is typically present at high concentrations in mature female fish and very low or undetectable concentrations in juveniles and mature male fish.

If mature male or juvenile fish are exposed to estrogenic compounds, vitellogenin can be produced and detected in the blood. Increased vitellogenin levels in adult male fish are associated with intersex traits, including oocytes in the testis (Huang et al. 2000). In juvenile fish, exposure to EDCs before sexual differentiation is associated with higher ratios of females in exposed populations and decreased testis growth rates in males (Mikula et al. 2009; Orn et al. 2003; Jobling et al. 1996). Juvenile and male fish cannot store vitellogenin, so the production of this protein can cause kidney lesions and necrosis in males and hinder reproductive fitness (Schwaiger et al. 2000; Zha et al. 2007; Thorpe et al. 2007). Additionally, exposure to EDCs that impact reproductive success can have adverse population-level effects over generations (Spromberg & Meador 2005).

1.3.4 Na^+/K^+ ATPase activity

The Na^+/K^+ ATPase (NKA) enzyme is an essential active transport mechanism throughout the body, controlling ion homeostasis and regulating neuronal excitability. It requires energy to transport sodium and potassium across cell membranes against concentration gradients. By maintaining the ion potential of sodium and potassium, NKA controls resting membrane potential, cell volume regulation, neuronal activity, secondary active transport, and signal transduction (Evans 1987; McCormick 1993). The energy required for this could otherwise be used for growth, reproduction, behavior, and immune response. While this pump provides the same function in all parts of the body, it affects different pathways depending on its location.

NKA is ubiquitous in the brain, where it consumes approximately 40-50% of generated ATP (Zhang et al. 2012). Inhibiting brain NKA activity can cause weakened synaptic responses, learning impairment, memory deficits, and long-term depression in mammals (Baldissera et al. 2020; Zhang et al. 2012). Some

psychoactive pharmaceuticals can alter NKA activity in the brain. Sodium and potassium channel blocker medications like lidocaine, oxcarbazepine, and some antiarrhythmic agents directly inhibit brain NKA in human patients, and most medications are minimally removed during the wastewater treatment process (Monteiro & Boxall 2010). Lajeunesse et al. (2011) demonstrated that chronic *in vivo* exposure to WWE and acute *in vitro* exposure to commonly prescribed SSRIs (paroxetine and fluoxetine) readily inhibited NKA activity in the brains of brook trout (*Salvelinus fontinalis*). In addition to pharmaceuticals, previous studies have shown that biocides and metals can inhibit brain NKA (biocides: Sarma et al. 2010; Li et al. 2016; Das & Mukherjee 2003; Tabassum et al. 2015; metals: Shaw et al. 2012; Maiti et al. 2010).

In gills, proper NKA function is essential to fish health because they reside in an aquatic environment. In freshwater, fish need to actively replace sodium, potassium, and other ions; in saltwater, they need to actively excrete ions from their body. Gill NKA activity increases drastically as a juvenile salmonid migrates downstream in preparation for its transition to saltwater. As a result, increased NKA activity often indicates that salmonids are smolting (Schrock et al. 1994; Beeman et al. 1991; Madsen et al. 1995). Since gills are a primary site for toxicant exposure, their cellular functions can be easily disrupted in polluted waters. Toxicants in pulp mill effluent (Parvez et al. 2006), metals (Atli & Canli 2007), mercury (Stagg et al. 1992), and venlafaxine (Best et al. 2014) can inhibit gill NKA, potentially impairing a fish's fitness, particularly during migration. To date, no known studies have documented the effects of municipal WWE on gill NKA in fish.

1.3.5 Stress

Cortisol and glucose are commonly measured in fish blood as indicators of stress. Cortisol is a hormone synthesized in the kidney during a neuroendocrine cascade in response to a stressor. The production of cortisol and adrenaline (the primary catecholamine hormone produced) allows a fish to manage in a fight, flight, or coping response. These hormones can cause a slew of cardiovascular and respiratory responses, including increased oxygen distribution, changes in osmotic balances via increased NKA activity (Madsen et al. 1995; Liew et al. 2015), increased glucose, and changes in free fatty acids and proteins (Schreck & Tort 2016). Glucose is a monosaccharide energy source produced as a secondary stressor response. Adrenaline instantly mobilizes glucose for energy to respond in a fight-or-flight manner. In contrast, cortisol is an adjustment hormone that directs energy to restore pre-stress baseline physiological conditions over minutes to days.

While cortisol and stress-related glucose production can be beneficial for short periods, chronic production can take energy away from other necessary processes in the body. For example, long-term exposure to stressors in fish can cause immunosuppression (Yada & Tort 2016), decreased growth rates (Sadoul & Vijayan 2016), lower survival rates (Schreck & Tort 2016), and impaired predator avoidance behavior (Noakes & Jones 2016; Schreck & Tort 2016). Previous studies have shown that exposure to WWE and pharmaceuticals like fluoxetine and diazepam cause increased baseline cortisol and glucose levels and decreased cortisol and glucose spikes in response to external stressors (Gauthier et al. 2020; Pottinger et al. 2013; de Abreu et al. 2014; Ings et al. 2011; Sebire et al. 2015). These changes indicate an impaired neuroendocrine stress response, which could lead to fish not responding appropriately in a fight-or-flight response.

1.3.6 Metabolic Indicators

It is challenging to assess toxic effects on metabolism as there is no one reliable indicator, so several metrics must be evaluated simultaneously. Biochemical parameters in blood plasma in fish can be used to assess overall nutrition (e.g., total protein, cholesterol, calcium), organ status (e.g., amylase, alanine aminotransferase, creatinine, albumin), and lipid metabolism (e.g., triglycerides, lipase, albumin, phosphorous). Generally, a decrease in each of these parameters represents poor health, except for alanine aminotransferase and creatinine, for which an increase indicates damage to the liver and kidney, respectively. Different combinations of these parameters have been used extensively in the literature to assess fish health and nutrition.

Juvenile salmonids must maximize the ability to store lipids and accumulate protein to survive the energetically taxing bottlenecks of migration and their first winter in marine waters (Burrows 1969; Biro et al. 2004). Several studies have shown that migration through a contaminated estuary is associated with reduced growth, a critical survival endpoint for juvenile Chinook (Duffy et al. 2006; Zabel et al. 2004; Lundin et al. 2021; Meador et al. 2006). Fish exposed to contaminants like PAHs (Meador et al. 2006) and polyvinyl chloride microparticles (Iheanacho & Odo 2020) can induce starvation-like symptoms, reduce protein accumulation, and impede lipid storage (Meador et al. 2018). Exposure to metabolism-disrupting compounds, such as organotins, phthalates, flame retardants, bisphenol A (BPA), PCBs, organochlorine and neonicotinoid pesticides, and alkylphenols, is associated with a range of metabolic problems from the alteration of fat cell development, energy metabolism, and lipid homeostasis to neuroendocrine regulation of food intake in mammals and fish (Heindel et al. 2017; Saunders et al. 2015; Cocci et al. 2019). These studies indicate that metabolism is affected by exposure to xenobiotics, many of which are prevalent in WWE.

Studies of individual endogenous metabolites (small-molecule substrates and intermediates of cellular processes) have been informative for detecting differences in metabolic function for fish exposed to a variety of CECs and whole effluent (Al-Salhi et al. 2012; Jordan et al. 2012; Simmons et al. 2017; Meador et al. 2017). Consequently, metabolite profiling is a powerful indicator of altered physiological pathways necessary for homeostasis. These pathways are crucial for myriad functions including energy generation and utilization, reproductive function, growth, behavior, immune function, and many others. Minor alterations of these important metabolic functions, especially during the juvenile or early life stages for fish may lead to increased mortality, impaired growth, or reduced reproductive fitness (Beamish and Mahnken 2001). Comparisons of metabolite levels found in plasma or liver can be used to identify metabolic pathways altered by exposure to contaminants and provide insight to the mechanisms of adverse responses. Metabolomic analysis is important for assessing impacts from complex mixtures of environmental contaminants, such as wastewater effluent, because such data can help identify multiple impaired pathways that contribute to the overall adverse response (Samuelsson and Larsson 2008; Pomfret et al. 2020).

2 METHODS

2.1 Wastewater and Estuarine Water Collection and Analysis

Two treated WWE samples were collected by KCEL personnel from each of three municipal treatment plants operated by King County (South Treatment Plant, West Point Treatment Plant, and Brightwater;

Figure 1). Samples were collected at the NPDES compliance points (i.e., final effluent) of each facility. One sample was collected at each plant under high-flow conditions, and one sample was collected at each plant under low-flow conditions. All samples were collected as 24-hour composites. High-flow conditions were defined as having a sustained flow rate twice the average base flow for each facility and designed to characterize the inclusion of surface water runoff and/or infiltration in the combined systems of each facility. These were collected in 2021 on February 21-22 (South Plant and Brightwater) and March 24-25 (West Point). Low-flow samples were collected after at least five days of dry weather to capture only wastewater. These were collected on June 23-24, 2021.

Two ISCO 3710 autosamplers were set up at the NPDES Permit Compliance Points at the West Point and South Plant facilities (King County Location ID: FESD01 at WP, A5001 at South Plant). West Point and South Plant each had one ISCO collecting effluent samples in a 5-gal high-density polyethylene (HDPE) container and one ISCO collecting effluent samples in a 10-gal glass carboy. One ISCO sampler was setup at Brightwater (King County Location ID: A6101) to collect in a 10-gal glass carboy. Each ISCO was programmed to capture an aliquot of WWE every 15 minutes over the 24-hour sampling period (~ 380 mL per 15 minutes in the glass carboy and ~ 190 mL per 15 min in the HDPE container). All sample containers and sample collection tubing were cleaned with methanol and ultrapure deionized water before sampling. Sample containers were immersed in wet ice during the sample collection period. Following sample collection, all samples were transported from the WWTP facilities to the KCEL, by KCEL field staff, where they were transferred under chain-of-custody protocols to UW and WSU personnel. UW and WSU personnel split the samples from West Point and South Plant into pre-cleaned 0.5- or 1-L glass or HDPE containers, depending on the contaminant class, for analysis by SGS-AXYS Analytical Services Ltd. (SGS-AXYS; Sidney, British Columbia, Canada). Samples were kept on ice and shipped to SGS-AXYS in coolers via overnight delivery. Contaminant classes tested by SGS-AXYS and the methods used to test for them are listed in Table 2, and a complete list of contaminants is in Appendix A. Despite being packed with ice packs, some samples arrived at SGS-AXYS above 4^{o}C: West Point low flow (7.7-8.2^{o}C), South Plant low flow (4.7-5.9^{o}C).

Additionally, 4 L of each sample was split into a precleaned, 4-L glass amber jugs for analysis via HRMS at the UW Tacoma facilities at the Center for Urban Waters (CUW). Each sample was held on wet ice at <4°C for transport to the laboratory and processed within 48 hours. Brightwater samples were only analyzed at UW Tacoma facilities at CUW. Brightwater samples were not included in the SGS-AXYS analyses due to high costs of the SGS-AXYS analyses.

Estuarine samples were collected on June 9, 2021, from five locations throughout Puget Sound (Figure 2). Deep composite samples (30-50 meters at 5-meter intervals) were collected at each site; a shallow composite sample was collected at West Point North (5-20 meters at 2.5-meter intervals, see Figure 2). Samples were collected by King County personnel on the research vessel SoundGuardian with a CTD rosette deployment at each location. All samples were collected in 5-L polyvinyl chloride Niskin water samplers (General Oceania Model 1010). The Niskin samplers were precleaned and flushed with Puget Sound water before sampling. Following collection at each site, the 5-L samples from each Niskin were split evenly into a glass carboy and an HDPE carboy. All samples were held on wet ice during the collection period. A field blank was collected by filling three Niskin sampling bottles with ultrapure deionized water from the UW Tacoma at CUW and transferring it to cleaned sample containers.

All estuarine samples were split into precleaned sample containers for SGS-AXYS and the UW Tacoma CUW laboratories. Samples for SGS-AXYS were packed with ice packs and shipped via overnight delivery

services. The following samples arrived at SGS-AXYS above 4°C: West Point North (9.5-9.6°C), Reference (10.3°C), and Alki (5.1°C). Samples for HRMS were split into precleaned,4-L glass amber jugs (four per site) and held on wet ice until analysis. All water samples were processed within 48 hours of collection.

2.2 Laboratory Exposure Study

2.2.1 Laboratory Exposure Study Source Water and Sampling

Water used for the Chinook exposure study was collected from South Plant every other day from June 21 through June 29, 2021. Grab samples of WWE were collected post-chlorination at the end of the disinfection channel using a 3-L stainless steel bucket. Approximately 25 grab samples were composited in a 75-L stainless steel drum, which was transported to WSU-PREC for the initial tank fills or renewals of WWE diluted to one of five concentrations (20%, 5.3%, 1.4%, 0.4%, 0.1%). Water samples for analytical chemistry were taken from 20%, 5.3%, 1.4%, and the 0% WWE clean water control (described below) to assess the exposure of fish to targeted chemicals (analytes listed in Appendix A). Samples were composited across replicates and across time to estimate the average exposure concentrations across the study. Samples were taken when water was renewed (see *2.2.3 Juvenile Chinook Salmon Exposures*) and composited in a stainless-steel container, which was frozen between renewals. The two lowest-effluent concentrations were not sampled because of cost constraints and expected non-detections for most analytes.

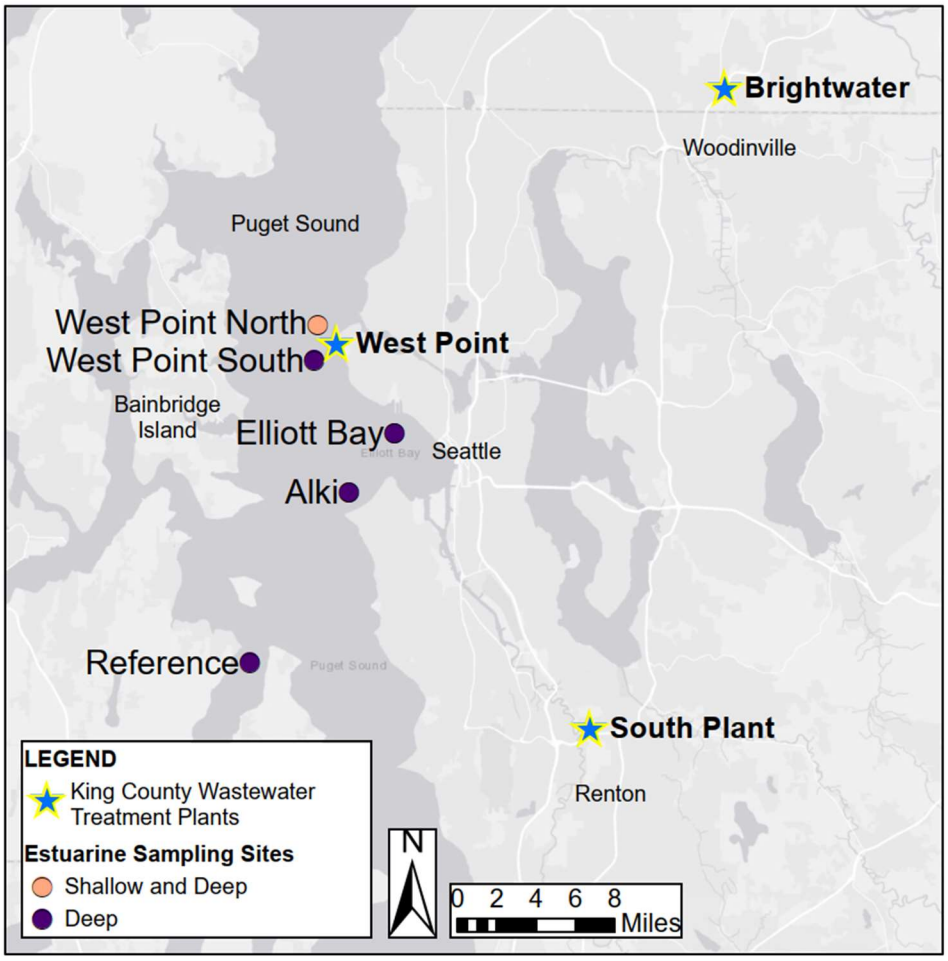

Figure 2. Sampling locations for estuarine waters and wastewater effluent from treatment plants operated by King County in Puget Sound, Washington, USA.

Table 2. Contaminant classes for samples submitted to SGS-AXYS for targeted analysis. Whole wastewater effluent (WWE) only refers to South Plant and West Point samples. Salmon exposures used only WWE from South Plant.

Contaminant Class	# of Analytes Measured	Water Sample Type		
		Estuarine	Whole WWE	Salmon Exposures
Alkylphenols	4	X	X	X
Bisphenols	6	X	X	X
Dioxins/ Furans	25		X	
PFAS	40	X	X	X
HFRs	25	X	X	X
Hormones	18		X	X
PAHs	76		X	
PBDEs	40		X	
PCBs	160		X	
Pesticides	62		X	
Phthalates	11	X	X	
PPCPs	141	X	X	X

Contaminant class abbreviations: PFAS = Per- and Polyfluorinated Substances, HFRs = Halogenated Flame Retardants, PAHs = Polycyclic Aromatic Hydrocarbons, PBDEs = Polybrominated Diphenyl Ethers, PCBs = Polychlorinated Biphenyls, PPCPs = Pharmaceuticals and Personal Care Products

2.2.2 Juvenile Chinook Salmon Pre-Exposure Husbandry

The laboratory study was conducted in the Aquatic Toxicology Lab at WSU-PREC. Approximately 500 young-of-year Chinook salmon (about 6 g each) were donated by the Puyallup Tribal Hatchery in May 2021. These fish were reared in three 50-L fiberglass tanks maintained at 12°C in a custom recirculating water system under a 12:12 h photoperiod. Water was dechlorinated municipal water, subject to reverse osmosis (RiOs; Millipore Sigma), and conditioned with buffered salt stocks to 80-120 mg/L total hardness as calcium carbonate, pH 7.4 ± 0.2, and 8.1 mg/L dissolved oxygen (DO). Fish were fed pellet food (Bio-Oregon) at a rate of 1.8% body weight (bw)*day^{-1} based on their average wet weight. On June 4, the fish were diagnosed with furunculosis (caused by the bacterium *Aeromonas salmonicida*), which is pervasive in surface waters and especially prevalent in hatcheries. All juvenile Chinook were treated with the antibiotic Romet TC (active drug ingredients: ormetoprim and sulfadimethoxine) for ten days starting on June 7 under the supervision of the WSU Office of the Campus Veterinary, which is very common among hatcheries raising juvenile salmonids before release into watersheds. We detected concentrations of these antibiotics in whole-body fish; however, the half-life for these is long in skin and fat but short in other tissues and likely not highly bioavailable. All fish were treated the same in terms of antibiotic administration and we cannot say with any certainty how this pretreatment affected fish. The

three tanks housing the Chinook were on the same recirculation system, so were connected and exposed to the same water. While all tanks were treated, 93% of the mortalities (n=58) occurred in Tank 2. After antibiotic treatment, we conducted the experiment using fish only from Tanks 1 and 3 (n=1 and n=3 mortalities, respectively) to reduce further any influence disease might have had on the exposure study. The wastewater exposure experiment began five days after the end of Romet TC treatment (June 21, 2021).

2.2.3 Juvenile Chinook Salmon Exposures

Exposures included six treatments: five concentrations of WWE (20%, 5.3%, 1.4%, 0.4%, and 0.1%), arrived at by using a dual geometric concentration series with a dilution factor of 3.8 (calculated via the equation $(20/0.1)^{1/4}$), and a 0% WWE clean water control. The dual geometric concentration series allowed us to pick the upper and lower bounds of desired effluent concentrations with evenly spaced concentrations between treatments when log transformed with a base 3.8. Exposure water was renewed (83% of volume) every other day with freshly collected WWE from South Plant (see Water Sample Collection section for collection procedures). Total residual chlorine (TRC) in collected WWE samples ranged from 0.27-0.55 mg/L (Table 2), using a HACH Pocket Colorimeter II. EPA recommends TRC <0.011 mg/L for acute exposure experiments with fish (U.S. EPA 2002). To neutralize TRC, we added anhydrous sodium thiosulfate (STS) at the recommended (stoichiometric) rate of 6.7 mg/L per 1 mg/L TRC (U.S. EPA 2002; Table 3). The 0% control tank received the same amount of STS as each 20% effluent tank. Collected WWE was also tested for total ammonia nitrogen (TAN, Table 3) using an API Ammonia Aquarium Test Kit to ensure unionized ammonia concentrations were below fish health effects thresholds in 20% exposure tanks. TAN concentrations were 25-50 mg/L in whole WWE, resulting in 5-10 mg/L in the 20% effluent tanks. Unionized ammonia would be 1% of the TAN at the pH of exposure waters (U.S. EPA 2002), resulting in a maximum unionized ammonia concentration of ~ 0.1 mg/L, which is below effects thresholds, even at the 20% effluent concentration (<0.1 mg/L unionized; Thurston and Russo, 1983). Anticipated TAN concentrations dictated the maximum concentration (20%) of WWE used in the experiment.

Three hundred thirty-six fish were exposed in 44 tanks, including seven replicate tanks per treatment, each containing eight juvenile Chinook (Appendix B). Each replicate tank (10-gallon glass aquarium) contained 32 L of exposure water. All tanks were held in water baths with submerged chillers to maintain the temperature of the exposure waters at approximately 11°C. Tank locations were randomly assigned a concentration and replicate number. At the start of the exposure, fish addition was randomized by adding two fish to each randomized tank location before adding more fish. Each tank was covered with a white plastic lid and supplied aeration via a dedicated air stone attached to polypropylene tubing.

The semi-static exposures ran for ten days with renewals of fresh WWE every two days (Days 2, 4, 6, 8). During renewal, 7 L of the aged water was maintained in each tank to reduce the stress on the fish. Clean renewal water was added immediately after the aged water was removed. Because it was not feasible to pre-mix the large volumes of renewal water needed, renewals proceeded by adding clean water, followed by the appropriate volume of STS-treated effluent within 30 minutes to achieve the desired final effluent concentration. Extra volume was added to tanks from which water was sampled

for analytical chemistry to achieve the same volume of water across replicates and treatments after renewal.

Table 3. Chlorine and ammonia concentrations in wastewater effluent (WWE) used for initial dosing and renewals during the exposure study, including total available chlorine (TAC), added sodium thiosulfate (STS), and total ammonia nitrogen (TAN) concentrations.

	TAC (mg/L)	STS (mg)		TAN (mg/L)
Date	100% WWE	Added to 75 L WWE	Added to each 32 L control tank	20% WWE
6/21	0.55	276.4	25	10
6/23	0.27	135.7	9.05	5
6/25	0.30	150.8	10.05	5
6/27	0.30	150.8	10.05	5
6/29	0.37	185.9	12.4	5

Water quality metrics, including dissolved oxygen, temperature, conductivity, and pH, were measured daily in each tank (Table 4)**.** All tanks were given the same amount of food daily (full or half ration) on all but day 10. Fish were initially fed a full ration (1.8% body weight), but when most fish did not appear to eat, all tanks were fed a half ration the following day. When fish appeared to eat most of the food, all tanks were fed a full ration the next day. Following feeding, excess food and waste were siphoned out of each tank using a glass pipette.

The tank room at the WSU-PREC Aquatic Toxicology Lab was not equipped with central air conditioning (A/C) at the time of the study, so the clean system water for renewing exposure waters is normally at ambient air temperatures. The middle of the experiment fell during a record heatwave in western WA (above 35^{o}C for several consecutive days), so multiple efforts were needed to prevent exposure waters from becoming too warm. Fans were installed to draw cool air down the hallway from rooms with central A/C. On days 7 and 8 of the experiment (June 28 and 29), tank room temperatures reached 29^{o}C. We pre-chilled the clean system water used for renewals in several empty, pre-cleaned fish tanks. However, the volume of water needed to renew all of the tanks exceeded the storage capacity of the pre-chilling system such that after renewing all treatments except 0.1% on day 6, the water bath on the pre-chilling system reached 17^{o}C. We saved the remaining WWE at 4^{o}C to renew the 0.1% treatment tanks on day 7 (June 28) to allow more system water to cool. To avoid this problem on subsequent days, we made ice blocks from system water and added them to the chilling water baths as chilled water was removed during renewals. These 4-L ice blocks were made using system water in pre-cleaned stainless-steel buckets to prevent contamination of the exposure water with chemicals that would leach from plastic buckets. Due to the excess heat, the temperature in some exposure tanks was elevated immediately after renewal on days that water was renewed. Despite the ambient heat, the water bath for the exposure tanks were maintained at temperatures near 11^{O}C during days without renewal (Table 4).

Table 4. Average water quality metrics from all exposure tanks. For temperature, All Days includes temperatures from all days, whereas Non-Renewal Days only includes data recorded on the initial fill day and remaining non-renewal days.

	Temperature (°C)				
	All Days	**Non-Renewal Days**	**DO (mg/L)**	**Conductivity (µS/cm)**	**pH**
Minimum	10.2	10.3	6.24	1,414	7.51
Maximum	15.3	12.0	16.2	1,654	8.85
Average	11.3	11.0	12.8	1,579	8.49
Standard Deviation	0.80	0.34	0.85	61	0.19

The experiment ended on Day 10, and fish were euthanized and processed across two days due to time constraints (Day 10 and Day 11). Tanks were processed in replicate order (1 through 7) for each treatment and treatments were processed in the following order: 0%, 20%, 5.3%, 1.4% on Day 10 and 0.4%, 0.1% on Day 11. At the end of the experiment, the average fish weight was 10.5 g (±1.6 g standard deviation). Upon being removed from their tanks, fish were chased with a net for approximately 10 seconds to ensure a stress response. Immediately after, fish were anesthetized four at a time with MS-222 (30-50 mg/L) in tanks immersed in an ice bath. Water and MS-222 were replaced between each group of anesthetized fish. Once anesthetized, the caudal vein was severed, and blood was extracted into heparin-coated vials (300 µL Microvette CB; Sarstedt Inc.). Vials were centrifuged (566 x *g* at 4°C for 10 minutes) to obtain plasma for targeted chemical analyses and various physiological tests. Fish were then euthanized by brainstem severance, and brains, gills, and livers were removed for subsequent analyses.

Per treatment, the number of replicates used, the number of fish per replicate, and the storage containers for each analysis are recorded in Table 5. Plasma was composited from all fish within a tank (8 fish per sample) to obtain sufficient plasma volumes. Four brains and three sets of gills were composited per tank, each in their own tube with 100 µL SEI buffer (0.25 M sucrose). All extracted tissues were saved in pre-labeled vials or bags and stored at -80°C. Any abnormal characteristics were recorded for each fish, such as liver anomalies. Liver anomalies include pale and enlarged livers and tumors in the liver. Fish tissue collection and euthanasia methods were approved by the WSU Institutional Animal Care and Use Committee (ASAF#6887).

Of the 344 fish in the study, 11 fish were excluded. Two were excluded due to poor physical quality (very small size with severely abraded fins) and nine due to accidental mortality for the following reasons: escape (3 fish), sucked into water removal hose on Day 2 (1 fish), and air stone was pulled out of tank water for ~12 hours on Day 3 (3 fish from replicate 7 of the 0.1% treatment and 2 fish from replicate 7 of the 0.4% treatment).

Table 5. Number of replicates, fish per replicate (in parenthesis), and sample container used for each analysis and tissue type. For each treatment, number of replicates and number of fish per replicate (in parenthesis) are shown.

Tissue (Analysis)	Treatment (% WWE)						Container Used
	0	0.1	0.4	1.4	5.3	20	
Brain (NKA)	5(4)	5(4)	5(4)	5(4)	5(4)	5(4)	2 mL Corning polypropylene tube
Gill (NKA)	15(1)	15(1)	15(1)	15(1)	15(1)	15(1)	2 mL Corning polypropylene tube
Livers (Metabolomics)	7(3)	7(3)	7(3)	7(3)	7(3)	7(3)	2 mL Corning polypropylene tube
Plasma (Vitellogenin)	4(8)	4(8)	4(8)	4(8)	4(8)	4(8)	0.6 mL Fisherbrand Premium Microcentrifuge tube
Plasma (Cortisol)	4(8)	4(8)	4(8)	4(8)	4(8)	4(8)	0.6 mL Fisherbrand Premium Microcentrifuge tube
Plasma (IDEXX, metabolic and stress indicators)	4(8)	4(8)	4(8)	4(8)	4(8)	4(8)	1.5 mL IDEXX white top tube
Plasma (SGS-AXYS)	--	--	2(56)	2(56)	2(56)	2(56)	2 mL Corning polypropylene tube
Whole Body (SGS-AXYS – Full Targeted Chemistry)	1(7)	--	--	1(7)	1(7)	1(7)	Aluminum foil in 710 mL Nasco Whirl-Paks
Whole Body (SGS-AXYS - PPCP Targeted Chemistry)	--	--	1(7)	1(7)	1(7)	1(7)	Aluminum foil in 710 mL Nasco Whirl-Paks

2.2.4 Plasma and Tissue Analyses

Plasma samples were shipped to IDEXX in West Sacramento, CA, on ice packs on July 7, 2021, and were processed on July 8, 2021. These samples were analyzed for: alanine aminotransferase, amylase, lipase, albumin, total protein, creatinine, cholesterol, glucose, calcium, phosphorous, and triglycerides (together identified as “IDEXX parameters”).

We measured cortisol and vitellogenin in plasma at WSU-PREC using commercial kits. Cortisol concentrations were measured using the Cortisol ELISA kit (Neogen Corporation, Item No. 402710). Plasma was diluted by a factor of 100, and each sample was run in duplicate. Vitellogenin concentrations were measured using the Ultra Sensitive Salmonid Vitellogenin ELISA (TECO, Catalog No. TE1049). Based on pilot tests with unexposed control fish, all samples were initially diluted five-fold for measuring vitellogenin, after which plasma samples were refrozen at -80°C. Because vitellogenin exceeded the calibration range for the higher % WWE treatments, plasma concentrations of vitellogenin were re-measured at dilution factors up to 100-fold. Vitellogenin concentrations were averaged between the two measurements for those concentrations that fell within the standard curve on both assay runs.

Gill and brain NKA activities were determined based on the coupled enzyme method of McCormick (1993). For each molecule of PEP (phosphoenol pyruvic acid) converted to lactate by LDH (lactate dehydrogenase), one molecule of ATP (adenosine 5-triphosphate) is generated by PK (pyruvate kinase)

for use by NKA while one molecule of NADH (nicotinamide adenine dinucleotide) is reduced by LDH. Therefore, the rate of use of ATP by NKA is measured by the reduction of NADH, which is detectable spectrophotometrically. For gill NKA activity, the composites of three distinct sets of gills ended up being too much tissue for the assay. Instead, filaments from one arch of each fish were removed with a scalpel and were run separately (n = 15 per treatment). Filaments were homogenized for 15 seconds in 200 µL SEI buffer (250 mM sucrose with 10 mM Na_2EDTA and 50 mM imidazole) with 0.1% sodium deoxycholate using a motorized Kimble Kontes pellet pestle. Homogenized samples were centrifuged at 5,000 x*g* for 2 minutes at 4°C. The resulting supernatant was added to quadruplicate wells (10 µL in each) of a 96-well plate. Two wells received 200 µL of assay mixture A with salt solution, while two received 200 µL of assay mixture B with salt solution. Assay mixtures were mixed with the salt solution before adding to the wells at a ratio of 150 µL assay mixture: 50 µL salt solution. Assay mixture A contained 5.25 U/mL pyruvate kinase, 4.2 U/mL lactate dehydrogenase, 0.22 mM NADH, 2.8 mM PEP, 0.7 mM ATP, and 50 mM imidazole. Assay mixture B contained the same ingredients, plus 0.7mM ouabain to inhibit ATPase activity. The salt solution contained 50 mM imidazole, 189 mM sodium chloride, 10.5 mM $MgCl_2 \bullet 6H_2O$, and 42 mM KCl. The linear rate of NADH reduction was measured at 340 nm every 10 seconds (or minimum interval) for 10 minutes on a microplate reader (Biotek Cytation 5). NKA activity was calculated between 3 and 10 minutes as the difference in ATP hydrolysis with and without ouabain, represented in µmol ADP/ mg protein/ hour. For brain NKA activity, composited samples were homogenized with 300 µL SEI buffer containing 0.1% sodium deoxycholate, followed by centrifugation at 5,000 x*g* for two minutes at 4°C (Kajimura et al. 2005). The resulting supernatant was diluted by a factor of 50 with SEI buffer and added in quadruplicate to a 96-well plate. The activity was measured in the same way as gills. Protein content for gills (50 dilution factor) and brains (100 dilution factor) was determined using a commercial BCA protein assay kit (Novagen, catalog number TB380).

2.2.5 Metabolomic analysis

Analysis of liver samples for endogenous metabolites was conducted by the Northwest Metabolomics Research Center in the UW School of Medicine. Their current assay attempts to measure 361 metabolites four spiked stable isotope-labeled internal standards. In our samples 185 metabolites and the four spiked standards were detected. The data were highly reproducible with a median coefficient of variation (CV) of under 5%.

Sample Preparation

Aqueous metabolites for targeted liquid chromatography mass spectrometry (LC-MS) analysis were extracted using a protein precipitation method similar to the one described elsewhere (Mathon et al. 2019, Meador et al. 2020). Salmon liver tissue samples were first homogenized in 200 µL purified deionized water at 4 ˚C, and then 800 µL of methanol containing $6C^{13}$-glucose and $2C^{13}$-glutamate (reference internal standards) was added. Afterwards samples were vortexed, stored for 30 minutes at -20 ˚C, sonicated in an ice bath for 10 minutes, centrifuged for 15 min at 14,000 rpm and 4 ˚C, and then 600 µL of supernatant was collected from each sample. Lastly, recovered supernatants were dried on a SpeedVac and reconstituted in 1.0 mL of LC-matching solvent containing $2C_{13}$-tyrosine and $3C_{13}$-lactate (reference internal standards). Protein pellets that were left over from the sample prep were saved for BCA protein assay.

Liquid Chromatography-Mass Spectrometry Assay

Targeted liquid chromatography – mass spectrometry (LC-MS) analysis of metabolites was performed on a duplex-LC-MS system composed of two Shimadzu UHPLC (ultra high performance liquid chromatography) pumps, CTC Analytics PAL HTC-xt temperature-controlled auto-sampler and AB Sciex 6500+ Triple Quadrupole MS equipped with ESI ionization source (2). UHPLC pumps were connected to the auto-sampler in parallel and were able to perform two chromatography separations independently from each other. Each sample was injected twice on two identical analytical columns (Waters XBridge BEH Amide XP) performing separations in hydrophilic interaction liquid chromatography (HILIC) mode. While one column was performing separation and MS data acquisition in ESI+ ionization mode, the other column was getting equilibrated for sample injection, chromatography separation and MS data acquisition in ESI- mode. Each chromatography separation was 18 minutes (total analysis time per sample was 36 minutes). MS data acquisition was performed in multiple-reaction-monitoring (MRM) mode. The LC-MS system was controlled using AB Sciex Analyst 1.6.3 software. Measured MS peaks were integrated using AB Sciex MultiQuant 3.0.3 software. The LC-MS assay targeted 361 metabolites (plus 4 spiked reference internal standards). In the addition to the study samples, two sets of quality control (QC) samples were used to monitor the assay performance as well as data reproducibility. One QC [QC(I)] was a pooled human serum sample used to monitor system performance and the other QC [QC(S)] was pooled study samples and this QC was used to monitor data reproducibility. Each QC sample was injected per every 10 study samples. The data were well reproducible with a median CV of 6.4 %. Generated MS data were normalized to BCA total protein count.

2.3 Bioaccumulation modeling and fish plasma model

2.3.1 Bioaccumulation Model

Simple bioaccumulation models were used to predict tissue concentrations for Chinook salmon from water exposure. We estimated steady-state bioconcentration factors (BCFs) from K_{ow} or D_{ow} values using the equation of Veith et al. (1979) as described and modified by Fu et al. (2009) and Schreiber et al. (2011) for pharmaceuticals (equation 1). In general, the K_{ow} or D_{ow} is a surrogate for partitioning into organic phases (e.g., protein and lipid). The D_{ow} is the pH-specific octanol-water partition coefficient that is needed for some ionizable compounds to more accurately reflect partitioning (Turner and Williamson 2005). Studies have shown that for all organic compounds below a $\log_{10} K_{ow}$ or D_{ow} value of 1, a whole-body tissue concentration is essentially equal to aqueous exposure concentration because the compound does not partition into lipid but does occur in the tissue's water compartment. Hence, the BCF was set to 1.41 for all $\log_{10} K_{ow}$ and $\log_{10} D_{ow}$ values below 1, which was the result of equation 1 when $\log_{10} D_{ow}=1$ (Fu et al. 2009). In general, these predictions are relatively accurate for compounds with relatively low K_{ow} values, when time is sufficient for bioaccumulation, and metabolism or passive elimination is not rapid. It is also important to note that many of the pharmaceuticals have low D_{ow} values; hence these bioaccumulation prediction equations can be relatively accurate because partitioning into organic phases is not a major factor. As with most models, variability for the predictions is expected. For bioaccumulation models, factors such as K_{ow} and D_{ow}, pH, uptake and elimination kinetics, and other factors will contribute to inherent variability of the predicted bioaccumulation factor. Some of the largest variability occurs for Kow or Dow values, whether they were determined empirically or estimate with Quantitative Structure–Activity Relationship (QSAR) models.

$$Log\ BCF = 0.85 * log\ D_{OW} - 0.70 \quad (1)$$

Values for D_{ow} were obtained with the plugin LogD within the program MarvinSketch (ChemAxon 2016). A D_{ow} for pH 8 was used for all calculations with analytes determined in estuarine samples, which is very close to the mean value for pH (= 8) determined at several Puget Sound sites (Lowe et al. 2019). For the analytes determined in laboratory water, a D_{ow} was determined for pH 8.4, which is close to the mean value for all laboratory samples (pH = 8.49). For most chemicals, structures in the form of SDF or MOL files from DrugBank and PubChem were imported to MarvinSketch for Log D_{ow} calculations. It should be noted that the term "bioconcentration" generally refers to accumulation from water only and "bioaccumulation" implies accumulation from all sources; however, bioaccumulation is used here to describe uptake during the predominantly water-based exposure.

Each predicted whole-body concentration was determined with equation 2 by multiplying the predicted BCF (equation 1) times the observed or predicted water concentration (ng/L) in diluted effluent or estuarine water. The result was divided by 1000 to convert to ng/g.

$$[Whole\ Body] = BCF * [water] \quad (2)$$

The BCFs generated by QSAR models assume steady state, which may or may not occur in fish exposed in these local estuaries. In general, the rate of elimination indicates how fast steady-state tissue concentrations will occur, and the faster the elimination the less time is required to achieve steady-state tissue concentrations for a continuous exposure (Meador et al. 1995). Because the half-life for many of these CECs is relatively rapid in humans and fish (Meador et al. 2016), steady-state bioaccumulation is expected to occur relatively quickly. Even though most of these pharmaceuticals exhibit relatively fast half-lives, they can be considered as pseudo-persistent in the environment as a result of their continuous input (Daughton 2002). For many compounds with slow rates of elimination or low rates of metabolism, the time to steady state can be very long (weeks to months), especially those exhibiting $\log_{10} K_{ow}$ values >5. Consequently, the accuracy of predicting steady-state tissue concentrations for very hydrophobic compounds ($\log_{10} K_{ow}$ >5) is often very low. This is generally the case for the perfluoro compounds, PCBs, PBDEs, dioxins, and nonylphenols.

2.3.2 Fish plasma model

Fish plasma concentrations can also be predicted with simple QSAR models and water exposure concentrations. Predicting blood:water partitioning (P_{bw}) was accomplished using the equation originally developed by Nichols et al. (1991) and modified by Fitzsimmons et al. (2001). Several authors have utilized this equation for ionizable pharmaceuticals (Du et al. 2014; Tanoue et al. 2015; Nichols et al. 2015), which was developed in the laboratory using water-only *in vivo* exposures. A factor of 0.16 accounts for the fraction of organic material in trout blood (Nichols et al. 2015), which we assumed was similar to that for Chinook salmon.

$$Log\ P_{bw} = log\ ((10^{0.73logDow} * 0.16) + 0.84) \quad (3)$$

In the same fashion as described by Fu et al. (2009) for the BCF, the P_{bw} was set to 1.70 for all $\log_{10} D_{ow}$ <1, which was the result of equation 3 when $\log_{10} D_{ow}$=1. Predicted plasma concentrations were determined by multiplying P_{bw} by water concentrations in ng/L.

$$[Plasma] = logP_{bw} * [water] \quad (4)$$

We selected a small group of CEC analytes for comparison of predicted fish plasma concentrations to C_{max} values for humans, which represent the maximum plasma concentration for the minimum therapeutic dose. C_{max} values were obtained from Mofatt et al. (2011) and DrugBank (2022). When comparing toxicity values in ecological risk assessment among disparate species, such as humans and fish, safety factors (also known as uncertainty factors and assessment factors) are usually applied. These safety factors have been discussed by several authors (Chapman et al. 1998; Duke and Taggart 2000; Huggett et al. 2003). In general, safety factors are usually applied for expected differences in toxicokinetics, pharmacodynamics, inter- and intraspecific differences (e.g., human to fish), internal partitioning, temporal sampling bias, and adjustments for converting low-effect to no-effect concentrations. As noted by Meador et al. (2016), the half-lives for many pharmaceuticals in fish are substantially lower than for humans, which also supports reduced C_{max} values for comparison. Another important factor supporting the 1% uncertainty factor comes from a study by Henneberger et al. (2022) demonstrating less binding between pharmaceuticals and plasma proteins for fish as compared to humans, meaning these compounds in fish plasma would be more bioavailable and cause effects at lower concentrations. For this evaluation, we compared each fish plasma analyte concentration to its respective 1% human C_{max} therapeutic value to determine potential adverse effects in juvenile Chinook.

We considered a select number of pharmaceuticals observed in this study that are considered likely to exhibit plasma concentrations occurring in the range of those known to elicit therapeutic or adverse effects. This was done with the Response Ratio, which is defined by equation 5.

$$\text{Response Ratio (RR)} = \text{FPCss/HtPC} \quad (5)$$

Where FPCss is the estimated fish plasma concentration at steady state and HtPC is the safety-factor adjusted human therapeutic concentrations (1%C_{max}). RR values provide a prediction for adverse effects. Ratios less than 1 indicate effects that are less likely in fish compared to ratios greater than 1 indicate a higher potential for adverse effects. At a glance, the reader can tell if an observed or predicted fish plasma concentration is likely to result in physiological effects in fish. This type of ratio also has greater utility for assessing mixtures and is more amenable to a toxic unit approach when adding ratios to determine the likelihood of adverse effects. Summed values that approach or exceed unity give the reader an easy way to quickly assess potential toxicity.

It is important to consider multiple compounds with the same mechanism of action, such as macrolide antibiotics, SSRIs, or endocrine disruptors. We can evaluate the combined effect of similar-acting compounds with an equation describing the sum of toxic units (TU) (equation 6), which assumes additivity.

$$\sum TU_{RR} = \sum_{i=1}^{n} \frac{[FPC_{ss}]_i}{HtPC_i} \quad (6)$$

For example, the SSRIs sertraline, fluoxetine, and citalopram detected in this study would be added together to generate a sum of toxic units for that class of pharmaceuticals.

2.4 Data Analysis

2.4.1 Analytical Data Review

2.4.1.1 Targeted Data QA/QC Review

The purpose of this data verification and validation process is to review how closely protocols and methods were followed during data generation. As described in the sampling report for the high-flow event, the low-flow event, the estuarine sampling, and the laboratory exposure study, all protocols and methods that were put in place were followed with one exception. The exception was that some of the water samples that were shipped to SGS-AXYS arrived at temperatures slightly above the specified holding temperatures. As described, there is the possibility of a low bias for some of the analytes in the MLA-075 (PPCP), though it is not possible to determine the extent of bias or exactly which analyte may have been affected. Based on our laboratory experience performing similar analyses on similar samples, we would expect the bias to be minimal.

Laboratory precision and bias were measured via calibration standards, check standards, and internal control samples. The reported relative percent difference of all replicate measurements of calibration standards and control standards was within method limits. These results are included in the data packages. Additional evaluation of bias was performed through a review of data flags, as described in Section 2.4.1.2.

The sensitivity of the methods, as determined by the evaluation of blanks, method detection limits, and reporting limits, was comparable to that described in the Sampling and Analysis Plan (Deliverable 2.0), and generally sufficient to meet project objectives.

The comparability of the data, based on a comparison of results obtained here to similar studies, has been done.

The representativeness of the data was addressed in the experimental design, as described in the Sampling and Analysis Plan. As noted, sampling approaches, such as the use of 24-hour composite samplers in the wastewater facilities, and depth-integrated sample collection in the estuarine samples, were used in order to maximize the representativeness of the samples. However, the systems of interest likely exhibit variability, and the ability to characterize this variability is limited in this study (as in any study) by limited sample numbers.

2.4.1.2 Targeted data - Flagged data review

A review of flagged data was performed. A description of each flag, and the results on usability associated with each flag, are included in Table 6. Note that data associated with three of the flags (V, N, and B) were evaluated further to determine if affected data were usable.

A V flag indicates that the recovery of an isotopically labelled surrogate was outside method limits. Such compounds are quantified with the isotope dilution method and recovery corrected. As such, a slight variance from the method acceptance criteria would not affect the quantification. Those compounds for which the isotopically labelled surrogate recovery was outside the method control limit for more than 50% of the control samples are listed in the Chemistry and Data Quality Report. Additional scrutiny may be warranted when evaluating associated data.

A N flag indicates that the analyte recovery was not within control limits in the spiked matrix sample. The results of the corresponding analyte in the field samples may have been similarly affected; i.e., a low recovery in the spiked matrix samples would suggest a low recovery (and low bias) in the field sample,

and a high recovery in the spiked matrix would suggest a high recovery (and a high bias) in the field sample. A list of analytes that were N-flagged in one or more control sample is listed in the Chemistry and Data Quality Report. If the spiked matrix recoveries were out of compliance for two or more samples, which may suggest a persistent, laboratory- or method-related bias, a H-flag was applied to the associated environmental sample data. If there was only one spiked matrix sample, the associated environmental sample data were H-flagged in cases of significant exceedances.

Table 6. List of analytical flags, flag definitions, and usability assessments for SGS-AXYS data.

Flag	Definition	Use
MAX	concentration is an estimated maximum value	no
B	analyte found in the associated blank and concentration in sample is less than 10X the concentration in the associated blank	per review
C	co-eluting congener	yes
J	concentration less than limit of quantification	yes
K	peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration	no
U	not detected at RL	yes
H	identifies a compound that hasn't been fully validated; the reported concentration value is for informational purposes only	yes
N	authentic recovery is not within method/contract control limits	per review
V	surrogate recovery is not within method/contract control limits	per review
D	dilution	yes
NQ	not quantified	no

2.4.1.3 Targeted data - Laboratory Blanks

Laboratory blanks were processed and run with each analytical set to determine if sample handling and processing in the laboratory resulted in specific contamination and potential results bias. Separate blanks were run for each schedule, and for the low-flow, high-flow, and estuarine samples. Detectable levels (i.e., above the method reporting limit) were reported for the analytes (or analyte groups). For each of these analytes, the blank information and the corresponding concentrations in the environmental samples were reviewed and adjusted as necessary. There were four general outcomes of this review:

- In cases where the environmental samples were reported as non-detect (U-flagged) or there was some other QA/QC issue with the data, such as a K-flag, there was no change.
- In cases where an analyte was detected in one or more of the environmental samples, the reporting limit (RL) was reviewed and adjusted based on the measured values in the blanks. The RL adjustment was based on the distribution of laboratory blanks specifically from the project (n=4) and/or from additional laboratory blank sample data provided by SGS-AXYS (n=20). The adjusted RL was calculated by:
    - removing the data for blanks that were R-flagged, indicating that the ion fragment ratio was outside compliance limits,
    - replacing non-detect values with a random number between 0 and the blank detection limit,

    - calculating the upper 95% confidence interval of the blanks as: mean + 1.96*standard deviation.
- The adjusted RL was used to screen results from environmental samples, where appropriate. Reported environmental concentrations that were less than the adjusted RL were replaced and reported as non-detect (U-flagged) above the adjusted RL.
- No blank correction or blank subtraction was performed for any analyte.

2.4.1.4 Targeted data - Field and Equipment Blanks

In addition to the laboratory blanks, a set of field blanks were collected and analyzed for a subset of analytes. 4-nonylphenol was the only analyte present above the reporting limit in any of the field blank samples, at a reported concentration of 4.91 ng/L at the West Point field blank. This is significantly lower than the level reported in the laboratory blank and significantly less than the values reported in any of the wastewater treatment plant effluent samples (100-887 ng/L).

Unless otherwise noted, the analytical data received from SGS-AXYS are suitable for use.

2.4.1.5 High Resolution Mass Spectrometry Data Verification and Validation

All samples were collected, stored, and processed according to the QA/QC procedures described in the Chemistry and Data Quality Report and associated UW Tacoma Standard Operating Procedures. The processed samples were analyzed in March and April 2021 and a QA/QC review of the control standards that are included in every analytical batch indicated that there were issues with the instrument performance. The mass accuracy error of spiked surrogates in both the control samples and environmental samples was > 5 ppm, and the instrument response had decreased by more than 25% for the control samples compared to previous runs, and the instrument response for the continuously injected reference mass standards was below desired limits. As such, the initial data files were deemed to be unusable. Following instrument troubleshooting, instrument maintenance, and recalibration, several test runs were performed, and the instrument performance met QA/QC requirements. All samples were re-run and the resulting data files met QA/QC limits. The resulting data are thus suitable for use.

2.4.2 High Resolution Mass Spectrometry Data Reduction and Analysis

The data analysis workflow was based on Du et al. (2017) and used MassHunter Profinder (B.08.00) for nontarget feature extraction and alignment across samples. Valid features were identified based on the application of replicate filters and blank subtraction in Mass Profiler Professional (B.13.00, MPP) by replicate filters and blank subtraction. Retained features had peak area >5000, occurred in all replicates, and had peak area five-fold greater than solvent, method, and field blanks. Formula assignment, suspect screening, and feature identification were performed using MassHunter ID Browser (B.07.00) and MassHunter Qualitative Analysis (B.08.00).

Suspect screening was based on MS-only and MS2 fragmentation. The MS-only screening was performed based on an in-house database with molecular formula, accurate mass, and retention time (RT) information for ~1100 compounds (CUW database). It includes chemicals from EPA's ToxCast library and a range of wastewater and stormwater-derived contaminants (Du et al. 2017). Molecular features that were considered matches fell within mass and RT control limits (<5 ppm, <0.3 min) and with a matching score, which accounts for isotopic patterns, exact mass, and RT, of >85 (maximum score = 100) (Tian et al. 2020).

To achieve a more comprehensive screening, all MS2 files were compared to the Global Natural Products (GMPS) molecular libraries (Wang et al. 2016). All HRMS MS2 data files were acquired as Agilent .d files, and subsequently converted to .mzXML format using ProteoWizard MSConvert software with 32-bit encoding and zlib compression (Chambers et al. 2012), and then .mzML format using GNPS Conversion Drag and Drop, which makes scans sequential in Agilent MS2 files, enabling compatibility with all GNPS functionality.

Acquired MS2 spectra sets were compared with a suite of MS2 spectra libraries using GNPS Library Search with the following settings: parent mass peak tolerance = 0.025 Da, MS2 peak tolerance = 0.02 Da, cosine score >0.7, spectra peak match ≥3. This process generated a total of >7300 matches for >250 individual compounds. The high number of repeated matches is because: 1) each parent ion is fragmented three times at 10eV, 20eV, and 40 eV, 2) the parent ion can be fragmented multiple times during a single run, and 3) a given compound may show up in multiple libraries in the GNPS system, providing multiple opportunities for a match between acquired and library spectra.

Each spectra match was manually evaluated to compare measured versus library spectra and poor matches were discarded. Further confirmation was performed by evaluating chromatographic peak shape, retention time consistency, and isotopic spacing abundance patterns utilizing Mass using MassHunter Qualitative Analysis 10.0. Compounds that lacked suitable peaks or RT match were discarded. In some cases, an additional evaluation was performed in MassHunter Qualitative Analysis based on formula and retention time.

The confidence level of identification was based on accurate mass, isotopic spacing and abundance patterns, retention time, and MS/MS fragmentation patterns, as described by Schymanski et al. (2014). All level 1 identifications are confirmed with reference standards run on CUW instrumentation; no new standards were purchased or run during this work. Level 2 identifications are based on matches with existing MS/MS spectra libraries (GNPS).

2.4.3 Fish Exposure Study – Biological Response Data

All analyses were conducted in the statistical programming language R (version 4.0.5) using the "stats" and "drc" packages (R Core Team 2021, Ritz et al. 2015). The alpha level of 0.1 used to determine significant differences between treatments.

We used a principal component analysis (PCA) to explore relationships among plasma endpoints in multivariate space, with the goal of determining if a subset of parameters were similarly impacted by wastewater effluent exposure. All data was $\log_{10}$-transformed in addition to scaling and centering since numeric ranges varied among endpoints. A parallel analysis (Patil et al. 2017) was used to determine the number of principal components (PCs) to retain. We used a threshold PC loading value of 0.4 to determine which endpoints accounted for the most variability in each retained PC.

Dose-response relationships between each retained PC and effluent concentration were tested by regression analysis. Subsequently, each parameter highly loading to that PC was examined for a relationship with effluent concentration to determine which parameters were driving the group relationships. Based on a visual assessment, PC1 appeared to follow a hormesis response with effluent concentration, whereas PC2 appeared to follow a linear response with effluent concentration. Hormesis is defined by a stimulatory, often beneficial, response at low exposure concentrations compared with a decreasing, often detrimental, response at higher concentrations. This relationship is well documented in the toxiciology literature (Calabrese & Baldwin 2001). To test for a hormesis relationship with effluent

concentration for PC1, we used a five-parameter Cedergreen-Ritz-Streibig (CRS, α = 0.75) model with Tukey's biweight robust estimation to reduce the effects of outliers (Cedergreen et al. 2005, Ritz et al. 2015). The significance of the CRS model is determined via the significance of the *f* parameter (Equation 7). The CRS hormesis model is defined by Equation 7, where *y* is the dependent parameter (PC1 or individual plasma parameter), *α* is the rate of hormetic effect (manually defined as 0.25-1), *b* is the slope around the *e* parameter, *c* is the response at infinite concentration (lower asymptote), *d* is the response of the control group, *e* is the concentration at which *d-c* is reduced by 50% (approximating an EC50), *f* is the rate of increase in response at low concentrations, and *x* is the independent parameter (% WWE).

$$y = c + \frac{d - c + f(\exp(-1/(x^{\alpha})))}{1 + \exp(b(\log(x)-\log(e)))} \quad (7)$$

The α parameter governs the rate at which the hormetic effect is applied without overparameterizing the model (Cedergreen et al. 2005). For the model to be viable, α must be a positive value between 0.25-1 and *b* and *f* must be positive. We added a constant of 8 to the PC1 values because the CRS model required all dependent values to be positive. We also ran CRS models with Tukey's biweight robust estimation for each of the plasma parameters heavily loading onto PC1.

The relationship between PC2 or its heavily loading parameters and % WWE was defined using simple linear regression (lm() in stats package, R Core Team, 2021). To linearize data for regression, some parameters were logarithmically transformed and some were not. When % WWE was transformed, we used a base of 3.8 because of the dual geometric concentration series for % WWE. For all parameters modeled with simple linear regressions, we added a constant of 0.025 to all % WWE values to allow inclusion of control values (0% WWE).

Plasma parameters lacking a significant regression relationship with % WWE and those that did not load heavily onto either retained PC axis were assessed for differences among % WWE treatments using non-parametric Kruskal Wallis tests with Dunn's pairwise comparison post-hoc tests for non-normal parameters and parametric ANOVAs with Dunnett's post hoc tests for normal parameters. Creatinine and lipase were excluded from analyses because creatinine was not detected in any sample, and lipase was only detected in one 0.1% and one 20% replicate sample.

Percent lipid content of fish among treatments was compared graphically because there was only one measured value per treatment from the tissue chemistry performed by SGS-AXYS (one composite sample of n = 7 per treatment). NKA activity and the number of liver anomalies among %WWE treatments were compared using non-parametric Kruskal Wallis tests with Dunn's pairwise comparison post-hoc tests.

2.4.4 Data analysis for metabolomics

The metabolite data were analyzed in two phases to evaluate alterations. We examined the dataset as a whole for all treatments to search for overall patterns of change caused by exposure to WWE. For the second phase we evaluated pairwise comparisons of control versus treatment to highlight the numerous altered metabolites and reliant pathways allowing us to characterize important physiological pathways that were altered by CECs in WWE.

All analyses were conducted using MetaboAnalyst 5 (Pang et al. 2021). Data were transformed with the Pareto algorithm to achieve a normal distribution. Pareto scaling subtracts each value from the mean (centering) and divides that value by the square root of standard deviation of each variable, which highlights the differences over the similarities in the data. The threshold for removing analytes with excessive zero or non-detect values was set at 50%. For analytes with less than 50% missing, blanks were imputed with the K-Nearest Neighbors method (KNN), which is an algorithm to impute a missing value for a target sample. The *k* most similar values are identified and a defined distance metric is calculated using the weighted average of the values of the target and neighboring samples.

In general, analytes that may be biologically important were identified based on a p-values and a false discovery rate (FDR) (q-value) from control versus treatment comparisons. The FDR is based on the Benjamini-Hochberg correction was used to correct for multiple comparisons and reduce the likelihood of false positives.

We examined the coefficient of variation (CV) among replicates within treatments to determine outliers. One of the 42 replicates was identified as an outlier and was removed from analysis. In replicate 1 of treatment 1.4%, 30% of the analytes were greater than five-fold different than the mean analyte value for that treatment. All other replicates in treatment 1.4% exhibited 8 - 10% of their values with the five-fold difference. The overall CV based on all analytes for treatment 1.4% was reduced from 65% to 47% by removal of the outlier. All other replicates within their respective treatment exhibited much less variation, with only 0-3% of analytes exhibiting a more than five-fold difference.

We used the Significance Analysis of Microarray (SAM) method to highlight important analytes. This method assigns a significance score to each variable based on its change relative to the standard deviation of repeated measurements. SAM use moderated t-tests to compute a statistic (delta) for each analyte that measures the strength of the relationship between analyte concentration and response (class membership). The procedure accounts for correlations in analytes and avoids normal assumptions about the distribution of individual genes (Xia et al. 2016). The SAM method is recommended for high-dimensional data analysis for small sample sizes (<8 per group) because variance tends to be unstable when the sample size is small (Xia and Wishart 2016). Delta is a tuning parameter to balance the FDR and the number of false positives (Roxas et al. 2008). We used a delta value that identified the highest number of important analytes with a false positive rate under 15% and an FDR <0.2, which we considered as potentially biologically important.

We used Partial Least Squares – Discriminant Analysis (PLS-DA) for determining separation among treatments and forming classes using all detected analytes. PLS-DA is a supervised method using multivariate regression to predict classes. The significance of the PLS-DA output was checked with a permutation test using the between-group to within-group (B/W) ratio to calculate the test statistic. Ellipses showing the 95% confidence interval were constructed around each treatment. We also calculated an r^2 and Q^2 for the PLS-DA model. Q^2 is an estimate of the predictive ability of the model, and is calculated via cross-validation.

To estimate the importance of each variable in the PLS-DA plots, a Variable Importance in Projection (VIP) plot was also constructed. The VIP is a weighted sum of squares of the PLS loadings based on the amount of variation for Y in each dimension. In studies with less than 100 variables, a VIP cutoff of 1.0 is used to select analytes for additional analysis.

Volcano plots were constructed showing the fold change in analytes for control versus treatment comparisons. Fold-change is denoted by the control value over the value for each treatment (control/treatment). Heatmaps were also constructed to show treatment and replicate groupings. We used Euclidean for the distance and the Ward clustering method. The 50 most impacted metabolites of the 184 detected were selected for plotting each heatmap, based on T-tests (control v. treatment) or ANOVA results (all treatments).

Metabolite Set Enrichment Analysis (MSEA) was used to explore pathway alteration. MSEA is conceptually similar to Gene Set Enrichment Analysis and is able to highlight changes among related metabolites and identify biologically important patterns. For this analysis MetaboAnalyst5 performed Quantitative Enrichment Analysis (QEA) with the R package Globaltest. This estimates a Q statistic for each metabolite set using a general linear model, which is an aggregate of squared covariance between concentration changes and the phenotypes. Metabolites with large variance have much more influence on the Q statistic than compounds with small variance. The Q statistic essentially describes the correlation between metabolite alterations (X) and phenotype (Y) or pathway result. FDRs and p-values and were calculated for each Q statistic. With QEA, a list of low p-value metabolites is not required and enriched metabolite sets can be identified when only a few compounds are substantially changed or when many compounds are only slightly (but consistently) changed (Xia and Wishart 2010). Enrichment analysis evaluates the number of observed metabolite differences in a pathway to the expected difference that would occur by random chance within the dataset. The enrichment ratio for QEA is calculated by dividing the observed Q statistic by the expected Q statistic ("Statistic" / "Expected").

We focused the dataset to those metabolites from the entire set that exhibited significant differences between control and treatment. A T-test for each control versus treatment pair was conducted and only those analytes that exhibited a p-value ≤ 0.1 were selected for the focused datasets. These filtered values resulted in a dataset of a variable number of metabolites from the total 184 detected analytes ranging from 26 to 46 for each pairwise comparison. We used Human metabolome database (HMDB) identifiers for our metabolites in the concentration table. It is advantageous to reduce the number of analytes because the FDR corrects for all comparisons as a function of the number of analytes. Reducing those analytes that do not exhibit variability among treatments facilitates discovery of the most important differences and analytes driving the biological response. Filtering to increase power in such analyses has been discussed by Hackstadt and Hess (2009).

Our data were compared to metabolite set libraries to examine for differences and predict altered pathways. For QEA, KEGG and SMPDB databases were used, which are based on human metabolite pathways. The Small Molecule Pathway Database contains more than 30,000 small molecule pathways and most are not found in any other database. KEGG (Kyoto Encyclopedia of Genes and Genomes) is widely used and it contains 372 metabolic pathways (372 reference pathways) from over 700 species. We combined the results from the SMPBD and KEGG analyses for a more complete picture of altered pathways for fish exposed to WWTP effluent. We also compared our data to disease-associated metabolites for blood (344 pathway), drug pathway metabolite sets (461 pathways), and chemical classes. Enrichment analysis was used to evaluate whether the observed metabolites in a particular pathway appear more frequently than expected by random chance within a given dataset, which is reflected in the number of hits and FDR level. Pathways identified as significantly altered was based on the overlap of pathways and metabolites, as a function of what would be expected by chance alone.

Given that there are thousands of metabolites and pathways, even one hit can often return a low p-value or FDR. In general, the larger the number of hits for a given pathway, the lower the FDR or p-value.

3 RESULTS

3.1 Water Quality Monitoring

The following section presents a summary of results of the water quality monitoring including a comparison of loadings and/or concentrations from other facilities, where data are available to support such calculations. A screening level toxicity evaluation is also presented. Complete water quality monitoring data are included in the appendix.

3.1.1 PCBs in wastewater treatment plant effluent

PCBs were present in wastewater treatment plant effluent from the West Point and South Plant facilities under low flow and high flow conditions at total concentrations ranging from 0.94-1.87 ng/L (Table 7). The PCB concentrations in the West Point effluent were higher (1.44-1.87 ng/L) compared to the South Plant effluent (0.94-1.17 ng/L). The PCB concentration in the high flow sample was higher than the low flow sample at West Point; at South Plant the PCB concentration was higher in the low flow compared to high flow (Table 7). The analytical QA/QC information indicates that all congener measurements were within control limits and that the range of recovery for the individual congeners was 103-115% (median 111%) for the high flow samples and 101-113% (median 107%) for the low flow samples, where 100% would indicate a perfect recovery; these results suggest a consistent high bias. Using a conservative assumption of an analytical uncertainty of 15%, which is probably an overestimation based on matrix spike recovery values, the difference in concentration between sample points was West Point high flow > West Point low flow > South Plant high flow ~ South Plant low flow.

Figure 3 presents the total PCB concentrations measured in this study, with those measured at other WWTP facilities and in other potential pathways that may lead to PCB loading to Puget Sound.

Raw data for all PCBs are included in the Chemistry and Data Quality Report.

Table 7. Total PCB and PBDE concentrations in WWTP effluent from the West Point and South Plant facilities under high flow and low flow conditions.

Facility	Flow Regime	Total PCB Concentration (pg/L)	$\sum_{11}$PBDE Concentration (pg/L)
West Point	High Flow	1,870	8,935
West Point	Low Flow	1,440	5,039
South Plant	High Flow	940	9,170
South Plant	Low Flow	1,170	10,743

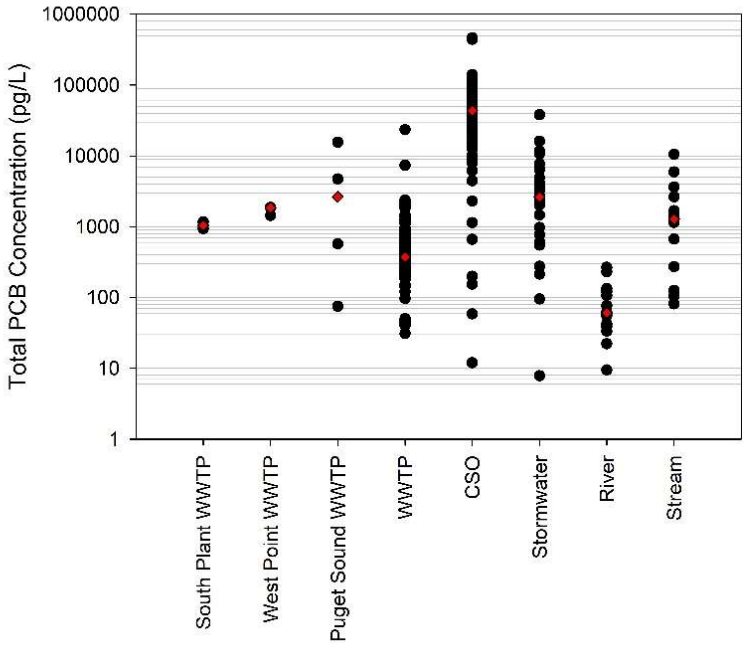

Figure 3. Total PCB concentration (pg/L) measured in different potential pathways, including data from this study (South Plant and West Point WWTPs). Puget Sound WWTP from Washington State Department of Ecology (2010), WWTP data from Washington State collected by Spokane River Regional Toxics Task Force in 2014 and 2015, CSO data from King County (2011) and King County (2013a), and Stormwater, River, and Creek data from King County (2013a). Creeks are from urbanized watersheds in King County and are likely not representative of creeks in less developed watershed. Individual data points shown in black. Median for each category shown in red.

Concentration and flow data were used to estimate mass loading of total PCBs to Puget Sound from the South Plant and West Point WWTPs, as well as other WWTPs in the region and other potential pathways (Figure 4). WWTP flows were annual average flow rates as reported in the NPDES Permit Fact Sheets for each facility. Concentration data were the median of all reported values (see Figure 3). Error bars represent the 25th- and 75th-percentile of the concentration distributions. The Diagonal drainage is a 2,686-acre basin in south Seattle that extends from Beacon Hill to lower east Duwamish River industrial area and is one of the largest drainage basins in the City of Seattle. The Diagonal drainage enters Puget Sound through an outfall on the lower Duwamish River. It was included as an example of potential loading from urban stormwater runoff through a residential/commercial/industrial basin. Loadings from the basin were calculated based on median reported PCB concentrations for storm-flow and base-flow conditions for CSOs in the Duwamish basin as reported in King County (2011). Basin flow rates were estimated from Aqualize (2018) and storm-flow occurrence based on rainfall data from 2010-2018 from Seattle Public Utility rainfall gauge RG15, located adjacent to the lower Duwamish Waterway.

While the values in Figure 4 should be considered as estimates only, based on a limited number of samples from the individual facilities and average annual flows, they do suggest that PCB loadings from West Point and South Plant WWTPs are within the same order-of-magnitude as other large WWTPs in

the region, comparable to stormwater runoff from a large urbanized basin, and probably greater than loadings from individual rivers and streams.

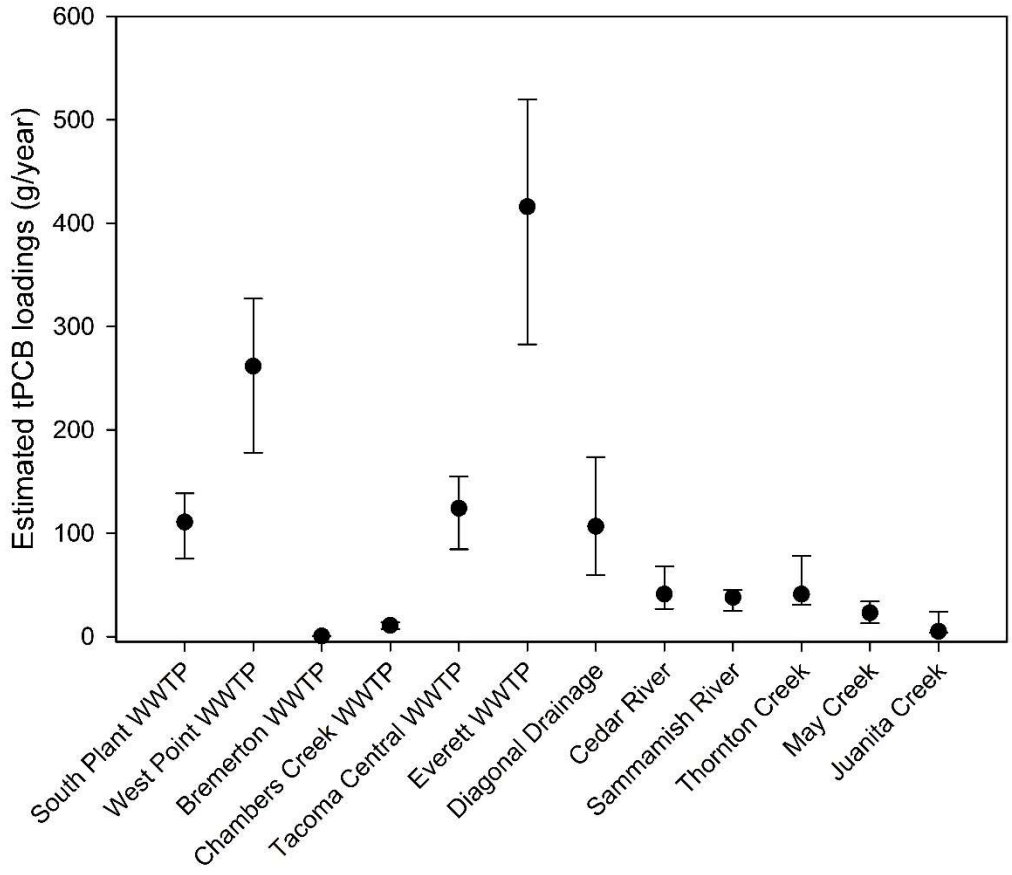

Figure 4. Estimated total PCB loadings from South Plant and West Point to Puget Sound based on the data obtained in this study. Estimated loadings for other WWTPs to Puget Sound based on information reported in Washington State Department of Ecology (2010). Loadings from Diagonal Drainage to the Lower Duwamish River estimated based on data in King County (2011) and Aqualize (2018). Loadings from the rivers and creeks to Lake Washington from King County (2013b).

3.1.2 PBDEs in wastewater treatment plant effluent

PBDEs were present in the effluent samples collected from the West Point and South Plant facilities under high flow and low flow conditions. The total concentration of PDBEs was estimated by summing a suite of 11 PBDE congeners (BDE-028, -047, -049, -066, -085, -099, -100, -153, -154, -183, -209; $\sum_{11}$PBDE) which collectively account for approximately 97% of the PBDEs measured in the WWTP effluent samples collected in this study. Note that the 11 PBDE congeners account for >90% of total PBDEs in the other WWTP effluent samples, and ~80% of total PBDEs in industrial effluent samples shown in Figure 5. This approach has been used to estimate total PBDEs present in marine organisms in the Puget Sound (West

et al. 2017). Summary results are shown in Table 7. Complete results are included in the project data reports.

These data were compared to estimated $\sum_{11}$PBDE concentrations reported for other facilities to provide a basis for comparison with other potential pathways to Puget Sound (Figure 5). It should be noted that there has been a widespread overall decline in PBDE concentrations in marine organisms over the last 20 years that was attributed to efforts to remove or reduce these chemicals from use (West et al. 2017). These management activities might result in a high bias in the 2009 WWTP samples relative to current samples, suggesting that they are not perfectly comparable to current WWTP effluent. As a point of comparison, the $\sum_{11}$PBDE concentration from West Point in 2021 is approximately 40% of the $\sum_{11}$PBDE concentration from West Point in 2009.

Additional considerations include that the 2020 WWTP samples are from a single facility that had been identified as a potential high-loading source of PBDEs to Puget Sound (O'Neill et al. 2019). The 2020 industrial samples are from discharges to a wastewater conveyance system and contribute to the municipal wastewater. These discharges do not go directly to Puget Sound.

3.1.3 Other Water Quality Monitoring Data

A summary of the concentrations of detected chemicals in WWTP effluent sample and the estuary samples is shown in Tables 8 and 9. Only compounds that were present above the detection limit in at least one sample are shown.

Complete data results are shown in the project data reports.

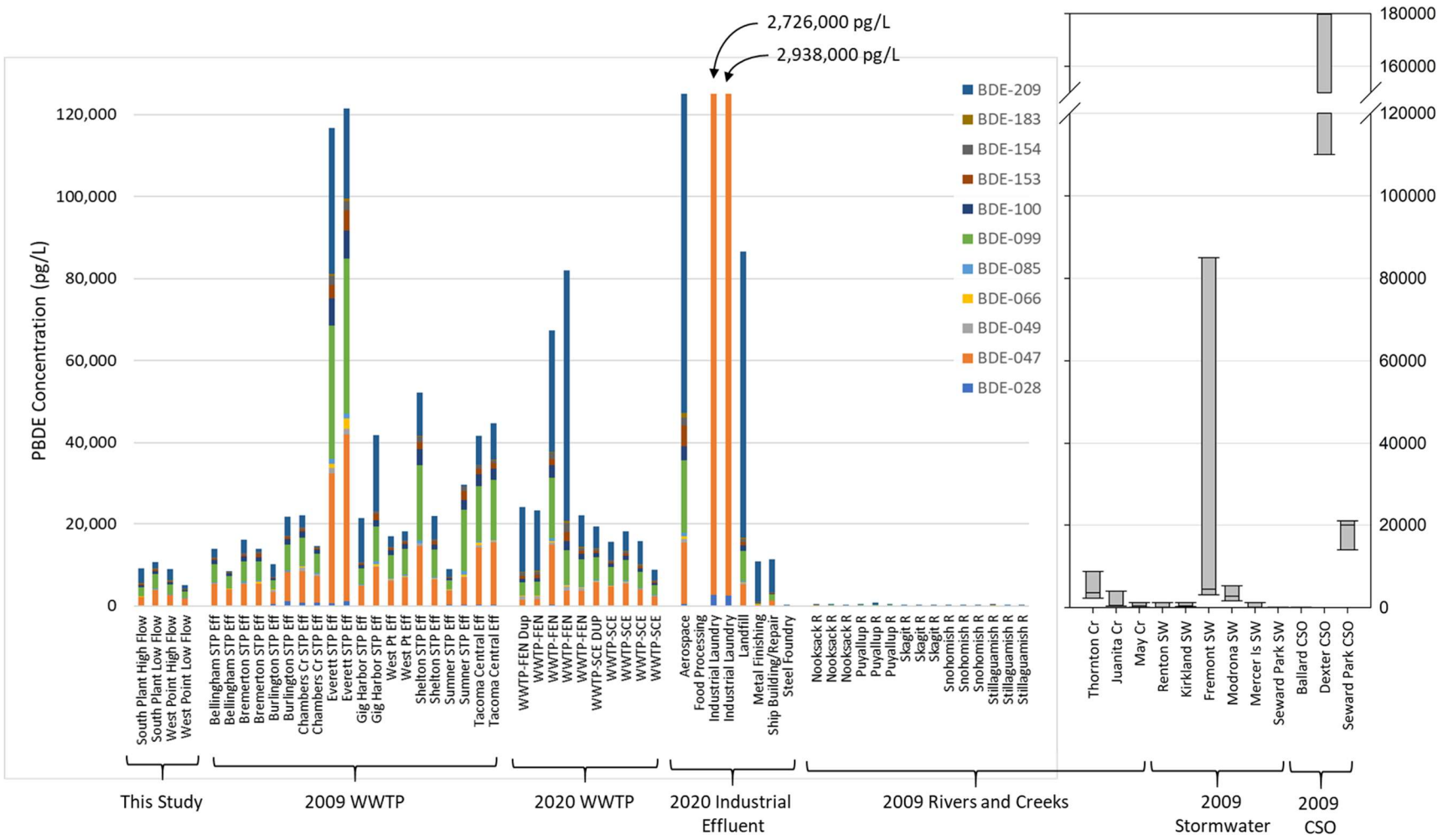

Figure 5. $\sum_{11}$PBDE *concentration (left) and reported total PBDE concentrations (grey bars, right) for effluent water from different facilities, and surface waters in the Puget Sound. The 2009 WWTP data are from Washington State Department of Ecology (2010). 2009 rivers, creeks, and CSO data are from King County (2013b) and Washington State Department of Ecology (2011). 2020 WWTP and 2020 Industrial Effluent are from Washington State Department of Ecology.*

Table 8. Summary of chemical monitoring in WWTP effluent and estuarine water samples for pharmaceuticals and personal care products, alkylphenols, and (per)fluorinated compounds. Shown are compounds that were present above the detection limit in at least one sample across the entire study. All concentrations in ng/L. Other WWTP and Other Estuary sample data from Meador et al. (2016) and are included for reference. Note that not all compounds measured in this study were analyzed for in Meador et al (2016). nd – not present above the detection limit. "-" – not measured.

Compound	CAS	WWTP Effluent (this study)				Other WWTP		Estuary (this study)						Other Estuary	
		South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow	Min	Max	West Point North deep	West Point North shallow	West Point south	Alki	Elliott Bay	Ref site	Min	Max
Pharmaceuticals and Personal Care Products (Contaminants of Emerging Concern)															
1,7-Dimethylxanthine	611-59-6	327	190	4360	399	873	2060	nd	nd	nd	nd	nd	nd	nd	nd
10-hydroxy-amitriptyline	1159-82-6	16.3	11.9	10.5	12.1	60.2	42.8	nd	nd	nd	nd	nd	nd	0.16	0.21
17 beta-Estradiol	50-28-2	18.2	43.5	nd	6.97	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2-Hydroxy-ibuprofen	51146-55-5	1070	149	3450	578	1160	4550	nd	nd	nd	nd	nd	nd	nd	nd
Acetaminophen	103-90-2	nd	nd	159	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Albuterol	18559-94-9	10.8	12.9	11.8	8.63	36	41	nd	nd	nd	nd	nd	nd	nd	nd
Alprazolam	28981-97-7	1.54	2.09	1.11	1.54	2.99	4	nd	nd	nd	nd	nd	nd	nd	nd
Amitriptyline	50-48-6	20.9	21.9	15.6	20.3	87.5	119	nd	nd	nd	nd	nd	nd	nd	nd
Amlodipine	88150-42-9	14.1	13.7	12.8	12.8	9.65	26.3	nd	nd	nd	nd	nd	nd	nd	nd
Amphetamine	300-62-9	3.84	nd	30.9	4.51	67.1	164	nd	nd	nd	nd	nd	nd	2.2	28.5
Androstenedione	63-05-8	nd	17.1	nd	14	nd	8.4	nd	nd	nd	nd	nd	nd	nd	nd
Atenolol	29122-68-7	550	78.8	413	468	1700	2440	0.552	0.382	0.428	0.332	0.39	0.31	3.4	22.1
Atorvastatin	134523-00-5	16	99.2	6.34	74.4	nd	68	nd	nd	nd	nd	nd	nd	nd	nd
Azithromycin	83905-01-5	352	170	474	227	261	629	nd	nd	nd	nd	nd	nd	nd	2.2
BEHTBP	26040-51-7	1.68	1.91	1.25	1.1	-	-	nd	nd	nd	nd	nd	nd	-	-
Benzoylecgonine	519-09-5	371	40.1	384	85	151	293	0.421	0.337	0.377	0.303	0.403	0.33	0.51	0.78
Bisphenol A	80-05-7	2985	963.5	191.5	183	350	4290	nd	nd	2.05	nd	nd	nd	2.8	4.3
Bisphenol E	2081-08-5	nd	nd	nd	39.6	-	-	nd	nd	nd	nd	nd	nd	-	-

Compound	CAS	WWTP Effluent (this study)				Other WWTP		Estuary (this study)						Other Estuary	
		South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow	Min	Max	West Point North deep	West Point North shallow	West Point south	Alki	Elliott Bay	Ref site	Min	Max
Bisphenol F	620-92-8	nd	nd	nd	39.5	-	-	nd	nd	nd	nd	nd	nd	-	-
Bisphenol S	80-09-1	1160	127	485	1050	-	-	nd	nd	nd	nd	nd	nd	-	-
Caffeine	58-08-2	nd	50.4	2760	169	152	1170	nd	nd	nd	nd	nd	nd	nd	nd
Carbadox	6804-07-5	nd	nd	nd	3.39	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Carbamazepine	298-46-4	127	206	101	159	510	735	nd	nd	nd	nd	nd	nd	nd	1.9
Ciprofloxacin	85721-33-1	30.8	40	44.3	54.9	158	192	nd	nd	nd	nd	nd	nd	nd	7.3
Citalopram	59729-33-8	208	212	207	254	-	-	nd	nd	nd	nd	nd	0.45	-	-
Clarithromycin	81103-11-9	128	142	85.2	94.6	52	181	nd	nd	nd	nd	nd	nd	nd	nd
Clotrimazole	23593-75-1	0.864	0.706	1.42	1.28	-	-	nd	nd	nd	nd	nd	nd	-	-
Cloxacillin	61-72-3	24.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cocaine	50-36-2	25.4	0.802	50.1	2.73	8.5	59	nd	nd	nd	nd	nd	nd	nd	nd
Codeine	76-57-3	126	93.2	81.3	110	178	290	nd	nd	nd	nd	nd	nd	nd	nd
Colchicine	64-86-8	3.21	3.65	1.77	3.04	-	-	nd	nd	nd	nd	nd	nd	-	-
Cotinine	486-56-6	53.4	24.5	91	62.7	115	340	0.792	0.731	0.702	0.655	0.836	0.65	nd	nd
Cyclophosphamide	50-18-0	1.1	2.64	4.97	3.46	-	-	nd	nd	nd	nd	nd	nd	-	-
Dec 603	13560-92-4	nd	0.021	0.008	nd	-	-	nd	nd	nd	nd	nd	nd	-	-
DEET	134-62-3	66.6	829	34.2	110	23.3	684	nd	5.41	4.93	nd	nd	nd	2.4	8.4
Dehydronifedipine	67035-22-7	nd	4	1.37	2.49	-	-	nd	nd	nd	nd	nd	nd	-	-
Desmethyldiltiazem	86408-45-9	37.2	39.8	25.9	34.8	81.8	148	nd	nd	nd	nd	nd	nd	nd	nd
Diatrizoic acid	117-96-4	5630	9970	12500	14000	-	-	nd	nd	nd	nd	nd	nd	-	-
Diazepam	439-14-5	0.588	1.08	nd	0.5	1.5	2.2	nd	nd	nd	nd	nd	nd	nd	nd
Diltiazem	34933-06-7	204	146	157	136	390	425	nd	nd	nd	nd	nd	nd	0.52	0.75
Diphenhydramine	58-73-1	970	941	578	677	1030	1240	0.634	nd	nd	nd	nd	nd	0.96	1.5
Doxycycline	564-25-0	nd	23	21.4	16.3	-	-	nd	nd	nd	nd	nd	nd	-	-
DP Anti	135821-74-8	nd	nd	0.148	nd	-	-	nd	0.108	nd	nd	nd	nd	-	-

Compound	CAS	WWTP Effluent (this study)				Other WWTP		Estuary (this study)						Other Estuary	
		South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow	Min	Max	West Point North deep	West Point North shallow	West Point south	Alki	Elliott Bay	Ref site	Min	Max
EHTBB	183658-27-7	2.15	3.91	2.61	2.53	-	-	nd	nd	nd	nd	nd	nd	-	-
Enalapril	75847-73-3	4.86	nd	5.31	1.64	nd	5.6	nd	nd	nd	nd	nd	nd	nd	nd
Enrofloxacin	93106-60-6	nd	nd	nd	3.76	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Erythromycin-H2O	114-07-8	27.9	17.5	28.7	21.7	87.3	138	nd	nd	nd	nd	nd	nd	nd	3.3
Estrone	53-16-7	38.3	170	5.09	47.9	4.5	58	nd	nd	nd	nd	nd	nd	nd	nd
Flumequine	42835-25-6	nd	5.15	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Fluoxetine	54910-89-3	25.3	13.6	37.1	46.8	56.8	59.5	nd	nd	nd	nd	nd	nd	nd	nd
Fluticasone propionate	80474-14-2	nd	nd	2.1	2.56	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Furosemide	54-31-9	62.2	173	5.04	124	994	1290	nd	nd	nd	nd	nd	nd	nd	nd
Gemfibrozil	25812-30-0	544	649	281	314	1360	1640	nd	nd	nd	nd	nd	nd	3.4	5.5
Glipizide	29094-61-9	14.6	16	7.49	7.92	nd	22	nd	nd	nd	nd	nd	nd	nd	nd
Glyburide	10238-21-8	2.44	2.93	2.65	1.43	7.6	11	nd	nd	nd	nd	nd	nd	nd	nd
HBB	87-82-1	0.052	nd	nd	nd	-	-	nd	nd	nd	nd	nd	nd	-	-
Hydrochlorothiazide	58-93-5	1270	1360	775	752	411	578	nd	nd	nd	nd	nd	nd	nd	nd
Hydrocodone	125-29-1	11.8	24.8	8.46	15.6	69	74	nd	nd	nd	nd	nd	nd	nd	nd
Ibuprofen	15687-27-1	228	13.3	524	20.2	116	1060	nd	nd	nd	nd	nd	nd	nd	nd
Iopamidol	60166-93-0	12100	31000	3900	5770	-	-	nd	nd	nd	nd	nd	nd	-	-
Lincomycin	154-21-2	4.65	5.5	nd	nd	nd	27	nd	nd	nd	nd	nd	nd	nd	nd
Meprobamate	57-53-4	53.1	64.5	27.1	26.4	513	623	nd	nd	nd	nd	nd	nd	nd	nd
Metformin	657-24-9	35600	2640	34500	30300	29300	82700	60.6	44.2	45.1	31.1	44.3	34.4	105	832
Metoprolol	51384-51-1	549	647	363	424	805	835	nd	nd	nd	nd	nd	nd	nd	nd
Metronidazole	443-48-1	162	67.9	152	105	-	-	nd	nd	nd	nd	nd	nd	-	-
Miconazole	22916-47-8	2.32	2.41	4.99	2.9	4.86	4.86	nd	nd	nd	nd	nd	nd	nd	nd
Moxifloxacin	151096-09-2	nd	nd	nd	5.56	-	-	nd	nd	nd	nd	nd	nd	-	-
Naproxen	22204-53-1	1190	84	905	241	106	701	nd	nd	nd	nd	nd	nd	nd	nd

Compound	CAS	WWTP Effluent (this study)				Other WWTP		Estuary (this study)						Other Estuary	
		South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow	Min	Max	West Point North deep	West Point North shallow	West Point south	Alki	Elliott Bay	Ref site	Min	Max
Norfluoxetine	83891-03-6	4.4	1.81	5.99	5.06	17	28.1	nd	nd	nd	nd	nd	nd	nd	nd
Norverapamil	67018-85-3	5.27	4.19	3.08	3.77	12.6	13.5	nd	nd	nd	nd	nd	nd	nd	nd
Ofloxacin	82419-36-1	22.6	48.3	42.6	85.3	108	387	nd	nd	nd	nd	nd	nd	nd	nd
Oxazepam	604-75-1	6.97	7.09	5.3	5.4	-	-	nd	nd	nd	nd	nd	nd	-	-
Oxolinic Acid	14698-29-4	nd	nd	3.39	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Oxycodone	76-42-6	36.8	52.3	22.8	38.3	158	231	nd	nd	nd	nd	nd	nd	nd	nd
Paroxetine	61869-08-7	3.47	3.36	2.36	4.5	6.6	42	nd	nd	nd	nd	nd	nd	nd	nd
PBBZ	608-90-2	0.025	nd	nd	nd	-	-	nd	nd	nd	nd	nd	nd	-	-
PBEB	85-22-3	0.017	nd	nd	nd	-	-	nd	nd	nd	nd	nd	nd	-	-
Progesterone	57-83-0	nd	1.59	nd	6.56	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Promethazine	60-87-7	nd	0.36	nd	nd	nd	3.77	nd	nd	nd	nd	nd	nd	nd	nd
Propranolol	525-66-6	71.9	72.3	51.8	81.1	76	109	nd	nd	nd	nd	nd	nd	nd	nd
Ranitidine	66357-35-5	5.07	2.92	nd	nd	nd	494	nd	nd	nd	nd	nd	nd	nd	0.75
Rosuvastatin	287714-41-4	511	358	334	291	-	-	nd	nd	nd	nd	nd	nd	-	-
Roxithromycin	80214-83-1	2.76	nd	3.14	1.39	nd	3.8	nd	nd	nd	nd	nd	nd	nd	nd
Sertraline	79617-96-2	49	45.7	69.2	98.5	89	116	nd	nd	nd	nd	nd	nd	nd	nd
Sulfadiazine	68-35-9	nd	10	1.44	nd	-	-	nd	nd	nd	nd	nd	nd	nd	nd
Sulfamethoxazole	723-46-6	259	370	175	193	nd	1380	nd	nd	0.674	nd	0.93	nd	nd	4.1
Sulfanilamide	63-74-1	30.2	74.6	nd	31.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Testosterone	58-22-0	4.64	3.31	nd	6.25	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.9
Theophylline	58-55-9	293	237	7410	436	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Thiabendazole	148-79-8	32.4	33.1	67.7	29.7	24	27	nd	nd	nd	nd	nd	nd	nd	nd
Triamterene	396-01-0	68.2	108	42	51	151	156	nd	nd	nd	nd	nd	nd	nd	nd
Triclocarban	101-20-2	2.51	2.5	nd	nd	11.9	16.9	nd	nd	nd	nd	nd	nd	nd	nd
Triclosan	3380-34-5	21.4	27.6	12.3	12	183	411	nd	nd	nd	nd	nd	nd	nd	5.2

Compound	CAS	WWTP Effluent (this study)				Other WWTP		Estuary (this study)						Other Estuary	
		South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow	Min	Max	West Point North deep	West Point North shallow	West Point south	Alki	Elliott Bay	Ref site	Min	Max
Trimethoprim	738-70-5	255	272	242	249	742	852	nd	nd	nd	nd	nd	nd	nd	2.3
Tylosin	1401-69-0	6.96		7.78	15.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Valsartan	137862-53-4	729	968	412	590	2010	3000	nd	nd	nd	nd	nd	nd	nd	5.4
Venlafaxine	93413-69-5	337	406	313	392	-	-	0.594	0.455	0.519	0.419	0.458	0.65	-	-
Verapamil	52-53-9	16	10.6	13.8	13.4	40.5	44.3	nd	nd	nd	nd	nd	nd	nd	nd
Zidovudine	30516-87-1	132	120	21.5	52.1	-	-	nd	nd	nd	nd	nd	nd	-	-
Alkyl Phenols															
4-Nonylphenol diethoxylates	20427-84-3	1520	940	1220	401	1690	2610	nd	nd	nd	nd	nd	nd	nd	nd
4-Nonylphenol monoethoxylates	104-35-8	2550	2960	550	770	1220	1760	nd	nd	nd	nd	nd	nd	nd	nd
4-Nonylphenols	104-40-5	630	887	100	252	506	1690	nd	nd	nd	nd	nd	nd	13.6	41.4
Perfluorinated Compounds															
5:3 FTCA	914637-49-3	11.3	27.6	nd	13.7	-	-	nd	nd	nd	nd	nd	nd	-	-
6:2 FTS	27619-97-2	2.35	3.01	4.4	2.46	-	-	nd	nd	nd	nd	nd	3.56	-	-
EtFOSAA	909405-49-8	0.402	0.979	0.493	nd	-	-	nd	nd	nd	nd	nd	nd	-	-
MeFOSAA	2355-31-9	1.9	nd	1.16	1.06	-	-	nd	nd	nd	nd	nd	nd	-	-
PFBA	375-22-4	11.1	9.08	9.13	5.38	nd	6.7	nd	nd	nd	nd	nd	nd	nd	nd
PFBS	375-73-5	18	12	20.8	3.08	nd	13	nd	nd	nd	nd	nd	nd	nd	nd
PFDA	335-76-2	1.2	1.37	0.677	0.528	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFHpA	375-85-9	3.59	2.26	4.12	2.27	3.0	7.5	nd	nd	nd	nd	nd	nd	nd	nd
PFHpS	375-92-8	nd	nd	6.28	nd	-	-	nd	nd	nd	nd	nd	nd	-	-
PFHxA	307-24-4	33.5	22.5	22.8	15.9	15	53	nd	nd	nd	nd	nd	nd	nd	nd
PFHxS	355-46-4	3.64	5.24	89.3	2.2	nd	55	nd	nd	nd	nd	nd	nd	nd	nd
PFNA	375-95-1	1.22	0.721	1.94	1.29	nd	2	nd	nd	nd	nd	nd	nd	nd	nd
PFOA	335-67-1	11.4	5.48	11.7	5.02	7.6	12	nd	nd	nd	nd	nd	nd	nd	nd

Compound	CAS	WWTP Effluent (this study)				Other WWTP		Estuary (this study)						Other Estuary	
		South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow	Min	Max	West Point North deep	West Point North shallow	West Point south	Alki	Elliott Bay	Ref site	Min	Max
PFOS	1763-23-1	6.37	12.9	127	8.3	nd	461	nd	nd	nd	nd	nd	nd	nd	nd
PFPeA	2706-90-3	10.5	7.69	9.71	5.86	3.4	4.7	nd	nd	nd	nd	nd	nd	nd	nd
PFPeS	2706-91-4	1.42	0.639	18.8	0.456	-	-	nd	nd	nd	nd	nd	nd	-	-

Table 9. Summary of chemical monitoring in WWTP effluent for dioxins and furans and pesticides. Shown are compounds that were present above the detection limit in at least one sample. All concentrations in ng/L.

Compound	CAS	South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow
Dioxins and Furans					
1,2,3,4,6,7,8-HPCDF	67562-39-4		0.000501	0.00265	
1,2,3,4,7,8,9-HPCDF	55673-89-7			0.00164	
1,2,3,4,7,8-HXCDD	39227-28-6			0.00144	
1,2,3,4,7,8-HXCDF	70648-26-9			0.00152	
1,2,3,6,7,8-HXCDD	57653-85-7			0.00162	
1,2,3,6,7,8-HXCDF	57117-44-9			0.00135	
1,2,3,7,8,9-HXCDD	19408-74-3			0.00186	
1,2,3,7,8,9-HXCDF	72918-21-9		0.000737	0.00191	
1,2,3,7,8-PECDD	40321-76-4			0.00127	
1,2,3,7,8-PECDF	57117-41-6			0.000759	
2,3,4,6,7,8-HXCDF	60851-34-5			0.00151	
2,3,4,7,8-PECDF	57117-31-4			0.00116	
4,4'-DDT	50-29-3			0.165	
Pesticides					
Aldrin	309-00-2		0.346		
Chlordane, alpha (cis)	5103-71-9		0.045		
Chlorpyriphos	2921-88-2		0.129	0.224	0.31
Cypermethrin	52315-07-8	1.27	1.99	1.98	1.17
Diazinon	333-41-5	0.738	0.569		
Dieldrin	60-57-1	0.195	0.433	0.316	0.164
HCH, alpha	319-84-6		0.014		0.018
HCH, beta	319-85-7	0.155	0.202		0.102
HCH, gamma	58-89-9	0.153	0.158		
Hexachlorobenzene	118-74-1	0.044	0.046	0.052	0.037
Nonachlor, cis-	5103-73-1		0.059		
Nonachlor, trans-	39765-80-5	0.063	0.046		0.148
OCDD	3268-87-9	0.0141	0.00684	0.0372	0.00428
OCDF	39001-02-0	0.000957	0.000655	0.00447	
Permethrin	52645-53-1	11.1	14.5	9.9	4.58
Quintozene	82-68-8			0.641	
Simazine	122-34-9	0.642		1.6	

3.1.3.1 Toxicity Screening

In addition to the evaluation based on the fish plasma model and bioaccumulation study, a prioritization/screening approach was applied in order to evaluate additional lines of evidence on the potential effects associated with chemical occurrence and exposure. This is performed by comparing the occurrence concentrations in effluent with a Predicted No Effects Concentration (PNEC) and an Activity Concentration at Cutoff (ACC). The complete method is described in James and Sofield (2021) and references therein. Briefly, a biological response ratio (BRR) is calculated by comparing a measured environmental concentration with an effects threshold. If the measured environmental concentration exceeds the threshold then there is a likely biological response. The greater the magnitude of exceedance, the higher the likelihood. In this exercise, two different BRRs were calculated as summarized by:

$$Toxicity\ Quotient\ (TQ) = \frac{C_{envr}}{PNEC} \quad (8)$$

$$Exposure\ Activity\ Ratio\ (EAR) = \frac{C_{envr}}{ACC} \quad (9)$$

where, C_{envr} = measured environmental concentration.

CECs are categorized based on a comparison of the TQ and EAR values to separate threshold values as described in Corsi et al. (2019); James and Sofield (2021) and shown in Table 10. The results provide a categorization of the CEC into one of three categories: Category 1 – Likely Biological Effects (High Priority); Category 2 – Potential for Biological Effects (Watch List), and Category 3 – Low Potential for Biological Effects (Low Priority).

Table 10. Threshold values used to categorize CECs.

Category	TQ threshold	EAR threshold
High Priority	>100	>1
Watch List	>1	>0.01
Low Priority	<1	<0.01

Additional notation was added based on the number and consistency of the lines of evidence that resulted in the categorization where: A – multiple lines of evidence support categorization, B – only one measure/line of evidence available to support categorization, and C – lines of evidence are split.

This approach resulted in the identification of nine high priority compounds (Table 11). These include: 17β-estradiol, azithromycin, bisphenol A, diatrizoic acid, estrone, iopamidol, theophylline, triamterene, and venlafaxine.

Table 11. Summary of screening approach based on evaluation of Biological Response Ratios, utilizing Toxicity Quotient (TQ) and Exposure Activity Ratio (EAR). Yellow shaded cells indicate a "Watch List" compound for the given comparison. Red shaded cells indicate "High Priority" compounds. Category reflects grouping where A – multiple and consistent lines of evidence, B – only single line of evidence available, C- split lines of evidence.

Compound	CAS	Toxicity Quotient Wastewater Treatment Plant Effluent				∑EAR Max WWTP	Category
		South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow		
Pharmaceuticals and Personal Care Products/Contaminants of Emerging Concern							
1,7-Dimethylxanthine	611-59-6	0.2	0.1	2.0	0.2	0.002	Watch List - C
10-hydroxy-amitriptyline	1159-82-6	1.0	0.7	0.7	0.8		Watch List - B
17 beta-Estradiol	50-28-2	182.0	435.0		69.7	4.751	High Priority - A
2-Hydroxy-ibuprofen	51146-55-5	1.4	0.2	4.4	0.7		Watch List - B
Acetaminophen	103-90-2			0.0		0.000	-
Albuterol	18559-94-9	0.0	0.0	0.0	0.0	0.009	-
Alprazolam	28981-97-7	0.2	0.3	0.1	0.2	0.000	-
Amitriptyline	50-48-6	1.5	1.6	1.1	1.5		Watch List - B
Amlodipine	88150-42-9	0.6	0.6	0.6	0.6		-
Amphetamine	300-62-9	0.0		0.0	0.0		-
Androstenedione	63-05-8		0.0		0.0	0.032	Watch List - C
Atenolol	29122-68-7	0.0	0.0	0.0	0.0	0.000	-
Atorvastatin	134523-00-5	15.8	97.7	6.2	73.3	0.004	Watch List - C
Azithromycin	83905-01-5	185.3	89.5	249.5	119.5	0.001	High Priority - C
BEHTBP	26040-51-7	17.7	20.1	13.2	11.6	0.000	Watch List - C
Benzoylecgonine	519-09-5						-
Bisphenol A	80-05-7	298.5	96.4	19.2	18.3	7.002	High Priority - A
Bisphenol E	2081-08-5				0.2	0.002	-
Bisphenol F	620-92-8				0.1	0.051	Watch List - C
Bisphenol S	80-09-1	0.9	0.1	0.4	0.8	0.069	Watch List - C
Caffeine	58-08-2		0.4	23.0	1.4	0.209	Watch List - A

Compound	CAS	Toxicity Quotient Wastewater Treatment Plant Effluent				∑EAR Max WWTP	Category
		South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow		
Carbadox	6804-07-5				0.0	0.000	-
Carbamazepine	298-46-4	25.4	41.2	20.2	31.8	0.104	Watch List - A
Ciprofloxacin	85721-33-1	3.5	4.5	5.0	6.2		Watch List - B
Citalopram	59729-33-8	0.1	0.1	0.1	0.2		-
Clarithromycin	81103-11-9	10.7	11.8	7.1	7.9	0.000	Watch List - C
Clotrimazole	23593-75-1	0.3	0.2	0.5	0.4	0.002	-
Cloxacillin	61-72-3	15.2					Watch List - B
Cocaine	50-36-2	0.1	0.0	0.2	0.0		-
Codeine	76-57-3	0.2	0.1	0.1	0.2	0.000	-
Colchicine	64-86-8	4.5	5.1	2.5	4.2	0.018	Watch List - A
Cotinine	486-56-6					0.022	Watch List - B
Cyclophosphamide	50-18-0	0.0	0.0	0.0	0.0		-
Dec 603	13560-92-4		0.4	0.2			-
DEET	134-62-3	0.0	0.1	0.0	0.0	0.007	-
Dehydronifedipine	67035-22-7		1.9	0.7	1.2		Watch List - B
Desmethyldiltiazem	86408-45-9						-
Diatrizoic acid	117-96-4	771.2	1365.8	1712.3	1917.8		High Priority - B
Diazepam	439-14-5	0.0	0.0		0.0	0.002	-
Diphenhydramine	58-73-1	9.8	9.5	5.8	6.8	0.001	Watch List - C
Doxycycline	564-25-0		1.1	1.1	0.8		Watch List - B
EHTBB	183658-27-7	2.3	4.2	2.8	2.7		Watch List - B
Enalapril	75847-73-3	0.0		0.0	0.0		-
Enrofloxacin	93106-60-6				0.0	0.053	Watch List - C
Erythromycin-H2O	114-07-8	1.4	0.9	1.4	1.1	0.000	Watch List - C
Estrone	53-16-7	106.4	472.2	14.1	133.1	6.168	High Priority - A
Flumequine	42835-25-6		0.0				-

Compound	CAS	Toxicity Quotient Wastewater Treatment Plant Effluent				∑EAR Max WWTP	Category
		South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow		
Fluoxetine	54910-89-3	2.5	1.4	3.7	4.7	0.001	Watch List - C
Fluticasone propionate	80474-14-2			0.0	0.0	0.140	Watch List - C
Furosemide	54-31-9	0.9	2.4	0.1	1.8	0.000	Watch List - C
Gemfibrozil	25812-30-0					0.001	-
Glipizide	29094-61-9	1.6	1.8	0.8	0.9		Watch List - B
Glyburide	10238-21-8	0.4	0.5	0.4	0.2	0.000	-
HBB	87-82-1	0.0				0.000	-
Hydrochlorothiazide	58-93-5	1.5	1.6	0.9	0.9	0.005	Watch List - C
Hydrocodone	125-29-1	0.0	0.1	0.0	0.0		-
Ibuprofen	15687-27-1	2.3	0.1	5.2	0.2	0.001	Watch List - C
Iopamidol	60166-93-0	933.9	2392.7	301.0	445.4	0.103	High Priority - A
Lincomycin	154-21-2	0.0	0.0				-
Meprobamate	57-53-4	0.0	0.0	0.0	0.0		-
Metformin	657-24-9	2.3	0.2	2.2	1.9	0.011	Watch List - A
Metoprolol	51384-51-1					0.001	-
Metronidazole	443-48-1	0.0	0.0	0.0	0.0	0.000	-
Miconazole	22916-47-8	0.9	1.0	2.0	1.1	0.000	Watch List - C
Moxifloxacin	151096-09-2				0.7		-
Naproxen	22204-53-1	6.5	0.5	5.0	1.3		Watch List - B
Norfluoxetine	83891-03-6	0.0	0.0	0.0	0.0		-
Norverapamil	67018-85-3	0.5	0.4	0.3	0.4		-
Ofloxacin	82419-36-1	0.2	0.3	0.3	0.6	0.000	-
Oxazepam	604-75-1	0.2	0.2	0.1	0.1	0.000	-
Oxolinic Acid	14698-29-4			0.0		0.001	-
Oxycodone	76-42-6	0.0	0.1	0.0	0.0		-
Paroxetine	61869-08-7	0.0	0.0	0.0	0.0	0.000	-

Compound	CAS	Toxicity Quotient Wastewater Treatment Plant Effluent				∑EAR Max WWTP	Category
		South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow		
PBBZ	608-90-2	0.0					-
PBEB	85-22-3	0.9				0.000	-
Progesterone	57-83-0		0.0		0.1	0.019	Watch List - C
Promethazine	60-87-7		0.0			0.000	-
Propranolol	525-66-6	1.7	1.8	1.3	2.0		Watch List - B
Ranitidine	66357-35-5	0.0	0.0			0.040	Watch List - C
Rosuvastatin	287714-41-4	19.0	13.3	12.4	10.8		Watch List - B
Roxithromycin	80214-83-1	0.3		0.4	0.2	0.000	-
Sertraline	79617-96-2	5.4	5.0	7.6	10.8		Watch List - B
Sulfadiazine	68-35-9		0.0	0.0			-
Sulfamethoxazole	723-46-6	4.3	6.2	2.9	3.2		Watch List - B
Sulfanilamide	63-74-1	0.0	0.0		0.0	0.000	-
Testosterone	58-22-0	0.0	0.0		0.0	0.515	Watch List - C
Theophylline	58-55-9	0.2	0.2	5.0	0.3	1.378	High Priority - A
Thiabendazole	148-79-8	0.1	0.1	0.2	0.1	0.034	Watch List - C
Triamterene	396-01-0	68.1	107.8	41.9	50.9	0.002	High Priority - C
Triclocarban	101-20-2	22.4	22.3			0.000	Watch List - C
Triclosan	3380-34-5	10.7	13.8	6.2	6.0	0.002	Watch List - C
Trimethoprim	738-70-5	0.0	0.0	0.0	0.0	0.000	-
Tylosin	1401-69-0	0.3		0.3	0.6		-
Valsartan	137862-53-4	0.0	0.0	0.0	0.0		-
Venlafaxine	93413-69-5	88.7	106.8	82.4	103.2		High Priority - B
Verapamil	52-53-9	0.1	0.0	0.1	0.1	0.000	-
Zidovudine	30516-87-1	0.3	0.3	0.0	0.1	0.000	-
Alkyl Phenols							
4-Nonylphenol diethoxylates	20427-84-3	41.4	25.6	33.2	10.9		Watch List - B

Compound	CAS	Toxicity Quotient Wastewater Treatment Plant Effluent				∑EAR Max WWTP	Category
		South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow		
4-Nonylphenol monoethoxylates	104-35-8	3.2	3.7	0.7	1.0		Watch List - B
4-Nonylphenols	104-40-5	1.9	2.7	0.3	0.8	0.023	Watch List - A
Perfluorinated Compounds							
5:3 FTCA	914637-49-3	0.1	0.3		0.2		-
6:2 FTS	27619-97-2	0.0	0.0	0.1	0.0		-
MeFOSAA	2355-31-9	0.0		0.0	0.0		-
PFBA	375-22-4	0.0	0.0	0.0	0.0		-
PFBS	375-73-5	0.0	0.0	0.1	0.0		-
PFDA	335-76-2	0.1	0.1	0.0	0.0	0.000	-
PFHpA	375-85-9	0.1	0.0	0.1	0.0	0.000	-
PFHpS	375-92-8			0.1			-
PFHxA	307-24-4	0.0	0.0	0.0	0.0	0.187	Watch List - C
PFHxS	355-46-4	0.0	0.1	1.0	0.0		Watch List - C
PFNA	375-95-1	0.0	0.0	0.0	0.0	0.000	-
PFOA	335-67-1	0.6	0.3	0.7	0.3	0.000	-
PFOS	1763-23-1	0.0	0.0	0.1	0.0	0.069	Watch List - C
PFPeA	2706-90-3	0.0	0.0	0.0	0.0		-
PFPeS	2706-91-4	0.0	0.0	0.1	0.0		-

Results of this evaluation indicate that several of the CECs present in effluent have the potential to elicit biological responses based on the concentrations measured in wastewater treatment plant effluent. This is clearly a worst-case exposure scenario and an unlikely measure of actual environmental concentrations due to the dilution of wastewater in the receiving water. However, there were several compounds that, based on measured concentrations in treatment system effluent, exceeded biological response thresholds by 100-1000x (venlafaxine, iopamidol, estrone, erythromycin, diatrizoic acid, bisphenol A, and 17β-estradiol). As such, a dilution of that same magnitude (100-1,000x) in estuarine waters would still leave the potential to elicit a biological response to exposed organisms.

3.2 High Resolution Mass Spectrometry analytical results

Samples from wastewater treatment facilities effluent, the Puget Sound estuary, and laboratory exposure were all process and analyzed via HRMS instrumentation. The primary intent of this was to screen wastewater effluent for compounds that had not been previously monitored or reported. Additional analysis was performed, as presented below. It should be noted that all of the resulting analytical data files have been archived for future potential evaluation to, for example, compare these results against new or expanded spectral libraries, and/or evaluate with future computational tools to support identifications. Current results are reported below.

3.2.1 Suspect screening of wastewater effluent samples

As described in Section 2.4.2, effluent samples were screened against a spectral library containing >500,000 different entries. There were > 7,500 individual spectral matches representing >250 identified compounds including many that, to our knowledge, have not previously been reported in effluent samples. The identifications should be considered as a confidence level 2, which indicates that there is a high-quality match with an existing spectra library (e.g., GNPS) but that confirmation standards were not run on CUW instrumentation for final confirmation (Schymanski et al. 2014).

A summary of all compounds identified, including MS/MS spectra acquired in this work and the corresponding library matches, are included in Appendix C. To provide some context with regard to their biological relevance, a preliminary screening of the compounds was performed based on the PNEC values provided in the European Union NORMAN database (Moermond et al. 2016; NORMAN Network, 2021) by highlighting those with a PNEC value less than 10 ng/L (Table 11; James et al. 2015). While the concentrations of these compounds were not explicitly quantified in the effluent, based on our quantification of similar compounds in similar samples, the detection limit for this instrumentation generally ranges from 1-100 ng/L. It is then reasonable to expect that detected compounds would occur within that same general concentration range. Based on this approach, 33 compounds were identified for potential future screening and monitoring. Note that some compounds have been the subject of monitoring in wastewater effluent, including in this study. Seven are nominally naturally occurring compounds that might not be easily managed or otherwise controlled.

Table 11. List of compounds identified by high resolution mass spectrometry analysis and subsequent MS/MS fragment spectra match. Compounds shown have reported PNEC values < 10 ng/L (Moermond et al. 2016; NORMAN Network 2021). Some of the compounds identified in this approach were included on the targeted analytical schedule from AXYS, and the results of the estuarine samples collected in this study were used to provide estimated concentrations. These are shown, with median estimated estuary marine water concentration. The complete results are included in Appendix C.

Compound Name	CAS Number	CEC Category	PNEC marine water (ng/L)	Estuarine Concentration (this study; ng/L)
Candesartan	139481-59-7	Pharmaceutical	0.3	-
Bis(2-ethylhexyl) phthalate	117-81-7	Industrial	0.5	-
Dioctyl phthalate	117-84-0	Industrial	0.6	-
Disodecyl phthalate	26761-40-0	Industrial	0.7	-
Erucamide	112-84-5	Industrial	0.7	-
DL-alpha-Tocopherol acetate	7695-91-2	Commercial	0.9	-
13-Docosenamide	3061-72-1	Natural	1.0	-
Nobiletin	478-01-3	Pharmaceutical	1.3	-
Octadecylamine	124-30-1	Industrial	1.6	-
Hexa(methoxymethyl)melamine	3089-11-0	Industrial	1.7	-
Edifenphos	17109-49-8	Pesticide (Current Use)	2.0	-
Arachidonic acid	506-32-1	Commercial	2.2	-
Tangeritin	481-53-8	Pharmaceutical	2.5	-
Cholecalciferol	67-97-0	Commercial	2.5	-
Stearidonic acid	20290-75-9	Natural	3.1	-
5alpha-Cholestan-3-one	566-88-1	Sterol	3.5	-
Venlafaxine	93413-69-5	Pharmaceutical	3.8	0.5
Cholest-4-en-3-one	601-57-0	Natural	3.8	-
n-Oleoylethanolamine	111-58-0	Commercial	3.8	-
linolenic acid	463-40-1	Natural	4.2	-
Benzyltetradecyldimethylammo-nium	139-08-2	Commercial	4.3	-
Carbamazepine	298-46-4	Pharmaceutical	5.0	<1.5
Oleic acid	112-80-1	Commercial	5.3	-
Meclizine hydrochloride	1104-22-9	Pharmaceutical	5.5	-
N,N-Dimethyltetradecylamine	112-75-4	Commercial	5.9	-
Hexadecanamide	629-54-9	Commercial	7.4	-
Elaidamide	4303-70-2	Pharmaceutical	7.9	-
Mitragynine	4098-40-2	Pharmaceutical	8.3	-
Sertraline	79617-96-2	Pharmaceutical	9.1	<0.3
Glyceryl monooleate	111-03-5	Natural	9.3	-
Tetradecylamine	2016-42-4	Natural	9.6	-
20-Hydroxyeicosatetraenoic acid	79551-86-3	Pharmaceutical	9.9	-
N-Methyldodecylamine	7311-30-0	Natural	10.4	-

3.2.2 Comparative Analysis of Wastewater Effluent Samples

Acquired data files for the wastewater treatment effluent samples were compared using a hierarchical cluster analysis to understand similarities in waste stream between WWTPs and flow condition and differences in chemical occurrence patterns (Figure 6). Results suggest that Brightwater high flow and low flow samples were similar, clustering closely to each other rather than to the West Point or South Plant samples. In contrast, low flow samples from West Point and South Plant were more similar to each other than to their respective high flow samples. Finally, there was variation amongst the high flow samples from West Point and South Plant with the difference between high and low flow being more different from West Point than for South Plant. This is likely related to the relatively small contribution of stormwater to South Plant. Note that this is a preliminary analysis and that the full, non-targeted evaluation of chemical occurrence in wastewater effluent is beyond the scope of the current project.

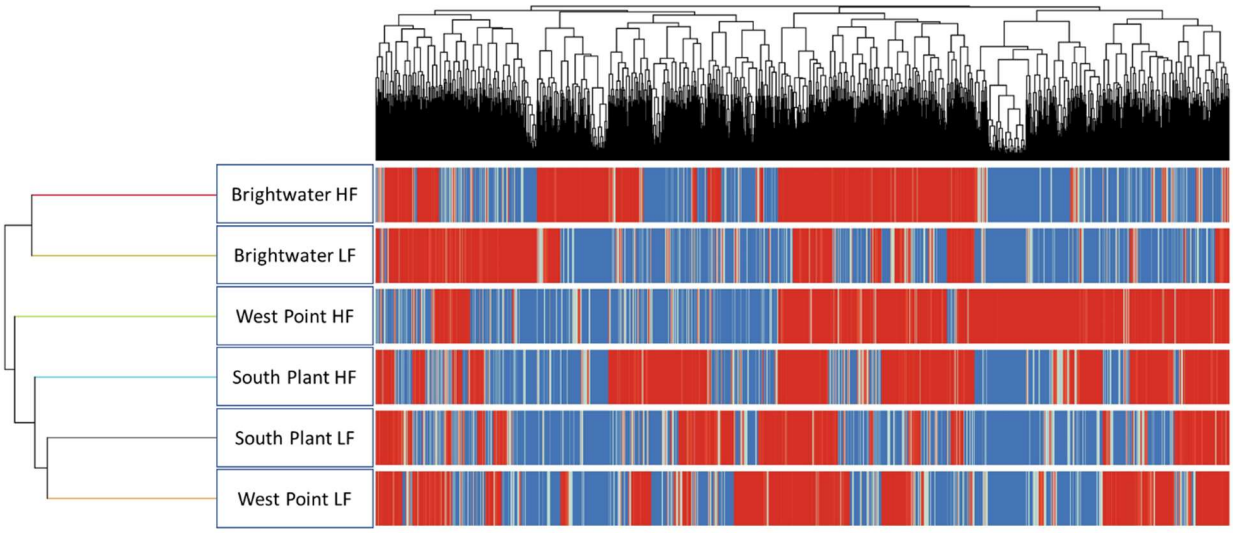

Figure 6. Hierarchical cluster analysis (Euclidian distance, Wards clustering) of wastewater treatment plant effluent samples for high flow (HF) and low flow (LF) conditions. All high flow samples were collected on Feb 22, 2022. Color indicates abundance of feature with red being abundant and blue being absent.

3.2.3 Comparative Analysis of Laboratory Exposure Water

The laboratory exposure water was sampled several times over the duration of the study to determine the consistency of the exposures and to confirm the laboratory sampling procedures and protocols. Results generally supported the experimental protocol in that:

- The approach of freezing individual grab samples did not appear to affect the chemical composition compared to samples that were immediately processed after collection. Samples that were frozen for ~10 days were essentially identical to those that were processed immediately. This supports the use of the previously untested protocol of freezing individual time-point grab samples to create a final representative composite.
- The approach to managing the exposure water provided a generally-consistent exposure profile over the 10-day exposure period. The individual grab samples, which were collected at six time-

points over the duration of the exposure, were analyzed for sucralose. Sucralose has been identified as a conservative tracer of wastewater as it is generally ubiquitous in wastewater effluent and undergoes limited degradation through treatment systems or in the environment (James et al. 2016; Tran et al. 2014). Based on all measures, the sucralose concentration varied by ~6% (relative standard deviation) over the course of the exposure.

- The relative concentrations of other compounds varied likely based on chemical fate and transport properties. For example, the abundance of 4-tert octylphenol (4-OP) appeared to increase over the exposure period. 4-OP is formed by the degradation of the non-ionic surfactants octylphenol ethoxylates which are present in wastewater (Kovarova et al. 2013). It is possible that this degradation pathway is active in the exposure tanks resulting in an increase in 4-OP. Escitalopram, conversely, was only present in the samples collected just after the water refresh (when 'new' wastewater was added to the exposure tanks) but not present two days later. This suggests that escitalopram is degraded or transformed by fish or in the environment.
- A comparison of water in the tanks with fish to water collected from the no-fish control tanks revealed substantial differences in water chemistry. The chemical differences are expected to result from a loss of anthropogenic compounds due to uptake by fish as well as the excretion by of biotransformed compounds and fish-derived organic compounds.

3.3 Laboratory Exposure Study

3.3.1 Stress, Endocrine Function and Metabolism

From the principal components analysis (PCA) conducted on endocrine disruption, stress, and metabolism endpoints, we retained two principal components (PCs). PC1 explained 35.9% of the variance in the dataset and was heavily contributed to by four metabolic parameters: albumin, calcium, cholesterol, and total protein (Figure 7) that collectively followed a hormesis relationship with WWE concentration ($t_{(1,19)} = 3.2$, $p = 0.002$, Figure 7B, Table 12). Typically, a hormesis response would show stimulus, return to baseline, and finally an inhibitory response with increasing contaminant concentrations. This model captured a stimulating response and return to baseline; the inhibiting portion of the hormesis dose-response relationship may have required more concentrated WWE (>20%) or longer exposure durations. To further explore the hormesis response, individual endpoints loading on PC1 (albumin, calcium, cholesterol, total protein) were compared to treatment using the same model. While the relationship for each parameter appeared to follow the pattern of stimulatory responses at intermediate WWE concentrations (Figure 8), a viable and statistically significant model was only produced for total protein ($t(1,19) = 23$, $p < 0.001$, Figure 8A, Table 12). In Kruskal Wallis and ANOVA tests, there were significant differences among treatments for albumin ($\chi^2(5,18) = 10.4$, $p = 0.066$), and cholesterol ($F(5,18) = 4.43$, $p = 0.008$) but effluent treatments were not significantly different from controls for any parameter (Figure 8). There were no significant differences among %WWE treatments for calcium ($F(5,18) = 2.00$, $p = 0.13$).

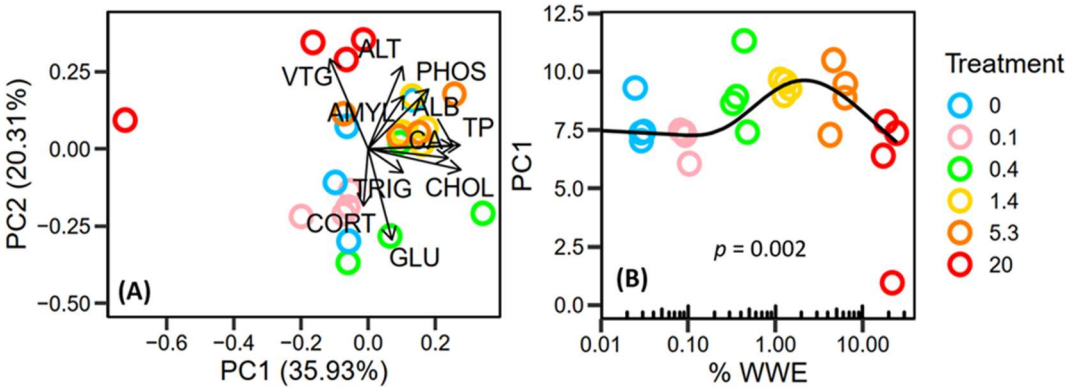

Figure 7. A) Principal Component Analysis biplot showing all samples (circles) and plasma endpoints (vectors) associated with the two retained principal components (PCs). Percent values associated with each PC are the percent of variance explained by that PC. Four overlapping vectors on the positive end of the PC1 axis are TP (total protein), ALB (albumin), CA (calcium), and CHOL (cholesterol). The remaining vectors are: VTG (vitellogenin), ALT (alanine aminotransferase), AMYL (amylase), PHOS (phosphorous), TRIG (triglycerides), GLU (glucose), and CORT (cortisol). B) Cedergreen-Ritz-Streibig (CRS) regression model of PC1 on wastewater effluent concentration (% WWE) with the associated p-value. Defined equation parameters are listed in Table 4. A jitter effect was applied to PC1 values to exhibit where each data point lies; all data points within a treatment were exposed to the same WWE concentration.

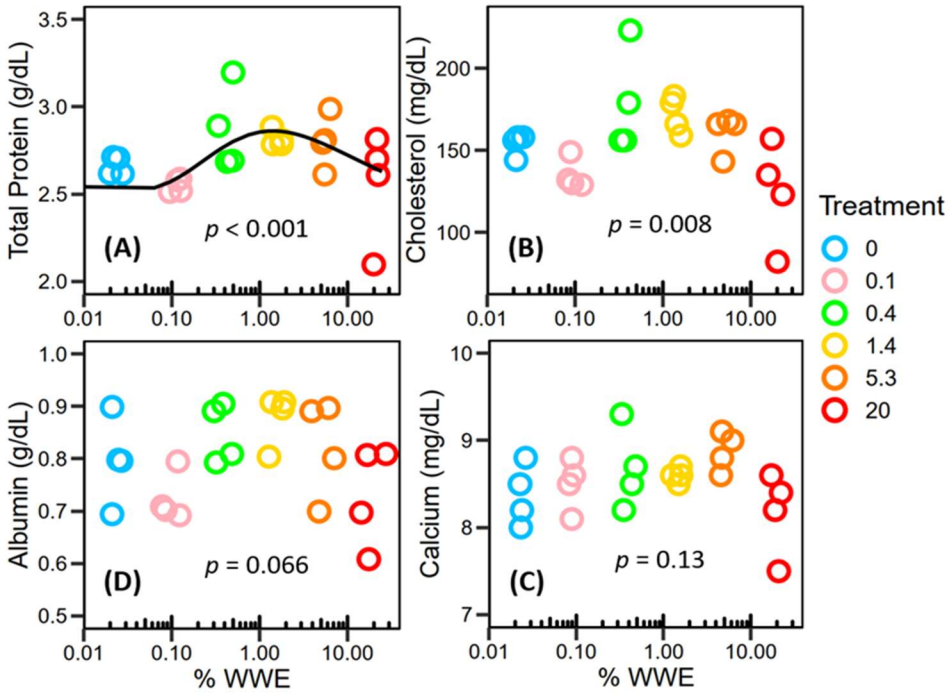

Figure 8. Relationship with %WWE for plasma parameters loading strongly onto PC1 showing A) CRS regression (α = 0.5) for total protein; ANOVA results for B) Cholesterol and C) Calcium; D) Kruskall-Wallis result for albumin. A jitter effect was applied to all values to exhibit where each data point lies; all data points within a treatment were exposed to the same WWE concentration.

Table 12. Parameter definitions in regression equations for significant Cedergreen-Ritz-Streibig (CRS) models.

	α	*b*	*c*	*d*	*e*	*f*
PC1	0.75	0.649	3.93	7.55	3.84	10.5
Total Protein	0.5	0.458	2.34	2.65	0.0475	6.26

PC2 explained 20.3% of the constrained variance in the PCA, with strongly loading parameters of vitellogenin, alanine aminotransferase, and glucose (Figure 7A). PC2 followed a monotonic relationship with %WWE $\log_{3.8}$ WWE ($F(1,22) = 19.9$, $p < 0.001$, Figure 9A). Of the strongly loading parameters for PC2, glucose and vitellogenin had a significant monotonic relationship with %WWE. Glucose concentration decreased linearly with $\log_{3.8}$(%WWE) ($F(1,22) = 20.4$, $p < 0.001$, Figure 9B) whereas $\log_{10}$(vitellogenin) increased linearly with WWE concentration ($F(1,22) = 225$, $p < 0.001$, Figure 9C). In 20% effluent, fish showed a 383-fold increase in vitellogenin over controls Finally, alanine aminotransferase did not have a significant dose-dependent relationship with WWE concentration ($F(1,22) = 0.938$, $p = 0.34$, Figure 9D) and did not show significant differences between WWE concentrations in an ANOVA ($F(5,18) = 0.630$, $p = 0.68$).

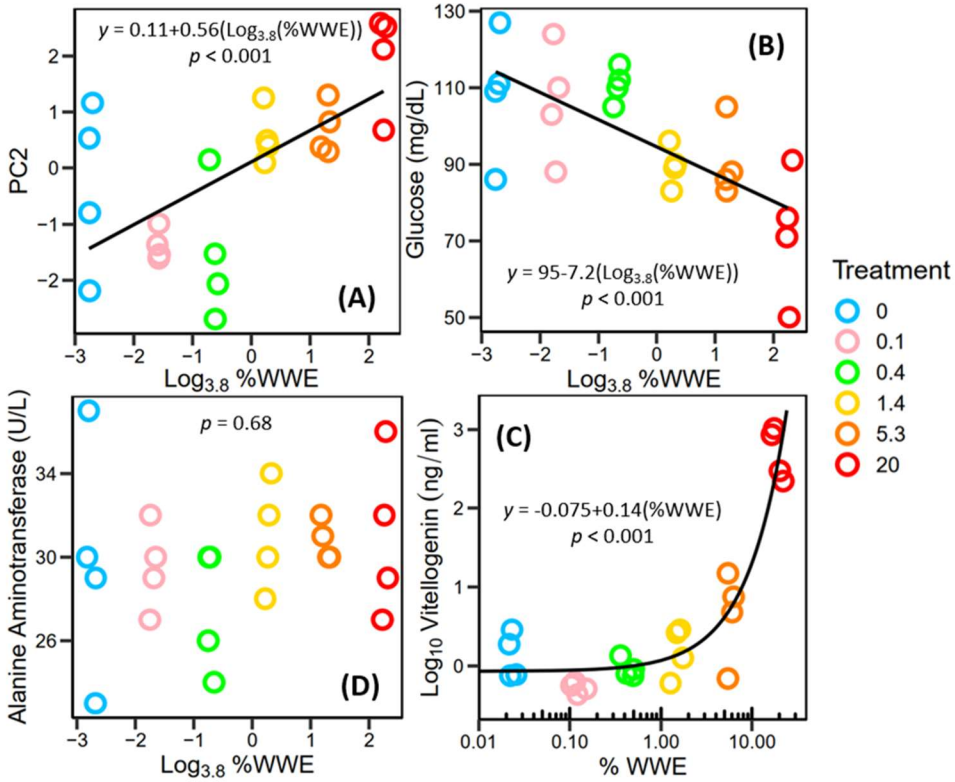

Figure 9. Linear relationships with % wastewater effluent (WWE) for A) PC2; B) glucose concentration C) $\log_{10}$ of vitellogenin concentration; D) alanine aminotransferase (ALT) concentration. Equations, regression lines, and p-values are shown for significant linear regression models. For ALT, the p-value for the ANOVA is shown.

Four plasma endpoints did not load heavily onto either retained PC. Using ANOVAs (amylase, phosphorous) and Kruskal Wallis (cortisol, triglycerides) tests, only phosphorus was significantly different between treatments ($F(5,18) = 2.7$, $p = 0.053$). Still, no treatments were significantly different from controls in the Dunn's post-hoc test (Figure 10).

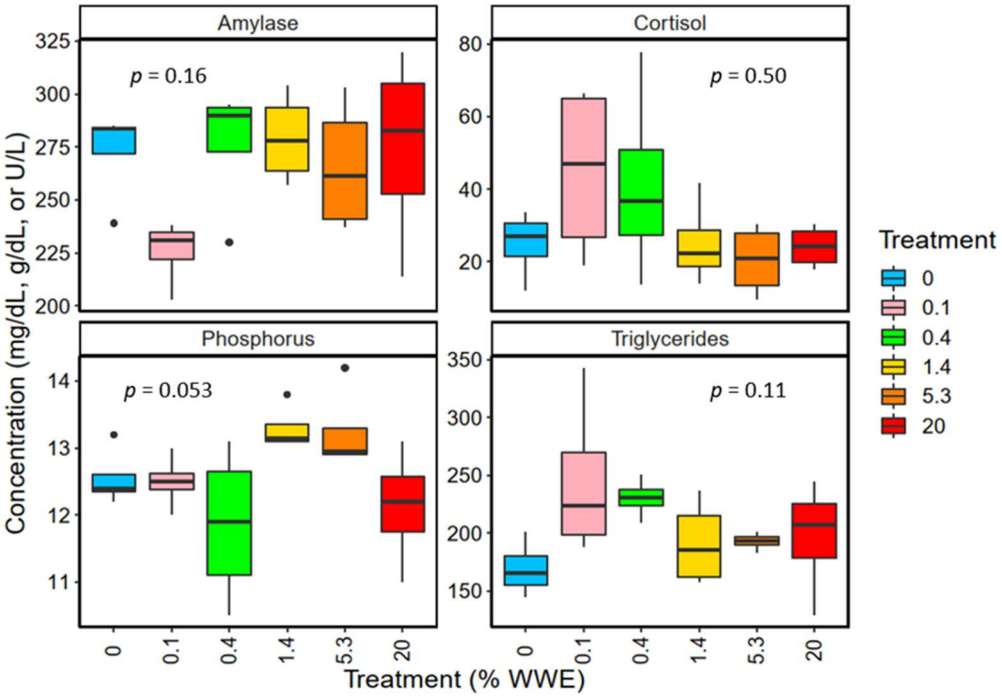

Figure 10. Concentrations of the plasma endpoints that did not load significantly in the principal component analysis versus % wastewater effluent (% WWE). Boxes represent the 25th percentile, median (middle) and 75th percentile, while lines extending from boxes represent the ranges excluding outliers. Dots represent outliers. Units for each endpoint are mg/dL for phosphorus, triglycerides; U/L for amylase; ng/ml for cortisol. P-values are shown for ANOVA (amylase, phosphorous) and Kruskal-Wallis (cortisol, triglycerides) tests.

3.3.2 Na+/K+ ATPase

Tissue data included Na^+/K^+ ATPase activity levels (NKA) in gills and brains. The Chinook used for this study (June-July 2021) are ocean-type and would likely have been smolting around May 2021 when the remainder of the cohort was released from the hatchery. Smolting is associated with increased gill NKA (e.g., post-smolt NKA range of 23-49 µmol ADP*mg protein^{-1}*hr^{-1}; Beeman et al. 1991, Madsen et al. 2004), in preparation for the transition to higher ambient ion concentrations in saltwater. The gill NKA activity levels measured in this study (0.57-2.85 µmol ADP*mg protein^{-1}*hr^{-1}) was indicative of freshwater phase juveniles, to which anadromous fish will revert if held back in freshwater. WWE treatments did not have a significant effect on gill NKA ($\chi^2(5,18) = 6.0$, $p = 0.31$, Figure 11A). In contrast, brain NKA values were significantly different between treatments ($\chi^2(5,18) = 11.5$, $p = 0.040$, Figure

11B), with all treatments except 1.4% being significantly reduced from the controls in a Dunnett's post-hoc test.

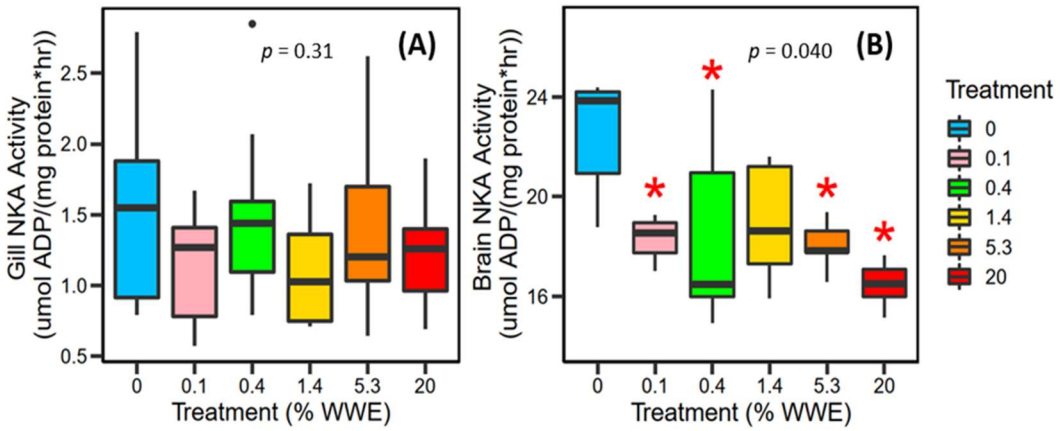

Figure 11. Activity levels of sodium/potassium ATPase (NKA) from different concentrations of wastewater effluent (% WWE) for gills (A) and brains (B) of juvenile Chinook. Boxes represent the 25th percentile, median (middle) and 75th percentile, while lines extending from boxes represent the ranges excluding outliers. Dots represent outliers. Treatments that were significantly different from controls are marked with an asterisk. P-values represent the results from the Kruskal-Wallis tests.

3.3.3 Apical Endpoints

Treatments also affected percent lipid content and the number of fish with liver anomalies. Percent lipid content decreased dramatically in the 5.3% and 20% treatments, with 22% and 41% declines from the control value, respectively (Figure 12A). The number of tanks of fish with visible liver anomalies differed significantly between treatments using a Kruskal Wallis test ($\chi^2(5) = 9.26$, $p = 0.099$, Figure 12B). The 20% treatment had five times more liver anomalies than the control and was the only treatment for which we could detect a statistically significant difference from the control (Dunnett's post hoc test). While the 0.4% treatment had double the liver anomalies from controls, this was not significantly different in a Dunnett's post hoc test.

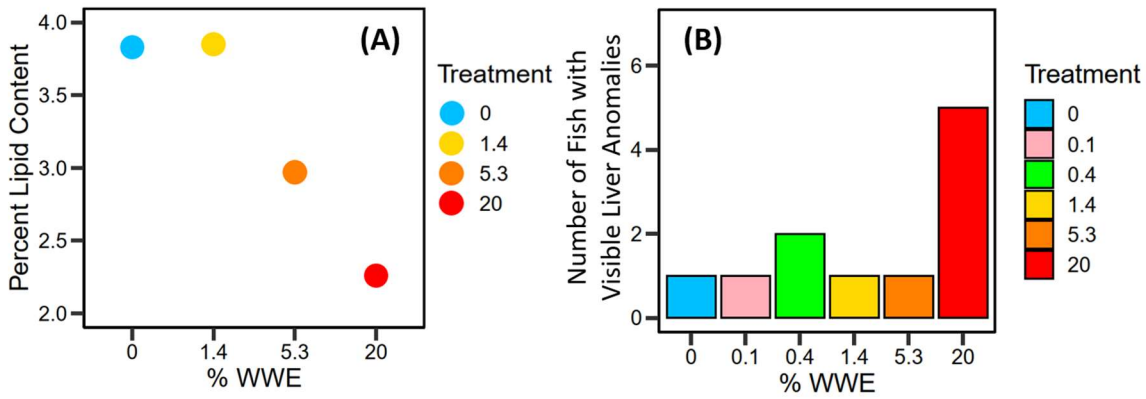

Figure 12. Lipid content and liver anomalies for fish exposed to wastewater effluent (WWE). (A) Percent lipid content of composited samples (n=7 fish) for treatments with tissue chemistry analysis. (B) Total number of fish in each treatment with a visible liver anomaly. Each fish with a visible liver anomaly was from a different replicate tank and there were a total of 7 tanks per treatment and 56 fish per treatment.

3.4 Metabolomics Results

The first phase of analysis (3.4.1 Analysis with all treatments) presents our statistical evaluation for all treatments combined that was conducted to show how the treatments responded to our dilution series of WWE. We present the results showing the total number of altered metabolites, the PLS-DA plot, and heatmap, which give an overview of relationships among various treatments and their separation over the dilution series. The second phase (3.4.2 Analysis for control versus treatment comparisons) shows the same statistical analyses for all control versus pairwise companions, in addition to altered physiological pathways and drug pathways that are based on altered metabolite concentrations. The altered pathways were determined by algorithms that were premised on observed versus expected observations. The tables contain all relevant results. Example figures are included to show graphical representation of the results for selected comparisons.

3.4.1 Analysis with all treatments

In order to reduce systemic bias and improve data consistency, data normalization was performed for all comparisons. Normalization was achieved with the Pareto scaling algorithm (Figure 13), which provided the best fit for all datasets analyzed. Only 1 analyte was removed as a result of using a 50% threshold for missing values. With a delta factor of 1.1 and FDR of 0.03, the Significance Analysis of Microarray (SAM) analysis detected 46 metabolites (of 184) with a false positive rate (FDR) of 14%, indicating a significant difference for at least one treatment compared with controls (Figure 14). A list of those 46 metabolites is shown in Table 13.

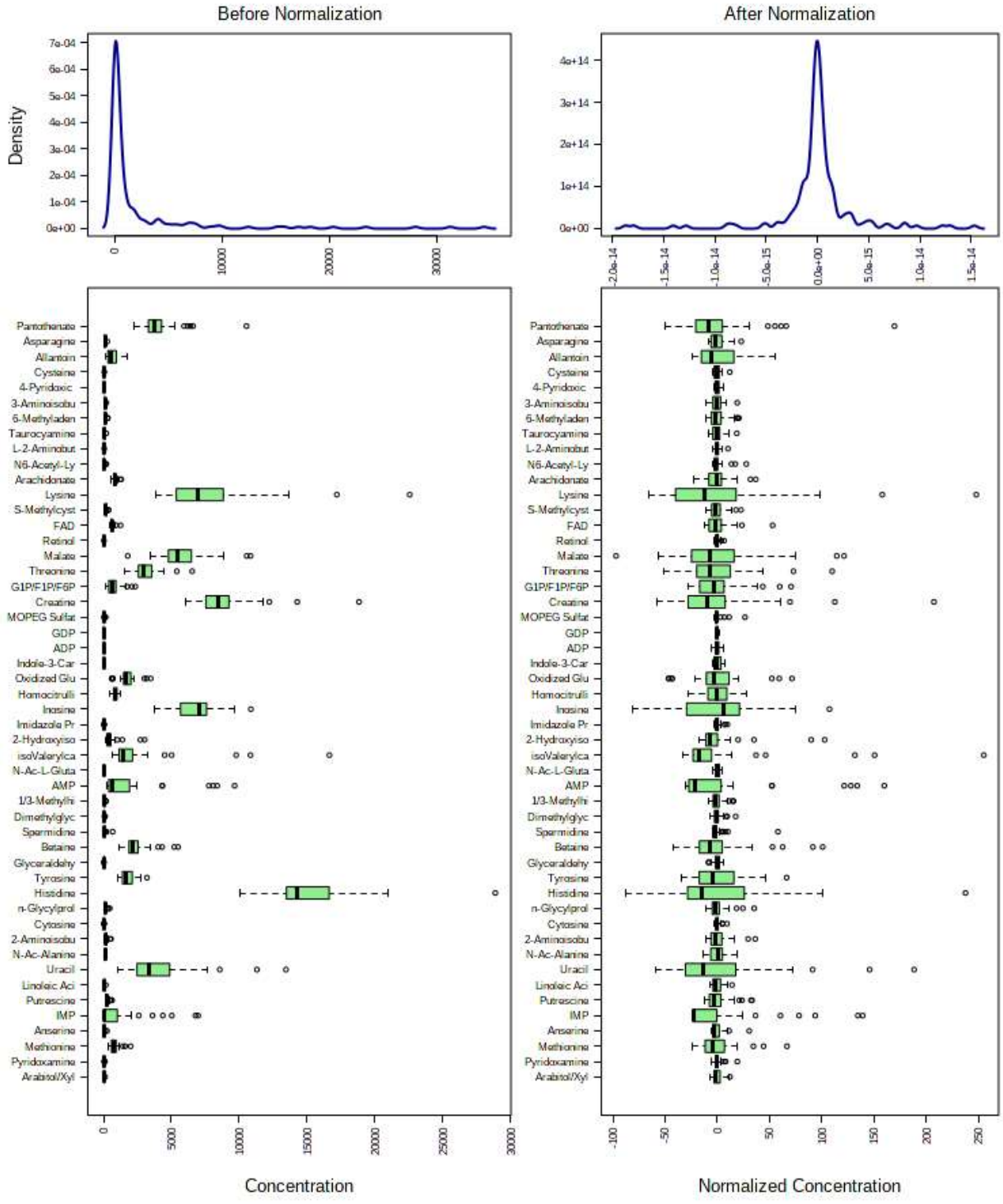

Figure 13. Normalization of data with Pareto algorithm for analysis of all treatments and the full set of analytes. Similar results were obtained for all pairwise comparisons.

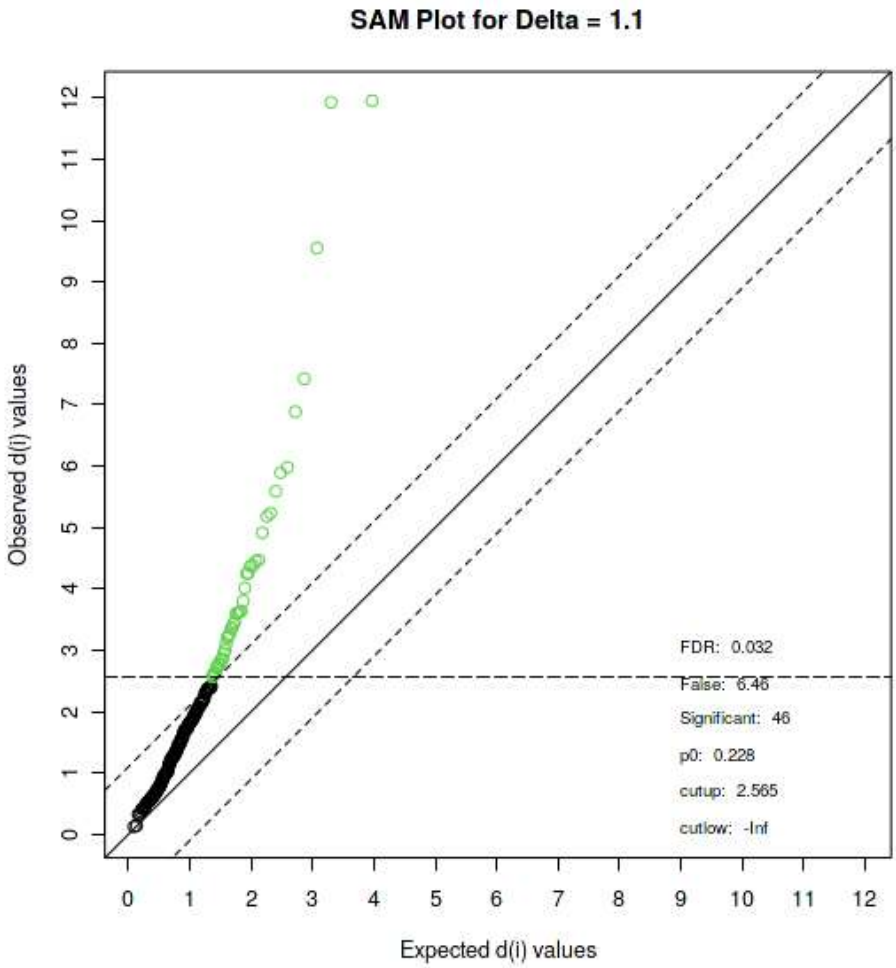

Figure 14. Plot showing results from the Significance Analysis of Microarray (SAM) test, showing in green circles above the dashed horizontal line the number of analytes for which there was a significant difference from controls for at least one treatment. FDR is the false discovery rate. X and Y axes are expected and observed delta values (d(i)). Slanted dashed lines show optimized delta (e.g., 1.1)

Table 13. Metabolites in juvenile Chinook with a significant difference from controls for at least one treatment, determined with Significance Analysis of Microarray (SAM).

Analyte	*p*-values	FDR	Analyte	*p*-values	FDR
1-Methylimidazole Acetate	0.012	0.018	IMP	0.031	0.030
2-Aminoadipate	0.010	0.016	Indole-3-Carboxylic Acid	<0.0001	<0.0001
5,6-Dihydrouracil	0.004	0.009	Inositol	0.017	0.023
7-Methylguanine	0.024	0.027	Lactate	0.001	0.006
Acetylcholine	0.031	0.030	L-Kynurenine	0.004	0.009
Allantoin	0.002	0.007	Lysine	0.033	0.031
AMP	0.032	0.030	MOPEG Sulfate	0.026	0.027
Arabitol/Xylitol	0.006	0.014	N6-Trimethyllysine	0.001	0.006
Beta Alanine	<0.001	0.001	Ornithine	0.007	0.014
Creatinine	<0.0001	<0.0001	Oxalacetate	0.013	0.018
Cytidine	0.021	0.025	Picolinic Acid	<0.001	0.001
Dimethylarginine	0.001	0.006	Riboflavin	0.008	0.014
Dimethylglycine	0.003	0.009	SAH	0.012	0.018
G1P/F1P/F6P	0.015	0.020	Sarcosine	0.022	0.025
G6P	0.010	0.016	Sedoheptulose 7-Phosphate	0.005	0.011
Glucosamine-6-Phosphate	0.018	0.023	Serine	0.007	0.014
Glycerate	0.003	0.009	Sorbitol	0.026	0.027
Glycerol-3-P	0.007	0.014	Succinate	0.024	0.027
Glycine	0.003	0.009	Threonine	0.035	0.032
Homocitrulline	0.010	0.016	UDP-Glucose	0.009	0.016
Hydroxyproline	0.002	0.007	UMP	0.003	0.009
Hypotaurine	0.022	0.025	Uridine	0.002	0.007
Imidazoleacetic Acid	<0.001	0.001	Valine	0.027	0.028

Delta = 1.1, includes all treatments. See Figure 14.

The PLS-DA plot for all detected metabolites is shown in Figure 15. The r^2 for this model is 0.82 and the Q^2 was 0.45. The permutation test (p = 0.015) indicated substantial discrimination among treatments. The plot shows clean separation of the control and 20% treatment and the high variability of the 1.4% treatment. The 5.3% treatment groups mostly with the 20% treatment and the 0.4% and 0.1% treatments grouped closer to the control treatment.

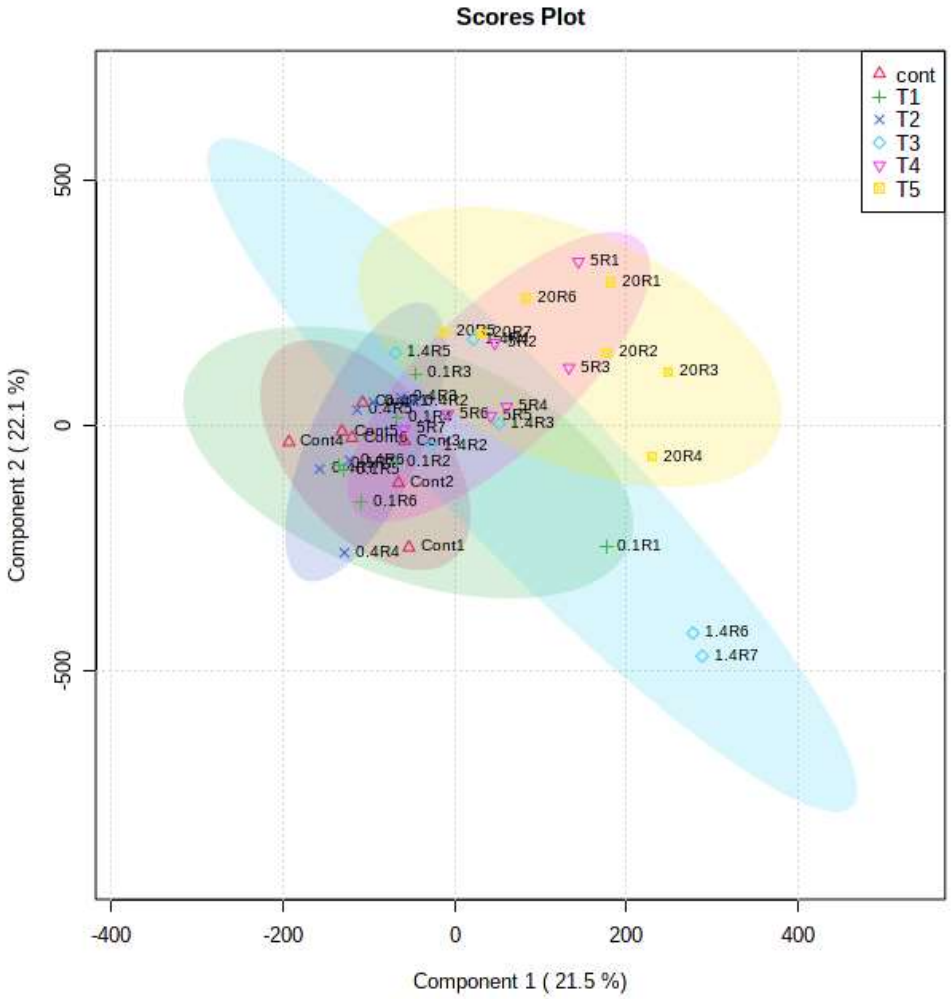

Figure 15. Scores plot from the PLS-DA. Ellipses around each treatment show the 95% confidence interval. Treatments are T1=0.1%, T2=0.4%, T3=1.4%, T4=5.3%, and T5=20% dilution.

A heatmap for the 50 most altered metabolites from all treatments is shown in Figure 16. Based on the degree of metabolite response, treatments separated into three main groups along the x-axis, with the control treatment in one group (middle), plus replicates from the 0.1, 0.4, and 1.4% treatments. The right group is mostly high effluent treatments (20% and 5.3%) and the far-left group is comprised of mostly low effluent replicates (0.1% and 0.4%). Two of the 1.4% treatment replicates were so different they grouped separately from the others.

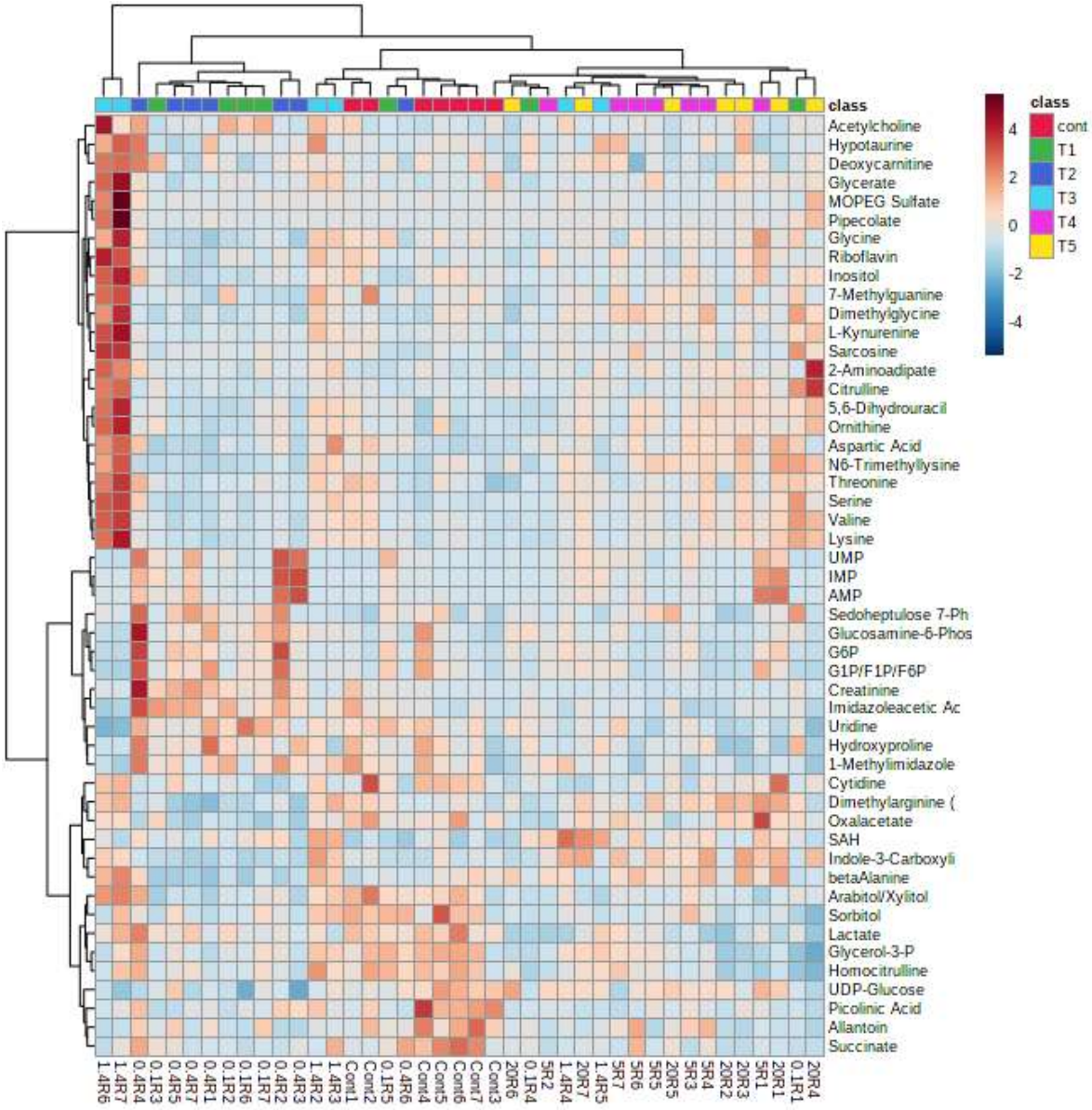

Figure 16. Heatmap showing the 50 most impacted analytes of the 184 detected. Replicate names shown at bottom. Cont is the control replicate and T1 – T5 are the 0.1%, 0.4%, 1.4%, 5.3% and 20% treatments, respectively.

3.4.2 Analysis for control versus treatment comparisons

3.4.2.1 Basic statistics

To highlight important differences and altered pathways, pairwise comparisons were made between the control group and each WWE treatment using a SAM analysis (Table 14). The most altered metabolites occurred at the highest doses and the fewest in the lowest dose (0.1%). Differences were also evident at intermediate doses; a high number (n=13) occurred in the 0.4% dose, which may have resulted from lower variability among replicates.

We examined each control versus treatment comparison for the magnitude of difference (fold change) for each metabolite as determined by volcano plot analysis (Table 15). A number of analytes exhibited relatively large fold change differences with low p-values. These values highlight analytes that were elevated or reduced in relation to the control values. Many of the metabolites were altered in each treatment and exhibited the same fold change direction (increase or decrease) across treatments. These patterns provide some insight to the results of the pathway analysis in terms of important analytes that were altered. The most important metabolites across treatments occur at the top of the table and were altered from controls in 3-5 of the treatments. A number of substantially different metabolites (n=18) with low p-values in the fold change analysis were observed only for the highest treatment (20%).

Table 14. Results of the Significance of Microarray Analysis (SAM) highlighting metabolites that were different from controls in each of the wastewater effluent treatments.

	Cont v. 20%		Cont v. 5.3%		Cont v. 1.4%		Cont v. 0.4%		Cont v. 0.1%	
	p-value	FDR	p-value	FDR	p-value	FDR	p-value	FDR	p-value	FDR
Picolinic Acid	0.0006	0.018	0.003	0.16			0.01	0.09	0.001	0.05
Indole-3-Carboxylic Acid			0.004	0.16	0.003	0.05	0.0001	0.07		
2-Aminoadipate			0.006	0.16	0.003	0.05				
4-Pyridoxic Acid	0.0001	0.01	0.0002	0.04						
Allantoin	0.0007	0.02					0.02	0.10		
Glycerophosphocholine							0.02	0.11	0.01	0.17
Inosine	0.0200	0.12	0.003	0.16						
Kynurenic Acid	0.0017	0.04					0.01	0.09		
Lactate	5.5E-05	0.01	0.002	0.16						
S-Adenosylhomocysteine SAH			0.007	0.16	0.005	0.07				
UDP-Glucose							0.02	0.10	0.001	0.10
1-Methylimidazole Acetate	0.0220	0.12								
2-Hydroxyglutarate	0.0133	0.11								
5-Aminovaleric Acid	0.0304	0.13								
Alpha-Ketoglutaric Acid					0.002	0.05				
Arabitol/Xylitol	0.0037	0.05								
Carnitine	0.0296	0.13								
Citrulline							0.01	0.07		
Cysteine	0.0272	0.13								
Cytidine							0.01	0.07		
Dimethylglycine							0.01	0.07		
Glucoronate	0.0223	0.12								

	Cont v. 20%		Cont v. 5.3%		Cont v. 1.4%		Cont v. 0.4%		Cont v. 0.1%	
	p-value	FDR	p-value	FDR	p-value	FDR	p-value	FDR	p-value	FDR
Glutarylcarnitine	0.0162	0.11								
Glycerol-3-Phosphate	0.0026	0.04								
Glycine							0.004	0.07		
Homocitrulline	0.0024	0.04								
Hydroxyproline	0.0040	0.05								
N6-Trimethyllysine							0.01	0.09		
N-Acetylneuraminate	0.03	0.13								
NADH	0.03	0.13								
Oxalacetate							0.0001	0.06		
Phosphorylcholine	0.003	0.05								
Reduced Glutathione	0.01	0.11								
Ribulose 5-Phosphate	0.02	0.12								
Sorbitol	0.01	0.09								
Succinate	0.01	0.10								
Succinylcarnitine	0.0006	0.02								
Thiamine	0.03	0.14								
Urate	0.02	0.11								
Uridine	0.02	0.11								
Xanthine							0.02	0.10		

Analytes with low p-values and FDRs determined with Significance Analysis of Microarray (SAM) analysis. Treatment 5.3 is shown without replicate 1, which was determined to be an outlier with the Random Forest algorithm.

Table 15. Fold change (FC) results for each analyte for control versus treatment.

Analyte	Cont v. 20%		Cont v. 5.3%		Cont v. 1.4%		Cont v. 0.4%		Cont v. 0.1%	
	FC	p-value	FC	p-value	FC	p-value	FC	p-value	FC	p-value
Alpha-Ketoglutarate	0.67	0.015	0.61	0.019	0.65	0.003	0.68	0.073	0.71	0.020
Indole-3-Carboxylic Acid	0.57	0.032	0.57	0.005	0.53	0.005	1.84	0.005	1.63	0.009
Picolinic Acid	2.93	0.001	2.19	0.003	2.09	0.010	1.82	0.018	2.84	0.001
Allantoin	3.37	0.001			2.62	0.004	1.94	0.025	2.11	0.010
Arabitol/Xylitol	1.64	0.005	1.53	0.018			1.46	0.066	1.51	0.025
Sorbitol	2.02	0.010	1.60	0.044			1.51	0.081	1.66	0.050
Succinate	4.49	0.015	2.92	0.051	2.41	0.100			3.24	0.027
4-Pyridoxic Acid	1.90	0.000	1.48	0.083					1.53	0.020
Creatinine	2.36	0.067	2.20	0.081			0.31	0.010		
Cytidine			1.79	0.037			2.31	0.011	1.95	0.020
Dimethylglycine			0.71	0.028	0.57	0.047	1.47	0.010		
N6-Trimethyllysine	0.55	0.003			0.50	0.034	1.79	0.017		
Tryptamine	0.71	0.037			0.59	0.083			0.74	0.044
UDP-Glucose					1.41	0.039	1.58	0.021	1.68	0.003
1-Methylimidazole Acetate	1.82	0.026	1.66	0.048						
2-Aminoadipate			0.58	0.013	0.31	0.005				
5,6-Dihydrouracil	0.76	0.079			0.55	0.023				
AMP							0.10	0.015	0.44	0.098

Analyte	Cont v. 20%		Cont v. 5.3%		Cont v. 1.4%		Cont v. 0.4%		Cont v. 0.1%	
	FC	p-value	FC	p-value	FC	p-value	FC	p-value	FC	p-value
Beta Alanine					0.73	0.031	1.34	0.066		
Citrulline					0.53	0.076	1.34	0.010		
Glucose	1.34	0.093			1.38	0.096				
Glycerophosphocholine							1.66	0.027	1.83	0.005
Lactate	1.53	0.00003	1.31	0.003						
Oxalacetate							1.77	0.001	1.44	0.022
SAH			0.67	0.005	0.55	0.008				
Sedoheptulose 7-Phosphate							0.55	0.014	0.71	0.084
Succinylcarnitine	1.88	0.001	1.39	0.034						
UMP			0.39	0.046			0.18	0.008		
Uridine	1.49	0.020			1.53	0.049				
2-Hydroxyglutarate	1.40	0.017								
5-Aminovaleric Acid	1.73	0.035								
Cysteine	1.72	0.031								
Glucoronate	1.36	0.026								
Glutamine	0.66	0.032								
Glutarylcarnitine	1.66	0.020								
Glycerol-3-Phosphate	1.43	0.004								
Homocitrulline	1.43	0.003								

Analyte	Cont v. 20%		Cont v. 5.3%		Cont v. 1.4%		Cont v. 0.4%		Cont v. 0.1%	
	FC	p-value	FC	p-value	FC	p-value	FC	p-value	FC	p-value
Hydroxyproline	1.88	0.006								
Kynurenic Acid	1.47	0.002								
N-Acetylneuraminate	1.34	0.031								
NADH	2.37	0.031								
Phosphorylcholine	1.63	0.005								
Ribose-5-Phosphate	1.61	0.054								
Ribulose 5-Phosphate	1.46	0.023								
Thiamine	1.67	0.039								
Trimethylamine-N-Oxide	1.75	0.077								
Urate	1.71	0.018								

Fold change is control / treatment; the cutoff was set at 1.3 (+1.3 or 0.7). *P*-values used to select most important analytes. All analytes were shown if they occurred in 2 or more treatments and exhibited *p*-values ≤0.1. Single occurrence analytes were shown if they exhibited a fold-change value >1.5 or <0.5 or a *p*-value <0.05.

Heatmaps for each control versus treatment comparison were generated and they generally showed complete separation between the control and treatment analytes by clustering, except for the highly variable 1.4% treatment. Even though many of the analytes may not be considered significantly different in these comparisons, they show distinct patterns of high and low values, which is important for Pathway and Enrichment analysis. The heatmap for the 0.4% treatment is shown (Figure 17), which is representative for all pairwise comparisons between controls and wastewater treatments.

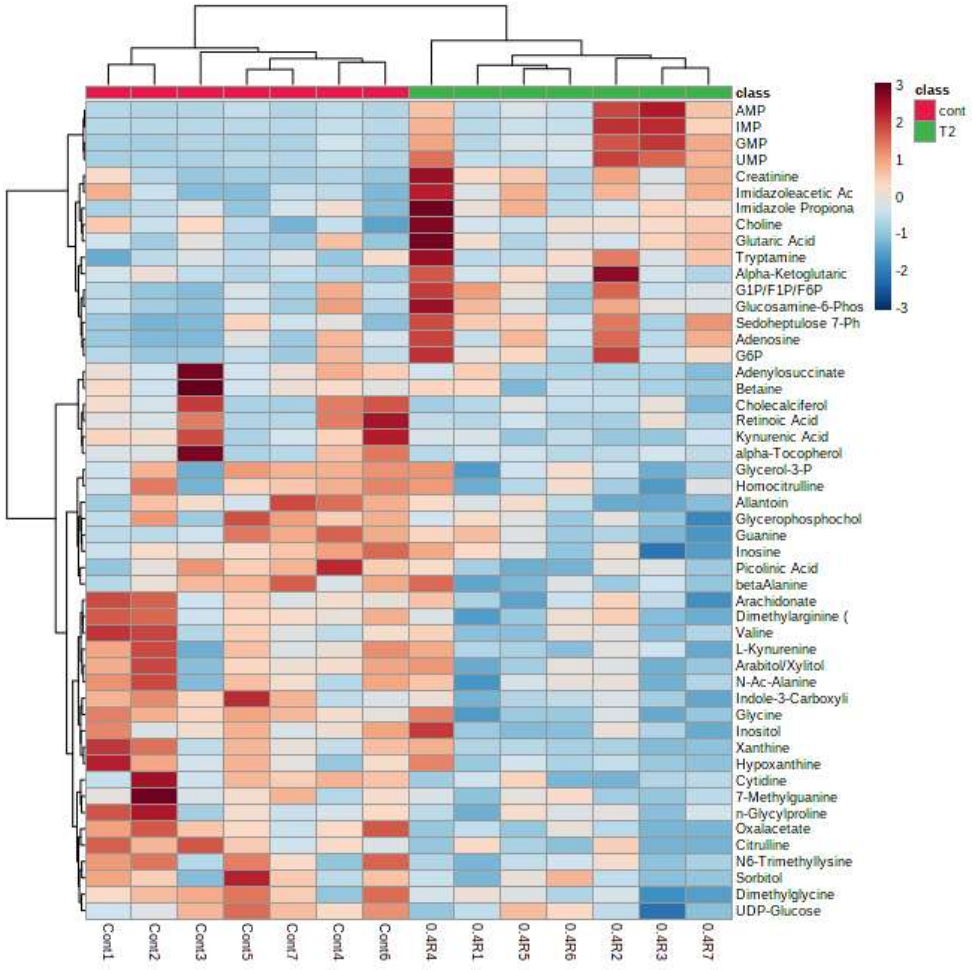

Figure 17. Heatmap showing the 50 most impacted analytes for the 0.4% treatment, based on significant difference in metabolite concentration compared with controls.

3.4.2.2 Pathway analysis

We combined the results for metabolic pathway analyses from the SMPDB and KEGG database queries showing altered pathways for an FDR <0.1 (Table 16). Approximately 90 metabolic pathways were altered when all control versus treatment comparisons were considered and 45 of those occurred for all 5 comparisons. An additional 33 pathways were altered in 3 or 4 of the control versus treatment comparisons. Figure 18 shows a select group of altered pathways as determined with the SMPDB for the control versus 0.4% treatment. Many pathways important for energy generation and utilization, lipid metabolism and biosynthesis, amino acid metabolism, growth, and oxidative stress were altered.

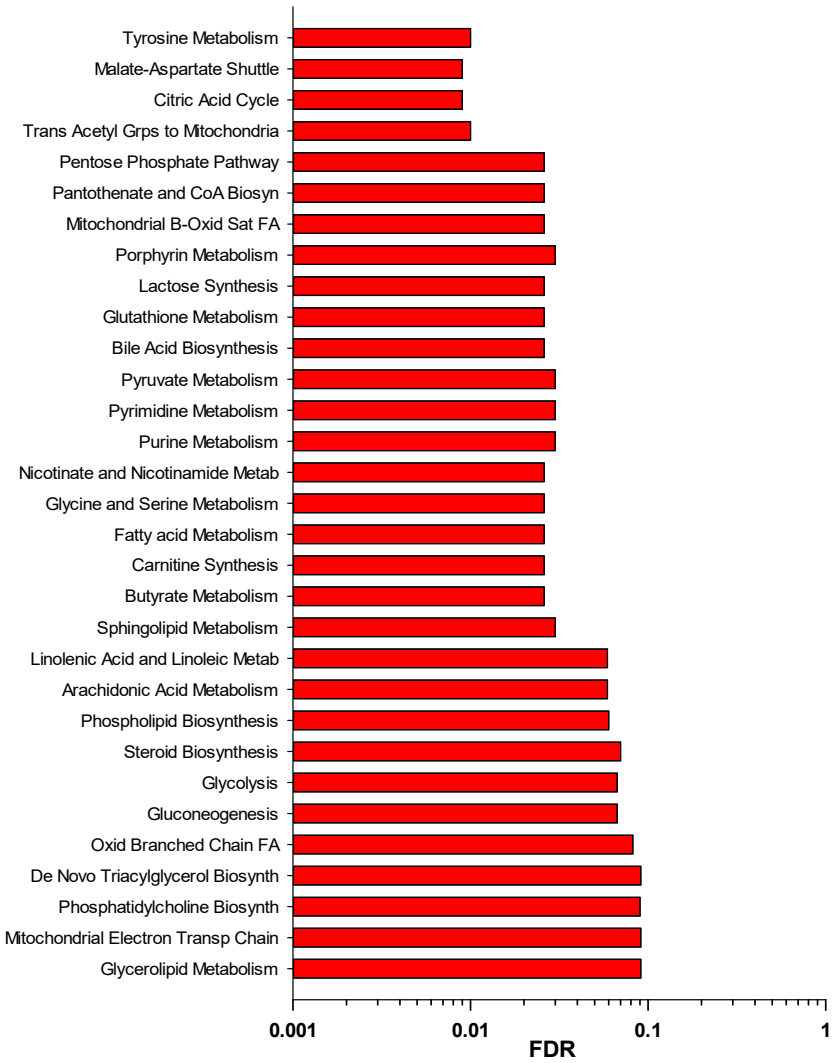

Figure 18. Altered metabolic pathways exhibiting an FDR<0.1 as determined with the SMPDB for the control versus 0.4% treatment comparison. The most impacted pathways are located at the top.

Table 16. Summary of pathway analysis for all control versus treatment comparisons.

Pathway	Cont v. 20%			Cont v 5.3			Cont v. 1.4%			Cont v. 0.4%			Cont v. 0.1%		
	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR
Alanine metabolism	17	2	0.04	17	2	0.09	17	3	0.09	17	4	0.03	17	3	0.10
Alanine, aspartate and glutamate metabolism	28	3	0.04	28	5	0.08	28	5	0.07	28	3	0.01	28	3	0.04
Amino sugar and nucleotide sugar metabolism	33	2	0.04	33	2	0.09	33	2	0.07	37	3	0.03	37	1	0.02
Ammonia Recycling	32	4	0.04	32	5	0.09	32	7	0.05	32	3	0.03	32	2	0.10
Arginine and Proline metabolism	53	4	0.04	53	6	0.09	53	10	0.05	53	5	0.03	53	4	0.05
Arginine biosynthesis	14	2	0.04	14	4	0.08	14	6	0.07	14	2	0.03	14	1	0.04
Aspartate metabolism	35	3	0.04	35	7	0.09	35	7	0.06	35	7	0.03	35	4	0.10
Beta-Alanine metabolism	34	3	0.04	34	5	0.09	34	7	0.07	34	2	0.07	34	2	0.05
Betaine metabolism	21	1	0.04	21	3	0.04	21	4	0.10	21	3	0.07	21	1	0.05
Butanoate metabolism	15	3	0.04	15	3	0.08	15	3	0.08	15	1	0.08	15	2	0.04
Butyrate metabolism	19	2	0.04	19	2	0.09	19	1	0.10	19	1	0.03	19	2	0.05
Carnitine Synthesis	22	6	0.03	22	3	0.09	22	7	0.08	22	3	0.03	22	2	0.05
Krebs cycle (TCA cycle)	32	4	0.04	32	4	0.09	32	3	0.10	32	2	0.01	32	3	0.05
Cysteine & methionine metabolism	33	1	0.04	33	1	0.05	33	4	0.07	26	2	0.03	33	1	0.05
D-Glutamine and D-glutamate metabolism	6	3	0.04	6	5	0.08	6	5	0.07	6	1	0.08	6	1	0.04
Fatty acid metabolism	43	2	0.03	43	1	0.09	43	1	0.10	43	1	0.03	43	1	0.10
Galactose metabolism	38	3	0.04	38	1	0.09	38	2	0.08	38	4	0.03	38	3	0.04
Glucose-Alanine Cycle	13	3	0.05	13	2	0.09	13	3	0.09	13	1	0.08	13	1	0.05
Glutamate metabolism	49	7	0.03	49	6	0.09	49	6	0.05	49	5	0.03	49	4	0.05
Glycerolipid metabolism	25	2	0.03	25	2	0.07	25	2	0.09	25	1	0.09	16	1	0.04

Pathway	Cont v. 20%			Cont v 5.3			Cont v. 1.4%			Cont v. 0.4%			Cont v. 0.1%		
	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR
Glycerophospholipid metabobilsm	36	2	0.03	36	1	0.08	36	2	0.09	36	3	0.04	36	3	0.03
Glycine and Serine metabobilsm	59	4	0.04	59	5	0.09	59	11	0.05	59	4	0.03	33	1	0.04
Glycolysis / Gluconeogenesis	35	5	0.002	35	3	0.04	35	4	0.10	35	4	0.07	35	3	0.05
Histidine metabolism	43	2	0.04	43	5	0.09	43	6	0.07	43	3	0.03	43	2	0.10
Lactose Synthesis	20	1	0.09	20	1	0.09	20	2	0.08	20	3	0.03	20	1	0.04
Lysine Degradation	30	2	0.04	30	4	0.09	30	3	0.08	25	1	0.04	30	1	0.05
Malate-Aspartate Shuttle	10	2	0.04	10	3	0.09	10	3	0.08	10	2	0.01	10	2	0.04
Methionine metabolism	43	3	0.04	43	3	0.04	43	8	0.09	43	5	0.03	43	2	0.05
Nicotinate and Nicotinamide metabobilsm	37	2	0.04	37	4	0.09	37	4	0.06	37	1	0.03	37	1	0.10
Oxid Branched Chain Fatty Acids	26	3	0.04	26	2	0.09	26	2	0.10	26	1	0.08	26	2	0.05
Pantothenate and CoA biosyn	21	2	0.04	19	2	0.08	19	3	0.08	21	1	0.03	21	1	0.10
Phenylacetate metabolism	9	1	0.04	9	1	0.09	9	1	0.10	9	1	0.03	9	1	0.10
Phenylalanine and Tyrosine metabobilsm	28	1	0.04	28	3	0.09	28	2	0.09	28	2	0.03	28	2	0.10
Phosphatidylcholine Biosyn	14	2	0.03	14	1	0.04	14	3	0.10	14	1	0.09	14	1	0.05
Phospholipid Biosynthesis	29	3	0.03	29	2	0.07	29	2	0.10	29	2	0.06	29	2	0.05
Phytanic Acid Peroxisomal Oxidation	26	3	0.04	26	2	0.09	26	2	0.10	26	1	0.08	26	2	0.05
Propanoate metabolism	42	2	0.04	42	4	0.09	42	3	0.09	42	4	0.03	23	2	0.04
Purine metabolism	74	7	0.04	74	5	0.09	74	6	0.05	74	9	0.03	65	3	0.02
Pyrimidine metabolism	59	4	0.04	59	6	0.09	59	4	0.10	59	3	0.03	39	2	0.04
Pyruvate metabolism	48	4	0.03	48	3	0.04	48	2	0.10	48	2	0.03	22	2	0.03

Pathway	Cont v. 20%			Cont v 5.3			Cont v. 1.4%			Cont v. 0.4%			Cont v. 0.1%		
	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR
Tryptophan metabolism	60	4	0.04	60	5	0.09	60	6	0.08	60	4	0.03	41	1	0.05
Tyrosine metabolism	72	2	0.04	72	5	0.09	72	5	0.08	72	2	0.01	72	2	0.04
Urea Cycle	29	3	0.04	29	4	0.09	29	6	0.06	29	4	0.03	29	3	0.10
Valine, Leucine and Isoleucine Degradation	60	3	0.04	60	4	0.09	60	3	0.08	60	2	0.07	60	2	0.05
Warburg Effect	58	9	0.03	58	7	0.07	58	7	0.05	58	4	0.06	58	5	0.04
Aminoacyl-tRNA biosynthesis	48	3	0.04	48	3	0.08	48	8	0.07	48	2	0.07			
Arachidonic Acid metabolism	69	1	0.04	69	1	0.09	69	1	0.09	69	1	0.06			
Ascorbate and aldarate metabobilsm	8	1	0.04				8	1	0.08	8	1	0.05	8	2	0.02
Bile Acid Biosynthesis	65	2	0.04	65	1	0.09	65	2	0.10	65	1	0.03			
Cardiolipin Biosynthesis	11	3	0.03	11	1	0.07				11	1	0.09	11	1	0.05
De Novo Triacylglycerol Biosyn	9	2	0.03	9	1	0.07				9	1	0.09	9	1	0.05
Fructose and Mannose Degrad	32	2	0.04	32	1	0.09				32	1	0.09	32	1	0.08
Glutathione metabolism	21	3	0.04	21	2	0.09	28	4	0.08	21	1	0.03			
Glycerol Phosphate Shuttle	11	2	0.03	11	2	0.07				11	1	0.09	11	1	0.05
Glyoxylate dicarboxylate metabobilsm	32	2	0.04	32	2	0.08				32	2	0.01	32	1	0.04
Ketone Body metabolism	13	2	0.04	13	1	0.09	13	1	0.10				13	1	0.05
Mito B-Oxid Long Chain Sat Fatty Acids	28	2	0.03	28	1	0.09				28	1	0.03	28	1	0.10
Mito B-Oxid Med Chain Sat Fatty Acids	27	1	0.04	27	1	0.09				27	1	0.03	27	1	0.10
Mito B-Oxid short Chain Sat Fatty Acids	27	2	0.03	27	1	0.09				27	1	0.03	27	1	0.10

Pathway	Cont v. 20%			Cont v 5.3			Cont v. 1.4%			Cont v. 0.4%			Cont v. 0.1%		
	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR
Mito Electron Transport Chain				19	3	0.07	19	1	0.10	19	1	0.09	19	2	0.05
Nucleotide Sugars metabolism	20	1	0.04				20	1	0.08	20	3	0.03	20	1	0.04
Pentose and glucuronate interconversions	18	2	0.04				18	1	0.08	18	2	0.04	18	1	0.02
Pentose phosphate pathway	29	2	0.04				22	1	0.09	29	3	0.03	29	2	0.07
Porphyrin metabolism	40	2	0.04	40	1	0.09	30	2	0.09	40	1	0.03			
Sphingolipid metabolism	40	2	0.04				21	1	0.08	40	1	0.03	40	1	0.04
Steroid Biosynthesis	43	1	0.04	48	1	0.09	48	1	0.10	48	1	0.07			
Transfer of Acetyl Groups into Mitochondria	22	2	0.05				22	1	0.10	22	1	0.01	22	1	0.05
Estrone metabolism	24	1	0.04	24	1	0.04	24	2	0.02						
Folate metabolism	29	1	0.04	29	2	0.09	29	1	0.09						
Inositol metabolism	33	1	0.04							33	1	0.07	33	1	0.05
Inositol Phosphate metab	30	1	0.04							26	1	0.07	26	1	0.05
Nitrogen metabolism	6	1	0.04	6	2	0.08	6	2	0.07						
Primary bile acid biosynthesis	46	1	0.09				46	1	0.09	46	1	0.03			
Retinol metabolism	37	1	0.04							37	2	0.04	37	1	0.04
Riboflavin metabolism				20	1	0.09				20	1	0.03	20	1	0.10
Thiamine metabobilsm (Vit. B1)	9	1	0.04							9	1	0.03	9	1	0.10
Vitamin B6 metabolism	20	1	0.002	20	2	0.09									
Alpha Linolenic Acid and Linoleic Acid metab	19	1	0.04							19	1	0.06			
Biosynthesis of unsaturated fatty acids	36	1	0.04				36	1	0.09						

Pathway	Cont v. 20%			Cont v 5.3			Cont v. 1.4%			Cont v. 0.4%			Cont v. 0.1%		
	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR	Total Cmpd	Hits	FDR
Caffeine metabolism	24	1	0.04	24	1	0.09									
Catecholamine Biosynthesis				20	1	0.04	20	2	0.02						
Fatty Acid Elongation In Mitochondria	35	1	0.04				35	1	0.10						
Homocysteine Degradation	9	1	0.04				9	1	0.08						
Lactose Degradation	9	1	0.09				9	1	0.10						
Methylhistidine metab				4	1	0.04	4	3	0.10						
Phosphatidylinositol Phosphate metabobilsm	17	1	0.05										17	1	0.05
Plasmalogen Synthesis	26	2	0.04	26	1	0.09									
Pyruvaldehyde Degradation	10	1	0.04	10	1	0.09									
Taurine and Hypotaurine metabolism	12	1	0.04				12	1	0.07						
Androgen and Estrogen metabolism	33	1	0.04												
Androstenedione metabobilsm	24	1	0.04												

Altered pathways determined with the Small Molecule Pathway Database (SMPBD) and KEGG database. Determined with Q statistic, which is the aggregate of squared covariance between concentration changes and the phenotypes. FDR is the false discovery rate. FDR values $\leq$0.1 shown. Total cmpnd is the total number of compounds in a pathway and hits is the number of altered metabolites in this study association with the pathway. Each control versus treatment comparison shown.

Drug pathway analysis revealed a large number of altered pathways characteristic of drug action. For all control versus treatment comparisons, 199 drug pathways were considered impacted based on an FDR <0.1 (Table 17). Some of these were presented as groups, such as the Antibiotic Action or Metabolism pathways (n=29 pathways). For the 0.4% treatment, 79 drugs pathways were altered with and FDR <0.05. Figure 19 shows the enrichment factors for the top 25 pathways from the control versus 0.4% treatment, most occurring in the Antibiotic group of pathways. Table 17 lists the altered pathways for all control versus treatment comparisons.

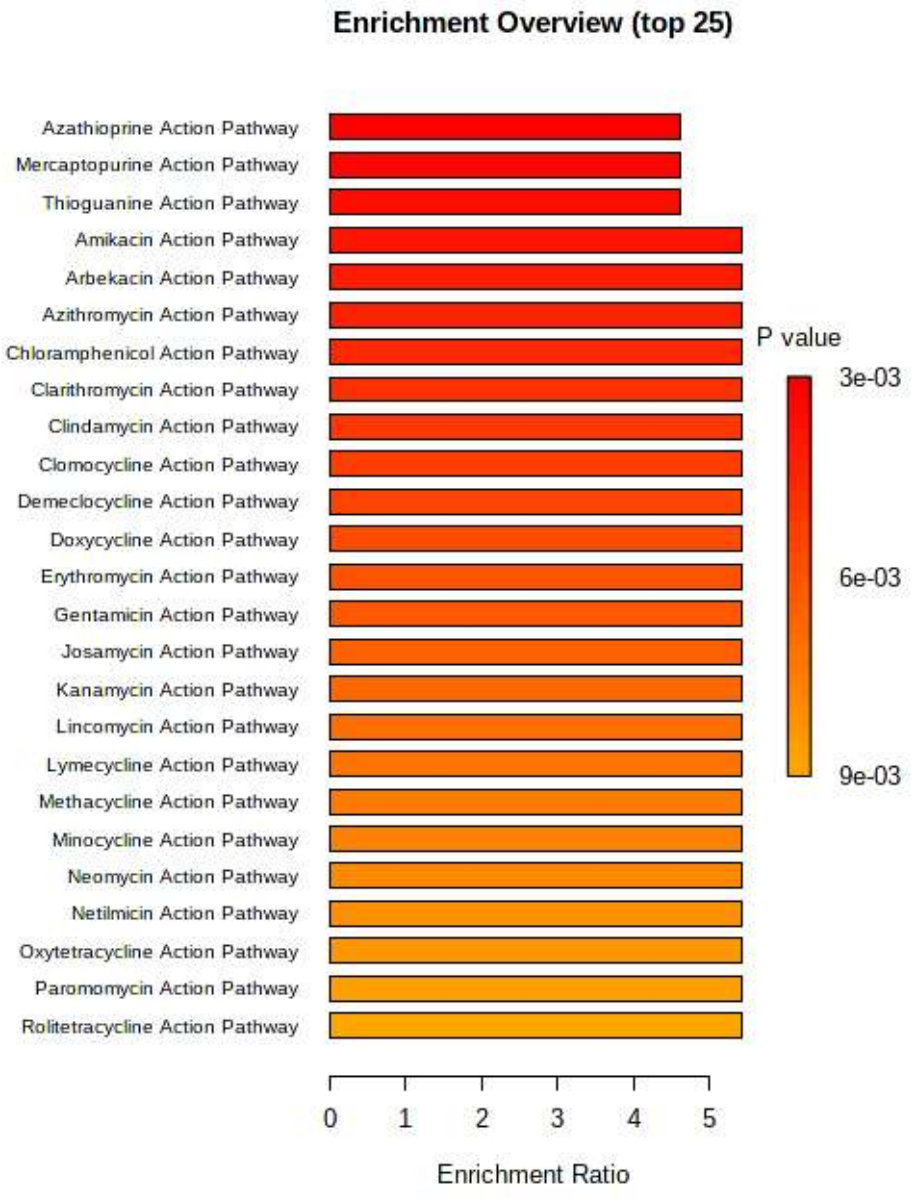

Figure 19. Enrichment ratios for drug pathways for the 0.4% treatment identified with Quantitative Enrichment Analysis (QEA). The enrichment ratio is determined by dividing the observed Q statistic for each pathway by the expected Q statistic for the dataset.

Table 17. Drug pathways determined by analysis of metabolites altered by exposure to wastewater effluent (%).

	Cont v. 20%			Cont v. 5.3%			Cont v. 1.4%			Cont v. 0.4%			Cont v. 0.1%		
	Total Cmpd	**Hits**	**FDR**	**Total Cmpd**	**Hits**	**FDR**	**Total Cmpd**	**Hits**	**FDR**	**Total Cmpd**	**Hits**	**FDR**	**Total Cmpd**	**Hits**	**FDR**
Antibiotic Action or Metabolism pathways (n=29 drugs)	20	1	0.049	20	1	0.091	20	3	0.079	20	4	0.027	20	1	0.098
Mercaptopurine metabolism pathway	30	2	0.049	30	3	0.091	30	3	0.079	30	1	0.031	30	1	0.098
Disulfiram Action pathway	79	2	0.049	79	5	0.091	79	5	0.079	79	3	0.031	79	3	0.098
Mercaptopurine Action pathway	90	7	0.049	90	6	0.091	90	8	0.079	90	9	0.027	90	3	0.098
Thioguanine Action pathway	91	7	0.049	91	6	0.091	91	8	0.079	91	9	0.027	91	3	0.098
Azathioprine Action pathway	92	7	0.049	92	6	0.091	92	8	0.079	92	9	0.027	92	3	0.098
Valproic Acid metabolism pathway	37	1	0.049	37	1	0.091				37	1	0.031	37	1	0.098
Rofecoxib Action pathway	66	1	0.049	66	1	0.091	66	1	0.079	66	1	0.046			
Pathways affected by glutathione (n=32 drugs)	67	1	0.049	67	1	0.091	67	1	0.079	67	1	0.046			
Diclofenac Action pathway	68	1	0.049	68	1	0.091	68	1	0.079	68	1	0.046			
Naproxen Action pathway	68	1	0.049	68	1	0.091	68	1	0.079	68	1	0.046			
Piroxicam Action pathway	68	1	0.049	68	1	0.091	68	1	0.079	68	1	0.046			
Celecoxib Action pathway	74	1	0.049	74	1	0.091	74	1	0.079	74	1	0.046			
Ibuprofen Action pathway	76	1	0.049	76	1	0.091	76	1	0.079	76	1	0.046			
H1-Antihistamine Action (n=81 drugs)	8	1	0.049	8	1	0.091	8	1	0.079						
Methotrexate Action pathway	30	1	0.049	30	2	0.091	30	1	0.079						
Statin Pathway (n=6 drugs)				48	1	0.091	48	1	0.091	48	1	0.048			

	Cont v. 20%			Cont v. 5.3%			Cont v. 1.4%			Cont v. 0.4%			Cont v. 0.1%		
	Total Cmpd	**Hits**	**FDR**	**Total Cmpd**	**Hits**	**FDR**	**Total Cmpd**	**Hits**	**FDR**	**Total Cmpd**	**Hits**	**FDR**	**Total Cmpd**	**Hits**	**FDR**
Doxorubicin metabolism pathway	16	1	0.049	16	1	0.091									
Citalopram metab. pathway	19	1	0.049	19	1	0.091									
Nicotine metabolism pathway	26	1	0.049	26	1	0.091									
Citalopram Action pathway	28	1	0.049	28	1	0.091									
Nicotine Action pathway	36	1	0.049	36	1	0.091									
L-Cysteine affected pathways (n=22 drugs)	9	1	0.049												
Fluoxetine metabolism pathway	10	1	0.049												
Codeine metabolism pathway	12	1	0.049												
Venlafaxine metabolism pathway	13	1	0.049												
Acetaminophen metabolism pathway	17	2	0.049												
Methadone Action pathway	18	1	0.049												
Tramadol metabolism pathway	18	1	0.049												
Carbamazepine metabolism pathway	19	1	0.049												
Fluoxetine Action pathway	19	1	0.049												
Codeine Action pathway	21	1	0.049												
Lidocaine Action pathway	27	1	0.049												
Etoricoxib Action pathway	67	1	0.049												

Potentially affected drug related pathways. Determined with Q statistic, which is the aggregate of squared covariance between concentration changes and the phenotypes. FDR is the false discovery rate, shown for FDR $\leq$ 0.1. Total cmpnd is the total number of compounds in a pathway and hits is the number of altered

metabolites in this study association with the pathway. Each control versus treatment comparison shown. Several pathways were reduced because a large number of drugs could be responsible. For example, 29 different antibiotics were identified that may result in alteration of the Antibiotic action or metabolism pathways (n=29 drugs).

3.5 Bioaccumulation Modeling

Bioaccumulation modeling was performed based on the results of the laboratory study and the field data. Results are presented separately below.

3.5.1 Lab study

Several analytes were detected both in dilutions of effluent water and in tissue from the laboratory exposure study. For water, 74, 59, 39, and 13 analytes were detected in the 20%, 5.3%, 1.4%, and 0% treatments, respectively. For whole-body tissue, we detected 26, 18, 11, and 9 analytes for the 20%, 5.3%, 1.4%, and 0.4% treatments, respectively (Table 18).

Five analytes were detected in whole body composites of fish from the 0% control treatment (Table 18). Hydrocortisone may be a contaminant because there is no relationship between dilution and occurrence in tissue. The source for bisphenol A and miconazole is also not clear and these may be contaminants from within the lab. Miconazole was not detected in effluent water. Ormetoprim and sulfadimethoxine are part of the antibiotic mixture Romet TC that was provided to all fish before the experiment commenced to control for a bacterial infection.

Of the 13 analytes detected in the 0% control treatment water, many were considered potential laboratory contaminants. As mentioned above, ormetoprim and sulfadimethoxine were used prior to the experiment to treat fish. Nonylphenols, PFAS, and bisphenol A are ubiquitous compounds that are difficult to exclude from lab equipment and supplies. Several of the analytes detected in the 0% treatment water were flame retardants (Syn and anti declorane, and HBB) and their source is unknown. The source of the remaining compounds (metformin, cotinine, DEET, hydrocortisone, and theophylline) is unknown; however, they occurred at relatively high concentrations in the other treatments; hence some minor cross contamination with the 0% control may have occurred.

Table 18 also shows the expected tissue concentration as determined with the predicted bioconcentration factor (equation 1) times the observed water concentration using equation 2. For those samples with observed tissue concentrations, we also list the observed to predicted concentrations (obs/pred) ratios to highlight the degree of predictability of the bioaccumulation model. The geometric mean of the obs/pred tissue concentrations was 0.97 (SD=2.9, n=29), excluding two metabolites (desmethyldiltiazem and benzoylecgonine) that would occur in tissue due to water uptake and biotransformation of their respective parent compound. Based on the mean ratio, our predictive model for tissue concentrations was fairly accurate, notwithstanding the high variability among analytes, which was expected due to the inherent variability in predicting K_{ow} (or D_{ow}) and the extended time needed for steady-state tissue concentrations to occur for some analytes.

We compared the observed BCF based on measured results, with the predicted BCF values (equation 1). Only a limited group of analytes were detected in both water and tissue samples (Table 19). The geometric mean of all comparisons was 1.5 (SD=3.3, N=45) indicating good predictive ability of equation 1 for the BCF of CECs with a $\log_{10}K_{ow}$ <5.

An additional evaluation of expected or predicted tissue concentrations was conducted to estimate the occurrence of analytes in whole-body tissue that would occur below the limit of detection (quantification), as determined by the tissue reporting limit (RL) (Table 20). The RL was chosen as the maximum potential concentration that could occur between 0 and the detection (reporting) limit. We identified 117 occurrences where a chemical was detected water but not in tissue. Predicted tissue

concentrations for those occurrences were mostly below the mean RL, with only 15 occurrences for which the predicted tissue concentration was greater than the RL. The geometric mean of all analyte/treatment combinations for the ratio of predicted tissue concentrations/each respective RL was 0.07 (SD=7.2, N=117), excluding the very hydrophobic 4-nonylphenol and the flame retardants. This mean highlights the relatively low predicted tissue concentration for those analytes with a water-only detection. As seen at the bottom of Table 20, the sum of predicted whole-body tissue concentrations range from 12.1 to 85.0 ng/g for those analytes without observed tissue concentrations.

Detectable plasma concentrations were observed for 21 analytes (Table 21). Several of those may not be reflective of actual bioaccumulation from exposure water because levels show little variation over the range of doses. This includes all analytes except diphenhydramine, gemfibrozil, sertraline, and citalopram. It should be noted however, that some drugs are regulated by plasma-membrane transporters, which may affect their absorption and distribution (ITC 2010). Interestingly, many of the ratios between observed whole-body and plasma concentrations are relatively consistent across the 100-fold range of exposure doses, which may indicate consistent partitioning between whole-body and plasma in detected compounds. Also shown in this table are the predicted blood/water partition ratios (Pbw), the predicted BCFs, and the predicted volumes of distribution (Vd). Vd is the ratio between tissue and plasma concentrations and is also the ratio between the BCF and Pbw. The Pbw was predicted with equation 3 and the BCF with equation 1. The Vd can be predicted with the BCF/Pbw or the whole-body/plasma concentration. For most analytes, the observed plasma concentration was greater than predicted indicating an under estimation of plasma levels. For those analytes showing a correlation to treatment dilution (specified above), the observed plasma concentrations were generally below their predicted value. Interestingly, a few analytes (virginiamycin, benztropine, and cocaine) exhibited detectable plasma concentrations but were not detected in whole-body tissue or water. These, and a few others, may be potential contaminants (e.g., caffeine and bisphenol A), or were present before the experiment started (ormetoprim and sulfadimethoxine). Hydroxy-ibuprofen is a metabolite and was not expected to follow predictions for partitioning based on tissue or water concentrations. Some of the factors that may impact the high variability for the ratio of observed to predict plasma concentrations include insufficient time to steady state, action by membrane transporters, and inaccurate concentrations.

An analysis of the observed and predicted plasma concentrations in relation to the therapeutic levels for humans (1% C_{max}) shows a large range of potential adverse effects among analytes (Table 22). Based on the observed plasma concentrations, many of the Response Ratios (RR; equation 5) for several of the treatments exceeded 1, indicating the potential for adverse effects. Also, many are far below 1 and would be considered unlikely to cause effects in fish. The same patterns are noted for the RR determined with predicted plasma concentrations from either observed water or observed tissue concentrations. Noted CECs that exceeded a RR value of 1, include estrone, bisphenol A, citalopram, benztropine, hydrocortisone, sertraline, and triclosan. Several others were just below a value of 1; these compounds could potentially cause adverse effects, especially when considering additive or more-than-additive effects from the mixture.

It is important to note that many of the analytes in the lab exposure water do not have plasma Cmax values we can use to gauge potency and potential effects. Notable groups occurring at detected concentrations include nonylphenols, bisphenols, and PFAS.

An additional table shows the RR values sorted by magnitude from highest to lowest (Table 23), highlighting analytes with RR >0.5. Even with the limited data for observed plasma concentrations, some of the analytes occurred in all 3 lists (observed, predicted from water, and predicted from tissue). Also, several pharmaceuticals occurred in both the water and tissue approaches for predicted plasma concentrations. The plasma values predicted from tissue concentrations are considered less uncertain than those from water because of fewer assumptions, such as the time to steady state and variable uptake and elimination kinetics.

3.5.2 Field study

3.5.2.1 Detected chemicals

Only 10 analytes were detected in the estuarine samples from the six sites sampled. Five of those analytes were detected at each site (atenolol, cotinine, metformin, venlafaxine, and benzoylecgonine), and the others were detected at one to three sites. A PFAS, 6:2 FTS, was excluded from the analysis because the field concentration (reference site) was reported to be higher than the full effluent concentration (Table 24). It is important to note that the observed concentrations for these 10 chemicals were relatively similar among all sites, which suggests that 1) effluent plume was not captured in the sampling, and 2) the 10 chemicals are present at a pseudo steady-state due to factors such as continual inputs, high persistence, and a well-mixed water mass.

To get an estimation of percent wastewater contribution at the estuarine sampling locations, the mean concentration of the analytes in WWE was compared to the mean concentration of the estuarine samples for those 10 analytes. Results indicated that WWE contribution at the sampling sites ranged from 0.08% to 1.9% (geometric mean = 0.36%). The range of estimates likely reflects the range of degradability (i.e., labile vs persistent) and potential biotransformation. Many of the detected analytes are common drugs, including two antidepressants (citalopram and venlafaxine), an antihistamine (diphenhydramine), a diabetes drug (metformin), and a drug for blood pressure (atenolol).

We should note that most of the detected compounds in estuarine waters (Table 24) were pharmaceuticals; hence the likely source being effluent from the wastewater treatment facilities, with perhaps the exception of BPA. The West Point and South Plant outfalls are the most proximate to the estuarine sample locations. Other major WWE outfalls are a considerable distance from our estuarine sampling sites, with the plants in Tacoma and Everett being more than 40 km away and the Bremerton plant more than 20 km. While it is not possible to directly attribute a particular contaminant with a particular outfall or source, based on proximity and relative flows of each system (West Point and South Plant are amongst the largest facilities in the region), it is reasonable to conclude that effluent from West Point and South Plant contribute a majority of the estimated 0.36% of wastewater at our sampling locations in the Central Basin. Also, when our ratio is applied to legacy compounds and other CECs (e.g., PCBs, PBDEs, and PFAS) that likely have additional sources to WWE, our predicted values are considered as the sole contribution from effluent emanating from South Plant and West Point to the total concentration in the nearby water mass. As noted below, both PCBs and PBDEs were observed to be substantially higher than our predicted values; hence our predictions likely reflect the contribution of these compounds from WWE to the total found in the central basin derived from all sources.

As an additional exercise, we compared effluent concentrations from the current study to observed CEC concentrations reported for outer Elliott Bay sampled in 2014 (King County 2017). We selected data from station LSCW02 for both depths (1 m and 175 m) (n=6 samples), which was closest to our field

sampling locations. Ten CECs (all pharmaceuticals or over-the-counter medicine) were detected in these estuarine samples and compared to the mean value for high and low flow effluent from West Point and South Plant as determined in the present study. Outer Elliott Bay was sampled three times (late summer, fall, and winter), representing low and high flow conditions. Sulfadimethoxine was detected in the Elliott Bay samples but not in our effluent samples. Albuterol and amphetamine were detected in Elliott Bay and eliminated because the percentage ratio of observed field values to effluent values were 84% and 181%, respectively, and were likely a result of contamination. For the remaining seven CECs, the geometric mean ratio of observed field values to effluent values was 0.6% for the surface samples and 0.8% for the deep samples. The range of values was 0.1 to 3.5%. These values are not substantially different from the ratio calculated in the present study for observed field and effluent concentrations (0.36%). Additionally, there were five CECs detected in the estuarine samples common to both studies (benzoylecgonine, DEET, diphenhydramine, metformin, and sulfamethoxazole). The geometric mean ratio between observed field values for the 2017 study and the present study without metformin was 1.1. With metformin, the geometric mean ratio increased to 1.7 because this CEC was 6.7 – 11 times higher in the 2017 King County study. Of course, effluent concentrations from these wastewater treatment plants in 2014 may be substantially different than those measured in the present study (2021).

3.5.2.2 Predicted estuarine concentrations

From the data presented in Table 24, we predicted estuarine concentrations for all analytes detected in full low-flow effluent from West Point and South Plant (Table 25). We then compared those predicted estuarine concentrations to their sample-specific reporting limit (RL). Only 15 of those analytes exhibited a predicted concentration-to-RL ratio that was >1, meaning that the predicted concentration for most analytes was below their analytical reporting limit (Table 26). Values below the RL would have been reported as non-detected. Of those 15 analytes, six exhibited a ratio >1, but were not detected in estuarine samples (4-nonylphenol, 4-nonylphenol monoethoxylates, diatrizoic acid, diltiazem, gemfibrozil, and metoprolol). Most ratios for those 6 analytes were close to a value of 1. Only cotinine was detected in estuary samples and exhibited a ratio <1. Of the 15 analytes in Table 26, nine were detected at the six field sites in estuarine waters. Table 25 also shows the sum of analytes by class for all predicted field concentrations (mean of all sites). Most of the analytes were predicted to occur at very low concentrations at the estuarine sampling locations. Predicted concentrations of compounds in many classes summed to less than 1 ng/L, including PAHs, PCBs, pesticides, flame retardants, and BDEs. The PPCPs (lists 1 – 6) exhibited the highest sum as a group at 204 ng/L. It is important to note that many of these chemicals, such as PCBs and flame retardants, already occur at low levels system-wide before discharge of King County effluent. Therefore, our estimation of estuarine concentrations is actually only the additional contribution from WWTP effluent. Concentrations of these chemicals in the estuary are likely higher than our estimated levels, but below the analytical reporting limit for our samples.

3.5.2.3 Predicted tissue concentrations

A further analysis of predicted tissue concentrations for those analytes using predicted estuarine concentrations is shown in Table 27. Only predicted tissue concentrations greater than 0.05 ng/g are shown to limit the number of compounds in the table and concentrations below this level are likely not toxic. Most of the concentrations predicted to exceed 1 ng/g were for compounds with the highest K_{ow} values, such as BDEs, some PAHs, nonylphenols, and flame retardants. The sum of PAHs and PCBs are

also listed and fish whole-body concentrations were predicted to be 12.9 ng/g and 0.32 ng/g, respectively. A number of pharmaceuticals were also predicted to bioaccumulate at levels of 0.05 ng/g or greater, including estrone, diphenhydramine, miconazole, lopamidol, metformin, carbamazepine, diatrizoic acid, and valsartan.

To gauge the potential for adverse effects in fish, we used the fish plasma model and highlighted those results in Table 28. The upper half of the table shows predicted plasma concentrations based on the mean observed estuarine concentration and the lower section of Table 28 shows predicted plasma based on the predicted estuarine concentrations from Table 25. A response ratio (RR) (equation 5) was determined for each plasma concentration for evaluating potential effects on fish at these estuarine sites. Most of the RR values were far below 1; however, RR values may be additive for those analytes with the same or similar mechanism of action. In the upper section of Table 28, bisphenol A exhibited the highest RR and should be considered in conjunction with similar acting compounds (especially other endocrine disruptors) and for those estuarine areas exhibiting higher ambient concentrations. The lower section of Table 28 highlights potent PPCPs that should be considered. The most notable RR values are for hormones (estradiol, estrone, progesterone, and testosterone), which is mostly due to their extremely low-effect concentrations when based on plasma concentrations. Also noteworthy is the ubiquitous triclosan and several antidepressants (citalopram, sertraline, and fluoxetine). Other noteworthy PPCPs include the commonly occurring statins for blood lipid control (atorvastatin and rosuvastatin). It is important to note that the predicted estuarine concentrations for those analytes in the lower section of Table 28 were far below their respective quantitative reporting limit (RL), and therefore could occur in the field at those concentrations but likely not be detected. The exception is sertraline, which was predicted to occur just below its RL with a ratio of 0.9 (predicted concentration / RL).

3.5.3 Comparing lab and field results

A comparison of laboratory and field water concentrations indicates that the 0.4% dilution treatment for the laboratory exposures is comparable to the observed field concentrations. Concentrations of analytes were not measured for the 0.4% treatment due to budgetary constraints and were estimated based on results of the 1.4% treatment (1.4% concentration x 0.28). A comparison of the 10 analytes detected in the field samples to those predicted in the 0.4% treatment shows close agreement with a geometric mean ratio of 0.8 (SD=1.3) (Table 29). Based on the predicted concentrations for the 0.4% treatment and the sample RL, most of the 10 compounds in Table 29 would have likely been detected in the 0.4% treatment. An additional 10 analytes predicted to occur in the 0.4% lab exposure treatment (concentration to RL ratios greater than 1.0) but not detected in the field samples are also listed in Table 29. Most of these values were close to 1.0 indicating uncertainty regarding potential detection in the field samples, except for metoprolol, gemfibrozil, and nonylphenol monoethoxylates. An additional 17 analytes were predicted to occur in the 0.4% treatment; however, their concentration/RL ratios were <1.0 and would likely not have been detected in the 0.4% treatment or the field samples. We believe these results support our predictions of potential adverse effects to fish in the field based on our metabolomics and blood chemistry results for the 0.4% lab exposure treatment.

Table 18. Observed whole-body and water concentrations for the laboratory exposure study in addition to predicted whole body concentrations and their comparison to observed.

Analyte	Observed whole body (ng/g)					Observed Water (ng/L)				Predicted whole body (ng/g)			Obs/Pred whole body		
	20% Mean (sd)	5.3% Mean (sd)	1.4% Mean (sd)	0.4%	0%	20%	5.3%	1.4%	0%	20%	5.3%	1.4%	20%	5.3%	1.4%
10-OH amitriptyline						2.58	0.77	0.17		0.05	0.015	0.003			
2-Hydroxy-ibuprofen						29.1	8.18			0.04	0.011				
4-Nonylphenol diethoxylates	21.2	10.6	3.4			85.7				119					
4-Nonylphenol monoethoxylates	31.1	15.3	6.7			454	133	41		878	257	79	0.04	0.06	0.09
4-Nonylphenols	59.1					158	63.6	2.55	17.2	1,226	494	20	0.05		
Albuterol						2.14	0.75			0.003	0.001				
Alprazolam						0.38				0.02					
Amitriptyline	0.56 (0.01)					4.82	1.35	0.35		0.81	0.226	0.058	0.69		
Amlodipine						3.88	1.16			0.01	0.002				
Atenolol						10.7	3.56	0.88		0.01	0.005	0.001			
Atorvastatin						7.37	1.54			0.05	0.010				
Azithromycin						67.6	12.9	2.99		0.09	0.018	0.004			
Benzoylecgonine		0.08		0.06		8.08	2.38	0.59		0.01	0.003	0.001		24.6	
Bisphenol A (method 1)	13.3 (1.5)	6.2 (2.5)	4.8 (0.2)	4.0	3.6	99.2	47	21.9	17.1	52.7	25.0	11.6	0.25	0.25	0.41
Bisphenol A (method 2)	6.7					145	69.1	39.9	22.9	131	62	36	0.05		
Bisphenol F						11.8		7.2		2.31		1.4			
Bisphenol S						31.6	15.5			0.04	0.022				
Caffeine						16.6				0.02					
Carbamazepine						31.1	9.23	2.2		3.39	1.005	0.242			
Ciprofloxacin						21.9	9.99	6.7		0.03	0.014	0.009			
Citalopram	1.2 (0.08)	0.27 (0.05)	4.8 (0.2)			51.8	14.4	4.1		0.71	0.197	0.056	1.75	1.35	
Clarithromycin						14.3	4.66			0.92	0.299				

Analyte	Observed whole body (ng/g)					Observed Water (ng/L)				Predicted whole body (ng/g)			Obs/Pred whole body		
	20% Mean (sd)	5.3% Mean (sd)	1.4% Mean (sd)	0.4%	0%	20%	5.3%	1.4%	0%	20%	5.3%	1.4%	20%	5.3%	1.4%
Clotrimazole						0.44				5.58					
Cloxacillin	4.5	6.1	6.9												
Codeine						11.4	2.35			0.02	0.003				
Cotinine						8.0	2.5	0.9	0.7	0.01	0.004	0.001			
Dechlorane plus anti						0.22	0.13	0.35	0.31	272	166	444			
Dechlorane plus syn						0.23	0.14	0.28	0.42	15.8	9.4	19.2			
DEET						159	53.8	19.1	5.66	4.23	1.4	0.51			
Dehydronifedipine						0.79				0.057					
Desmethyldiltiazem	0.3 (0.03)	0.09 (0.01)				4.48	1.4	0.35		0.02	0.005	0.001	20.1	18.9	
Diatrizoic acid						110	29.8			0.15	0.042				
Diltiazem	2.3 (0.2)	0.41 (0.03)	0.2 (0.03)			27.7	5.74	1.58		0.77	0.159	0.044	3.05	2.58	4.96
Diphenhydramine	29.6 (8.5)	5.5 (0.3)	1.8 (0.2)	0.52		174	39.2	8.17		13.85	3.1	0.65	2.14	1.76	2.77
EHTBB						0.49				265,106					
Erythromycin-H2O						7.05	3.4	2.69		0.13	0.062	0.049			
Estrone						36.6	11.9	4.09		51.8	16.8	5.8			
Flumequine	2.2 (0.6)	1.2	1.6 (0.7)												
Fluoxetine	0.89 (0.1)					8.44	4.76	1.58		0.39	0.219	0.073	2.29		
Furosemide						5.44	6.58			0.01	0.009				
Gemfibrozil	2.1 (0.01)	0.42 (0.07)				122	36.4	9.03		0.32	0.094	0.023	6.67	4.43	
Glipizide						3.38				0.00					
HBB							0.097		0.07		312				
Hydrochlorothiazide						245	79.1	21.9		0.34	0.111	0.031			
Hydrocodone						4.65	1.27			0.02	0.007				

Analyte	Observed whole body (ng/g)					Observed Water (ng/L)				Predicted whole body (ng/g)			Obs/Pred whole body		
	20% Mean (sd)	5.3% Mean (sd)	1.4% Mean (sd)	0.4%	0%	20%	5.3%	1.4%	0%	20%	5.3%	1.4%	20%	5.3%	1.4%
Hydrocortisone	13.6 (3.3)	27 (16)	15.9 (5.5)	16.9	16.9	14.3	10	8.9	10.9						
Ibuprofen						4.33				0.01					
Iopamidol						5,020	1380	320		7.03	1.93	0.45			
Meprobamate						12.3	3.16			0.02	0.004				
Metformin	5.6					526	160	38.3	0.74	0.74	0.22	0.05	7.63		
Methyl Triclosan	0.18	0.049													
Metoprolol	0.47 (0.08)					118	34.4	8.11		0.17	0.05	0.01	2.84		
Metronidazole						15	4.54			0.02	0.006				
Miconazole	1.8 (0.5)	1.4 (0.3)	0.95 (0.1)	0.77	0.68										
Naproxen						19.1	5.1			0.03	0.007				
Norfloxacin						82				0.11					
Norfluoxetine	0.8 (0.01)														
Norverapamil						1.1	0.3			0.02	0.005				
Ofloxacin						24.2	9.02	2.8		0.03	0.013	0.004			
Ormetoprim	1570 (311)	2405 (785)	1760 (57)	1750	1960	122	135	141	218						
Oxacillin	3.8 (0.6)	2.9	1.5	1.2											
Oxolinic Acid		0.32													
Oxycodone						9.22	2.58	0.70		0.01	0.004	0.001			
PBBZ							0.024				0.62				
Penicillin V	2.7	2.0 (0.2)													
PFBS						1.92	0.47			0.005	0.001				
PFHpA						0.47				0.002					
PFHxA						3.41	1.04			0.005	0.002				

Analyte	Observed whole body (ng/g)					Observed Water (ng/L)				Predicted whole body (ng/g)			Obs/Pred whole body		
	20% Mean (sd)	5.3% Mean (sd)	1.4% Mean (sd)	0.4%	0%	20%	5.3%	1.4%	0%	20%	5.3%	1.4%	20%	5.3%	1.4%
PFHxS						0.94				0.010					
PFOA						1.3				0.022					
PFOS						3.07	0.52			0.87	0.15				
PFOSA						1.38	2.8	1.7	5.0	2.57	5.2	3.07			
PFPeA						1.48				0.002					
Propranolol	0.34 (0.1)					11.7	3.43	0.89		0.03	0.007	0.002	13.5		
Rosuvastatin						100	31.4	7.74		0.14	0.044	0.011			
Sertraline	8.7 (0.9)	1.9 (0.07)	0.58	0.13		15	4.66	1.12		5.29	1.64	0.39	1.7	1.1	1.5
Sulfadimethoxine (Romet TC)	67 (17)	61 (9.5)	68 (13)	70	93.4	72.1	96.6	86.1	183						
Sulfamethazine			0.96												
Sulfamethoxazole						48.6	19.7	5.7		0.07	0.028	0.008			
Theophylline						47.9	14.9		7.1	0.07	0.021				
Thiabendazole						6.69	2.49			0.10	0.036				
Triamterene						18.4	5.47	1.32		0.05	0.016	0.004			
Triclocarban						0.56				2.21					
Triclosan (method 2)	5.1	1.08	0.34												
Triclosan	4.4 (0.3)					10.2				4.73			0.94		
Trimethoprim						62.3	16	3.66		0.09	0.022	0.005			
Valsartan						182	55	14.4		2.17	0.656	0.172			
Venlafaxine	0.44 (0.02)					79.7	22.7	5.76		0.48	0.136	0.035	0.92		
Verapamil	0.07 (0.01)					3.76	1.05	0.26		0.44	0.124	0.030	0.17		

Predicted tissue concentration determined with equation 2 using the BCF times the observed water concentration. Obs/Pred is the observed whole-body tissue concentration over the predicted tissue concentration. The geometric mean of the obs/pred tissue concentrations was 0.97 (SD=2.9; n=29), excluding two metabolites (desmethyldiltiazem and benzoylecgonine).

Table 19. Observed over predicted BCF values for laboratory exposure.

Analyte	K_{ow} or D_{ow} (pH 8.4)	Pred BCF	T20% Rep 1 obs/pred BCF	T20% Rep2 obs/pred BCF	T5.3% Rep 1 obs/pred BCF	T5.3% Rep 2 obs/pred BCF	T1.4% obs/pred BCF
4-Nonylphenol diethoxylates	5.6	11,482	0.022				
4-Nonylphenol monoethoxylates	5.8	16,982	0.004		0.007		
4-Nonylphenols	5.4	7,762	0.048		0.061		0.10
Amitriptyline	3.44	167	0.71	0.68			
Benzoylecgonine (M)	0.41	1.4			24.6		
Bisphenol A	4.04	542	0.23	0.27	0.31	0.18	0.54
Citalopram	1.98	10	2.6	2.4	2.2	1.7	
DEET	2	10	0.45	0.45	1.1	1.4	11.1
Desmethyldiltiazem (M)	1.45	3	21.5	18.7	20.5	17.2	
Diltiazem	2.5	27	3.0	3.4	2.5	2.8	
Diphenhydramine	3.06	80	1.7	2.6	1.7	1.8	
Fluoxetine	2.78	46	2.1	2.5			
Gemfibrozil	1.31	3	6.64	6.71	4.9	3.9	
Metformin	-3.5	1.4		7.63			
Metoprolol	-0.17	1.4	2.5	3.2			
Norfluoxetine (M)	2.05	11	0.9				
PFOS	6.3	45,186	0.003		0.006		
Propranolol	1.22	2	10.9	16.2			
Sertraline	3.82	352	1.8	1.5	1.2	1.1	
Triclosan	3.96	463	0.98	0.89			
Venlafaxine	1.74	7	0.9	0.8			
Verapamil	3.26	118	0.2	0.2			

Values are observed/predicted BCFs for analytes detected in both water and fish whole-body. K_{ow} is the octanol-water partition coefficient and where appropriate the pH-specific K_{ow} (D_{ow}). D_{ow} was determined for pH 8.4, which was close to the mean pH for all replicate tanks (pH = 8.49). The geometric mean for all values < K_{ow} = 5 was 1.5 (SD=3.3; SE=0.4, n=45), excluding metabolites (M). Several analytes occurred in whole-body fish but not lab exposure water (Cloxacillin, Flumequine, Ofloxacin, Oxacillin, Oxolinic acid, and Penicillin V). Ormetoprim occurred in both lab water and tissue, but not in whole effluent. Oxacillin, Oxolinic acid, and penicillin did not occur in whole effluent.

Table 20. Observed and predicted tissue concentrations for detected water concentrations and undetected tissue concentrations.

Analyte	Observed whole body (ng/g)						Predicted whole body (ng/g)			Predicted whole body/RL		
	20% Mean (sd)	5.3% Mean (sd)	1.4%Mean (sd)	0.4%	0%	Mean RL	20%	5.3%	1.4%	20%	5.3%	1.4%
10-OH amitriptyline						0.11	0.05	0.015	0.003	0.47	0.14	0.03
2-Hydroxy-ibuprofen						1.56	0.04	0.011		0.025	0.007	
4-Nonylphenol diethoxylates	21.2	10.6	3.4			0.46						
4-Nonylphenol monoethoxylates	31.1	15.3	6.7			0.46						
4-Nonylphenols	59.1					0.46		494	20		1073	43
Albuterol						0.25	0.003	0.001		0.012	0.004	
Alprazolam						0.12	0.02			0.147		
Amitriptyline	0.56 (0.01)					0.12			0.058			0.48
Amlodipine						0.39	0.01	0.002		0.014	0.004	
Atenolol						0.25	0.01	0.005	0.001	0.060	0.020	0.005
Atorvastatin						1.0	0.05	0.010		0.047	0.010	
Azithromycin						8.9	0.09	0.018	0.004	0.011	0.002	0.0005
Benzoylecgonine		0.08		0.06		0.06	0.01		0.001	0.189		0.014
Bisphenol A	13.3 (1.5)	6.2 (2.5)	4.8 (0.2)	4.0	3.6	2.3						
Bisphenol F						NA	2.31		1.4			
Bisphenol S						NA	0.04	0.022				
Caffeine						5.8	0.02			0.004		
Carbamazepine						0.58	3.39	1.0	0.24	5.84	1.73	0.42
Ciprofloxacin						7.9	0.03	0.014	0.009	0.004	0.002	0.001
Citalopram	1.2 (0.08)	0.27 (0.05)	4.8 (0.2)			0.16						
Clarithromycin						0.58	0.92	0.299		1.58	0.52	
Clotrimazole						0.16	5.58			35		
Cloxacillin	4.5	6.1	6.9			2.2						
Codeine						1.0	0.02	0.003		0.016	0.003	

Analyte	Observed whole body (ng/g)						Predicted whole body (ng/g)			Predicted whole body/RL		
	20% Mean (sd)	5.3% Mean (sd)	1.4%Mean (sd)	0.4%	0%	Mean RL	20%	5.3%	1.4%	20%	5.3%	1.4%
Cotinine						0.25	0.01	0.004	0.001	0.045	0.014	0.005
Dechlorane plus Anti						0.11	272	166	444	2,472	1511	4,040
Dechlorane plus Syn						0.13	15.8	9.4	19.2	122	72	147
DEET						0.12	4.23	1.43	0.51	35.3	11.9	4.2
Dehydronifedipine						0.24	0.057			0.24		
Desmethyldiltiazem	0.3 (0.03)	0.09 (0.01)				0.06			0.001			0.019
Diatrizoic acid						4.7	0.15	0.042		0.033	0.009	
Diltiazem	2.3 (0.2)	0.41 (0.03)	0.2 (0.03)			0.15						
Diphenhydramine	29.6 (8.5)	5.5 (0.3)	1.8 (0.2)	0.52		0.23						
EHTBB						1.1	265,106			241,005		
Erythromycin-H2O						0.9	0.13	0.062	0.049	0.144	0.069	0.055
Estrone						NA	51.8	16.8	5.8			
Flumequine	2.2 (0.6)	1.2	1.6 (0.7)			0.83						
Fluoxetine	0.89 (0.1)					0.58		0.219	0.073		0.38	0.13
Furosemide						1.6	0.01	0.009		0.005	0.006	
Gemfibrozil	2.1 (0.01)	0.42 (0.07)				0.3			0.023			0.075
Glipizide						0.3	0.005			0.015		
HBB						0.12		312			2603	
Hydrochlorothiazide						3.4	0.34	0.111	0.031	0.101	0.033	0.009
Hydrocodone						1.0	0.02	0.007		0.024	0.007	
Hydrocortisone	13.6 (3.3)	27 (16)	15.9 (5.5)	16.9	16.9	2.4						
Ibuprofen						1.6	0.01			0.004		
Iopamidol						31.1	7.03	1.93	0.45	0.23	0.062	0.014
Meprobamate						0.58	0.02	0.004		0.030	0.008	

Analyte	Observed whole body (ng/g)						Predicted whole body (ng/g)			Predicted whole body/RL		
	20% Mean (sd)	5.3% Mean (sd)	1.4%Mean (sd)	0.4%	0%	Mean RL	20%	5.3%	1.4%	20%	5.3%	1.4%
Metformin	5.6					0.32		0.22	0.05		0.70	0.17
Metoprolol	0.47 (0.08)					0.2		0.05	0.01		0.24	0.057
Metronidazole						0.78	0.02	0.006		0.027	0.008	
Miconazole	1.8 (0.5)	1.4 (0.3)	0.95 (0.1)	0.77	0.68	0.59						
Naproxen						0.78	0.03	0.007		0.034	0.009	
Norfloxacin						14.7	0.11			0.008		
Norfluoxetine	0.8 (0.01)					0.2						
Norverapamil						0.06	0.02	0.005		0.340	0.086	
Ofloxacin						0.70	0.03	0.013	0.004	0.048	0.018	0.006
Ormetoprim	1570 (311)	2405 (785)	1760 (57)	1750	1960	2.7						
Oxacillin	3.8 (0.6)	2.9	1.5	1.2		1.7						
Oxolinic Acid		0.32				0.23						
Oxycodone						0.5	0.01	0.004	0.001	0.026	0.007	0.002
PBBZ						0.19		0.62			3.2	
Penicillin V	2.7	2.0 (0.2)				1.3						
PFBS						0.11	0.005	0.001		0.04	0.01	
PFHpA						0.11	0.002			0.014		
PFHxA						0.11	0.005	0.002		0.043	0.013	
PFHxS						0.11	0.010			0.09		
PFOA						0.11	0.022			0.20		
PFOS						0.11	0.87	0.15		7.9	1.3	
PFOSA						0.11	2.57	5.2	3.07	23.2	46.8	27.9
PFPeA						0.23	0.002					
Propranolol	0.34 (0.1)					0.12		0.007	0.002		0.062	0.016

Analyte	Observed whole body (ng/g)						Predicted whole body (ng/g)			Predicted whole body/RL		
	20% Mean (sd)	**5.3% Mean (sd)**	**1.4%Mean (sd)**	**0.4%**	**0%**	**Mean RL**	**20%**	**5.3%**	**1.4%**	**20%**	**5.3%**	**1.4%**
Rosuvastatin						1.6	0.14	0.044	0.011	0.088	0.027	0.007
Sertraline	8.7 (0.9)	1.9 (0.07)	0.58	0.13		0.12						
Sulfadimethoxine (Romet TC)	67 (17)	61 (9.5)	68 (13)	70	93.4	0.2						
Sulfamethazine			0.96			0.80						
Sulfamethoxazole						0.23	0.07	0.028	0.008	0.34	0.14	0.040
Theophylline						2.3	0.07	0.021		0.12	0.036	
Thiabendazole						0.58	0.10	0.036		0.17	0.062	
Triamterene						0.25	0.05	0.016	0.004	0.21	0.064	0.015
Triclocarban						0.16	2.21			13.8		
Triclosan	4.4 (0.3)					2.3						
Trimethoprim						0.58	0.09	0.022	0.005	0.15	0.039	0.009
Valsartan						1.6	2.17	0.656	0.172	1.36	0.410	0.107
Venlafaxine	0.44 (0.02)					0.16		0.136	0.035		0.46	0.118
Verapamil	0.07 (0.01)					0.06		0.124	0.030		2.06	0.51
Sum	217.1	138.9	104.5				85.0	28.7	12.1			

Predicted tissue concentrations based on the equation 2 using the D_{ow} pH-specific octanol-water partition coefficient (D_{ow}), which are shown for all detected water concentrations and non-detected tissue concentrations. Predicted whole body/RL is the predicted tissue concentration over the reporting limit (RL) for that analyte. All predicted whole body/RL values shown for detected water concentrations and the absence of detected tissue concentrations. The geometric mean of the predicted whole body /RL values is 0.07 (SD=7.2, N=117). Only 15 predicted whole body /RL values were > 1. Sum of obs compounds, excluding ormetoprim shown. Sum of predicted tissue concentrations shown for those analytes with a detected water concentration and a non-detected tissue concentration (excluding the hydrophobic compounds, declorane, HBB, EHTBB, PBBZ, and 4-nonylphenol). Two replicates for whole-body tissue were analyzed for treatments 20%, 5.3%, and 1.4% dilution. NA denotes not analyzed for tissue.

Table 21. Plasma concentrations in juvenile Chinook and predicted partitioning.

Analyte	D_{ow} pH 8.4	Obs plasma (ng/g)				Pred Pbw	Pred BCF	Pred Vd	Obs/Pred Pbw				Obs whole body/plasma			
		20%	5.3%	1.4%	0.4%				20%	5.3%	1.4%	0.4%	20%	5.3%	1.4%	0.4%
Caffeine	-0.79	1.4	1.4	1.4	1.3	1.7	1.4	0.82	50.7							
Diphenhydramine	3.06	0.06				28.3	79.6	2.82	0.013				477			
Erythromycin-H2O	2.31	1.36	1.25	0.92	1.23	8.6	18.3	2.13	22.4	42.7	39.5					
Ormetoprim		1.76	2.72	2.54	2.32	3.9	6.1	1.58	0.002	0.002	0.002	0.002	892	884	693	754
Sulfadimethoxine (Romet TC)	0.57	1.46	0.82	0.88	1.28	1.3	1.4	1.11	0.024	0.015	0.014	0.020	46	74	78	55
Thiabendazole	2.19		0.15		0.14	7.2	14.5	2.02		8.3						
Virginiamycin M1	2.32	1.2		1.8	0.63	8.7	18.7	2.14								
Bisphenol A	4.03	2.62	1.8	4.2	2.67	141	531	3.77	0.19	0.27	1.36		5.1	3.5	1.1	1.5
Furosemide	-1.36	0.40	0.40	0.35	0.33	1.7	1.4	0.82	43.6	35.9						
Gemfibrozil	1.31	0.66	0.28	0.10		2.3	2.6	1.13	2.4	3.3	5.0		3.2	1.5		
Hydrochlorothiazide	-0.23	1.67	1.41	1.28	1.21	1.7	1.4	0.82	4.0	10.5	34.4					
Hydroxy-ibuprofen	-1.02	30.6	35.2	42.5	13.1	1.7	1.4	0.82	619	2,531						
Ibuprofen	0.85	1.29	0.95	1.27	0.86	1.5	1.4	0.93	198							
Metformin	-3.33	0.90	0.23	23.4	4.03	1.7	1.4	0.82	1.0	0.8	359		6.3			
Benztropine	2.91	0.16	0.13	0.13	0.12	22	59.4	3								
Cocaine	1.7	0.02	0.02		0.05	3.6	5.6	1.53								
DEET	2.5	0.81	0.52	0.50	0.36	11.5	26.6	2.31	0.4	0.8	2.3					
Hydrocortisone	1.28	56.3	84.8	58.2	69.2	2.2	2.4	1.10	**4.57**	**3.47**	**4.04**	**4.52**	0.24	0.32	0.27	0.24
Sertraline	3.82	0.19	0.07			99.2	352.4	3.55	0.13	0.16			45.2	25.3		
Theophylline	-1.35	0.70	0.54	0.62	0.54	1.7	1.4	0.82	8.6	21.2						
Citalopram	2.16		0.04			6.9	13.7	1.99		0.4				6.0		

Observed (obs) plasma concentrations in ng/g wet weight. Predicted (pred) blood-water partitioning (Pbw) determined with equation 3 and the predicted BCF determined with equation 1. Vd is the volume of distribution, which is the ratio between tissue and plasma concentrations and is also the ratio between the BCF and Pbw. Observed tissue/plasma concentrations determined for those analytes with both values. Predicted plasma concentration determined for those analytes with Pbw*water concentrations or with tissue/Vd (in bold). Obs/pred plasma concentration is equivalent to the obs/pred Pbw values. The geometric mean of obs/pred plasma concentrations was 3.9 (SD=67, N=33), excluding the two antibiotics sulfamethoxine and ormetoprim, and the metabolite hydroxy-ibuprofen.

Table 22. Plasma concentrations compared to human therapeutic levels (1% Cmax) and Response Ratios (RR).

Analyte	Dow pH 8.4	1% Cmax ng/ml (ppb)	Ratio Plasma/1% Cmax (RR) for Obs plasma				Ratio Plasma/1% Cmax (RR) for pred plasma (water Pbw)			Ratio Plasma/1% Cmax (RR) for pred plasma (tissue Vd)			
			T20%	T5.3%	T1.4%	T0.4%	T20%	T5.3%	T1.4%	T20%	T5.3%	T1.4%	T0.4%
10-hydroxy-amitriptyline	2.35	0.24					0.10	0.03	0.01				
2-Hydroxy-ibuprofen	-1.02	150	0.20	0.23	0.28	0.09	0.0003	0.0001					
Albuterol	-0.66	0.1					0.04	0.01					
Alprazolam	2.79	0.05					0.14						
Amitriptyline	3.44	0.24					1.06	0.30	0.08	0.74			
Amlodipine	0.57	0.05					0.10	0.03					
Atenolol	-0.85	0.1					0.18	0.06	0.01				
Atorvastatin	1.77	0.12					0.24	0.05					
Azithromycin	0.65	0.4					0.29	0.05	0.01				
Benzoylecgonine	0.4	2.5					0.005	0.002	0.0004		0.04		0.03
Benztropine	2.91	0.1	1.6	1.3	1.3	1.2							
Bisphenol A	4.03	1.95	1.3	0.9	2.2	1.4	7.16	3.39	1.58	1.80	0.84	0.65	0.55
Caffeine	-0.79	25	0.057	0.056	0.055	0.053	0.001						
Carbamazepine	3.22	20					0.06	0.02	0.004				
Ciprofloxacin	-0.06	0.5					0.07	0.03	0.02				
Citalopram	2.16	0.1		0.440			3.56	0.99	0.28	6.24	1.33		
Clarithromycin	2.95	8					0.04	0.01					
Clotrimazole	5.65	200					0.005						
Cloxacillin	-1.7	280								0.02	0.03	0.03	
Cocaine	1.7	2.5	0.006	0.009		0.018							
Codeine	0.49	1.3					0.01	0.002					
Cotinine	-0.3	0.4					0.03	0.01	0.0039				
DEET	2.5	30	0.027	0.017	0.017	0.012	0.06	0.02	0.01				
Dehydronifedipine	3.01	0.25					0.08						
Desmethyldiltiazem	1.45	0.4					0.03	0.01	0.0023	0.60	0.18		
Diatrizoic acid	-0.49	200,000											

Analyte	Dow pH 8.4	1% Cmax ng/ml (ppb)	Ratio Plasma/1% Cmax (RR) for Obs plasma				Ratio Plasma/1% Cmax (RR) for pred plasma (water Pbw)			Ratio Plasma/1% Cmax (RR) for pred plasma (tissue Vd)			
			T20%	T5.3%	T1.4%	T0.4%	T20%	T5.3%	T1.4%	T20%	T5.3%	T1.4%	T0.4%
Diltiazem	2.52	0.5					0.66	0.14	0.04	2.01	0.35	0.19	
Diphenhydramine	3.06	0.8	0.078				6.14	1.38	0.29	13.13	2.44	0.80	1.84
Erythromycin	2.31	19	0.072	0.066	0.048	0.065	0.003	0.002	0.001				
Estrone	4.3	0.002					4,048	1,316	452				
Flumequine	-0.3	21								0.13	0.07	0.06	
Fluoxetine	2.78	0.1					1.5	0.9	0.28	3.5			
Furosemide	-1.36	20	0.020	0.020	0.018	0.017	0.0005	0.0006					
Gemfibrozil	1.31	300	0.002	0.001	0.0003		0.0009	0.0003	0.0001	0.0062			
Glipizide	0.87	1					0.01						
Hydrochloro-thiazide	-0.23	0.7	2.39	2.01	1.83	1.73	0.60	0.19	0.05				
Hydrocodone	1.67	0.18					0.09	0.02	0.00				
Hydrocortisone	1.28	0.1	563	848	582	692				123	244	144	153
Ibuprofen	0.85	150	0.009	0.006	0.008	0.006							
Iopamidol	-1.01	1,130					0.008	0.0021	0.0005				
Meprobamate		50					0.0004	0.0001					
Metformin	-3.33	1	0.89	0.23	23.4	4.0	0.89	0.27	0.07	6.82			
Metoprolol	0.21	0.35					0.36	0.10	0.02	1.02			
Metronidazole	-0.57	30					0.0009	0.0003					
Miconazole	5.53	150								0.002	0.002	0.001	0.0009
Naproxen	-0.25	200					0.0002	0.00004					
Norfloxacin	-1.31	5					0.0279						
Norfluoxetine	2.41	0.12								2.99	0.76	0.00	0.00
Norverapamil	2.33	0.1					0.095	0.024					
Ofloxacin	-1.18	25					0.0016	0.0006	0.0002				
Ormetoprim		12	0.15	0.23	0.21	0.19				83	127	93	92
Oxacillin	-2.2	430								0.01	0.01	0.004	0.003
Oxolinic Acid	-1.4	36									0.01		
Oxycodone	0.69	0.05					0.25	0.07	0.02				

Analyte	Dow pH 8.4	1% Cmax ng/ml (ppb)	Ratio Plasma/1% Cmax (RR) for Obs plasma				Ratio Plasma/1% Cmax (RR) for pred plasma (water Pbw)			Ratio Plasma/1% Cmax (RR) for pred plasma (tissue Vd)			
			T20%	T5.3%	T1.4%	T0.4%	T20%	T5.3%	T1.4%	T20%	T5.3%	T1.4%	T0.4%
Penicillin V	-3.07	1								3.3	2.4		
Propranolol	1.22	0.2					0.12	0.04	0.01	1.7			
Rosuvastatin	-1.56	0.1					1.7	0.53	0.13				
Sertraline	3.82	0.5	0.38	0.15			3.0	0.92	0.22	4.9	1.0	0.33	0.07
Sulfadimethoxine (Romet TC)	0.57	500	0.003	0.002	0.002	0.003				0.16	0.15	0.17	0.17
Sulfamethazine	-0.26	500											
Sulfamethoxazole	0.3	300					0.0003	0.0001					
Theophylline	-1.35	200	0.004	0.003	0.003	0.003	0.0004	0.0001					
Thiabendazole	2.19	33		0.005		0.004	0.0015	0.0005					
Triamterene	1.37	0.3					0.15	0.04	0.01				
Triclocarban	5.06	1.7					0.26						
Triclosan	3.96	0.04					31.9			29.9			
Trimethoprim	1	15					0.01	0.002	0.0004				
Valsartan	2.09	8					0.14	0.04	0.01				
Venlafaxine	1.74	2					0.15	0.04	0.01	0.14			
Verapamil	3.26	0.1					1.47	0.41	0.10	0.25			
Virginiamycin M1	2.32												

Observed (obs) and predicted (pred) plasma concentrations compared to human therapeutic values. Values determined with equation 5 to determine the Response Ratio (RR). Values >1 indicate a potential for adverse effects in fish and values <1 indicate a lower probability of effects. Predicted plasma concentrations determined with observed water concentrations and the blood:water partition coefficient (Pbw). Predicted plasma concentrations with observed tissue concentrations determined with the volume of distribution (Vd), which can be approximated with BCF/Pbw (Table 21). C_{max} is the maximum plasma concentration for the minimum therapeutic effect in humans.

Table 23. Response Ratios (RR) determined for observed and predicted plasma. Values >0.1, ordered from high to low.

Analyte	1% Cmax (ng/mL) ppb	Ratio Plasma/1% Cmax (RR) Obs plasma				Analyte	Ratio Plasma/1%Cmax (RR) pred plasma - from water			Analyte	Ratio Plasma/1%Cmax (RR) pred plasma – from tissue			
		T20%	T5.3%	T1.4%	T0.4%		T20%	T5.3%	T1.4%		T20 %	T5.3 %	T1.4 %	T0.4%
Hydrocortisone	0.1	563	848	582	692	**Estrone**	4,048	1,316	452	**Hydrocortisone**	123	244	144	153
Hydro-chlorothiazide	0.7	2.4	2.0	1.8	1.7	**Triclosan**	31.9			**Ormetoprim**	83	127	93	92
Benztropine	0.1	1.6	1.3	1.3	1.2	**Bisphenol A**	7.2	3.4	1.6	**Triclosan**	30			
Bisphenol A	1.95	1.3	0.9	2.2	1.4	**Diphen-hydramine**	6.1	1.4	0.29	**Diphenhydramine**	13	2.4	0.80	1.4
Metformin	1	0.89	0.23	23.4	4.0	**Citalopram**	3.6	0.99	0.28	**Metformin**	6.8			
Sertraline	0.5	0.39	0.15			**Sertraline**	2.9	0.92	0.22	**Citalopram**	6.2	1.33		
2-Hydroxy-ibuprofen	150	0.20	0.23	0.28	0.09	**Rosuvastatin**	1.7	0.53	0.13	**Sertraline**	4.9	1.0	0.33	0.07
Ormetoprim	12	0.15	0.23	0.21	0.19	**Fluoxetine**	1.5	0.9	0.28	**Fluoxetine**	3.5			
Diphen-hydramine	0.8	0.08				**Verapamil**	1.5	0.41	0.10	**Penicillin V**	3.3	2.4		
Erythromycin	19	0.07	0.07	0.05	0.065	**Amitriptyline**	1.1	0.30	0.08	**Norfluoxetine**	3.0	0.76	0.00	0.00
Caffeine	25	0.06	0.06	0.05	0.053	**Metformin**	0.89	0.27	0.07	**Diltiazem**	2.0	0.35	0.19	
DEET	30	0.03	0.02	0.02	0.012	**Diltiazem**	0.66	0.14	0.04	**Bisphenol A**	1.8	0.84	0.65	0.55
Furosemide	20	0.02	0.02	0.02	0.017	**Hydro-chlorothiazide**	0.60	0.19	0.05	**Propranolol**	1.7			
Ibuprofen	150	0.009	0.006	0.008	0.006	Metoprolol	0.36	0.10	0.02	**Metoprolol**	1.0			
Cocaine	2.5	0.006	0.009		0.018	Azithromycin	0.29	0.05	0.01	**Amitriptyline**	0.74			
Theophylline	200	0.004	0.003	0.003	0.003	Triclocarban	0.26			**Desmethyl-diltiazem**	0.60	0.18		
Sulfadi-methoxine	500	0.003	0.002	0.002	0.003	Oxycodone	0.25	0.07	0.02	Verapamil	0.25			
						Atorvastatin	0.24	0.05		Venlafaxine	0.14			
						Atenolol	0.18	0.06	0.01	Flumequine	0.13	0.07	0.06	
						Venlafaxine	0.15	0.04	0.01	Sulfadimethoxine	0.16	0.15	0.17	0.17

Analyte	1% Cmax (ng/mL) ppb	Ratio Plasma/1% Cmax (RR) Obs plasma				Analyte	Ratio Plasma/1%Cmax (RR) pred plasma - from water			Analyte	Ratio Plasma/1%Cmax (RR) pred plasma – from tissue			
		T20%	T5.3%	T1.4%	T0.4%		T20%	T5.3%	T1.4%		T20 %	T5.3 %	T1.4 %	T0.4%
						Triamterene	0.15	0.04	0.01					
						Valsartan	0.14	0.04	0.01					
						Alprazolam	0.14							
						Propranolol	0.12	0.04	0.01					
						10-OH - amitriptyline	0.10	0.03	0.01					
						Amlodipine	0.10	0.03						
						Norverapamil	0.01	0.02						
						Hydrocodone	0.01	0.02						

Values are Response Ratios (RR) as determined with equation 5. RR values >0.5 in bold. Values based on observed plasma concentration, or predicted plasma concentration based on observed water (Pbw) or tissue (Vd) concentrations.

Table 24. Compounds detected in estuarine waters and WWTP effluent.

Analyte	Obs Mean field sites	SD field sites	Pred mean field sites	Mean Obs / pred field	SP low flow conc	WP low flow conc	Mean ratio field to SP	SD ratio field to SP	Mean ratio field to WP	SD ratio field to WP	Mean ratio field to SP (%)	Mean ratio field to WP (%)	Mean ratio field/WWTPs (%)
Atenolol	0.39	0.09	0.99	0.40	79	468	0.0051	0.0011	0.0008	0.0002	0.51	0.085	0.20
Benzoylecgonine	0.36	0.05	0.23	1.6	40	85	0.0090	0.0012	0.0042	0.0005	0.89	0.42	0.61
Bisphenol A	2.1		2.5	0.80	1,160	258	0.0018		0.0079		0.18	0.79	0.38
Citalopram	0.45		0.84	0.50	212	254	0.0021		0.0018		0.21	0.18	0.19
Cotinine	0.72	0.07	0.37	4.6	25	63	0.029	0.0030	0.0116	0.0012	3.0	1.2	1.9
DEET	4.9	0.49	1.7	2.9	829	110	0.0059	0.0006	0.0446	0.0044	0.59	4.5	1.6
Diphenhydramine	0.63	-	2.9	0.22	941	677	0.0007		0.0009		0.07	0.09	0.08
Metformin	42	10.3	59	0.71	2,640	30,300	0.016	0.0039	0.0014	0.0003	1.6	0.14	0.47
Sulfamethoxazole	0.79	0.18	1.0	0.78	370	193	0.0022	0.0005	0.0042	0.0009	0.22	0.42	0.29
Venlafaxine	0.51	0.09	1.44	0.35	406	392	0.0013	0.0002	0.0013	0.0002	0.13	0.13	0.13
	Overall mean			0.83							Overall mean %		0.36
	SD			1.4							SD		0.63

Analytes detected in estuarine samples and WWTP effluents shown (SP = South Plant and WP = West Point WWTPs). Effluent data for low-flow sampling event. Geometric mean and standard deviation (SD) concentrations shown for all sampled estuarine sites (n=6). Mean ratio shows the geometric mean ratio of observed field value to low-flow effluent concentration for each WWTP. Data also shown in percentages. Obs/pred is the geometric mean for observed field concentrations over the predicted values for all sites (n=6). Predicted field values were determined by multiplying the overall mean ratio (0.36) times the mean effluent concentration for the low-flow samples from both WP and SP. Mean predicted values were calculated for each analyte among the 6 estuarine sites because of the low standard deviation for observed values over the 6 sites. Last column shows geometric mean percentage of the ratios for each analyte in estuary water over mean effluent. All concentrations as ng/L.

Table 25. Predicted field concentrations (ng/L).

Analyte	Pred field conc	Mean field RL	Mean Effluent conc	Mean effluent RL	Est/RL WP N. shallow	Est/RL WP N. deep	Est/RL WP south	Est/RL Elliott	Est/RL Alki	Est/RL Ref site
Phenols										
4-Nonylphenols	2.1	3.1	570	4.17	1.0	1.2	0.6	0.4	0.8	0.5
4-Nonylphenol monoethoxylates	6.8	8.7	1865	8.22	1.3	0.9	0.5	0.9	0.7	0.8
4-Nonylphenol diethoxylates	2.4	4.9	671	4.18	0.9	0.7	0.3	0.6	0.5	0.4
Sum phenols	11.2									
Br and Cl Fire Retardants										
Dec 603	0.0001	0.007	0.02	0.01	0.007	0.014	0.010	0.013	0.012	0.009
BEHTBP	0.0054	0.33	1.51	0.51	0.018	0.023	0.014	0.023	0.015	0.013
EHTBB	0.012	0.19	3.22	0.47	0.075	0.082	0.058	0.076	0.049	0.046
Sum fire retardants	0.02									
PFAS										
PFBA	0.026	1.53	7.23	1.47	0.017	0.017	0.017	0.017	0.017	0.017
PFPeA	0.024	0.77	6.78	0.73	0.032	0.032	0.032	0.032	0.032	0.032
PFHxA	0.069	0.38	19.2	0.37	0.182	0.182	0.182	0.181	0.181	0.181
PFHpA	0.008	0.38	2.27	0.37	0.022	0.022	0.021	0.021	0.021	0.021
PFOA	0.019	0.38	5.25	0.37	0.050	0.050	0.050	0.049	0.050	0.049
PFNA	0.004	0.38	1.01	0.37	0.010	0.010	0.010	0.009	0.010	0.009
PFDA	0.003	0.38	0.95	0.37	0.009	0.009	0.009	0.009	0.009	0.009
PFBS	0.027	0.38	7.54	0.37	0.072	0.072	0.071	0.071	0.071	0.071
PFPeS	0.0017	0.38	0.46	0.37	0.004	0.004	0.004	0.004	0.004	0.004
PFHxS	0.014	0.38	3.72	0.37	0.035	0.035	0.035	0.035	0.035	0.035
PFOS	0.038	0.38	10.6	0.37	0.101	0.101	0.100	0.100	0.100	0.100
6:2 FTS	0.0099	1.38	2.74	1.32	0.007	0.007	0.007	0.007	0.007	0.007
MeFOSAA	0.004	0.38	1.06	0.37	0.010	0.010	0.010	0.010	0.010	0.010
5:3 FTCA	0.075	9.57	20.6	9.1	0.008	0.008	0.008	0.008	0.008	0.008
Sum PFAS	0.32									
Pesticides										
Hexachlorobenzene	0.0002		0.04	0.010	0.015	0.015	0.015	0.015	0.015	0.015

Analyte	Pred field conc	Mean field RL	Mean Effluent conc	Mean effluent RL	Est/RL WP N. shallow	Est/RL WP N. deep	Est/RL WP south	Est/RL Elliott	Est/RL Alki	Est/RL Ref site
HCH, alpha	0.0001		0.02	0.012	0.005	0.005	0.005	0.005	0.005	0.005
HCH, gamma	0.0006		0.16	0.016	0.037	0.037	0.037	0.037	0.037	0.037
HCH, beta	0.0006		0.15	0.011	0.048	0.048	0.048	0.048	0.048	0.048
Aldrin	0.001		0.35	0.010	0.122	0.122	0.122	0.122	0.122	0.122
Chlordane, alpha (cis)	0.0002		0.05	0.030	0.006	0.006	0.006	0.006	0.006	0.006
Nonachlor, trans-	0.0004		0.10	0.032	0.011	0.011	0.011	0.011	0.011	0.011
Nonachlor, cis-	0.0002		0.06	0.038	0.006	0.006	0.006	0.006	0.006	0.006
Dieldrin	0.002		0.43	0.017	0.093	0.093	0.093	0.093	0.093	0.093
Diazinon	0.002		0.57	0.334	0.006	0.006	0.006	0.006	0.006	0.006
Chlorpyriphos	0.0008		0.22	0.103	0.008	0.008	0.008	0.008	0.008	0.008
Permethrin	0.034		9.54	0.475	0.073	0.073	0.073	0.073	0.073	0.073
Cypermethrin	0.006		1.58	0.200	0.029	0.029	0.029	0.029	0.029	0.029
Sum Pesticides	0.05									
PAHs										
Naphthalene	0.051		14.0	2.62	0.019	0.019	0.019	0.019	0.019	0.019
Acenaphthylene	0.002		0.66	0.18	0.013	0.013	0.013	0.013	0.013	0.013
Acenaphthene	0.038		10.4	0.52	0.073	0.073	0.073	0.073	0.073	0.073
2-Methylfluorene	0.007		1.95	0.23	0.030	0.030	0.030	0.030	0.030	0.030
C2 Phenanthrenes/Anthracenes	0.033		9.20	0.19	0.173	0.173	0.173	0.173	0.173	0.173
Fluorene	0.036		10.0	0.117	0.310	0.310	0.310	0.310	0.310	0.310
Phenanthrene	0.038		10.5	0.83	0.046	0.046	0.046	0.046	0.046	0.046
Anthracene	0.003		0.80	0.18	0.016	0.016	0.016	0.016	0.016	0.016
C1 Phenanthrenes/Anthracenes	0.028		7.81	0.23	0.122	0.122	0.122	0.122	0.122	0.122
Fluoranthene	0.019		5.36	0.51	0.038	0.038	0.038	0.038	0.038	0.038
Pyrene	0.021		5.90	0.47	0.045	0.045	0.045	0.045	0.045	0.045
Benz[a]anthracene	0.003		0.74	0.17	0.015	0.015	0.015	0.015	0.015	0.015
Chrysene	0.005		1.41	0.35	0.015	0.015	0.015	0.015	0.015	0.015
Benzo[b]fluoranthene	0.001		0.33	0.11	0.010	0.010	0.010	0.010	0.010	0.010
Benzo[j,k]fluoranthenes	0.001		0.30	0.13	0.008	0.008	0.008	0.008	0.008	0.008
Benzo[e]pyrene	0.002		0.50	0.17	0.011	0.011	0.011	0.011	0.011	0.011

Analyte	Pred field conc	Mean field RL	Mean Effluent conc	Mean effluent RL	Est/RL WP N. shallow	Est/RL WP N. deep	Est/RL WP south	Est/RL Elliott	Est/RL Alki	Est/RL Ref site
Benzo[a]pyrene	0.001		0.32	0.17	0.007	0.007	0.007	0.007	0.007	0.007
Benzo[ghi]perylene	0.002		0.55	0.16	0.012	0.012	0.012	0.012	0.012	0.012
2-Methylnaphthalene	0.012		3.26	1.12	0.011	0.011	0.011	0.011	0.011	0.011
1-Methylnaphthalene	0.024		6.76	0.71	0.034	0.034	0.034	0.034	0.034	0.034
C1-Naphthalenes	0.036		10.00	1.84	0.020	0.020	0.020	0.020	0.020	0.020
Biphenyl	0.011		3.09	2.92	0.004	0.004	0.004	0.004	0.004	0.004
C1-Biphenyls	0.006		1.68	0.95	0.006	0.006	0.006	0.006	0.006	0.006
C2-Biphenyls	0.013		3.74	4.05	0.003	0.003	0.003	0.003	0.003	0.003
C2-Naphthalenes	0.065		17.9	2.3	0.028	0.028	0.028	0.028	0.028	0.028
1,2-Dimethylnaphthalene	0.008		2.30	0.52	0.016	0.016	0.016	0.016	0.016	0.016
2,6-Dimethylnaphthalene	0.011		3.16	0.44	0.026	0.026	0.026	0.026	0.026	0.026
C3-Naphthalenes	0.068		18.9	1.13	0.061	0.061	0.061	0.061	0.061	0.061
2,3,6-Trimethylnaphthalene	0.022		6.24	0.25	0.090	0.090	0.090	0.090	0.090	0.090
2,3,5-Trimethylnaphthalene	0.017		4.75	0.20	0.086	0.086	0.086	0.086	0.086	0.086
C4-Naphthalenes	0.050		13.8	0.49	0.101	0.101	0.101	0.101	0.101	0.101
C1-Fluorenes	0.027		7.55	0.41	0.067	0.067	0.067	0.067	0.067	0.067
1,7-Dimethylfluorene	0.004		1.12	0.65	0.006	0.006	0.006	0.006	0.006	0.006
C2-Fluorenes	0.067		18.6	0.65	0.105	0.105	0.105	0.105	0.105	0.105
C3-Fluorenes	0.045		12.5	0.66	0.069	0.069	0.069	0.069	0.069	0.069
Dibenzothiophene	0.014		3.95	0.17	0.082	0.082	0.082	0.082	0.082	0.082
C1-Dibenzothiophenes	0.009		2.69	0.31	0.031	0.031	0.031	0.031	0.031	0.031
2/3-Methyldibenzothiophenes	0.005		1.53	0.31	0.018	0.018	0.018	0.018	0.018	0.018
C2-Dibenzothiophenes	0.019		5.19	0.25	0.075	0.075	0.075	0.075	0.075	0.075
2,4-Dimethyldibenzothiophene	0.002		0.45	0.20	0.008	0.008	0.008	0.008	0.008	0.008
4,6-Dimethyldibenzothiophene	0.003		0.84	0.17	0.018	0.018	0.018	0.018	0.018	0.018
C3-Dibenzothiophenes	0.014		3.87	0.25	0.056	0.056	0.056	0.056	0.056	0.056
C4-Dibenzothiophenes	0.005		1.48	0.26	0.021	0.021	0.021	0.021	0.021	0.021
3-Methylphenanthrene	0.015		4.09	0.23	0.063	0.063	0.063	0.063	0.063	0.063
2-Methylphenanthrene	0.007		1.99	0.24	0.030	0.030	0.030	0.030	0.030	0.030
9/4-Methylphenanthrene	0.007		1.95	0.23	0.030	0.030	0.030	0.030	0.030	0.030

Analyte	Pred field conc	Mean field RL	Mean Effluent conc	Mean effluent RL	Est/RL WP N. shallow	Est/RL WP N. deep	Est/RL WP south	Est/RL Elliott	Est/RL Alki	Est/RL Ref site
1-Methylphenanthrene	0.006		1.83	0.23	0.029	0.029	0.029	0.029	0.029	0.029
3,6-Dimethylphenanthrene	0.003		0.77	0.20	0.014	0.014	0.014	0.014	0.014	0.014
2,6-Dimethylphenanthrene	0.004		1.09	0.19	0.021	0.021	0.021	0.021	0.021	0.021
1,7-Dimethylphenanthrene	0.003		0.83	0.19	0.016	0.016	0.016	0.016	0.016	0.016
1,8-Dimethylphenanthrene	0.001		0.36	0.19	0.007	0.007	0.007	0.007	0.007	0.007
C3-Phenanthrenes/Anthracenes	0.027		7.48	0.22	0.122	0.122	0.122	0.122	0.122	0.122
Retene	0.005		1.27	0.28	0.016	0.016	0.016	0.016	0.016	0.016
C4-Phenanthrenes/Anthracenes	0.030		8.30	0.28	0.107	0.107	0.107	0.107	0.107	0.107
C1-Fluoranthenes/Pyrenes	0.013		3.49	0.09	0.137	0.137	0.137	0.137	0.137	0.137
3-Methylfluoranth/Benzo[a]fluorene	0.004		1.20	0.09	0.047	0.047	0.047	0.047	0.047	0.047
C2-Fluoranthenes/Pyrenes	0.009		2.48	0.12	0.077	0.077	0.077	0.077	0.077	0.077
C3-Fluoranthenes/Pyrenes	0.002		0.63	0.09	0.024	0.024	0.024	0.024	0.024	0.024
C1-Benzo[a]anthracenes/Chrysenes	0.004		1.23	0.14	0.032	0.032	0.032	0.032	0.032	0.032
1-Methylchrysene	0.0008		0.22	0.14	0.006	0.006	0.006	0.006	0.006	0.006
C2-Benzo[a]anthracenes/Chrysenes	0.004		0.99	0.15	0.024	0.024	0.024	0.024	0.024	0.024
5,9-Dimethylchrysene	0.001		0.30	0.15	0.007	0.007	0.007	0.007	0.007	0.007
C3-Benzo[a]anthracenes/Chrysenes	0.0007		0.18	0.19	0.003	0.003	0.003	0.003	0.003	0.003
C4-Benzo[a]anthracenes/Chrysenes	0.007		2.05	0.10	0.076	0.076	0.076	0.076	0.076	0.076
C2-Benzofluoranth/Benzopyrenes	0.003		0.94	0.45	0.008	0.008	0.008	0.008	0.008	0.008
1,4,6,7-Tetramethylnaphthalene	0.009		2.62	0.49	0.019	0.019	0.019	0.019	0.019	0.019
Sum PAHs	1.02									
PCBs										
PCB-1	0.00004		0.0103	0.0077	0.005	0.005	0.005	0.005	0.005	0.005
PCB-6	0.00003		0.0084	0.0037	0.008	0.008	0.008	0.008	0.008	0.008
PCB-8	0.00006		0.0163	0.0103	0.006	0.006	0.006	0.006	0.006	0.006
PCB-11	0.00024		0.0676	0.021	0.012	0.012	0.012	0.012	0.012	0.012
PCB-15	0.00005		0.0125	0.0093	0.005	0.005	0.005	0.005	0.005	0.005
PCB-16	0.00004		0.0110	0.0038	0.010	0.010	0.010	0.010	0.010	0.010
PCB-17	0.00006		0.0175	0.0038	0.017	0.017	0.017	0.017	0.017	0.017
PCB-18 + 30	0.00009		0.0249	0.015	0.006	0.006	0.006	0.006	0.006	0.006

Analyte	Pred field conc	Mean field RL	Mean Effluent conc	Mean effluent RL	Est/RL WP N. shallow	Est/RL WP N. deep	Est/RL WP south	Est/RL Elliott	Est/RL Alki	Est/RL Ref site
PCB-19	0.00002		0.0054	0.0017	0.012	0.012	0.012	0.012	0.012	0.012
PCB-20 + 28	0.00012		0.0320	0.0129	0.009	0.009	0.009	0.009	0.009	0.009
PCB-21 + 33	0.00006		0.0163	0.0084	0.007	0.007	0.007	0.007	0.007	0.007
PCB-22	0.00005		0.0129	0.0054	0.009	0.009	0.009	0.009	0.009	0.009
PCB-25	0.00001		0.0032	0.0011	0.010	0.010	0.010	0.010	0.010	0.010
PCB-26 + 29	0.00002		0.0059	0.0025	0.008	0.008	0.008	0.008	0.008	0.008
PCB-27	0.000005		0.0013	0.0007	0.006	0.006	0.006	0.006	0.006	0.006
PCB-31	0.00010		0.0281	0.0103	0.010	0.010	0.010	0.010	0.010	0.010
PCB-32	0.00003		0.0073	0.0026	0.010	0.010	0.010	0.010	0.010	0.010
PCB-35	0.00001		0.0019	0.0009	0.008	0.008	0.008	0.008	0.008	0.008
PCB-37	0.00003		0.0080	0.0039	0.007	0.007	0.007	0.007	0.007	0.007
PCB-40 + 41 + 71	0.00005		0.0128	0.0034	0.014	0.014	0.014	0.014	0.014	0.014
PCB-42	0.00002		0.0066	0.0026	0.009	0.009	0.009	0.009	0.009	0.009
PCB-43	0.00000		0.0008	0.0005	0.006	0.006	0.006	0.006	0.006	0.006
PCB-44 + 47 + 65	0.00049		0.1354	0.0436	0.011	0.011	0.011	0.011	0.011	0.011
PCB-45 + 51	0.00023		0.0646	0.0066	0.035	0.035	0.035	0.035	0.035	0.035
PCB-48	0.00002		0.0048	0.0021	0.008	0.008	0.008	0.008	0.008	0.008
PCB-49 + 69	0.00006		0.0170	0.0056	0.011	0.011	0.011	0.011	0.011	0.011
PCB-52	0.00016		0.0447	0.0174	0.009	0.009	0.009	0.009	0.009	0.009
PCB-56	0.00003		0.0074	0.0031	0.009	0.009	0.009	0.009	0.009	0.009
PCB-59 + 62 + 75	0.00001		0.0024	0.0009	0.010	0.010	0.010	0.010	0.010	0.010
PCB-60	0.00002		0.0046	0.0022	0.008	0.008	0.008	0.008	0.008	0.008
PCB-61 + 70 + 74 + 76	0.00015		0.0419	0.0161	0.009	0.009	0.009	0.009	0.009	0.009
PCB-63	0.00000		0.0009	0.0008	0.004	0.004	0.004	0.004	0.004	0.004
PCB-64	0.00004		0.0102	0.0032	0.012	0.012	0.012	0.012	0.012	0.012
PCB-66	0.00006		0.0175	0.0045	0.014	0.014	0.014	0.014	0.014	0.014
PCB-68	0.00013		0.0366	0.0018	0.074	0.074	0.074	0.074	0.074	0.074
PCB-77	0.00001		0.0016	0.0013	0.005	0.005	0.005	0.005	0.005	0.005
PCB-82	0.00002		0.0060	0.0013	0.017	0.017	0.017	0.017	0.017	0.017
PCB-83 + 99	0.00010		0.0273	0.0063	0.016	0.016	0.016	0.016	0.016	0.016

Analyte	Pred field conc	Mean field RL	Mean Effluent conc	Mean effluent RL	Est/RL WP N. shallow	Est/RL WP N. deep	Est/RL WP south	Est/RL Elliott	Est/RL Alki	Est/RL Ref site
PCB-84	0.00005		0.0129	0.0045	0.010	0.010	0.010	0.010	0.010	0.010
PCB-85 + 116 + 117	0.00003		0.0087	0.0041	0.008	0.008	0.008	0.008	0.008	0.008
PCB-86 + 87 + 97 + 109 + 119 + 125	0.00014		0.0383	0.0104	0.013	0.013	0.013	0.013	0.013	0.013
PCB-88 + 91	0.00003		0.0077	0.0005	0.055	0.055	0.055	0.055	0.055	0.055
PCB-90 + 101 + 113	0.00019		0.0513	0.0099	0.019	0.019	0.019	0.019	0.019	0.019
PCB-92	0.00003		0.0090	0.0015	0.022	0.022	0.022	0.022	0.022	0.022
PCB-93 + 95 + 98 + 100 + 102	0.00016		0.0429	0.0143	0.011	0.011	0.011	0.011	0.011	0.011
PCB-103	0.00000		0.0006	0.0005	0.004	0.004	0.004	0.004	0.004	0.004
PCB-105	0.00008		0.0218	0.0051	0.015	0.015	0.015	0.015	0.015	0.015
PCB-107	0.00001		0.0034	0.0005	0.023	0.023	0.023	0.023	0.023	0.023
PCB-108 + 124	0.00001		0.0023	0.0006	0.015	0.015	0.015	0.015	0.015	0.015
PCB-110 + 115	0.00021		0.0583	0.0108	0.020	0.020	0.020	0.020	0.020	0.020
PCB-114	0.00001		0.0016	0.0006	0.010	0.010	0.010	0.010	0.010	0.010
PCB-118	0.00020		0.0545	0.0118	0.017	0.017	0.017	0.017	0.017	0.017
PCB-128 + 166	0.00003		0.0087	0.0017	0.019	0.019	0.019	0.019	0.019	0.019
PCB-129 + 138 + 160 + 163	0.00020		0.0565	0.0069	0.030	0.030	0.030	0.030	0.030	0.030
PCB-132	0.00007		0.0199	0.0022	0.033	0.033	0.033	0.033	0.033	0.033
PCB-133	0.00000		0.0014	0.0007	0.007	0.007	0.007	0.007	0.007	0.007
PCB-134 + 143	0.00001		0.0021	0.0007	0.011	0.011	0.011	0.011	0.011	0.011
PCB-135 + 151 + 154	0.00006		0.0170	0.0005	0.122	0.122	0.122	0.122	0.122	0.122
PCB-136	0.00002		0.0058	0.0005	0.041	0.041	0.041	0.041	0.041	0.041
PCB-137	0.00001		0.0032	0.0007	0.016	0.016	0.016	0.016	0.016	0.016
PCB-141	0.00003		0.0083	0.0012	0.025	0.025	0.025	0.025	0.025	0.025
PCB-144	0.00001		0.0034	0.0005	0.024	0.024	0.024	0.024	0.024	0.024
PCB-147 + 149	0.00013		0.0357	0.0046	0.028	0.028	0.028	0.028	0.028	0.028
PCB-153 + 168	0.00016		0.0431	0.011	0.014	0.014	0.014	0.014	0.014	0.014
PCB-155	0.00001		0.0040	0.0005	0.029	0.029	0.029	0.029	0.029	0.029
PCB-156 + 157	0.00003		0.0080	0.0016	0.018	0.018	0.018	0.018	0.018	0.018
PCB-158	0.00002		0.0056	0.0007	0.029	0.029	0.029	0.029	0.029	0.029
PCB-164	0.00001		0.0037	0.0008	0.017	0.017	0.017	0.017	0.017	0.017

Analyte	Pred field conc	Mean field RL	Mean Effluent conc	Mean effluent RL	Est/RL WP N. shallow	Est/RL WP N. deep	Est/RL WP south	Est/RL Elliott	Est/RL Alki	Est/RL Ref site
PCB-167	0.00001		0.0023	0.0007	0.012	0.012	0.012	0.012	0.012	0.012
PCB-171 + 173	0.00001		0.0031	0.0005	0.022	0.022	0.022	0.022	0.022	0.022
PCB-174	0.00003		0.0093	0.0005	0.067	0.067	0.067	0.067	0.067	0.067
PCB-176	0.00001		0.0016	0.0005	0.011	0.011	0.011	0.011	0.011	0.011
PCB-179	0.00001		0.0034	0.0005	0.024	0.024	0.024	0.024	0.024	0.024
PCB-180 + 193	0.00008		0.0216	0.0005	0.154	0.154	0.154	0.154	0.154	0.154
PCB-184	0.00001		0.0029	0.0005	0.020	0.020	0.020	0.020	0.020	0.020
PCB-194	0.00001		0.0039	0.0005	0.028	0.028	0.028	0.028	0.028	0.028
PCB-195	0.00001		0.0015	0.0005	0.010	0.010	0.010	0.010	0.010	0.010
PCB-198 + 199	0.00002		0.0051	0.0012	0.016	0.016	0.016	0.016	0.016	0.016
PCB-202	0.00001		0.0024	0.0005	0.017	0.017	0.017	0.017	0.017	0.017
PCB-203	0.00001		0.0041	0.0008	0.019	0.019	0.019	0.019	0.019	0.019
PCB-206	0.00001		0.0030	0.0018	0.006	0.006	0.006	0.006	0.006	0.006
PCB-209	0.00001		0.0029	0.0019	0.006	0.006	0.006	0.006	0.006	0.006
Sum PCBs	0.005									
PPCPs list 1										
Azithromycin	0.72	1.50	198	2.92	0.489	0.482	0.467	0.479	0.482	0.479
Caffeine	0.39	14.9	109	14.7	0.027	0.027	0.027	0.026	0.027	0.026
Carbadox	0.012	1.49	3.4	2.57	0.008	0.008	0.008	0.008	0.008	0.008
Carbamazepine	0.66	1.49	182	1.47	0.449	0.443	0.443	0.440	0.443	0.440
Ciprofloxacin	0.17	5.96	47.5	8.77	0.029	0.029	0.029	0.029	0.029	0.029
Clarithromycin	0.43	1.49	118.3	1.47	0.291	0.287	0.287	0.285	0.287	0.285
Dehydronifedipine	0.012	0.60	3.2	0.68	0.020	0.020	0.020	0.020	0.020	0.020
Diphenhydramine	2.9	0.60	809	2.95	4.989	4.914	4.897	4.889	4.922	4.889
Diltiazem	0.51	0.30	141.0	0.49	1.736	1.713	1.707	1.701	1.719	1.701
Enrofloxacin	0.014	2.98	3.8	2.95	0.005	0.005	0.005	0.005	0.005	0.005
Erythromycin-H2O	0.071	2.29	19.6	2.26	0.032	0.031	0.031	0.031	0.031	0.031
Flumequine	0.019	1.50	5.2	2.83	0.012	0.013	0.013	0.012	0.013	0.012
Fluoxetine	0.11	4.96	30.2	4.91	0.022	0.022	0.022	0.022	0.022	0.022
Lincomycin	0.02	2.98	5.5	2.95	0.007	0.007	0.007	0.007	0.007	0.007

Analyte	Pred field conc	Mean field RL	Mean Effluent conc	Mean effluent RL	Est/RL WP N. shallow	Est/RL WP N. deep	Est/RL WP south	Est/RL Elliott	Est/RL Alki	Est/RL Ref site
Miconazole	0.01	1.49	2.7	1.47	0.007	0.006	0.006	0.006	0.006	0.006
Ofloxacin	0.24	1.49	66.8	2.59	0.165	0.162	0.162	0.161	0.162	0.161
Roxithromycin	0.005	0.30	1.4	0.48	0.017	0.017	0.017	0.017	0.017	0.017
Sulfadiazine	0.036	1.49	10.0	1.69	0.025	0.024	0.024	0.024	0.024	0.024
Sulfamethoxazole	1.02	0.60	281.5	2.03	1.736	1.710	1.704	1.701	1.713	1.701
Sulfanilamide	0.19	14.9	53.2	14.7	0.013	0.013	0.013	0.013	0.013	0.013
Thiabendazole	0.11	1.49	31.4	1.47	0.077	0.076	0.076	0.076	0.076	0.076
Trimethoprim	0.94	1.49	260.5	2.02	0.642	0.633	0.633	0.629	0.633	0.629
Tylosin	0.06	5.96	15.7	5.89	0.010	0.010	0.010	0.009	0.010	0.009
1,7-Dimethylxanthine	1.06	59.6	294.5	58.9	0.018	0.018	0.018	0.018	0.018	0.018
PPCPs list 2 tetracyclines										
Doxycycline	0.07	5.96	19.7	5.89	0.012	0.012	0.012	0.012	0.012	0.012
PPCPs list 3										
Bisphenol A	1.6	5.96	437.5	15.77	0.270	0.266	0.265	0.264	0.266	0.264
Furosemide	0.54	3.97	148.5	7.90	0.137	0.135	0.135	0.135	0.135	0.135
Gemfibrozil	1.7	0.79	481.5	1.58	2.226	2.192	2.187	2.182	2.198	2.182
Glipizide	0.04	0.79	12.0	1.58	0.055	0.054	0.054	0.054	0.055	0.054
Glyburide	0.008	0.79	2.2	1.58	0.010	0.010	0.010	0.010	0.010	0.010
Hydrochlorothiazide	3.8	8.74	1056	17.3	0.444	0.437	0.436	0.435	0.438	0.435
2-Hydroxy-ibuprofen	1.3	3.97	363.5	7.90	0.336	0.331	0.330	0.330	0.331	0.330
Ibuprofen	0.061	3.97	16.8	7.90	0.015	0.015	0.015	0.015	0.015	0.015
Naproxen	0.59	1.99	162.5	4.10	0.300	0.296	0.296	0.294	0.297	0.294
Triclocarban	0.009	0.40	2.5	0.79	0.023	0.023	0.023	0.023	0.023	0.023
Triclosan	0.072	5.96	19.8	11.82	0.012	0.012	0.012	0.012	0.012	0.012
PPCPs list 4										
Albuterol	0.039	0.29	10.8	0.28	0.132	0.132	0.135	0.133	0.131	0.133
Amphetamine	0.016	0.29	4.5	0.58	0.055	0.055	0.057	0.056	0.055	0.056
Atenolol	0.99	0.29	273.4	0.28	3.355	3.355	3.436	3.378	3.321	3.389
Atorvastatin	0.31	1.17	86.8	1.12	0.266	0.266	0.273	0.269	0.264	0.269
Codeine	0.37	1.17	101.6	1.42	0.312	0.312	0.320	0.314	0.309	0.314

Analyte	Pred field conc	Mean field RL	Mean Effluent conc	Mean effluent RL	Est/RL WP N. shallow	Est/RL WP N. deep	Est/RL WP south	Est/RL Elliott	Est/RL Alki	Est/RL Ref site
Cotinine	0.16	0.29	43.6	0.28	0.535	0.535	0.548	0.539	0.530	0.541
Enalapril	0.006	0.90	1.6	0.9	0.007	0.007	0.007	0.007	0.007	0.007
Hydrocodone	0.073	1.17	20.2	1.12	0.062	0.062	0.064	0.062	0.061	0.062
Metformin	59.6	0.29	16470	1.61	202	202	207	203	200	204
Oxycodone	0.16	0.59	45.3	0.62	0.278	0.278	0.285	0.280	0.275	0.281
Ranitidine	0.011	0.59	2.9	0.56	0.018	0.018	0.018	0.018	0.018	0.018
Triamterene	0.29	0.29	79.5	0.28	0.976	0.976	0.999	0.982	0.966	0.986
PPCPs list 5										
Alprazolam	0.007	0.30	1.8	0.29	0.022	0.022	0.022	0.022	0.022	0.022
Amitriptyline	0.076	0.30	21.1	0.29	0.260	0.256	0.255	0.255	0.257	0.255
Amlodipine	0.048	1.00	13.3	0.99	0.049	0.048	0.048	0.048	0.048	0.048
Benzoylecgonine	0.23	0.15	62.6	0.15	1.540	1.520	1.520	1.510	1.520	1.510
Cocaine	0.006	0.15	1.8	0.15	0.043	0.043	0.043	0.043	0.043	0.043
DEET	1.7	0.30	469	0.29	5.781	5.703	5.684	5.665	5.723	5.665
Desmethyldiltiazem	0.14	0.15	37.3	0.15	0.919	0.906	0.906	0.900	0.906	0.900
Diazepam	0.003	0.50	0.8	0.49	0.006	0.006	0.006	0.006	0.006	0.006
Fluticasone propionate	0.009	2.00	2.6	1.98	0.005	0.005	0.005	0.005	0.005	0.005
10-hydroxy-amitriptyline	0.043	0.15	12.0	0.50	0.296	0.292	0.292	0.290	0.292	0.290
Meprobamate	0.16	1.49	45.5	1.47	0.112	0.110	0.110	0.110	0.110	0.110
Metoprolol	1.9	0.50	535	0.49	3.948	3.885	3.877	3.869	3.893	3.869
Norfluoxetine	0.012	0.50	3.4	0.49	0.025	0.025	0.025	0.025	0.025	0.025
Norverapamil	0.014	0.15	4.0	0.15	0.098	0.097	0.097	0.096	0.097	0.096
Paroxetine	0.014	1.00	3.9	0.99	0.014	0.014	0.014	0.014	0.014	0.014
Promethazine	0.001	0.30	0.4	0.29	0.004	0.004	0.004	0.004	0.004	0.004
Propranolol	0.28	0.30	76.7	0.41	0.944	0.932	0.929	0.926	0.935	0.926
Sertraline	0.26	0.30	72.1	0.29	0.888	0.876	0.873	0.870	0.879	0.870
Theophylline	1.2	5.96	336	5.89	0.208	0.204	0.204	0.203	0.205	0.203
Valsartan	2.8	3.97	779	3.93	0.719	0.709	0.707	0.707	0.710	0.707
Verapamil	0.043	0.15	12.0	0.15	0.296	0.292	0.292	0.290	0.292	0.290
PPCPs list 6										

Analyte	Pred field conc	Mean field RL	Mean Effluent conc	Mean effluent RL	Est/RL WP N. shallow	Est/RL WP N. deep	Est/RL WP south	Est/RL Elliott	Est/RL Alki	Est/RL Ref site
Diatrizoic acid	43.4	11.9	11985	79.5	3.708	3.646	3.615	3.615	3.646	3.615
Iopamidol	66.6	79.4	18385	291.5	0.850	0.837	0.835	0.833	0.839	0.833
Citalopram	0.84	0.40	233	1.97	2.152	2.119	2.114	2.114	2.125	2.114
Cyclophosphamide	0.011	0.40	3.1	0.39	0.028	0.028	0.028	0.028	0.028	0.028
Venlafaxine	1.4	0.40	399	1.97	3.685	3.629	3.620	3.620	3.638	3.620
Clotrimazole	0.004	0.40	1.0	0.39	0.009	0.009	0.009	0.009	0.009	0.009
Colchicine	0.012	0.79	3.3	0.79	0.015	0.015	0.015	0.015	0.015	0.015
Metronidazole	0.31	1.99	86.5	1.97	0.160	0.157	0.157	0.156	0.158	0.156
Moxifloxacin	0.020	3.97	5.6	3.93	0.005	0.005	0.005	0.005	0.005	0.005
Oxazepam	0.023	3.97	6.2	3.93	0.006	0.006	0.006	0.006	0.006	0.006
Rosuvastatin	1.18	3.97	324.5	3.93	0.300	0.295	0.294	0.294	0.296	0.294
Zidovudine	0.31	5.96	86.1	5.89	0.053	0.052	0.052	0.052	0.052	0.052
Sum PPCPs (list 1-6)	204									
Bisphenols										
Bisphenol E	0.14	4.88	39.6	5.34	0.030	0.030	0.029	0.029	0.029	0.029
Bisphenol F	0.14	4.88	39.5	6.18	0.030	0.029	0.029	0.029	0.029	0.029
Bisphenol A	2.5	1.95	709	5.51	1.33	1.32	1.30	1.31	1.32	1.30
Bisphenol S	2.1	12.5	589	12.5	1.76	1.76	1.73	1.73	1.75	1.73
Sum Bisphenols	5.0									
Dioxins and Furans										
OCDD	0.00002		0.0056	0.0005	0.040	0.040	0.040	0.040	0.040	0.040
1,2,3,4,6,7,8-HPCDF	0.000002		0.0005	0.0005	0.004	0.004	0.004	0.004	0.004	0.004
OCDF	0.000002		0.0007	0.0005	0.005	0.005	0.005	0.005	0.005	0.005
TOTAL HEXA-FURANS	0.000003		0.0007	0.0005	0.005	0.005	0.005	0.005	0.005	0.005
TOTAL HEPTA-FURANS	0.000002		0.0005	0.0005	0.004	0.004	0.004	0.004	0.004	0.004
Sum Dioxins and Furans	0.00003									
Polybrominated diphenylethers										
BDE-15	0.00002		0.005	0.0018	0.010	0.010	0.010	0.010	0.010	0.010
BDE-17 + 25	0.00016		0.041	0.0037	0.051	0.051	0.051	0.051	0.051	0.051
BDE-28 + 33	0.00021		0.057	0.0026	0.077	0.077	0.077	0.077	0.077	0.077

Analyte	Pred field conc	Mean field RL	Mean Effluent conc	Mean effluent RL	Est/RL WP N. shallow	Est/RL WP N. deep	Est/RL WP south	Est/RL Elliott	Est/RL Alki	Est/RL Ref site
BDE-47	0.010		2.770	0.014	0.716	0.716	0.716	0.716	0.716	0.716
BDE-49	0.00026		0.071	0.0024	0.107	0.107	0.107	0.107	0.107	0.107
BDE-66	0.00023		0.063	0.0031	0.074	0.074	0.074	0.074	0.074	0.074
BDE-71	0.00002		0.0057	0.0023	0.009	0.009	0.009	0.009	0.009	0.009
BDE-79	0.00003		0.0091	0.0020	0.016	0.016	0.016	0.016	0.016	0.016
BDE-85	0.00051		0.140	0.0058	0.086	0.086	0.086	0.086	0.086	0.086
BDE-99	0.0094		2.610	0.01	0.945	0.945	0.945	0.945	0.945	0.945
BDE-100	0.0019		0.537	0.0030	0.636	0.636	0.636	0.636	0.636	0.636
BDE-138 + 166	0.00014		0.039	0.0051	0.025	0.025	0.025	0.025	0.025	0.025
BDE-140	0.00003		0.009	0.0043	0.008	0.008	0.008	0.008	0.008	0.008
BDE-153	0.00085		0.236	0.0056	0.152	0.152	0.152	0.152	0.152	0.152
BDE-154	0.00065		0.179	0.0026	0.249	0.249	0.249	0.249	0.249	0.249
BDE-155	0.00006		0.016	0.0030	0.019	0.019	0.019	0.019	0.019	0.019
BDE-183	0.00015		0.040	0.0037	0.039	0.039	0.039	0.039	0.039	0.039
BDE-203	0.00008		0.022	0.0078	0.010	0.010	0.010	0.010	0.010	0.010
BDE-209	0.00445		1.230	0.101	0.044	0.044	0.044	0.044	0.044	0.044
Sum BDEs	0.03									
Hormones										
17 beta-Estradiol	0.091	3.82	25.2	7.24	0.024	0.024	0.024	0.024	0.023	0.023
Estrone	0.394	3.06	109.0	2.96	0.128	0.132	0.129	0.131	0.127	0.126
Androstenedione	0.056	0.96	15.6	0.93	0.059	0.060	0.059	0.060	0.058	0.057
Progesterone	0.015	0.38	4.08	0.71	0.038	0.040	0.039	0.039	0.038	0.038
Testosterone	0.017	0.38	4.78	0.84	0.045	0.046	0.045	0.046	0.044	0.044
Sum Hormones	0.52									

Column 1 shows the predicted (pred) field concentration (conc) for all estuarine sites. Value based on the ratio of observed field concentrations to the mean value for low-flow effluent observed for West Point and South Plant (Mean Effluent concentration). Estuarine (Est)/RL is the predicted estuary concentration over the reporting limit for each specific sample. All concentrations as ng/L.

Table 26. Ratio of predicted field concentration to reporting limit for analytes with one or more values >1.

Analyte	Ratio conc/RL WP Nor shallow	Ratio conc/RL WP Nor deep	Ratio conc/RL WP south	Ratio conc/RL Elliott B.	Ratio conc/RL Alki	Ratio conc/RL Ref site
4-Nonylphenol monoethoxylates	1.3	0.9	0.5	0.9	0.7	0.8
4-Nonylphenols	1.0	1.2	0.6	0.4	0.8	0.5
Atenolol	3.4	3.4	3.4	3.4	3.3	3.4
Benzoylecgonine	1.5	1.5	1.5	1.5	1.5	1.5
Bisphenol A	1.3	1.3	1.3	1.3	1.3	1.3
Citalopram	2.2	2.1	2.1	2.1	2.1	2.1
DEET	5.8	5.7	5.7	5.7	5.7	5.7
Diatrizoic acid	3.7	3.6	3.6	3.6	3.6	3.6
Diltiazem	1.7	1.7	1.7	1.7	1.7	1.7
Diphenhydramine	5.0	4.9	4.9	4.9	4.9	4.9
Gemfibrozil	2.2	2.2	2.2	2.2	2.2	2.2
Metformin	202	202	207	203	200	204
Metoprolol	3.9	3.9	3.9	3.9	3.9	3.9
Sulfamethoxazole	1.7	1.7	1.7	1.7	1.7	1.7
Venlafaxine	3.7	3.6	3.6	3.6	3.6	3.6

Values are the ratio of predicted estuary concentrations to the sample-specific reporting limit (RL) for that analyte. All were detected in estuarine samples, except 4-nonylphenol, 4-nonylphenol monoethoxylates, diatrizoic acid, diltiazem, gemfibrozil, and metoprolol. Cotinine was detected in estuary samples; however, the ratio was <1. Analytes in bold detected at estuarine sites.

Table 27. Predicted whole-body tissue concentration for fish in the field.

Analyte	Mean pred. est. conc. (ng/L)	Mean effluent conc. (ng/L)	K_{ow} or D_{ow}	BCF	Pred WB conc. field (ng/g)
BDE-209	0.0045	1.2	11.3	8.2E+08	3,650
BEHTBP	0.0054	1.5	9.5	2.4E+07	129
4-Nonylphenol monoethoxylates	6.8	1,865	5.8	16,982	115
4-Nonylphenol diethoxylates	2.4	671	5.6	11,482	27.9
4-Nonylphenols	2.1	570	5.4	7,762	16.0
Sum PAH					12.9
EHTBB	0.012	3.2	7.7	7.0E+05	8.2
C4-Benzo[a]anthracenes /Chrysenes	0.007	2.1	7.6	575,440	4.3
BDE-99	0.009	2.6	7.32	3.3E+05	3.1
Permethrin	0.035	9.5	6.5	66,834	2.3
PFDA	0.003	0.95	7.65	634,600	2.2
C4-Phenanthrenes/Anthracenes	0.030	8.3	6.5	66,834	2.0
Bisphenol A	1.6	438	4.32	938	1.5
BDE-47	0.010	2.8	6.81	122,603	1.2
BDE-153	0.001	0.24	7.9	1.0E+06	0.88
C3-Phenanthrenes/Anthracenes	0.027	7.5	6.0	25,119	0.68
C2-Benzofluoranthenes/ Benzopyrenes	0.003	0.94	7.0	177,828	0.60
BDE-154	0.001	0.18	7.82	8.9E+05	0.57
BDE-100	0.002	0.54	7.24	2.8E+05	0.55
C4-Naphthalenes	0.050	13.9	5.55	10,411	0.52
PFNA	0.004	1.0	6.82	125,026	0.45
C3-Fluorenes	0.045	12.5	5.5	9,441	0.43
Estrone	0.394	109	4.31	919	0.36
Benzo[ghi]perylene	0.002	0.55	7.0	1.8E+05	0.36
C2-Fluorenes	0.068	18.7	5.2	5248	0.35
TOTAL PCBs					0.32
BDE-183	0.0001	0.040	8.27	2.1E+06	0.31
Retene	0.005	1.3	6.5	66,834	0.31
BDE-203	0.00008	0.02	8.45	3.0E+06	0.24

Analyte	Mean pred. est. conc. (ng/L)	Mean effluent conc. (ng/L)	K_{ow} or D_{ow}	BCF	Pred WB conc. field (ng/g)
C2-Benzo[a]anthracenes/ Chrysenes	0.004	0.99	6.5	66,834	0.24
C3-Dibenzothiophenes	0.014	3.87	5.73	14808	0.21
C1-Benzo[a]anthracenes /Chrysenes	0.004	1.2	6.3	45,186	0.20
C3-Naphthalenes	0.069	19	4.9	2,917	0.20
C4-Dibenzothiophenes	0.005	1.5	6.2	37,154	0.20
C1-Fluoranthenes/Pyrenes	0.013	3.5	5.72	14,521	0.18
C2-Dibenzothiophenes	0.019	5.2	5.5	9,441	0.18
Cypermethrin	0.006	1.6	6.0	25,119	0.14
C1 Phenanthrenes/Anthracenes	0.028	7.8	5.14	4,667	0.13
5,9-Dimethylchrysene	0.001	0.30	6.8	120,226	0.13
C2-Fluoranthenes/Pyrenes	0.009	2.5	5.72	14,521	0.13
Diphenhydramine	2.9	809	2.73	42	0.12
Miconazole	0.010	2.7	5.52	9,817	0.09
Fluoranthene	0.019	5.4	5.16	4,853	0.09
Iopamidol	67	18,385	-1.01	1.4	0.09
Pyrene	0.021	5.9	5.1	4,315	0.09
C1-Fluorenes	0.027	7.6	4.97	3,346	0.09
Metformin	60	16,470	-3.51	1.4	0.08
3-Methylphenanthrene	0.015	4.1	5.2	5,248	0.08
Chrysene	0.005	1.4	5.71	1.4E+04	0.07
Carbamazepine	0.661	183	3.22	109	0.07
Benzo[j,k]fluoranthenes	0.001	0.30	6.5	6.7E+04	0.07
C2-Naphthalenes	0.065	18	4.37	1,034	0.07
Benzo[b]fluoranthene	0.001	0.33	6.4	5.5E+04	0.06
Diatrizoic acid	43	11,985	-0.49	1.4	0.06
Triclosan	0.07	19.8	4.26	834	0.06
C2-Biphenyls	0.01	3.7	5.1	4315	0.06
Bisphenol E	0.14	39.6	3.88	396	0.06
Benzo[e]pyrene	0.00	0.50	6.1	3.1E+04	0.06
Valsartan	2.8	779	2.34	19	0.05

Analyte	Mean pred. est. conc. (ng/L)	Mean effluent conc. (ng/L)	K_{ow} or D_{ow}	BCF	Pred WB conc. field (ng/g)
Benzo[a]pyrene	0.001	0.32	6.3	4.5E+04	0.05

Whole body (WB) concentrations based on predicted BCF and predicted water concentrations for estuarine field sites (est). Only values 0.05 ng/g or greater are shown. BCF predicted with equation 1 using the pH specific K_{ow} (D_{ow}). Mean predicted estuary and mean effluent concentrations in ng/L. See Table 25 for predicted estuary concentration.

Table 28. Predicted plasma concentrations and relationship to C_{max} values.

Analytes	Mean field sites (ng/L)	SD sites	D_{ow} pH 8.0	Pred PBw	Pred BCF	Pred plasma (ng/L)	1% Cmax (ng/L)	Plasma/ 1% Cmax
	Plasma based on observed estuary concentrations							
Atenolol	0.39	0.09	-1.2	1.7	1.4	0.66	1,000	0.0007
Benzoylecgonine	0.36	0.05	0.41	1.7	1.4	0.61	2,500	0.0002
Bisphenol A	2.05		4.0	143	542	294	1,950	0.15
Citalopram	0.45		1.98	5.3	9.6	2.4	100	0.024
Cotinine	0.72	0.07	-0.3	1.7	1.4	1.2	400	0.003
DEET	4.9	0.49	2.5	11.5	27	57	30,000	0.002
Diphenhydramine	0.63	-	2.73	16.6	42	10	800	0.013
Metformin	42.3	10.3	-3.5	1.7	1.4	72	1,000	0.072
Sulfamethoxazole	0.79	0.18	0.32	1.7	1.4	1.3	300,000	0.000004
Venlafaxine	0.51	0.09	1.78	4.0	6.5	2.1	2,000	0.001
	Plasma based on predicted estuary concentrations							
17 beta-Estradiol	0.091		3.75	88	307	8.1	0.00063	12,798
Estrone	0.394		3.93	119	437	47	2	23.5
Triclosan	0.072		4.26	207	834	15	42	0.353
Sertraline	0.261		3.44	53	167	14	500	0.028
Colchicine	0.012		1.46	2.7	3.5	0.03	1.3	0.025
Progesterone	0.015		4.15	172	672	2.5	120	0.021
Rosuvastatin	1.175		-1.48	1.7	1.4	2.0	100	0.020
Testosterone	0.017		3.54	62	204	1.1	80	0.013
Atorvastatin	0.314		1.86	4.5	7.6	1.4	120	0.012
Fluoxetine	0.109		2.43	10.3	23.2	1.1	100	0.011

Observed estuarine concentrations shown in top half of table and predicted estuarine concentrations in lower half. Also shown are the predicted BCFs and blood:water partition coefficients (Pbw). Plasma concentrations were compared to the 1% C_{max} value and a Response Ratio (RR) was calculated (equation 5) for evaluating potential effects to fish (last column). Entries in the lower half of the table show those analytes with predicted plasma concentrations and RR > 0.01.

Table 29. Comparison of lab and field analytes in water (ng/L).

Analytes detected in field samples	Mean field conc	RL for field	Pred lab T0.4%	Pred lab/ RL	Field/ lab	Analytes not detected in field	Pred field conc	RL for field	Pred lab T0.4%	Pred lab/RL	Pred field/ lab
Atenolol	0.39	0.29	0.25	0.81	1.6	Metoprolol	1.9	0.5	2.3	4.5	0.84
Benzoylecgonine	0.36	0.15	0.16	1.1	2.2	Gemfibrozil	1.7	0.79	2.5	3.1	0.67
Bisphenol A	2.1	2.0	11.1	5.8	0.18	4-Nonylphenol monoethoxylates	6.8	8.7	11.5	2.2	0.59
Citalopram	0.45	0.40	1.14	2.8	0.39	Diltiazem	0.5	0.3	0.44	1.5	1.1
Cotinine	0.72	0.29	0.26	0.85	2.8	Estrone	0.39	3.1	1.15	1.4	0.34
DEET	4.9	0.30	5.4	17.7	0.92	Triamterene	0.29	0.29	0.37	1.2	0.78
Diphenhydramine	0.63	0.60	2.3	3.8	0.28	PFOSA	ND		0.46	1.1	ND
Metformin	42.3	0.29	10.7	35.3	3.9	Iopamidol	66.6	79.4	89.6	1.1	0.74
Sulfamethoxazole	0.79	0.60	1.6	2.6	0.50	Sertraline	0.26	0.30	0.31	1.0	0.83
Venlafaxine	0.51	0.40	1.6	4.0	0.32	Valsartan	2.8	4.0	4.03	1.0	0.69

Pred lab shows the predicted (pred) water concentrations for the 0.4% treatment based on observed values for the 1.4% treatment (x 0.28). Ratios of the predicted concentrations to the reporting limit (RL) in column 4. Ratio of the observed field values in water to the predicted lab values for the 0.4% treatment shown in column 5. The geometric mean for the ratio of field to lab values in column 6 is 0.8 (SD=1.3). Column 6 shows the 10 analytes that were predicted to occur in the 0.4% lab treatment with predicted concentrations/RL >1.0 (Pred lab water/RL), but were not detected in the field samples. Last column shows the ratio of the predicted field concentrations to the predicted lab concentrations (geometric mean = 0.70, SD=0.21). RL values for these analytes essentially identical for the lab and field samples. ND is not detected in effluent.

4 DISCUSSION

4.1 Chemical loadings associated with WWTP effluent

4.1.1 PCBs

PCBs are present in Puget Sound biota at concentrations that are known to be harmful to their health (Meador et al. 2002; West et al. 2017) and has led to fish consumption advisories for several fish species in the Puget Sound (Washington State Department of Health, 2006). Additionally, levels of PCBs have remained persistently elevated, particularly in pelagic fish from the urbanized central basin of Puget Sound (West et al. 2017). Despite a 1979 ban on PCB production that also limited use, there are still many legacy PCB-containing products in circulation and certain new products contain some PCBs due to inadvertent production (Washington State Department of Ecology 2016). PCBs enter Puget Sound through multiple pathways including release from sediments, WWTP effluent, stormwater runoff, and atmospheric deposition.

There has previously only been limited high resolution data for PCBs entering Puget Sound via WWTP effluent. Washington State Department of Ecology (2010) reported effluent concentration of PCBs for several WWTPs in the region, including the West Point facility. While data are limited, the results from the present study are relatively consistent with what was reported earlier. The current data also demonstrate that, while there are likely some differences in PCB concentrations between high flow and low flow conditions, these differences are not consistent between facilities. This could be reflective of different inputs to the system based on land-use in the service area, differences between inputs under high flow and low flow conditions, or differences in treatment plant performance for the different conditions. Our findings suggest that PCBs are present in both municipal wastewater (as observed in samples collected under low flow conditions) and stormwater runoff (as observed in samples collected under high flow conditions).

The loading estimates (Figure 4) suggest that effluent from wastewater treatment facilities are a pathway for PCBs entering Puget Sound. While concentrations in wastewater effluent are generally lower than reported for stormwater runoff, particularly from industrial basins, the continual flow from wastewater facilities results in annual loadings from individual WWTPs that are comparable to loadings via stormwater runoff estimated from a single, large industrialized basin.

4.1.2 PBDEs

Although PDBE levels have been declining in Puget Sound biota, there remain several specific areas where concentrations are above predicted health effects thresholds, suggesting the ongoing potential to harm fish (O'Neill et al. 2019; West et al. 2017). Our results indicate that wastewater treatment system effluent remains a potential pathway for PBDEs to enter Puget Sound. Comparisons between high flow and low flow samples reflect inconsistent differences. This could reflect differences in the PBDE concentration in the influent to each facility under differing conditions, or could reflect differences in the extent of degradation under different conditions. Effluent from King County wastewater treatment facilities does not appear to be a greater source pathway of PBDEs than from other wastewater facilities (Figure 5). Additionally, PBDE concentrations in wastewater effluent may have declined due to widespread management activities against PBDEs, though data are not sufficient to verify this trend.

4.1.3 Contaminants of emerging concern

Up to 121 unique contaminants were detected in WWTP effluent samples (Table 8). This includes a wide range of pharmaceuticals, hormones, antibiotics, and compounds found in commercial products. This suggests that effluent from wastewater treatment systems is a pathway for a large variety of human-associated compounds to the marine environment.

Samples were collected under both high flow and low flow conditions at the West Point and South Plant facilities. There were 15 compounds that were consistently found at greater concentrations under high flow conditions (concentration in high flow > 1.5x concentration in low flow for both facilities). This could be due to changes in treatment plant performance and/or that the compounds are specifically associated with stormwater runoff entering the treatment facilities. These included illicit drugs (amphetamine, cocaine, and benzoylecgonine, a major metabolite of cocaine), some over-the-counter medications and their metabolites (ibuprofen, hydroxy-ibuprofen and naproxen), and some perfluorinated compounds. There were 14 compounds consistently found at greater concentrations under low flow conditions, suggesting municipal sewage is their primary conveyance to wastewater effluent. These included hormones (17β-estradiol, androstenedione, estrone, and progesterone) and several medications (atorvastatin, carbamazepine, diazepam, and hydrocodone). These results might indicate that selected compounds are associated more with stormwater runoff (i.e., high flow conditions) or municipal sewage (i.e., low flow), although differences in treatment plant performance may also result in systematic differences in concentrations.

4.2 Implications to juvenile Chinook health from laboratory exposures

4.2.1 Endocrine Disruption

Fish exposed to WWE exhibited increased vitellogenin, indicating endocrine disruption along the hypothalamus-pituitary-gonadal axis. Endocrine disruption during development can lead to improper gonad development and, therefore, long-term impacts on reproduction (Chen et al. 2009; Mikula et al. 2009; Orn et al. 2003; Jobling et al. 1996). Reproductive impairment can be detrimental to fish populations already in decline, including Puget Sound Chinook salmon (NWIFC 2020).

Several contaminant classes are known to be estrogenic to fish, including hormones, bisphenols, alkylphenols, and PFAS. Hormones are the most potent of the known endocrine disruptors that fish were exposed to in the laboratory study and the most likely to have caused the observed vitellogenin induction. The synthetic estrogen 17α-ethinylestradiol is known as the most potent estrogenic hormone, followed by 17β-estradiol, the hormone that naturally binds to estrogen receptors, and finally estrone (Thorpe et al. 2003; Bjerregaard et al. 2008; Routledge et al. 1998; Harding et al. 2016). In 20% WWE, estrone and 17β-estradiol were present at approximately half of the concentrations reported to induce vitellogenin in salmon in other studies (estrone: ~60 ng/L in Thorpe et al. 2003; 17β-estradiol: ~15 ng/L in Bjerregaard et al. 2008). The synthetic estrogen, 17α-ethinylestradiol, was below the analytical detection limits of the current study (4.7 ng/L), but could also have been present near its effect concentration (~1.4 ng/L in Thorpe et al. 2003). Collectively, these hormones were sufficient to induce vitellogenin in the laboratory study. Importantly, it is likely that the vitellogenin response in our study underestimated the response of chronic exposure to estrogenic hormones in Puget Sound. In fish exposed for 21 days to 20 ng/L of 17α-ethinylestradiol vitellogenin continued to increase over the exposure, peaking beyond the end of the exposure with a half-life of two to four weeks among the species tested (Craft et al. 2004). Therefore, although vitellogenin was not significantly elevated at the

lower WWE concentrations in our 10-day exposure, we would expect that chronic exposure would approximate the response of juvenile Chinook exposed to higher WWE concentrations in our study.

In comparison to hormones, bisphenols and alkylphenols were present in laboratory exposure waters at concentrations two to four orders of magnitude lower than effects concentrations in the literature (Meucci & Arukwe 2005; Li et al. 2012). Exposures to PFAS tended to be even lower; approximately six orders of magnitude lower than concentrations that induce vitellogenin (Perfluorooctanesulfonic acid (PFOS): effect concentration 3,000,000 ng/L in Oakes et al. 2005). Conversely, another PFAS (PFDA) was far more potent resulting in a lowest observed effect concentration (LOEC) of 12.8 µg/kg bw/day (Benninghoff et al. 2011). Even though comparing dietary and aqueous exposure concentrations is challenging, this value for PFDA is relatively high for a PFAS. Overall, measured compounds in the bisphenol, alkylphenol, and PFC classes were not expected to have contributed significantly to the observed vitellogenin induction. Many other compounds are known to be estrogenic and are likely present in WWE and throughout Puget Sound. For example, dioxins, furans, PCBs, PBDEs, metals, and some phthalates are weakly estrogenic. However, these compounds are known for causing other adverse effects, so regulations of these compounds are not often related to endocrine disruption. Based on the available data, the impact of EDCs in estuaries is likely far more complicated than it appears.

EDCs have diverse chemical structures but typically contain phenyl rings, like 17β-estradiol (Routledge & Sumpter 1997). However, not all compounds containing phenyl rings are estrogenic, and the number of phenyl rings does not indicate potency. For example, all phthalates have at least one phenyl ring, but not all phthalates cause endocrine disruption (Kennedy et al. 2013). Additionally, while PFAS are only weakly estrogenic, they do not contain phenyl rings, showing exceptions to the phenyl rule (Benninghoff et al. 2011). The chemical structures of EDCs may vary, but the most potent ones appear to be phenolic.

4.2.2 Na^+/K^+ ATPase

The enzyme NKA plays an essential role in the brain by establishing and maintaining an electrochemical gradient, facilitating neuronal signaling and rapid transmission of action potentials, and likely modulating dendritic growth in developing neurons (Desfrere et al. 2009). Most studies demonstrating inhibition of brain NKA from exposure to chemicals focus on biocides (Sarma et al. 2010; Li et al. 2016; Das and Mukherjee 2003; Tabassum et al. 2015) and metals (Shaw et al. 2012; Maiti et al. 2010). The reduction in brain NKA activity in our study confirmed the findings of Lajeunesse et al. (2011), who first documented the inhibitory effect of WWE exposure on brain NKA.

Recent studies have shown that psychoactive pharmaceuticals may also cause detrimental effects on brain function in fish (Ajima et al. 2017; Lajeunesse et al. 2011; Xie et al. 2015). Pharmaceuticals are often more potent than other contaminants as they are designed to elicit specific biological effects at relatively low concentrations. Additionally, many pharmaceuticals require repeated consumption, meaning that they are continually introduced into local waterways via WWE. The SSRIs paroxetine and fluoxetine significantly inhibited brain NKA in brook trout (*Salvelinus fontinalis*) synaptosomes in an *in vitro* study (Lajeunesse et al. 2011). During chronic WWE exposures, the SSRIs paroxetine, fluoxetine, sertraline, and metabolites bioconcentrate in brain tissue by several orders of magnitude (Schultz et al. 2010, Lajeunesse et al. 2011), such that effects would be expected at lower water concentrations for *in vivo* exposures than are reported for *in vitro* studies. This has negative implications for wild Chinook, which are chronically exposed to WWE during migration. Further research is needed to confirm the potential effects of chronic exposure to psychoactive pharmaceuticals in WWE.

Although gill NKA is a common endpoint for testing osmoregulatory function in fish, this is the first known study to test the impact of WWE exposure on gill NKA. The lack of significant change in gill NKA between treatments is a positive finding from this study. The combination of contaminants present in diluted South Plant WWE did not inhibit gill NKA relative to controls. In comparison to known brain NKA inhibitors, many pollutants present in WWE have been associated with decreased gill NKA activity such as venlafaxine (Best et al. 2014), carbamazepine (Li et al. 2009), cypermethrin (Begum 2014), para-nonylphenol (Robertson and McCormick 2012), and metals (Atli and Canli 2007). Among these, venlafaxine, a serotonin-norepinephrine reuptake inhibitor, was 12.5 times lower in 20% WWE than the effect concentration in the Best et al. study. Other detected gill NKA inhibitors were 3-5 orders of magnitude lower in 20% WWE than reported effects concentrations (cypermethrin: Begum 2014; carbamazepine: Li et al. 2009). The lack of observed effect may indicate that the contaminant concentrations were below additivity thresholds for gill NKA impairment, or they may have had an antagonistic effect on each other.

4.2.3 Stress

Stress responses associated with toxicant exposures are variable (i.e., increase or decrease in stressor endpoint in response to toxicant exposure), and changes in glucose and cortisol (both measures of stress response) do not always follow consistent trends (Cousineau et al. 2014; Gauthier et al. 2020; Ings et al. 2011). These differences in stress responses highlight the importance of measuring more than one stress indicator. In this study, glucose showed a dose-related decrease while cortisol did not follow a clear trend. The unclear cortisol response could be due to the inconsistent nature of cortisol production, which varies by the time of day, sex, and time after exposure to a stressor (Garcia & Meier 1973; Haddy & Pankhurst 1999). Fish in this study were sampled over 10 hours on two days, and fish sex was unknown. Glucose levels in response to a stressor are also affected by the time of day but decline after energy stores are depleted (Chen et al. 2009; Cousineau et al. 2014; Schreck & Tort 2016), which may be what was observed in the fish in higher wastewater concentrations. Stress responses to high toxicant loads, such as increased metabolism and vitellogenin production, can cause premature glucose metabolism. The increased vitellogenin response at higher effluent concentrations may have increased glucose depletion as estrogen hormones are known to rouse glucose transport (Chen et al. 2009). Long-term increases in glucose metabolism and heart rate in response to stressors, including toxicant exposures, can be detrimental to fish survival (Stephens et al. 1997). The altered stress response can be problematic and a warning sign for transformed physiology.

4.2.4 Metabolomics

All of the wastewater concentrations elicited changes in several important endogenous metabolites in juvenile Chinook. In some cases, several metabolites were altered in all treatments. There is also a clear trend with the highest number of altered metabolites occurring in the highest dose (20%) treatment and far fewer in the lower dose treatments. The 1.4% dilution treatment exhibited high variability among replicates (Figure 16) and changes in metabolite concentrations that exhibited minima and maxima concentrations indicating that this was a transitional dose. This implies that some fish were responding similarly to those in the lower dilution treatments and others were exhibiting responses more characteristic of the high dilution treatment.

Several altered physiological pathways were identified by enrichment analysis and many were altered in each control versus treatment comparison. Several of these pathways are crucial for energy generation

and utilization, lipid dynamics, amino acid utilization, genetic material generation, growth, and reduced oxidative stress, which could result in adverse effects.

Several endogenous metabolites were significantly altered in all or most of the treatments as seen in the fold-change analysis comparing control versus treatment (Table 15). In many cases metabolites important for energy generation in pathways such as the Krebs cycle were altered in several of the treatments. In some cases, a metabolite was altered in one or more of the lower dose treatments, but was not included in the list of low p-value metabolites as determined by the SAM analysis. If variability for that metabolite was high in the less dilute treatments it would not necessarily occur in the SAM list of metabolites.

A number of pharmaceuticals and personal care products (PPCPs) such as sertraline and diphenhydramine were detected in fish tissue from the 0.4% dilution, which may have resulted in some of the altered pathways. There were also likely a large number of PPCPs in the low dose treatments that occurred below the limit of analytical detection as noted in the bioaccumulation section of this report. Some of these are potent drugs that may cause effects at these very low concentrations. Additionally, there are several PPCPs found in WWTP effluent identified in the non-targeted analysis that were not part of the standard analytical analysis determinations, which may have contributed to altered metabolites and metabolic pathways.

The fold change analysis contains some compounds that were not found in the SAM analysis likely because of variability among treatment replicates that returned a higher FDR. Also, some of the metabolites identified by the SAM analysis were not listed in the fold change analysis (Table 15) because they did not exhibit a fold change of 30% or more even though they exhibited low p-values (<0.1). Even slight changes in metabolite abundance can indicate impacts to physiological homeostasis. Physiological systems have evolved to resist perturbation so there is a strong push to maintain homeostasis. It generally takes a severe insult to affect physiological pathways and the tendency is to adapt to insults and return to normal function.

As seen in Table 17, a large number of drug pathways were significantly altered in several of the treatments with the most changes occurring in the high dose (20%) and the fewest in the lowest dose treatment (0.1%). Several of the pathways that are involved in metabolism of pharmaceuticals or action/activation were altered and include those such as antibiotics, antidepressants, antihistamines, analgesics, and statins. Overall, these data provide an indication of the drugs in the effluent exposure water that are accumulated by the fish and able to affect pathways. There is overlap for many of these pathways that should be considered. For example, the alteration of glutathione is responsible for the hits for most of the analgesics listed in the drug pathway Table 17; however, we don't know which of these pain relievers is responsible for the altered pathway. A larger number of hits observed for a given pathway enhances the confidence of identifying the causative agent for that alteration.

4.2.5 Integrated physiological Impacts

Metabolism, endocrine system, stress, and brain function were altered following exposure to WWE. While proper metabolic function is essential at all life stages, it is particularly important in juveniles (Beamish et al. 2004). Juvenile salmonids must grow large enough to reduce their chances of predation and avoid starvation during their first winter in marine waters (Burrows 1969; Duffy & Beauchamp 2011; Tovey 1999). In the current study, a hormetic response with effluent exposure was observed for endpoints associated with metabolism, including total protein, cholesterol, calcium, and albumin. The

hormesis response to a contaminant is described as a stimulatory response at low concentrations followed by a harmful response at higher concentrations (Brain & Cousens 1989). This type of reaction has been widely observed in the toxicology literature in a variety of organisms, from plants and animals to microorganisms (Calabrese & Baldwin 2001; Calabrese 2003). Hormesis has not previously been reported for WWE fish exposure studies, and this lack of data highlights an area for further research.

Traveling through contaminated versus uncontaminated estuaries reduces the probability of survival for hatchery Chinook in Puget Sound by 45% (Meador 2014). Previous studies have shown that exposure to PAHs, PBDEs, and PCBs during outmigration into the Puget Sound induced symptoms mimicking starvation and potentially leads to population-level impacts (Meador et al. 2006; Meador 2014; Meador et al. 2018; O'Neill et al. 2015). Similar to previous studies, the substantial reduction in percent lipid content in the 5.3% and 20% treatments and increase in liver deformities in the 20% treatment potentially indicate starvation-like symptoms in Chinook. It is important to note that the severe reduction in whole-body total lipids occurred in the 5.3% effluent treatment, which is only 15-fold higher than our modeled open-water field concentrations (0.36%) (Figure 12). As seen in our table of altered pathways (Table 16), several are important for normal lipid homeostasis as a function of synthesis, degradation, and utilization. Overall, the findings from this study indicate that toxicants in WWE are potentially contributing to poor health in juvenile Chinook salmon in Puget Sound.

The present study is an example of how exposure to contaminants does not just alter one aspect of physiology but affects multiple pathways. For example, many agonists of endocrine receptors also affect metabolic pathways and behavior, highlighting that metabolism, the endocrine system, stress, and brain function are linked. Part of this linkage was observed in the Sohoni et al. (2001) study in which chronic exposure to BPA impacted growth and reproduction in fathead minnows, particularly in males. Chen et al. (2009) highlighted a similar critical linkage between estrogenic chemicals, metabolism, and stress. This cascade of effects can be explained by the fact that synthesizing vitellogenin requires energy, depleting glucose, and diverting resources from development.

Increases in vitellogenin may initiate a reproductive phase in juvenile fish resulting in excess energy expenditure in gamete production and the upregulation of related physiological pathways. Many energy pathways were affected by effluent exposure in the present study, which may be related to endocrine disruption. These include alteration of the Krebs cycle and pathways associated with lipid metabolism that are crucial for organism energetics. Additional energy related pathways altered in the present study include mitochondrial B oxidation; saturated fatty acid synthesis; carnitine synthesis; pentose phosphate pathway; gluconeogenesis; glycolysis; phospholipid biosynthesis, linoleic and linolenic acid metabolism; and fatty metabolism. In their review, Samuelsson et al. (2006) noted several studies that reported increased triglycerides in fish plasma when exposed to hormones. As we report in the present study, median triglyceride concentrations were elevated in each effluent treatment compared to the control (Figure 10). Additionally, Zhou et al. (2019) reported several pathways affected in carp exposed to 17α-ethinylestradiol including: valine, leucine and isoleucine degradation; aminoacyl-tRNA biosynthesis; alanine, aspartate and glutamate metabolism, and cysteine and methionine metabolism, all of which were altered in the present study. We also reported increases over controls for several precursors of pyrimidine and purine nucleotides (AMP, IMP, GMP, and UMP), which are necessary for biosynthesis. Related to this observation is the alteration of purine, pyrimidine, and related pathways. While EE2 was not detected in whole WWE from South Plant (<0.95 ng/L), the estrogenic effects of multiple hormones

are often additive so the expectation is that EE2 is acting with estrone and 17β-estradiol, as well as other less potent/present estrogenic chemicals, to elicit estrogenic effects.

Brain NKA levels were reduced in every effluent treatment. This crucial enzyme has many functions including neuronal health, regulation of membrane potential, regulation of ion fluxes, and cellular homeostasis (Evans 1987; McCormick 1993). SSRIs are known to inhibit brain NKA. Other chemicals such as propiconazole, have been shown to reduce brain NKA at exposure concentrations of 0.2 µg/L (Li et al. 2010). Propiconizole is not part of the targeted analytical schedule but was detected through the non-targeted evaluation of HRMS effluent data. Tebuconazole (detected via non-target evaluation) and clotrimazole, both triazole fungicides, were in both effluent and lab exposure water. There were likely other related fungicides present in effluent that were not identified in this study and may contribute to reduced NKA levels in brain. Triazole fungicide exposure also resulted in the alteration of antioxidant parameters including reduced glutathione (Li et al. 2010), which is consistent with our observation of altered glutathione metabolism and reduced glutathione.

Stress responses are known to have long term impacts on fish, including alterations in free fatty acids, proteins, glucose, immunosuppression, growth rates, predator avoidance behavior, and NKA activity (Schreck and Tort 2016; Yada and Tort 2016; Sadoul and Vijayan 2016; Noakes and Jones 2016). In many studies of osmoregulation and stress response, gill NKA and cortisol are tightly connected, as cortisol promotes gill NKA production and vice versa (Bonga 1997; Madsen et al. 1995; Liew et al. 2015; McDonald & Milligan 1997; Quinn 2005). Whereas gill NKA and cortisol were not affected by WWE in the current study, a similar relationship between brain NKA and stress responses might underlie the reduced brain NKA observed in this study. Effects of xenobiotics on one pathway are often reflected in others, so it is essential to study multiple pathways when testing the effects of contaminants.

Given that juvenile salmon are vulnerable to contaminant exposure, it is crucial to understand how anthropogenic pollution affects their physiology. Juvenile salmonid size while migrating through estuaries plays a pivotal role in salmonid survival (Burrows 1969; Tovey 1999; Beamish et al. 2004). Any decrease in growth or affected physiology that could decrease predator avoidance (reduced fitness, altered behavior, stress) could be fatal for a juvenile salmonid. Ocean type Chinook, used in this study, are more vulnerable to contaminated estuaries than stream type due to their increased use of this habitat (Quinn 2005). Contamination from multiple sources, including WWTPs, and the loss of critical rearing habitat exacerbate the threats to salmonid survival in Puget Sound (NWIFC 2020; O'Neill et al. 2015). Many previous studies have tried to model or mimic the effects of mixtures on salmon (Yeh et al. 2017; Meador et al. 2017; Meador et al. 2006; Laetz et al. 2013; Spromberg & Meador 2005), but it is difficult to accurately estimate what is occurring in nature. An interesting follow-up study would analyze the chronic effects of low concentrations of WWE exposure on growth to understand whether WWE contributes to reduced estuarine growth and, therefore, survival in exposed juvenile Chinook.

4.2.6 Water exposure and whole-body toxicity metrics

As noted, C_{max} plasma values are not available for many of the chemicals in this study so other lines of evidence regarding potential toxic effects are required. Below we provide a few examples of toxicity based on whole-body or water exposure concentrations that may be relevant for this study.

Nonylphenols should be considered in any risk assessment of WWTP effluent. The U.S. Environmental Protection Agency (2005b) chronic water quality criterion (WQC) for nonylphenol in marine systems is 1.7 µg/L (USEPA 2005b), which was the mean value of the observed effluent concentration in this study.

Based on the toxic equivalency factors (TEFs) for aquatic species, the nonylphenol mono- and diethoxalates are considered to be approximately 50% as potent as nonylphenol (USEPA 2010); therefore, all the nonylphenol compounds are considered additive at their designated potency factor. Additionally, the non-targeted evaluation of water samples from Puget Sound has demonstrated the presence of longer-chained alkylphenol ethoxylates, which can further contribute to the overall equivalent dose. While estuarine concentrations of nonylphenols were predicted to be relatively low (sum approx. 10 ng/L), estuarine areas with less diluted effluent (i.e., effluent plumes) may contain nonylphenol concentrations high enough to be of concern.

Even though we have plasma C_{max} data for bisphenol A, there are several additional bisphenol compounds that should be considered. A total of 16 bisphenol analogues have been identified and many of those are estrogenic (Chen et al. 2016). Only six bisphenols were analyzed by SGS-AXYS and all are considered estrogenic or antiandrogenic. Given the high propensity for bioaccumulation and potential toxicity, this is another class of compounds from wastewater effluent that should be further evaluated.

As noted in a recent review by Ankley et al. (2021) on perfluoroalkyl compounds/substances (PFAS), several of these compounds have regulatory thresholds or screening values in the ng/L to low µg/L range. We detected several PFAS in water in our laboratory study. These compounds were not detected in the field and predicted field concentrations were generally very low; however, the predicted sum of these compounds as a group totaled 0.32 ng/L, which is in the range for some of the listed regulatory threshold values. As noted by Ankley et al. (2021), it is important to study PFAS mixtures and adverse effects from mixtures may not be characterized by a single compound or small number of related compounds.

Two pesticides, permethrin and cypermethrin, were predicted to accumulate in fish at our sampled field sites at low ng/g levels. The Canadian water quality guideline for permethrin in marine water is 1 ng/L. Our predicted estuarine concentration for this pesticide is 0.035 ng/L (28x lower). The permethrin chronic water quality guideline for California is 0.3 ng/L. Our predicted estuarine concentration was 0.006 ng/L (50x lower). Additive toxicity, especially for other related pesticides, would be an important consideration here.

Even for those compounds with plasma effect concentrations, examination of toxicity studies is important for a more complete understanding of potential effects. One example is for metformin, one of the most abundant pharmaceuticals observed in wastewater effluent and in estuarine waters. This diabetes drug and its metabolite, guanylurea, are known to cause adverse effects in fish at environmentally relevant concentrations (Elizalde-Velazquez et al. 2022; Ussery et al. 2019; Niemuth and Klaper 2015). Niemuth and Klaper (2015) determined that metformin can cause severe reductions in fish fecundity at 40 µg/L and Jacob et al. (2018) observed reduced body weight in brown trout exposed to 1 µg/L metformin. Whole-body concentrations of metformin (4 ng/g) at 7°C and levels less than the limit of quantification (undefined) at 11°C in brown trout were demonstrated to reduce body weight (Jacob et al. 2018). These values are lower than our observed value of 5.6 ng/g in the 20% effluent treatment. Another study Barros et al. (2022) found significant physiological effects (COX I activity) and apical effects (increased hepatosomatic index) in zebrafish exposed to 390 ng/L of metformin, which is approximately two-fold higher than the observed concentration in our 5.3% treatment. This value is nine-fold greater than our observed field value for metformin, but lower than some of the values previously detected in Elliott Bay (King County 2017).

Guanylurea was detected in wastewater treatment system effluent samples via the non-target evaluation of HRMS data, but not quantified in this study. It was reported to be present at relatively high levels in WWE elsewhere (Elizalde-Velazques 2022). Elizalde-Velazques (2022) and Ussery et al. (2021) have demonstrated a variety of adverse effects in fish, including neurotoxicity, altered growth, and delayed hatching, which are supported by evidence of altered physiological parameters and biomarkers of effects in those studies. Guanylurea is toxic to fish at a concentration of 1 ng/L, inclusive of expected environmental concentrations, and is far more toxic than the parent compound (Ussery et al. 2021).

Additional ecotoxicological screening was performed to provide another line of evidence of potentially harmful compounds that were detected in the environment. This included the use of Biological Response Ratios determined by comparing measured concentrations of contaminants to levels of biological response as described by Predicted No Effects Concentrations and Activity Concentration at Cutoff. The latter are determined from *in vitro* exposure data. The approach is described in (James et al. 2015; James and Sofield, 2021). Results of the screening identified nine high priority compounds and 47 watch list compounds (Table 10) all of which have some potential to cause biological effects due to their presence in the aquatic environment. These high priority and watch list compounds should be the focus of follow up study in order to better understand their potential for adverse impacts on individuals and populations.

4.2.7 Contaminants of concern for Southern Resident Killer Whales and other wildlife

Based on our observed data for CECs, we do not expect many of these compounds to be of concern for Southern Resident Killer Whales (SRKW) in Puget Sound or other wildlife. Many CECs, especially pharmaceuticals, may cause adverse effects for fish; however, we don't expect these to bioaccumulate to any degree in higher trophic levels because of low partition coefficients. Conversely, some pharmaceuticals are hydrophobic, such as antidepressants, and will bioaccumulate to high levels in fish under continuous exposure; however, many exhibit relatively short half-lives that likely prevent them from bioaccumulating to high levels in wildlife, including SRKW.

There are, however, hydrophobic chemicals that were detected in effluent that may bioaccumulate to levels in fish and ultimately SRKW that should be considered. This list of chemicals includes polychlorinated biphenyls (PCBs), polybrominated biphenyl ethers (PBDEs), polyfluoroalkyl substances (PFAS), bisphenols, and nonylphenols. Many of these hydrophobic compounds are known to biomagnify because of the lack of metabolism or extremely slow elimination kinetics (Arnot and Gobas 2006). Biomagnification is the process whereby contaminants increase with increasing trophic level because of hydrophobicity and slow rates of elimination (Arnot and Gobas 2006). Continued ingestion of low levels of these compounds in prey will result in increasing accumulation in higher trophic level biota, including SRKWs.

Two of the many potential causes relating to the poor survival of the SRKW population include a reduced availability of prey, and exposure to contaminants. As noted by Wasser et al. (2017), low prey availability and potential increases in lipophilic toxicants may be responsible for the high rate of unsuccessful pregnancies for this population of *Orcinus orca*.

4.2.7.1 Polychlorinated biphenyls

As discussed by many authors, PCBs are considered the primary concern for contaminant toxicity in SRKWs. As reported by Mongillo et al. (2016), total PCBs (tPCBs) have been detected in SRKW blubber at high concentrations (up to 150 ppm lipid weight; lw). When considering the observed maximum tPCB

water concentration of 50 pg/L in central Puget Sound (Gries and Osterberg 2011), this represents a nine order of magnitude increase for biomagnification. Unfortunately, we do not know with any certainty the source(s) of these PCBs to SRKWs or concentrations that may be found in liver or other organs, which would be required for a more accurate risk assessment.

Even though the predicted estuarine PCB concentrations based on effluent levels were relatively low, these concentrations contribute to the existing load of compounds present in marine waters. One study found tPCBs in the Puget Sound main basin ranging from 10 to 50 pg/L (geometric mean = 27 pg/L) depending on depth and season (Gries and Osterberg 2011), which represents contributions from all sources. This location (main basin site) was between Bainbridge and Blake Islands (47.5616 N and -122.4759 W), which was relatively close to our reference site. These data were obtained in 2009 and 2010, which may not reflect current levels. Our results indicate that PCB loading from West Point and South Plant facilities is comparable to other large WWTPs in the region, comparable to loading from a single, large, industrialized basin, and likely greater than individual rivers or streams. Based on our evaluation of effluent input from South Plant and West Point, we estimate that these WWTPs may contribute a measurable percentage to the total load of PCBs in the central basin of Puget Sound.

The diet of SRKW is primarily Chinook salmon, ranging from 50 to 100%, depending on season (Hanson et al. 2021); therefore, this prey species is likely the primary sources of PCBs to this population of Orca. O'Neill et al. (2015) collected juvenile Chinook offshore in the central basin of Puget Sound and reported whole-body PCB concentrations of ≈ 20 µg/kg (wet weight; ww). Juvenile Chinook collected in the Green River/Duwamish system or nearshore contained higher concentrations of PCBs at 32-53 µg/kg ww. The offshore fish were substantially larger (17-39 grams ww) compared to the Green/Duwamish and nearshore juvenile Chinook (4.8-5.4 grams ww), which indicates that they bioaccumulated a substantial amount of PCBs in the offshore environment as they grew. While the specific sources of these compounds cannot be determined categorically, bioaccumulation is occurring in these Chinook occupying offshore waters via ventilation and ingestion of zooplankton and small fish. As noted by O'Neill et al. (2015), the mean percentage total body burden (ng/fish) of PCBs occurring in offshore juvenile Chinook that could be explained by burdens from river systems and nearshore juvenile Chinook was low ranging from 6-15%. These results indicate that a substantial amount of PCB bioaccumulation in juvenile Chinook occurs in offshore waters in Puget Sound where WWTP outfalls occur. This observation can also be extended to adult Chinook salmon, as noted by O'Neill and West (2009). In that study adult Chinook sampled in central Puget Sound exhibited mean concentrations of 86 µg/kg, which translated to 800 µg/fish. Most of these fish were considered resident within Puget Sound, indicating substantial bioaccumulation by adult fish.

As noted in the Mongillo et al. (2016) review, PCBs cause a variety of health effects in mammals at very low concentrations, including effects on reproduction, the immune system, nervous system, and disease susceptibility. Several studies have proposed PCB tissue residue effects thresholds for marine mammals. Kannan et al. (2000) derived a value of 17 ppm lw, for marine mammals, which is based on data for otter, mink, and harbor seals. Mos et al. (2010) derived a value of 1.3 mg/kg lw based on harbor seals and Hall et al. (2006) arrived at a value of 10 mg/kg lw for bottlenose dolphin. These values and the concentrations of PCBs determined in SRKW blubber above these values, indicate likely ongoing adverse effects that need to be reduced via source control. Gockel and Mongillo (2013) noted that for killer whales to have blubber concentrations below a 17 mg/kg threshold, their prey would need to be less than 8 µg/kg tPCBs, which is lower than the values for offshore juvenile Chinook reported by O'Neill et

al. (2015). A recent modeling effort evaluating the effects of PCBs on killer whale population growth predicts a decline for populations from various geographic locations with total PCB blubber concentrations ranging from 28-83 mg/kg lw (Desforges et al. 2018). All of the 14 SRKWs listed in Mongillo et al. (2012) exhibited concentrations of tPCBs in blubber close to or within this range considered by Desforges et al. (2018) to affect population growth of the world's Orca whales. Total PCB concentrations higher than this range were predicted to have stronger effects on population growth.

4.2.7.2 Polybrominated Diphenyl Ethers

PBDEs are similar to PCBs in that there are 209 possible congeners. They are also structurally similar and have similar physicochemical properties, such as K_{ow}. Even though some PBDEs were banned by the U.S. and other agencies, they still persist in the environment. PBDEs are readily accumulated by biota and can be found at high tissue concentrations. Total PBDEs (tPBDE) have been reported at concentrations up to 14 ppm lw in SRKW blubber (Mongillo et al. 2016).

PBDEs were detected in effluent from West Point and South Plant. For comparison, the mean field concentration of PBDEs in 2009 at the main basin site was reported to be 187 pg/L (Gries and Osterberg 2011). A similar modeling analysis was performed for PBDEs as PCBs and our predicted average tPBDE concentration in the current study was 30 pg/L for all field sampling sites, which is 16% of the observed field value in the Gries and Osterberg (2011) study.

O'Neill et al. (2015) reported on the sum of 11 PBDE congeners in offshore juvenile Chinook collected in the Whidbey, Central, and South basins of Puget Sound in the range of 2.6-4.1 µg/kg ww. Juvenile Chinook collected in the Green River/Duwamish system or nearshore were similar at 2.9-4.8 µg/kg ww. A similar pattern for PBDE bioaccumulation as that for PCBs was observed for the much larger offshore fish (17-39 g) compared to the smaller Green/Duwamish and nearshore juvenile Chinook (4.8-5.4 g), which means they also bioaccumulated a substantial amount of PBDEs in the offshore environment O'Neill et al. (2015). The mean percentage total body burden (ng/fish) for PBDEs occurring in offshore juvenile Chinook that could be explained by burdens from river system and nearshore juvenile Chinook was 11-20%, which was similar to that for PCBs.

As noted above for PCBs, Chinook prey would likely have to exhibit relatively low concentrations of tPBDEs to keep blubber concentrations below expected toxic thresholds. This value has not been determined for PBDEs, but may be similar as that noted above for PCBs.

PBDEs are similar to PCBs in that they are also endocrine disruptors and neurotoxicants. This group of compounds usually co-occurs with PCBs, so in the field it would be difficult to distinguish differences in toxic effects. We know of no toxicity effect concentrations or adverse threshold levels attributable to PBDEs; however, such toxicity values may be similar to those for PCBs. Alava et al. (2016) proposed using PCB toxic effect concentrations (TECs) for PBDE risk assessment, which would include the threshold values listed above for PCBs.

4.2.7.3 Polyfluoroalkyl substances (PFAS)

While manufacturing plants and fire suppression activities are considered to be the main source of PFAS to the environment, WWE is also considered an important pathway for PFAS (Tavasoli et al. 2021). Based on our detected total concentrations of PFAS in West Point and South Plant effluent, we calculated that the potential input of PFAS to the central basin of Puget Sound to be on the order of 17-62 kg per year, depending on the effluent flow rate and variations in concentration. The highest concentrations of PFAS occurred during the high-flow sampling event from the West Point facility,

suggesting that stormwater runoff and sanitary sewage likely contribute to PFAS in the WWE. PFAS concentration were in the order of West Point high flow >> South Plant high flow > West Point low flow ~ South Plant low flow. Our predicted total PFAS concentration for the field sites was 0.32 ng/L.

While there are a few studies of PFAS in Orca tissues, there are none for SRKW. One study reported mean liver concentrations for the sum of 24 PFAS in Orca liver of 353 µg/kg (SD = 101 µg/kg ww) (Schultes et al. 2020). These Orca were collected around East Greenland in an area that was presumably characterized by terrestrial input; however, input from global atmospheric transport likely contributed to the total concentration. Liver contained the highest levels and blood was second with a mean concentration of 116 µg/Kg (SD=21 µg/kg ww). Water concentrations for total PFAS the area of the Orca collections ranged from 0.050-0.2 ng/L (Busch et al. 2010), which is below our predicted PFAS concentration for the central basin field sites.

For PFAS, our predicted whole-body tissue concentrations for juvenile Chinook in the field were highest for PFDA (2.2 µg/kg ww) and PFNA (0.5 µg/kg ww), both of which are considered estrogenic in fish because they interact with the estrogen receptor and produced increases in vitellogenin, depending on the exposure concentration (Benninghoff et al. 2011). These predicted tissue concentrations are based on our predicted water concentration of 0.003 ng/L for PFDA and 0.004 ng/L for PFNA, which was calculated as a function of diluted effluent concentrations from the South Plant and West Point treatment facilities. PFAS are also known to be immunotoxic, mutagenic, and can affect the liver and thyroid (USEPA 2022a). The EPA has set a drinking water health advisory at 70 ng/L.

Meador et al. (2016) reported whole-body concentrations in juvenile Chinook for PFDA (0.78 µg/kg ww) and PFOS (1.4-34 µg/kg ww) in Sinclair inlet and similar concentrations in staghorn sculpin collected in Sinclair Inlet and the Puyallup River estuary. The source of these PFAS may have been effluent from nearby WWTP outfalls; however, this is uncertain. Effluent concentrations for some PFAS were very low and some likely below the limit of detection. As noted in Ankley et al. (2021), the dietary regulatory threshold value for PFOS to protect mammalian wildlife is 1.4 µg/kg ww in prey, which was promulgated in Canada, New Zealand, and Australia. Benninghoff et al. (2011) estimated a LOEC for PFDA in blood affecting vitellogenin production as 12.8 µg/kg bw/day, which was correlated with a blood plasma concentration of 1.03 mg/L (2 µM). These are very low threshold values that may occur in offshore juvenile Chinook and would be a potential source of PFAS to SRKWs.

Many PFAS, including PFOS, PFNA, and PFDA are considered to be estrogenic and have been shown to affect vitellogenin production, steroid synthesis, the hypothalamic-pituitary-gonadal-liver axis, and related endocrine system functions at exposure concentrations of 10 µg/L (Benninghoff et al. 2011, Zhang et al. 2016). This exposure concentration (10 µg/L) corresponded to low concentrations in zebrafish gonads (2.5 µg/kg for females and 5.1 µg/kg for males) (Zhang et al. 2016). One study determined competitive binding values (IC50) for PFAS compounds to the estrogen receptor in rainbow trout liver and determined that PFDA was one of the most active PFAS against the receptor (Benninghoff et al. 2011). A metric termed the relative binding affinity (RBA), which is based on the IC50, was calculated for several PFAS in relation to binding of estradiol to the estrogen receptor. It is important to note, that estrogenic responses are expected at levels below the IC50, which represents the 50% level of displacement of estradiol from the receptor. For PFDA the RBA was 0.006% of that for estradiol, which was the same value for PFOS and several other PFAS (Benninghoff et al. 2011). Additionally, many PFAS are known to have an additive effect on vitellogenin induction when fish are exposed to mixtures of

PFAS (Benninghoff et al. 2011). Given the high potency of estradiol, PFDA, in combination with other endocrine disruptors of similar potency, may be of concern.

As noted by Ankley et al. (2021) risk assessment and toxicological evaluation of PFAS should be considered as mixtures, not individual compounds. According to the EPA (USEPA 2022b) over 9,000 PFAS have been identified and more than 600 are currently in use (Ankley et al. 2021). Consequently, without occurrence and toxicity data for the hundreds of PFAS expected to occur in the environment, we are not able to adequately assess potential toxic effects to fish or SRKWs and believe the precautionary principle should be considered given the likelihood that a large number of PFAS may be present and several are expected to be estrogenic.

4.2.7.4 Nonylphenols

Nonylphenols are ubiquitous compounds occurring in a large number of products and they often occur at relatively high concentrations in water and tissue. We do not know of any values for this group of compounds for SRKW tissue; however, other studies have reported high levels in marine mammals. Diehl et al. (2012) reported high concentrations in sea lion, porpoise, and sea otter liver from central California ranging from 25-138 ppm lw. Biomagnification for hydrophobic compounds is difficult to demonstrate due to several uncontrolled factors; however one study determined biomagnification for 4-nonylphenol in sea otter liver (Diehl et al. 2012). Overall, we do know that low water concentrations can result in high tissue concentrations because these compounds are very hydrophobic (Korsman et al. 2015).

One study (Benninghoff et al. 2011) determined the IC50 for 4-nonylphenol to be 185 mg/L (842 µM) for the displacement of estradiol from the estrogen receptor. This value appears to be a high concentration; however, it is more similar to a serum concentration than a water exposure value and was expected to be high due to partitioning. Our predicted Pbw value for 4-nonylphenol is 2,743, therefore a water exposure concentration of 67 µg/L would be expected to result in a plasma concentration of 185 mg/L; however estrogenic effects are likely below this concentration because it was based on the 50% level of receptor inhibition. Based on this IC50 value, it was determined that this compound is 0.0017% as potent as 17β-estradiol. This study also determined that 17α-ethynylestradiol was 4 times less potent than 17β-estradiol for binding to the estrogen receptor. There is more toxicity data for 17α-ethynylestradiol and many studies have reported endocrine related effects at concentrations as low as 0.1–1.0 ng/L (USEPA 2008). Given the extremely low effect concentrations for estrogen hormones (sub ng/L); concentrations of nonylphenols in the low ppb range should be considered for additional analysis to determine potential effects on wildlife.

4.2.7.5 Bisphenols

Bisphenol A (BPA) was detected in South plant and West Point low flow effluent at relatively high concentrations (1,160 and 258 ng/L, respectively) and in estuarine waters at 2 ng/L. Predicted whole-body BPA concentrations for fish at the estuarine field sites were 1.5 µg/kg.

We could find no data quantifying bisphenol compounds in SRKW. Other studies have reported very high concentrations in blubber and liver for a variety of marine mammals. One study (Page-Karjian et al. 2016) reported dry weight (dw) blubber concentrations for bottlenose dolphin up to 250 mg/kg and higher (397 mg/kg dw) in a white beaked dolphin.

The predicted no effect water concentration for BPA of 0.18 µg/L to protect against ecological effects was promulgated by Environment Canada and Health Canada (Canada 2008), which was based on a

study with brown trout. The European Food Safety Authority set a Tolerable Daily Intake (TDI) value of 4 µg/kg body weight/day for the protection of human health (EFSA 2015). It is unknown how this TDI would relate to ingestion of prey contaminated with bisphenol A by SRKW. An older document lists several other regulatory values for BPA to protect against adverse effects for human and environmental health, some of which may have been updated (USEPA 2010). It is important to note that this TDI is only for BPA, and other estrogenic bisphenols should also be considered when assessing mammalian health from exposure to this group of compounds.

Bisphenol A is considered to be a weak ligand for the estrogen receptor (IC50 of 1 µM = 228 µg/L); however, its potency is only 1,000-fold lower (0.1%) than that for estradiol (Matsushima et al. 2010). The far higher concentrations expected for BPA should be weighed against the lower potency when evaluating potential endocrine effects. These authors also determined that bisphenol AF is a strong ligand for the estrogen receptor (ER) exhibiting an IC50 value of 19 – 50 nM (ERα and ERβ) (Matsushima et al. 2010). Interestingly, it was determined that bisphenol AF was an agonist for ERα and an antagonist for ERβ. Bisphenol AF was not detected in West Point or South Plant effluent.

A total of 16 bisphenol analogues have been identified and many of those are estrogenic (Chen et al. 2016). Of the nine bisphenol analogues tested, all were similar or more potent compared to BPA in terms of estrogenic, anti-androgenic, or anti-estrogenic activity (Chen et al. 2016). Of the untested compounds, some may be as potent as bisphenol AF. Only six bisphenols were analyzed by SGS-AXYS, which means we are potentially underestimating exposure to this group. Given the high propensity for bioaccumulation and potential toxicity, this is another class of compounds from wastewater effluent that should be further evaluated and considered for reduction.

4.2.7.6 Other contaminants of concern for wildlife

Triclosan and phthalates are also hydrophobic compounds that may accumulate to high levels in marine mammals from ingestion of contaminated prey. Blubber values up to 50 mg/kg dw have been reported for triclosan and values as high as 14 mg/kg dw for diethylphthalate in white beaked dolphin (Page-Karjian et al. 2016). Lower values for these hydrophobic compounds have also been reported (Page-Karjian et al. 2016), which highlights the variability among species that in many cases is determined by the contamination level in the environment where they reside.

4.2.7.7 Conclusions

In general, for many of the hydrophobic compounds we analyzed (other than PCBs and PBDEs) there is very little data on concentrations in marine mammals, especially for SRKW. Until we have more data on these contaminants in wildlife tissues, especially SRKW, a more detailed risk assessment regarding potential impacts from WWTP effluent will likely be impossible. Because many of these hydrophobic compounds can bioaccumulate to high levels in wildlife and many are estrogenic and suspected of adverse effects in marine mammals, it would be advantageous to reduce the discharge of these compounds to the environment to protect the declining SRKW population. For the pharmaceuticals and personal care products, most were observed or predicted to occur at relatively low concentrations and we do not expect many of these compounds to be of concern for SRKW in Puget Sound or other wildlife. Many CECs, especially pharmaceuticals, may cause adverse effects for fish; however, we don't expect these to bioaccumulate to any degree in higher trophic levels because of low partition coefficients and fast elimination rates.

5 STUDY UNCERTAINTIES AND LIMITATIONS

5.1 Water quality characterization

The water quality characterization performed in this study was based on a limited number of samples: one set of high-flow and low-flow samples were collected at West Point and South Plant, and one set of estuarine water samples were collected from different locations in central Puget Sound. It has been reported that there are variations in the concentrations of some pharmaceuticals and personal care products on hourly, daily, and seasonal time scales in wastewater effluents. Hong et al. (2015) collected and analyzed wastewater treatment systems influents sample on an hourly basis and noted significant variation for some compounds, such as acetaminophen and carbamazepine, over that time. Paiga et al. (2019) performed a similar evaluation on both WWTP influent and effluent and reported more variation in the influent compared to effluent. Ort el al. (2010) evaluated the variation ascribed to different sampling methods (e.g., grab samples, flow-weighted composites, time-weighted-composites) and demonstrated sampling error would be minimized with more frequent (<15 min intervals) composite sampling. In this study, wastewater effluent samples were time-weighted composites with aliquots collected every 15 minutes.

With regard to seasonal variation, Garbarino (2017) demonstrated that antibiotics such as erythromycin were found at significantly higher concentrations in autumn, winter, and spring compared to summer, though other medications such as SSRIs, acetaminophen, and diphenhydramine did not differ across seasons. Similarly, Yu et al (2013) reported higher pharmaceutical concentration in winter than in summer, and attributed the differences to changes in consumption patterns between summer and winter. In this study, samples were collected in early-spring and summer, providing some opportunity to capture the potential seasonal variation.

There is limited information on variation in estuarine systems. Miller-Schultz et al (2015) collected multiple samples in the Thea Foss waterway and analyzed samples for a suite of 20 CECs. They reported a range of concentration from ~ 3x (min concentration/max concentration) for sucralose to ~ 80x for mecoprop. It is not known if the variation in this terminal inlet is representative of variation in central Puget Sound. Results of estuarine water samples collected in this study were compared to other sample events in the region and were generally comparable.

Despite the aspects of the study design which were meant to minimize uncertainty, the data were collected with only a limited number of samples. While it is not known how the concentrations reported here compare with environmental minimums and maximums, they are generally comparable to results reported in other, similar studies, and so we feel they are generally representative of environmental conditions, and representative of environmental exposures.

There are also uncertainties related to the attribution of compounds detected in the Puget Sound estuary, and specifically the proportion of contribution from King County wastewater treatment facilities compared to other potential sources or pathways. It is well documented that WWE is a pathway for anthropogenic compounds entering into the marine environment, including the Puget Sound (e.g., Tian et al. 2021). Many of the chemicals detected in the estuary samples in this study were also detected in the West Point and South Plant sampling. However, there are more than 90 wastewater treatment facilities that discharge effluent to Puget Sound, some of which have been monitored, suggesting that the chemicals present in the estuary water sampling for this study could have come from outside the basin. However, there are several considerations that suggest that King County facilities contribute a

major fraction of detected chemicals. First, the King County effluent discharge outfalls are located much closer to the estuarine sampling locations compared to any other wastewater outfall in the region, and suggests a higher likelihood of influence. Additionally, this indicates that there is a much larger transport time for compounds released from distant outfalls compared to nearby outfalls, during which compounds will undergo some degradation (e.g., Benotti and Brownawell 2009, Cantwell et al. 2019). Finally, the effluent flow rate from the King County facilities is much higher than any of the other WWTPs, indicating a higher mass loading (based on comparable effluent concentrations). Again, while it is not possible to attribute an exact proportion of contaminant occurrences detected in this study to King County wastewater effluent, it is likely a significant fraction.

5.2 Uncertainty regarding predictions of bioaccumulation

The main determinant for bioaccumulation of organic compounds is hydrophobicity that can be characterized by the octanol-water partition coefficient (K_{ow}). This QSAR is, however, only relevant for the neutral or unionized phase. A substantial number of pharmaceuticals are ionizable and their K_{ow} will vary by pH, therefore a pH-specific K_{ow} was required for these compounds. In this study a pH-specific K_{ow} (= D_{ow}) was used for all pharmaceuticals in bioaccumulation modeling. Many K_{ow} and D_{ow} values are determined empirically; however, most are predicted with algorithms that are based on chemical structure. The QSAR values for hydrophobicity are generally considered to be fairly accurate; however, there is some degree of variation that is expected, and undefined, between observed and predicted values.

5.3 Freshwater versus saltwater toxicity

Even though our laboratory study was conducted in freshwater with freshwater-phase Chinook salmon, we believe our results are applicable for fish in marine waters. Hydrophobicity is the main factor controlling bioaccumulation for organic compounds and the resulting toxic effects. The ionic strength of exposure water has little or a minor effect on toxicant uptake, which is primarily through gill ventilation and dietary uptake. In general, bioaccumulation models do not include parameters for ionic strength or osmolarity (Arnot and Gobas 2006). In addition to hydrophobicity, an important factor to consider for assessing bioaccumulation of ionizable organic compounds for fish in fresh and marine waters is pH, which can vary among media. In this study we used separate pH-specific D_{ow} values for bioaccumulation modeling that matched the laboratory freshwater toxicity experiment or the marine waters of Puget Sound.

5.4 Ten-day exposures compared to chronic exposures

Ten days is not a long exposure relative to the time Chinook are in Puget Sound, so some of the effects reported in our study could be occurring in fish exposed for longer durations to lower concentrations. Juvenile Chinook salmon are known to spend considerable time in the estuary and nearshore waters before migrating to offshore waters (Healy 1991). Also, as noted by O'Neill and West (2009), an average of 45% of yearling Chinook exhibited resident behavior in Puget Sound and are known to bioaccumulate contaminants to higher levels than observed for other Pacific coast populations of Chinook residing in open water. The route of dietary exposure was not included in our study. This would result in even higher levels of bioaccumulation than observed in our study – particularly for more hydrophobic contaminants – further supporting that some effects would be expected in Chinook in Puget Sound at lower water concentrations than in our study.

6 CONCLUSIONS

This study helps set a baseline for King County South Plant WWE toxicity to juvenile Chinook and provides a detailed snapshot of effects of WWE on Chinook salmon. Exposure to WWE affected multiple metabolic pathways including brain function, the endocrine system, metabolism, and stress. This study highlighted that phenolic compounds in WWE, specifically estrogenic hormones, have a measurable effect on juvenile Chinook, which could cause long term reproductive effects in wild fish. Many of the contaminants primarily responsible for other effects we reported are still unknown. Further research is necessary to confirm the potential effects of chronic exposure to psychoactive pharmaceuticals in WWE on brain function and other aspects of physiology. A better understanding of metabolic disruption – including the contaminants driving those impacts – could also help explain limitations on recovery of PNW Chinook populations.

Even though we have identified several effluent compounds that may cause adverse effects in fish, this is by no means a complete picture. It should be noted that of the top 300 prescribed drugs in the United States, AXYS-SGS analytical labs can analyze approximately 25% of those. The non-targeted analysis of wastewater effluent samples performed in this study identified ~250 additional compounds, some of which are likely to affect biota at very low environmental concentrations. These compounds were identified based on comparisons with existing libraries and additional work could focus on elucidating the occurrence of metabolites or compounds with low effects thresholds. Our chemical screening approach did not include quantification of compounds identified through non-target analysis. Although effects from these chemicals would be captured in many of the endpoints evaluated in this study, a thorough evaluation of risk associated with these additional compounds would require focused quantification..

It is also important to note that we do not have plasma concentrations for a large number of the analytes in this study to predict effects with the fish plasma model, which uses human Cmax values to predict adverse effects. For those analytes other than pharmaceuticals, toxicity studies on individual compounds or classes of compounds were consulted. Also, predicted estuarine concentrations and tissue concentrations for several groups of hydrophobic compounds (e.g., PAHs, PCBs, and dioxins and furans) from King County WWE were very low and would not generally be considered high enough to cause toxic effects in juvenile Chinook at those levels (Meador et al. 2002; Berninger and Tillitt 2019; Meador et al. 2006; King-Heiden et al. 2009). However, when all sources of these hydrophobic compounds are considered, juvenile and adult Chinook can attain whole-body concentrations that exceed toxic threshold levels for PCBs (O'Neill and West 2009; O'Neill et al. 2015). Additionally, several individual PBDEs and flame retardants may occur in fish tissue at concentrations (Table 27) able to cause adverse effects in juvenile Chinook (Arkoosh et al. 2010; 2018). It is important to note that our bioaccumulation modeling is limited to uptake from water via gill ventilation; however dietary uptake is also a major contributor to body burdens, especially for hydrophobic compounds. These bioaccumulative compounds mentioned above should also be considered for fish and invertebrates that are sessile or have limited mobility and may accumulate high tissue concentrations given sufficient time, especially for biota that interact with sediment.

These classes of hydrophobic compounds, except PAHs, but including BDEs, are known to bioaccumulate in marine mammals to levels considered harmful. This is a key point because adult Chinook, which are the primary prey species for the SRKW, are commonly resident within Puget Sound and bioaccumulate these compounds. While there are many sources of these hydrophobic legacy compounds to Puget

Sound, the present study identified quantifiable levels of these compounds from King County WWE to the systemwide total that contributes to bioaccumulation by fish and other prey species and biomagnification in marine mammals to levels considered adverse.

Finally, measurements of selected classes of contaminants were used to support preliminary loading calculations in order to understand whether wastewater effluent is a significant pathway to Puget Sound. These estimates suggest that treated wastewater effluent is a measurable pathway for PCBs and may be comparable to ongoing loadings from individual, large, industrial basins via stormwater runoff.

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APPENDICES

Table A1. Targeted chemistry data from SGS-AXYS for whole wastewater effluent samples. All detected concentrations in ng/L.

Analyte	CAS#	South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow
Alkylphenol					
4-n-Octylphenol	1806-26-4	*<4.84*	*<0.938*	*<3.01*	*<1.4*
4-Nonylphenol diethoxylates	20427-84-3	1520	940	1220	401
4-Nonylphenol monoethoxylates	104-35-8	2550	2960	550	770
4-Nonylphenols	104-40-5	630 B	887	100 B	252
Bisphenol					
Bisphenol AF	1478-61-1	*<1.94*	*<2*	*<1.93*	*<1.97*
Bisphenol B	77-40-7	*<1.94*	*<2*	*<1.93*	*<1.97*
Bisphenol E	2081-08-5	*<4.85*	*<5.75*	*<4.81*	39.6
Bisphenol F	620-92-8	36.7 K	*<5.01*	14.9 K	39.5
Bisphenol S	80-09-1	1160	127	485	1050
Bisphenol A	80-05-7	2985	963.5	191.5	183
Dioxins and Furans					
1,2,3,4,6,7,8-HPCDD	35822-46-9	0.00217 B	0.00126 K	0.00641 B	0.00118 K
1,2,3,4,6,7,8-HPCDF	67562-39-4	0.000738 B K	0.000501	0.00265 B	*<0.000516*
1,2,3,4,7,8,9-HPCDF	55673-89-7	*<0.000578*	*<0.000501*	0.00164	*<0.000516*
1,2,3,4,7,8-HXCDD	39227-28-6	*<0.000578*	*<0.000501*	0.00144 K	*<0.000537*
1,2,3,4,7,8-HXCDF	70648-26-9	*<0.000578*	*<0.000501*	0.00152	*<0.000516*
1,2,3,6,7,8-HXCDD	57653-85-7	*<0.000578*	*<0.000501*	0.00162 K	*<0.000537*
1,2,3,6,7,8-HXCDF	57117-44-9	*<0.000578*	*<0.000501*	0.00135	*<0.000516*
1,2,3,7,8,9-HXCDD	19408-74-3	*<0.000578*	*<0.000501*	0.00186	*<0.000537*
1,2,3,7,8,9-HXCDD (225)	--	*NA*	*NA*	0.00123	*NA*
1,2,3,7,8,9-HXCDF	72918-21-9	0.000633 B K	0.000737 B	0.00191 B	0.000934 B K
1,2,3,7,8-PECDD	40321-76-4	*<0.000578*	*<0.000756*	0.00127 K	*<0.000713*
1,2,3,7,8-PECDF	57117-41-6	*<0.000578*	*<0.000501*	0.000759	*<0.000617*
2,3,4,6,7,8-HXCDF	60851-34-5	*<0.000578*	*<0.000501*	0.00151	*<0.000516*
2,3,4,7,8-PECDF	57117-31-4	*<0.000578*	*<0.000501*	0.00116 K	*<0.000617*
2,3,7,8-TCDD	1746-01-6	*<0.000578*	*<0.000501*	*<0.000522*	*<0.000645*
2,3,7,8-TCDF	51207-31-9	*<0.000578*	*<0.000501*	*<0.000522*	*<0.000516*
OCDD	3268-87-9	0.0141 B	0.00684 B	0.0372	0.00428 B
OCDF	39001-02-0	0.000957 B	0.000655 B	0.00447 B	*<0.000516*
TOTAL HEPTA-DIOXINS	--	0.00389	*<0.000501*	0.00979	*<0.000516*
TOTAL HEPTA-FURANS	--	*<0.000578*	0.000501	0.00428	*<0.000516*
TOTAL HEXA-DIOXINS	--	*<0.000578*	*<0.000501*	0.00186	*<0.000537*
TOTAL HEXA-FURANS	--	*<0.000578*	0.000737 B	0.00683	*<0.000516*
TOTAL PENTA-DIOXINS	--	*<0.000578*	*<0.000756*	*<0.000522*	*<0.000713*
TOTAL PENTA-FURANS	--	*<0.000578*	*<0.000501*	0.000759	*<0.000617*
TOTAL TETRA-DIOXINS	--	*<0.000578*	*<0.000501*	*<0.000522*	*<0.000645*
TOTAL TETRA-FURANS	--	*<0.000578*	*<0.000501*	*<0.000522*	*<0.000516*
Ether carboxylates					

Analyte	CAS#	South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow
ADONA	958445-44-8	*<1.59*	*<1.48*	*<1.58*	*<1.45*
HFPO-DA	13252-13-6	*<1.51*	*<1.41*	*<1.5*	*<1.37*
NFDHA	151772-58-6	*<0.793*	*NQ*	*<0.789*	*NQ*
PFMBA	--	*<0.396*	*<0.371*	*<0.395*	*<0.362*
PFMPA	--	*<0.793*	*<0.741*	*<0.789*	*<0.723*
Ether sulfonates					
11Cl-PF3OUdS	763051-92-9	*<1.59*	*<1.48*	*<1.58*	*<1.45*
9Cl-PF3ONS	--	*<1.59*	*<1.49*	*<1.58*	*<1.45*
PFEESA	113507-82-7	*<0.396*	*<0.371*	*<0.395*	*<0.362*
Fluorotelomer carboxylates					
3:3 FTCA	356-02-5	*<1.59*	*<1.48*	*<1.58*	*<1.45*
5:3 FTCA	914637-49-3	11.3	27.6	*<9.87*	13.7
7:3 FTCA	812-70-4	*<9.91*	*<9.26*	*<9.87*	*<9.04*
Fluorotelomer sulfonates					
4:2 FTS	757124-72-4	*<1.59*	*<1.48*	*<1.58*	*<1.45*
6:2 FTS	27619-97-2	2.35	3.01	4.4	2.46
8:2 FTS	39108-34-4	*<1.59*	*<1.48*	*<1.58*	*<1.45*
Halogenated flame retardant					
1,2,4,5/1,2,3,5-TBB	--	*<0.0193*	*<0.0312*	*<0.0177*	*<0.0206*
1,2,4-TriBB	615-54-3	*<0.235*	*<0.384*	*<0.247*	*<0.314*
1,2-DiBB	583-53-9	*<0.0693*	0.172 K	*<0.1*	0.072 K
1,4-DiBB	106-37-6	*<0.131*	*<0.134*	*<0.0967*	*<0.092*
ATE	3278-89-5	*<0.0548*	*<0.0541*	*<0.0511*	*<0.0391*
BATE	99717-56-3	*<0.107*	*<0.183*	*<0.133*	*<0.145*
BEHTBP	26040-51-7	1.68	1.91	1.25	1.1
BTBPE	37853-59-1	*<0.804*	*<0.837*	*<0.624*	*<0.6*
Dec 602	31107-44-5	*<0.0096*	*<0.0103*	*<0.0103*	*<0.0093*
Dec 603	13560-92-4	*<0.0093*	0.021	0.008	*<0.0068*
Dec 604	34571-16-9	*<0.337*	*<1.1*	*<0.244*	*<0.322*
Dechlorane	2385-85-5	*<0.1 B K*	*<0.0435*	*<0.0438*	*<0.0507*
DP Anti	135821-74-8	*<0.117*	*<0.105*	0.148	*<0.0908*
DP Syn	135821-03-3	*<0.0795*	*<0.0717*	*<0.0609*	*<0.0663*
DPTE	35109-60-5	*<0.409*	*<0.488*	*<0.951*	*<0.584*
EHTBB	183658-27-7	2.15	3.91	2.61	2.53
HBB	87-82-1	0.052 B	*<0.0401*	*<0.05 B*	*<0.0205*
HCDBCO	51936-55-1	*<0.0205*	*<0.0405*	*<0.0453*	*<0.077*
PBBB	38521-51-6	*<0.258*	*<0.214*	*<0.556*	*<0.228*
PBBZ	608-90-2	0.025	*<0.031*	*<0.0226*	*<0.0319*
PBEB	85-22-3	0.017	*<0.0197*	*<0.0362*	*<0.0277*
PBT	87-83-2	0.045 M	0.021 B M	0.071 M	0.03 B M
pTBX	23488-38-2	*<0.422*	*<0.93*	*<1.06*	*<0.792*
TBCT	39569-21-6	0.066 K	0.085 K	*<0.0699*	0.05 K
Total TBECH	3322-93-8	*<0.372*	*<0.35*	*<0.479*	*<0.448*
Hormone					
17 alpha-Dihydroequilin	651-55-8	*<1.93*	*<1.89*	*<1.97*	*<1.81*

Analyte	CAS#	South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow
17 alpha-Estradiol	57-91-0	*<7.74*	12.4 K	*<7.88*	*<7.24*
17 alpha-Ethinyl-Estradiol	57-63-6	*<18.5*	*<4.73*	*<4.93*	*<53.1*
17 beta-Estradiol	50-28-2	18.2	43.5	*<3.94*	6.97
17 beta-Estradiol 3-benzoate	50-50-0	*<0.774*	*<0.757*	*<0.788*	*<0.724*
Allyl Trenbolone	850-52-2	*<0.645*	*<0.756*	*<0.572*	*<0.609*
Androstenedione	63-05-8	16.6 K	17.1	5.76 K	14
Androsterone	53-41-8	*NQ*	*NQ*	*NQ*	*NQ*
Desogestrel	54024-22-5	*<114*	*<39.4*	*<63*	*<51.5*
Equilenin	517-09-9	*<0.418*	*<0.483*	*<0.394*	*<0.362*
Equilin	474-86-2	*<1.93*	*<1.89*	*<1.97*	*<1.81*
Estriol	50-27-1	*<25.3*	*<19*	*<20.6*	*<18.7*
Estrone	53-16-7	38.3	170	5.09	47.9
Mestranol	72-33-3	*<73.4*	*<36.1*	*<72.7*	*<27*
Norethindrone	68-22-4	*<4.05*	*<2.28*	*<2.56*	*<2.66*
Norgestrel	797-63-7	*<3.46*	*<1.7*	*<3.15*	*<2.13*
Progesterone	57-83-0	*<1.05*	1.59	3.22 K	6.56
Testosterone	58-22-0	4.64	3.31	1.87 K	6.25
Polyaromatic Hydrocarbons (PAH)					
1,2,6-Trimethylphenanthrene	30436-55-6	0.207	*<0.318*	0.91	*<0.267*
1,2-Dimethylnaphthalene	573-98-8	0.823	*<0.656*	2.91	2.3
1,4,6,7-Tetramethylnaphthalene	13764-18-6	2.41	2.06	8.76	3.18
1,7-Dimethylfluorene	442-66-0	*<0.642*	*<0.806*	2.16	1.12
1,7-Dimethylphenanthrene	483-87-4	0.538	0.571	2.53	1.08
1,8-Dimethylphenanthrene	7372-87-4	*<0.274*	*<0.307*	0.794	0.364
1-Methylchrysene	3351-28-8	0.16	0.229	0.552	0.204
1-Methylnaphthalene	90-12-0	3.24	2.32 B	10.8	11.2
1-Methylphenanthrene	832-69-9	0.705	0.748	3.82	2.91
2,3,5-Trimethylnaphthalene	2245-38-7	3.04	2.06	11.6	7.44
2,3,6-Trimethylnaphthalene	829-26-5	3.83	2.52	16	9.95
2,4-Dimethyldibenzothiophene	31317-18-7	*<0.432*	0.297	1.21	0.593
2,6-Dimethylnaphthalene	581-42-0	2.83	1.25	8.13	5.06
2,6-Dimethylphenanthrene	17980-16-4	0.904	0.899	3.58	1.29
2/3-Methyldibenzothiophenes	--	0.31 K	*<0.437*	2.43	1.53
2-Methylanthracene	613-12-7	*<0.286*	0.214 B K	0.659	*<0.325*
2-Methylfluorene	1430-97-3	1.41	1.65	3.82	2.24
2-Methylnaphthalene	91-57-6	2.39 B	2.49 B	5.56	4.02 B
2-Methylphenanthrene	2531-84-2	0.831	0.823	4.02	3.15
3,6-Dimethylphenanthrene	1576-67-6	0.711 K	0.77	3.13 K	1.13 K
3-Methylfluoranthene/Benzo[a]fluorene	--	0.665	1.06	1.83	1.34
3-Methylphenanthrene	832-71-3	1.54 K	1.78 K	4.87	4.09
4,6-Dimethyldibenzothiophene	1207-12-1	0.491 B	0.697	2.59	0.974
5,9-Dimethylchrysene	--	*<0.309*	0.356	0.552	0.249
5/6-Methylchrysene	--	*<0.133*	*<0.175*	*<0.284*	*<0.0971*

Analyte	CAS#	South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow
7-Methylbenzo[a]pyrene	--	*<0.35*	*<0.385*	*<0.362*	*<0.328*
9/4-Methylphenanthrene	--	0.935 K	0.853	4.12	3.04
Acenaphthene	83-32-9	3.36	2.5	18.3	18.4
Acenaphthylene	208-96-8	0.226	*<0.205*	0.596	0.656 B
Anthracene	120-12-7	0.359	0.397 B	1.47	1.21
Benz[a]anthracene	56-55-3	0.447	0.617	1.59	0.859
Benzo[a]pyrene	50-32-8	*<0.588*	0.198	1.12	0.449
Benzo[b]fluoranthene	205-99-2	*<0.353*	0.267	1.06	0.389
Benzo[e]pyrene	192-97-2	*<0.577*	0.502	1.44	0.427 K
Benzo[ghi]perylene	191-24-2	0.388	0.37 K	1.34	0.553
Benzo[j,k]fluoranthenes	--	*<0.406*	0.172	0.642 K	0.418
Biphenyl	92-52-4	*<2.92 B*	*<2.92 B*	3.39 B	3.69 B
C1 Phenanthrenes/Anthracenes	--	1.54	2.42	17.5	13.2
C1-Acenaphthenes	--	*<0.33 B*	0.526 K	0.969 B	1.05 K
C1-Benzo[a]anthracenes/Chrysenes	--	0.423	1.33	2.28	1.13
C1-Benzofluoranthenes/Benzopyrenes	--	*<0.35*	*<0.385*	*<0.362*	*<0.328*
C1-Biphenyls	--	2.56 B	1.72 B	4.36 B	1.64 B
C1-Dibenzothiophenes	--	2.13	0.496	7.89	4.89
C1-Fluoranthenes/Pyrenes	--	2.31	3.13	7.49	3.85
C1-Fluorenes	--	5.15	5.54	12.5	9.56
C1-Naphthalenes	--	5.63 B	4.8 B	16.4	15.2
C2 Phenanthrenes/Anthracenes	--	5.95	6.89	25.2	11.5
C2-Benzo[a]anthracenes/Chrysenes	--	*<0.309*	1.16	1.83	0.824
C2-Benzofluoranthenes/Benzopyrenes	--	*<0.45*	*<0.457*	*<0.526*	0.935
C2-Biphenyls	--	22.3 B	4.5 B	6.32 B	*<4.05 B*
C2-Dibenzothiophenes	--	4.12	3.99	14.2	6.38
C2-Fluoranthenes/Pyrenes	--	2.1	2.37	6.69	2.58
C2-Fluorenes	--	14.6	16.9	45.4	20.4
C2-Naphthalenes	--	13	7.6 B	39.6	28.3
C3-Benzo[a]anthracenes/Chrysenes	--	0.677	*<0.22*	1.59	0.181
C3-Dibenzothiophenes	--	1.95	3.31	11	4.42
C3-Fluoranthenes/Pyrenes	--	0.459	1	1.5	0.261
C3-Fluorenes	--	25.3	*<0.76*	49	12.5
C3-Naphthalenes	--	19.2	7.75 B	61.5	30.2
C3-Phenanthrenes/Anthracenes	--	5.71	7.49	21	7.47
C4-Benzo[a]anthracenes/Chrysenes	--	*<0.159*	*<0.0925*	*<0.117*	2.05
C4-Dibenzothiophenes	--	*<0.357*	1.48	*<0.414*	*<0.207*

Analyte	CAS#	South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow
C4-Fluoranthenes/Pyrenes	--	*<0.235*	*<0.202*	0.468	*<0.159*
C4-Naphthalenes	--	13.9	10.6	46.7	17.1
C4-Phenanthrenes/Anthracenes	--	3.98	8.81	18.1	7.78
Chrysene	218-01-9	0.847	1.26 B	2.72	1.56 B
Dibenz[a,h]anthracene	53-70-3	*<0.637*	*<0.411*	*<0.728*	*<0.327*
Dibenzothiophene	132-65-0	0.672 B K	0.577 B K	3.18	3.95
Fluoranthene	206-44-0	2.49 B	2.79 B	8.31	7.93
Fluorene	86-73-7	2.96	2.15 B	15.5	17.9
Indeno[1,2,3-cd]pyrene	193-39-5	*<0.428*	0.342 K	1.17 K	0.408 K
Naphthalene	91-20-3	5.29 B	5.34 B	23.2	22.7
Perylene	198-55-0	*<0.552*	*<0.16*	0.353	*<0.172*
Phenanthrene	85-01-8	3.58 B	3.15 B	13.4	17.9
Pyrene	129-00-0	3.78	4.31	10.9	7.49
Retene	483-65-8	1.44	1.21	2.63	1.32
Sum PAH	--	216.25937	657.025742	328.520097	1314.051484
Polybrominated Diphenyl Ethers (PBDE)					
BDE-10	51930-04-2	*<0.00537*	*<0.00308*	*<0.00691*	*<0.00386*
BDE-100	189084-64-8	0.474	0.736	0.501	0.338
BDE-105	373594-78-6	*<0.00686*	*<0.00635*	*<0.0138*	*<0.00951*
BDE-116	189084-65-9	*<0.00878*	*<0.00768*	*<0.0185*	*<0.0115*
BDE-119 + 120	--	*<0.00607*	0.00808 K	*<0.0126*	*<0.00776*
BDE-12 + 13	--	*<0.00321*	*<0.00196*	*<0.00392*	*<0.00245*
BDE-126	366791-32-4	*<0.00336*	*<0.0029*	*<0.00827*	*<0.00435*
BDE-128	182677-28-7	*<0.00881*	*<0.00386*	*<0.0099*	*<0.00346*
BDE-138 + 166	--	0.0223 K	0.0397	0.0193 K	0.0238 K
BDE-140	243982-83-4	*<0.00406*	0.00929	0.00706 K	0.00564 K
BDE-15	2050-47-7	0.00525 K	0.00732	0.00368	0.00322
BDE-153	68631-49-2	0.21 K	0.309	0.207	0.163
BDE-154	207122-15-4	0.161	0.236	0.164	0.123
BDE-155	35854-94-5	0.0125 K	0.0161	0.0121	0.00715 K
BDE-17 + 25	--	0.0353	0.0637	0.0398	0.0224
BDE-181	189084-67-1	*<0.0042*	*<0.00462*	*<0.00564*	*<0.00545*
BDE-183	207122-16-5	0.0238	0.0403	0.166	0.0198 K
BDE-190	189084-68-2	*<0.00727*	*<0.00771*	0.0107 K	*<0.00909*
BDE-203	337513-72-1	0.0389	0.0293	0.0647	0.0147
BDE-206	63936-56-1	0.233 M	0.141 M	0.0764 K	0.0725 M
BDE-207	437701-79-6	0.351 M	0.294 M	0.161 M	0.177 M
BDE-208	437701-78-5	0.247 M	0.17 M	0.11 M	0.0556 K
BDE-209	1163-19-5	3.67	1.67	2.67	0.791
BDE-28 + 33	--	0.0337 K	0.0763	0.0507 K	0.0384
BDE-30	155999-95-4	*<0.00214*	*<0.00241*	*<0.00426*	*<0.00342*
BDE-32	189084-60-4	*<0.0017*	*<0.00206*	*<0.00331*	*<0.00293*
BDE-35	147217-80-9	*<0.0014*	*<0.00183*	*<0.00259*	*<0.0026*
BDE-37	147217-81-0	0.0038 K	*<0.00171*	0.00334 K	*<0.00242*
BDE-47	5436-43-1	2.11	3.8	2.55	1.74

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BDE-49	243982-82-3	0.0654 K	0.105	0.0736	0.0382
BDE-51	189084-57-9	0.0114 K	0.0127 K	0.0114	0.00476 K
BDE-66	189084-61-5	0.0542	0.0904	0.0548 K	0.0362
BDE-7	171977-44-9	0.00569 K	0.004 K	*<0.00561*	*<0.00354*
BDE-71	189084-62-6	0.0125 K	0.014 K	0.0166	0.00569
BDE-75	189084-63-7	0.00397 K	0.0052 K	0.00611	0.00483 K
BDE-77	93703-48-1	*<0.00206*	*<0.0015*	*<0.003*	*<0.0021*
BDE-79	446254-48-4	0.0185	0.00914	0.0097	0.00421 K
BDE-8 + 11	--	*<0.00353*	*<0.00217*	*<0.00443*	*<0.00272*
BDE-85	182346-21-0	0.0879	0.14	0.0885	0.0716 K
BDE-99	60348-60-9	2.28	3.54	2.41	1.68
Polychlorinated Biphenyls (PCB)					
Decachloro Biphenyl	--	0.00318	0.00391	*NA*	0.00191
PCB-1	2051-60-7	*<0.0077 B*	*<0.0077 B*	0.0106 B	0.0103 B
PCB-10	33146-45-1	*<0.00207*	*<0.000906*	*<0.00202*	0.00153 K
PCB-103	60145-21-3	*<0.000613*	*<0.000507*	0.00104 K	0.000627
PCB-104	56558-16-8	0.00062 K	*<0.000507*	*<0.000539*	*<0.000504*
PCB-105	32598-14-4	0.0159 B	0.023 B	0.0363	0.0205 B
PCB-106	70424-69-0	*<0.0007*	*<0.000546*	*<0.000792*	*<0.000504*
PCB-107	70424-68-9	0.00217	0.00358	0.00468	0.00326
PCB-108 + 124	--	0.00206	0.0025 K	0.00338	0.00231
PCB-11	2050-67-1	0.0523 B	0.0824 B	0.0629 B	0.0527 B
PCB-110 + 115	--	0.0496	0.0628 B	0.111	0.0537 B
PCB-111	39635-32-0	*<0.000609*	*<0.000507*	*<0.000579*	*<0.000504*
PCB-112	74472-36-9	*<0.000609*	*<0.000507*	*<0.000536*	*<0.000504*
PCB-114	74472-37-0	0.00196 K	0.00142 K	0.00246 K	0.00157
PCB-118	31508-00-6	0.0408	0.0588 B	0.0963	0.0501 B
PCB-12 + 13	--	*<0.00223*	*<0.00094*	0.00312 K	*<0.00103*
PCB-120	68194-12-7	*<0.000609*	*<0.000507*	*<0.000542*	*<0.000504*
PCB-121	56558-18-0	*<0.000609*	*<0.000507*	*<0.000572*	*<0.000504*
PCB-122	76842-07-4	*<0.000739*	0.00105 K	*<0.000836*	0.000747 K
PCB-123	65510-44-3	0.00143 K	0.00149 K	0.00251 K	0.00118 K
PCB-126	57465-28-8	*<0.000762*	*<0.000624*	*<0.000828*	*<0.000504*
PCB-127	39635-33-1	*<0.000716*	*<0.000598*	*<0.000809*	*<0.000504*
PCB-128 + 166	--	0.00619	0.00988 B	0.0151	0.00757 B
PCB-129 + 138 + 160 + 163	--	0.0437	0.0638 B	0.0948	0.0492 B
PCB-130	52663-66-8	0.00303 K	0.00489 K	0.00625	0.00329 K
PCB-131	61798-70-7	*<0.000723*	0.00126 K	0.00101	0.000734 K
PCB-132	38380-05-1	0.0143	0.0232	0.0314	0.0166 B
PCB-133	35694-04-3	*<0.000688*	0.00201	0.00204	0.000728 K
PCB-134 + 143	--	0.00206	0.00256 K	0.0049 K	0.00214
PCB-135 + 151 + 154	--	0.0126	0.017	0.026	0.0139 K
PCB-136	38411-22-2	0.00363 K	0.00584	0.0103	0.00569
PCB-137	35694-06-5	0.00298 K	0.00339	0.00452	0.00309
PCB-139 + 140	--	0.00109	0.00117 K	0.00221 K	0.00129 K

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PCB-14	34883-41-5	*<0.00207*	*<0.000885*	*<0.00202*	*<0.000972*
PCB-141	52712-04-6	0.00714	0.0109 B K	0.0168	0.0083 B
PCB-142	41411-61-4	*<0.000725*	*<0.000924*	*<0.000995*	*<0.000504*
PCB-144	68194-14-9	0.00198 K	0.00335	0.00295 K	0.00147 K
PCB-145	74472-40-5	*<0.000609*	*<0.000507*	*<0.000506*	*<0.000504*
PCB-146	51908-16-8	0.00528	0.0104 K	0.0111	0.00755 B K
PCB-147 + 149	--	0.0297	0.0396	0.0667	0.0317 B
PCB-148	74472-41-6	*<0.000609*	*<0.000507*	*<0.000601*	*<0.000504*
PCB-15	2050-68-2	0.00784 B K	0.0125 B	0.0125 B K	0.0116 B K
PCB-150	68194-08-1	*<0.000609*	*<0.000507*	*<0.000506*	*<0.000504*
PCB-152	68194-09-2	*<0.000609*	*<0.000507*	*<0.000506*	*<0.000504*
PCB-153 + 168	--	0.0342	0.0475 B	0.0753	0.0386 B
PCB-155	33979-03-2	0.00413 K	0.00398	0.00323 K	0.00181 K
PCB-156 + 157	--	0.00705 B K	0.00923 B	0.014	0.00681 B
PCB-158	74472-42-7	0.00405	0.00571 B	0.00873	0.00544 B
PCB-159	39635-35-3	*<0.000609*	*<0.000626*	*<0.000675*	*<0.000504*
PCB-16	38444-78-9	0.0114 B	0.011 B	0.017	0.0106 B K
PCB-161	74472-43-8	*<0.000609*	*<0.000605*	*<0.000688*	*<0.000504*
PCB-162	39635-34-2	*<0.000609*	*<0.000637*	*<0.000694*	*<0.000504*
PCB-164	74472-45-0	0.00243	0.00372 B	0.00743	0.00305 B K
PCB-165	74472-46-1	*<0.000609*	*<0.000705*	*<0.000764*	*<0.000504*
PCB-167	52663-72-6	0.00183 B	0.00213 B	0.00417 B	0.00251 B
PCB-169	32774-16-6	*<0.000609*	*<0.000644*	*<0.000718*	*<0.000504*
PCB-17	37680-66-3	0.00994 B	0.00994 B	0.0151 B	0.0251 B
PCB-170	35065-30-6	0.00588 K	0.0103 K	0.0151	0.00705 B K
PCB-171 + 173	--	0.00188 K	0.00313	0.00585	0.00221 K
PCB-172	52663-74-8	0.000749 K	0.00134 K	0.0029	0.000577 K
PCB-174	38411-25-5	0.00591	0.0105	0.0165 K	0.00816
PCB-175	40186-70-7	*<0.000616*	*<0.000507*	0.00123 K	*<0.000504*
PCB-176	52663-65-7	*<0.000609*	0.00189	0.00271 K	0.00131
PCB-177	52663-70-4	0.00316	0.00516 B K	0.0098 K	0.00376 B K
PCB-178	52663-67-9	0.00151 K	0.00206 K	0.00375	0.00174 K
PCB-179	52663-64-6	0.00345 K	0.00481 K	0.00789	0.00339
PCB-18 + 30	--	0.0239 B	0.0202 B K	0.0367	0.0249 B
PCB-180 + 193	--	0.0188	0.0253	0.0422	0.0178
PCB-181	74472-47-2	*<0.000657*	*<0.000507*	*<0.000668*	*<0.000504*
PCB-182	60145-23-5	*<0.000609*	*<0.000507*	*<0.000612*	*<0.000504*
PCB-183 + 185	--	0.00586	0.00714 B K	0.0119	0.00648 B
PCB-184	74472-48-3	0.00776	0.00639 K	0.00626	0.00285
PCB-186	74472-49-4	*<0.000609*	*<0.000507*	*<0.000517*	*<0.000504*
PCB-187	52663-68-0	0.00983 B	0.0134 B	0.0231	0.0095 B
PCB-188	74487-85-7	*<0.000609*	*<0.000507*	*<0.000547*	*<0.000504*
PCB-189	39635-31-9	*<0.000609*	0.000808 K	0.000745 K	0.000712 K
PCB-19	38444-73-4	0.0049 B K	0.00426 B	0.0101 B	0.00659 B
PCB-190	41411-64-7	0.00134 K	0.00183 K	0.00269	*<0.000504*

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PCB-191	74472-50-7	*<0.000609*	*<0.000507*	*<0.000508*	*<0.000504*
PCB-192	74472-51-8	*<0.000609*	*<0.000507*	*<0.000562*	*<0.000504*
PCB-194	35694-08-7	0.00277 K	0.00628 K	0.0074 K	0.00388
PCB-195	52663-78-2	0.000962 K	0.00145	0.00336	0.00151 K
PCB-196	42740-50-1	0.0015 K	0.00241 K	0.0049	0.00163 K
PCB-197 + 200	--	*<0.000609*	0.00159 K	0.00188	0.000853 K
PCB-198 + 199	--	0.0041	0.00688 B K	0.0115	0.00514 B
PCB-2	2051-61-8	0.0015 B K	*<0.00263 B*	0.00167 B K	*<0.00263 B*
PCB-20 + 28	--	0.0192 B	0.0294 B	0.04	0.0345 B
PCB-201	40186-71-8	*<0.000609*	0.000869 K	0.000851 K	*<0.000504*
PCB-202	2136-99-4	0.00119	0.00237	0.00351	0.0019 K
PCB-203	52663-76-0	0.00203 K	0.0041 B	0.00643	0.00222 B K
PCB-204	74472-52-9	*<0.000609*	*<0.000507*	*<0.000506*	*<0.000504*
PCB-205	74472-53-0	*<0.000609*	*<0.000507*	0.000777 K	*<0.000504*
PCB-206	40186-72-9	0.00246 K	0.00321	0.00545	0.00277
PCB-207	52663-79-3	*<0.0011*	*<0.0017*	*<0.00139*	*<0.00098*
PCB-208	52663-77-1	0.00139 K	0.00201 K	0.00173 K	0.00146
PCB-209	2051-24-3	0.00318 B	0.00391 B	0.00455 B K	*<0.00193 B*
PCB-21 + 33	--	0.0111 B	0.0147 B	0.0112 B K	0.0178 B
PCB-22	38444-85-8	0.00793 B K	0.0125 B	0.017	0.0132 B
PCB-23	55720-44-0	*<0.000614*	*<0.000507*	*<0.000654*	*<0.000634*
PCB-24	55702-45-9	*<0.000609*	0.000728 K	*<0.000516*	*<0.000504*
PCB-25	55712-37-3	0.00147 K	0.0022 B	0.00365 K	0.00414 B
PCB-26 + 29	--	0.00347 B	0.0052 B	0.00863 B	0.00652 B
PCB-27	38444-76-7	0.00128	0.00125 B	0.0033	0.00254 B K
PCB-3	2051-62-9	0.0041 B K	0.0051 B K	*<0.0049 B*	*<0.00657 B*
PCB-31	16606-02-3	0.019 B	0.0274 B	0.036	0.0287 B
PCB-32	38444-77-8	0.00671 B	0.0063 B	0.0114	0.00828 B
PCB-34	37680-68-5	*<0.000609*	*<0.000507*	*<0.000622*	*<0.000613*
PCB-35	37680-69-6	0.0011 B K	0.00229 B	0.00156 B	0.00155 B
PCB-36	38444-87-0	*<0.000609*	*<0.000507*	*<0.000595*	*<0.000536*
PCB-37	38444-90-5	0.0043 B	0.00762 B	0.0107 B	0.0083 B
PCB-38	53555-66-1	*<0.000609*	*<0.000507*	*<0.000612*	*<0.000599*
PCB-39	38444-88-1	*<0.000609*	*<0.000507*	*<0.000592*	*<0.000569*
PCB-4	13029-08-8	0.0329	0.0177 B	0.0453	0.0272 B
PCB-40 + 41 + 71	--	0.0112 B	0.0126 B	0.0202 K	0.0129 B
PCB-42	36559-22-5	0.00536	0.00489 B	0.00874 K	0.00823 B
PCB-43	70362-46-8	*<0.000979*	0.00112	0.0013 K	0.000538
PCB-44 + 47 + 65	--	0.0472 B	0.0538 B	0.0604 B	0.217 B
PCB-45 + 51	--	0.00948 B	0.00826 B	0.0132 B	0.121
PCB-46	41464-47-5	0.00107 K	0.00183 B K	0.002 K	0.00295 B K
PCB-48	70362-47-9	0.00465 B	0.00469 B	0.00684	0.00497 B
PCB-49 + 69	--	0.0127 B	0.0145 B	0.0256	0.0195 B
PCB-5	16605-91-7	*<0.00226*	0.00168 B K	*<0.0022*	*<0.00108*
PCB-50 + 53	--	0.00277	0.00381 B K	0.00881	0.00591 B K

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PCB-52	35693-99-3	0.0356	0.045 B	0.066	0.0444 B
PCB-54	15968-05-5	*<0.00081*	*<0.000507*	0.00137 K	0.000694 K
PCB-55	74338-24-2	*<0.00093*	*<0.000647*	*<0.00108*	*<0.00094*
PCB-56	41464-43-1	0.00592 B	0.00767 B	0.0152	0.00706 B
PCB-57	70424-67-8	*<0.00091*	*<0.000643*	*<0.00105*	*<0.000933*
PCB-58	41464-49-7	*<0.000898*	*<0.000662*	*<0.00104*	*<0.000962*
PCB-59 + 62 + 75	--	0.00143	0.00239 B	0.00223 K	0.0027 B K
PCB-6	25569-80-6	0.00582 K	0.00571 B	0.0111	0.0111 B
PCB-60	33025-41-1	0.00398	0.00457 B	0.00851	0.00344 B K
PCB-61 + 70 + 74 + 76	--	0.0381 B	0.0413 B	0.0801	0.0425 B
PCB-63	74472-34-7	*<0.000847*	0.000851	0.00102 K	0.00109 K
PCB-64	52663-58-8	0.00846 B	0.00994 B	0.015	0.0105 B
PCB-66	32598-10-0	0.0137 B	0.0171 B	0.029	0.0178 B
PCB-67	73575-53-8	*<0.000764*	0.000578 K	*<0.000883*	*<0.000802*
PCB-68	73575-52-7	0.00226 B K	0.00263 B	0.00268 B K	0.0706
PCB-7	33284-50-3	*<0.07629 B*	*<0.07629 B*	0.00252 B K	0.00282 B K
PCB-72	41464-42-0	*<0.000825*	*<0.000615*	*<0.000954*	*<0.000894*
PCB-73	74338-23-1	*<0.000623*	*<0.000507*	*<0.000583*	*<0.000504*
PCB-77	32598-13-3	0.00145 B	0.00162 B	0.00368 B	0.00134 B K
PCB-78	70362-49-1	*<0.000918*	*<0.000641*	*<0.00106*	*<0.000932*
PCB-79	41464-48-6	*<0.000733*	0.000933 K	*<0.000848*	*<0.000781*
PCB-8	34883-43-7	0.0185 B	0.0158 B	0.0207 B	0.0167 B
PCB-80	33284-52-5	*<0.00079*	*<0.00057*	*<0.000914*	*<0.000828*
PCB-81	70362-50-4	*<0.000899*	*<0.000691*	*<0.00103*	*<0.00102*
PCB-82	52663-62-4	0.0053	0.006 B	0.0122	0.00679 K
PCB-83 + 99	--	0.0264	0.0275 B	0.0517	0.027 B
PCB-84	52663-60-2	0.0113 B	0.0138 K	0.0241	0.012 B
PCB-85 + 116 + 117	--	0.00734 B K	0.0087 B	0.0146 K	0.00865 B
PCB-86 + 87 + 97 + 109 + 119 + 125	--	0.0332 B	0.0405 B	0.069	0.0361 B
PCB-88 + 91	--	0.00482	0.00772	0.0131	0.00729 K
PCB-89	73575-57-2	*<0.000737*	*<0.000507*	*<0.000805*	*<0.000504*
PCB-9	34883-39-1	0.0024	0.00327 B K	0.00289	0.00286 B K
PCB-90 + 101 + 113	--	0.0466	0.0539 B	0.102	0.0487 B
PCB-92	52663-61-3	0.00716	0.00908 B	0.0159	0.00884 B
PCB-93 + 95 + 98 + 100 + 102	--	0.0356	0.0463 B	0.076	0.0395 B
PCB-94	73575-55-0	*<0.000757*	*<0.000507*	*<0.000827*	0.00055 K
PCB-96	73575-54-9	*<0.000609*	*<0.000507*	0.000584 K	*<0.000504*
Total Dichloro Biphenyls	--	0.116	0.135	0.143	0.108
Total Heptachloro Biphenyls	--	0.0513	0.0542	0.122	0.0495
Total Hexachloro Biphenyls	--	0.165	0.24	0.396	0.178
Total Monochloro Biphenyls	--	0.00663	0.00824	0.0148	0.0171
Total Nonachloro Biphenyls	--	*NA*	0.00321	0.00545	0.00423
Total Octachloro Biphenyls	--	0.00529	0.00792	0.0316	0.00902
TOTAL PCBs	--	0.94	1.17	1.87	1.44
Total Pentachloro Biphenyls	--	0.281	0.348	0.616	0.313

Analyte	CAS#	South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow
Total Tetrachloro Biphenyls	--	0.202	0.233	0.332	0.577
Total Trichloro Biphenyls	--	0.11	0.134	0.207	0.18
Perfluoroalkyl carboxylates					
PFBA	375-22-4	11.1	9.08	9.13	5.38
PFDA	335-76-2	1.2	1.37	0.677	0.528
PFDoA	307-55-1	*<0.396*	*<0.371*	*<0.395*	*<0.362*
PFHpA	375-85-9	3.59	2.26	4.12	2.27
PFHxA	307-24-4	33.5	22.5	22.8	15.9
PFNA	375-95-1	1.22	0.721	1.94	1.29
PFOA	335-67-1	11.4	5.48	11.7	5.02
PFPeA	2706-90-3	10.5	7.69 B	9.71	5.86 B
PFTeDA	376-06-7	*<0.396*	*<0.371*	*<0.395*	*<0.362*
PFTrDA	72629-94-8	*<0.396*	*<0.371*	*<0.395*	*<0.362*
PFUnA	2058-94-8	*<0.396*	*<0.371*	*<0.395*	*<0.362*
Perfluoroalkyl sulfonates					
PFBS	375-73-5	18	12	20.8	3.08
PFDoS	79780-39-5	*<0.396*	*<0.371*	*<0.395*	*<0.362*
PFDS	335-77-3	*<0.396*	*<0.371*	*<0.395*	*<0.362*
PFHpS	375-92-8	*<0.396*	*<0.371*	6.28	*<0.362*
PFHxS	355-46-4	3.64	5.24	89.3	2.2
PFNS	68259-12-1	*<0.396*	*<0.371*	*<0.395*	*<0.362*
PFOS	1763-23-1	6.37	12.9	127	8.3
PFPeS	2706-91-4	1.42 K	0.639 K	18.8	0.456
Perfluorooctane sulfonamides					
N-EtFOSA	4151-50-2	*<0.991*	*<0.926*	*<0.987*	*<0.904*
N-MeFOSA	31506-32-8	*<0.456*	*<0.426*	*<0.454*	*<0.416*
PFOSA	754-91-6	*<0.396*	*<0.371*	*<0.395*	*<0.362*
EtFOSAA	909405-49-8	0.402	0.979 K	0.493 K	*<0.362*
MeFOSAA	2355-31-9	1.9	2.36 K	1.16	1.06
N-EtFOSE	1691-99-2	*<2.96*	*<2.77*	*<2.95*	*<2.7*
N-MeFOSE	24448-09-7	*<3.96*	*<3.71*	*<3.95*	*<3.62*
Pesticides (MRES)					
2,4'-DDD	53-19-0	0.159 M	0.263 M	*<0.0449*	*<0.0428*
2,4'-DDE	3424-82-6	*<0.0145*	*<0.0143*	*<0.014*	*<0.0129*
2,4'-DDT	789-02-6	*<0.0864*	*<0.0778*	*<0.075*	*<0.0935*
4,4'-DDD	72-54-8	0.11 M	*<0.0488*	*<0.0395*	0.061 K
4,4'-DDE	72-55-9	0.138 M	0.197 B M	0.206 M	1.26 M
4,4'-DDT	50-29-3	*<0.0845*	*<0.0746*	0.165	*<0.0874*
Aldrin	309-00-2	*<0.013*	0.346	*<0.021*	0.023 B K
alpha-Endosulphan	959-98-8	0.757 B K	0.548 B K	0.795 B K	0.548 B K
Ametryn	834-12-8	*<0.26*	*<0.262*	*<0.24*	*<0.148*
Atrazine	1912-24-9	1.23 K	*<0.591*	1.26 K	0.632 K
Azinphos-Methyl	86-50-0	*<1.85*	*<1.96*	*<3.16*	*<1.9*
beta-Endosulphan	33213-65-9	0.348 B K	0.239 B K	0.315 B K	0.228 B K
Captan	133-06-2	*<1.57*	*<0.606*	*<1.46*	*<0.557*

Analyte	CAS#	South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow
Chlordane, alpha (cis)	5103-71-9	*<0.0388*	0.045	*<0.0444*	0.043 K
Chlordane, gamma (trans)	5103-74-2	0.072 K	0.081 K	0.231 K	0.276 K
Chlordane, oxy-	27304-13-8	*<0.0431*	0.039 B K	*<0.0736*	0.05 B K
Chlorothalonil	1897-45-6	*<0.115*	*<0.104*	*<0.103*	*<0.102*
Chlorpyriphos	2921-88-2	0.12 K	0.129	0.224	0.31
Chlorpyriphos-Methyl	5598-13-0	*<0.115*	*<0.104*	*<0.103*	*<0.102*
Chlorpyriphos-Oxon	5598-15-2	*<0.132*	*<0.104*	*<0.103*	*<0.102*
Cyanazine	21725-46-2	*<0.964*	*<1.07*	*<1.39*	*<0.92*
Cypermethrin	52315-07-8	1.27	1.99	1.98	1.17
Dacthal	1861-32-1	*<0.115*	*<0.104*	*<0.103*	*<0.102*
Desethylatrazine	6190-65-4	*<0.216*	*<0.257*	*<0.655*	*<0.566*
Diazinon	333-41-5	0.738	0.569	0.683 K	*<0.367*
Diazinon-Oxon	962-58-3	*<0.627*	*<0.303*	*<0.447*	*<0.374*
Dieldrin	60-57-1	0.195 B	0.433	0.316	0.164
Dimethoate	60-51-5	*<0.646*	*<0.633*	*<0.439*	*<0.514*
Disulfoton	298-04-4	*<0.165*	*<0.154*	*<0.163*	*<0.221*
Disulfoton Sulfone	2497-06-5	*<0.142*	*<0.104*	*<0.146*	*<0.102*
Endosulphan Sulphate	1031-07-8	*<0.091*	*<0.126*	*<0.122*	*<0.0822*
Endrin	72-20-8	0.071 B K	0.122 B K	0.068 B K	0.091 B K
Endrin Ketone	53494-70-5	*<0.0433*	*<0.0665*	*<0.0819*	*<0.119*
Ethion	563-12-2	*<0.143*	*<0.106*	*<0.117*	*<0.102*
Fenitrothion	122-14-5	*<0.137*	*<0.14*	*<0.127*	*<0.127*
Fonofos	944-22-9	*<0.115*	*<0.104*	*<0.103*	*<0.102*
HCH, alpha	319-84-6	*<0.0245*	0.014	*<0.0281*	0.018
HCH, beta	319-85-7	0.155	0.202 B	0.072 K	0.102 B
HCH, delta	319-86-8	*<0.0278*	*<0.011*	*<0.0263*	*<0.0134*
HCH, gamma	58-89-9	0.153	0.158	0.165 K	0.156 K
Heptachlor	76-44-8	*<0.0115*	0.011 K	0.019 K	0.014 K
Heptachlor Epoxide	1024-57-3	0.131 B K	0.052 B K	0.131 B K	0.055 B K
Hexachlorobenzene	118-74-1	0.044 B	0.046 B	0.052 B	0.037 B
Hexazinone	51235-04-2	*<0.602*	*<1.38*	*<1.04*	*<1.17*
Malathion	121-75-5	*<0.273*	*<0.234*	*<0.18*	*<0.234*
Methoxychlor	72-43-5	*<0.594*	*<0.641*	*<0.93*	*<0.529*
Metribuzin	21087-64-9	*<0.628*	*<0.848*	*<0.779*	*<0.391*
Mirex	2385-85-5	*<0.013*	0.013 K	*<0.0204*	0.021 K
Nonachlor, cis-	5103-73-1	*<0.0391*	0.059 B	*<0.0837*	0.053 B K
Nonachlor, trans-	39765-80-5	0.063 B	0.046	0.063 B K	0.148
Octachlorostyrene	29082-74-4	*<0.0115*	*<0.0104*	*<0.0103*	*<0.0102*
Parathion-Ethyl	56-38-2	*<0.141*	*<0.163*	*<0.149*	*<0.137*
Parathion-Methyl	298-00-0	*<0.686*	*<0.592*	*<0.882*	*<0.764*
Permethrin	52645-53-1	11.1	14.5	9.9	4.58
Perthane	72-56-0	*<0.967*	*<0.948*	*<11.2*	*<1.08*
Phorate	298-02-2	*<0.115*	*<0.104*	*<0.103*	*<0.102*
Phosmet	732-11-6	*<0.307*	*<0.263*	*<0.354*	*<0.267*
Pirimiphos-Methyl	29232-93-7	*<0.115*	*<0.104*	*<0.103*	*<0.102*

Analyte	CAS#	South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow
Quintozene	82-68-8	*<0.115*	*<0.104*	0.641	*<0.102*
Simazine	122-34-9	0.642	0.873 K	1.6	*<0.465*
Tecnazene	117-18-0	*<0.115*	*<0.104*	*<0.103*	*<0.102*
Terbufos	13071-79-9	*<0.115*	*<0.104*	*<0.103*	*<0.102*
Phthalate					
MBP	--	*<303*	*NA*	*<304*	*NA*
MBzP	2528-16-7	*<101*	*NA*	*<101*	*NA*
MCHP	7517-36-4	*<101*	*NA*	*<101*	*NA*
MCPP	66851-46-5	*<101*	*NA*	*<101*	*NA*
MECPP	40809-41-4	*<101*	*NA*	*<101*	*NA*
MEHHP	40321-99-1	*<151*	*NA*	*<152*	*NA*
MEHP	4376-20-9	*<101*	*NA*	*<101*	*NA*
MEOHP	40321-98-0	*<202*	*NA*	*<203*	*NA*
MEP	2306-33-4	*<202*	*NA*	*<203*	*NA*
MiNP	106610-61-1	*<101*	*NA*	*<101*	*NA*
MMP	4376-18-5	*<202*	*NA*	*<203*	*NA*
Pharmaceuticals and Personal Care Products (PPCP)					
1,7-Dimethylxanthine	611-59-6	327	190	4360	399
10-hydroxy-amitriptyline	1159-82-6	16.3	11.9	10.5	12.1
2-Hydroxy-ibuprofen	51146-55-5	1070	149	3450	578
4-Epianhydrochlortetracycline [EACTC]	81163-11-3	*<120*	*<59.4*	*<101*	*<58.4*
4-Epianhydrotetracycline [EATC]	7518-17-4	*<15.9*	*<14.8*	*<14.3*	*<14.6*
4-Epichlortetracycline [ECTC]	14297-93-9	*<15.9*	*<14.8*	*<14.3*	*<14.6*
4-Epioxytetracycline [EOTC]	14206-58-7	*<6.34*	*<5.94*	*<5.74*	*<5.84*
4-Epitetracycline [ETC]	79-85-6	*<6.34*	*<5.94*	*<5.74*	*<5.84*
Acetaminophen	103-90-2	*<15.9*	*<14.8*	159	*<14.6*
Albuterol	18559-94-9	10.8	12.9	11.8	8.63
Alprazolam	28981-97-7	1.54	2.09	1.11	1.54
Amitriptyline	50-48-6	20.9	21.9	15.6	20.3
Amlodipine	88150-42-9	14.1	13.7	12.8	12.8
Amphetamine	300-62-9	3.84	*<0.756*	30.9	4.51
Amsacrine	51264-14-3	*<0.0423*	*<0.0396*	*<0.0383*	*<0.039*
Anhydrochlortetracycline [ACTC]	4497-08-9	*<61.4*	*<14.8*	*<54.3*	*<14.6*
Anhydrotetracycline [ATC]	4496-85-9	*<15.9*	*<14.8*	*<14.3*	*<14.6*
Atenolol	29122-68-7	550	78.8	413	468
Atorvastatin	134523-00-5	16	99.2	6.34	74.4
Azathioprine	446-86-6	*<1.06*	*<0.99*	*<0.956*	*<0.974*
Azithromycin	83905-01-5	352	170	474	227
Benzoylecgonine	519-09-5	371	40.1	384	85
Benztropine	86-13-5	*<0.74*	*<0.693*	*<0.669*	*<0.682*
Betamethasone	378-44-9	*<1.59*	*<1.48*	*<1.43*	*<1.46*
Bisphenol A	80-05-7	2985	963.5	191.5	183
Busulfan	55-98-1	*<2.11*	*<1.98*	*<1.91*	*<1.95*

Analyte	CAS#	South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow
Caffeine	58-08-2	*<15.9*	50.4 B	2760	169 B
Carbadox	6804-07-5	*<3.04*	*<2.63*	*<1.43*	3.39
Carbamazepine	298-46-4	127	206	101	159
Cefotaxime	63527-52-6	*<29.8*	*<297*	*<42.2*	*<292*
Chlortetracycline [CTC]	57-62-5	*<6.34*	*<5.94*	*<5.74*	*<5.84*
Cimetidine	51481-61-9	*<0.588*	*<0.573*	*<0.595*	*<0.547*
Ciprofloxacin	85721-33-1	30.8	40	44.3	54.9
Citalopram	59729-33-8	208	212	207	254
Clarithromycin	81103-11-9	128	142	85.2	94.6
Clinafloxacin	105956-97-6	*<23.3*	*<22.3*	*<11.2*	*<19*
Clonidine	4205-90-7	*<1.18*	*<1.15*	*<1.19*	*<1.09*
Clotrimazole	23593-75-1	0.864	0.706	1.42	1.28
Cloxacillin	61-72-3	24.3	*<2.97*	*<2.87*	*<2.92*
Cocaine	50-36-2	25.4	0.802	50.1	2.73
Codeine	76-57-3	126	93.2	81.3	110
Colchicine	64-86-8	3.21	3.65	1.77	3.04
Cotinine	486-56-6	53.4	24.5	91	62.7
Cyclophosphamide	50-18-0	1.1	2.64	4.97	3.46
Daunorubicin	20830-81-3	*<2.11*	*<1.98*	*<1.91*	*<1.95*
DEET	134-62-3	66.6	829	34.2	110
Dehydronifedipine	67035-22-7	*<2.24*	4	1.37	2.49
Demeclocycline	127-33-3	*<15.9*	*<14.8*	*<14.3*	*<14.6*
Desmethyldiltiazem	86408-45-9	37.2	39.8	25.9	34.8
Diatrizoic acid	117-96-4	5630	9970	12500	14000
Diazepam	439-14-5	0.588	1.08	*<0.48*	0.5
Digoxigenin	1672-46-4	*<22.4*	*<108*	*<22.8*	*<24*
Digoxin	20830-75-5	*<6.34*	*<19.8*	*<5.74*	*<19.5*
Diltiazem	34933-06-7	204	146	157	136
Diphenhydramine	58-73-1	970	941	578	677
Doxorubicin	23214-92-8	*<6.34*	*<5.94*	*<5.74*	*<5.84*
Doxycycline	564-25-0	*<6.34*	23	21.4	16.3
Drospirenone	67392-87-4	*<8.46*	*<7.92*	*<7.65*	*<7.79*
Enalapril	75847-73-3	4.86	*<0.287*	5.31	1.64
Enrofloxacin	93106-60-6	*<3.17*	*<2.97*	*<2.87*	3.76
Erythromycin-H2O	114-07-8	27.9	17.5	28.7	21.7
Etoposide	33419-42-0	*<1.06*	*<0.99*	*<0.956*	*<0.974*
Flumequine	42835-25-6	*<1.59*	5.15	*<1.43*	*<2.34*
Fluocinonide	356-12-7	*<2.13*	*<1.99*	*<1.92*	*<1.96*
Fluoxetine	54910-89-3	25.3	13.6	37.1	46.8
Fluticasone propionate	80474-14-2	*<2.13*	*<1.99*	2.1	2.56
Furosemide	54-31-9	62.2	173	5.04	124
Gemfibrozil	25812-30-0	544	649	281	314
Glipizide	29094-61-9	14.6	16	7.49	7.92
Glyburide	10238-21-8	2.44	2.93	2.65	1.43
Hydrochlorothiazide	58-93-5	1270	1360	775	752

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Hydrocodone	125-29-1	11.8	24.8	8.46	15.6
Hydrocortisone	50-23-7	*<6.34*	*<5.94*	*<5.74*	*<5.84*
Ibuprofen	15687-27-1	228	13.3	524	20.2
Iopamidol	60166-93-0	12100	31000	3900	5770
Isochlortetracycline [ICTC]	514-53-4	*<6.34*	*<5.94*	*<5.74*	*<5.84*
Lincomycin	154-21-2	4.65	5.5	*<2.87*	*<2.92*
Lomefloxacin	98079-51-7	*<3.17*	*<2.97*	*<2.87*	*<2.92*
Medroxyprogesterone Acetate	71-58-9	*<4.23*	*<3.96*	*<3.83*	*<3.9*
Melphalan	148-82-3	*<25.4*	*<23.8*	*<23*	*<23.4*
Meprobamate	57-53-4	53.1	64.5	27.1	26.4
Metformin	657-24-9	35600	2640	34500	30300
Methylprednisolone	83-43-2	*<4.23*	*<4.41*	*<3.83*	*<5.81*
Metoprolol	51384-51-1	549	647	363	424
Metronidazole	443-48-1	162	67.9	152	105
Miconazole	22916-47-8	2.32	2.41	4.99	2.9
Minocycline	10118-90-8	*<63.4*	*<59.4*	*<57.4*	*<58.4*
Moxifloxacin	151096-09-2	*<4.23*	*<3.96*	*<3.83*	5.56
Naproxen	22204-53-1	1190	84	905	241
Norfloxacin	70458-96-7	*<20.5*	*<69.9*	*<14.3*	*<33.7*
Norfluoxetine	83891-03-6	4.4	1.81	5.99	5.06
Norgestimate	35189-28-7	*<4.82*	*<5.09*	*<4.91*	*<3.83*
Norverapamil	67018-85-3	5.27	4.19	3.08	3.77
Ofloxacin	82419-36-1	22.6	48.3	42.6	85.3
Ormetoprim	6981-18-6	*<0.634*	*<0.594*	*<0.574*	*<0.584*
Oxacillin	66-79-5	*<3.17*	*<2.97*	*<2.87*	*<2.92*
Oxazepam	604-75-1	6.97	7.09	5.3	5.4
Oxolinic Acid	14698-29-4	*<6.84*	*<3.98*	3.39	*<1.57*
Oxycodone	76-42-6	36.8	52.3	22.8	38.3
Oxytetracycline [OTC]	79-57-2	*<6.34*	*<5.94*	*<5.74*	*<5.84*
Paroxetine	61869-08-7	3.47	3.36	2.36	4.5
Penicillin G	61-33-6	*<3.17*	*<2.97*	*<2.87*	*<2.92*
Penicillin V	87-08-1	*<3.17*	*<3.24*	*<2.87*	*<2.92*
Prednisolone	50-24-8	*<4.23*	*<3.96*	*<3.83*	*<3.9*
Prednisone	53-03-2	*<6.34*	*<6.06*	*<5.74*	*<5.84*
Promethazine	60-87-7	*<0.317*	0.36	*<0.287*	*<0.292*
Propoxyphene	469-62-5	*<0.317*	*<0.297*	*<0.287*	*<0.292*
Propranolol	525-66-6	71.9	72.3	51.8	81.1
Ranitidine	66357-35-5	5.07	2.92	*<0.595*	*<0.547*
Rosuvastatin	287714-41-4	511	358	334	291
Roxithromycin	80214-83-1	2.76	*<0.655*	3.14	1.39
Sarafloxacin	98105-99-8	*<15.9*	*<14.8*	*<14.3*	*<14.6*
Sertraline	79617-96-2	49	45.7	69.2	98.5
Simvastatin	79902-63-9	*<2.13*	*<1.99*	*<1.92*	*<1.96*
Sulfachloropyridazine	80-32-0	*<1.59*	*<1.99*	*<1.43*	*<1.46*
Sulfadiazine	68-35-9	*<1.64*	10	1.44	*<1.46*

Analyte	CAS#	South Plant High Flow	South Plant Low Flow	West Point High Flow	West Point Low Flow
Sulfadimethoxine	122-11-2	*<8.21*	*<0.297*	*<14.9*	*<14.5*
Sulfamerazine	127-79-7	*<1.09*	*<1.38*	*<0.873*	*<0.973*
Sulfamethazine	57-68-1	*<4.84*	*<0.594*	*<2.65*	*<0.584*
Sulfamethizole	144-82-1	*<0.634*	*<0.594*	*<0.574*	*<0.584*
Sulfamethoxazole	723-46-6	259	370	175	193
Sulfanilamide	63-74-1	30.2	74.6	*<14.3*	31.7
Sulfathiazole	72-14-0	*<1.59*	*<3.74*	*<1.43*	*<1.46*
Tamoxifen	10540-29-1	*<0.423*	*<0.396*	*<0.383*	*<0.39*
Teniposide	29767-20-2	*<4.23*	*<3.96*	*<3.83*	*<3.9*
Tetracycline [TC]	60-54-8	*<6.34*	*<5.94*	*<5.74*	*<5.84*
Theophylline	58-55-9	293	237	7410	436
Thiabendazole	148-79-8	32.4	33.1	67.7	29.7
Trenbolone	10161-33-8	*<2.13*	*<1.99*	*<1.92*	*<1.96*
Trenbolone acetate	10161-34-9	*<0.317*	*<0.297*	*<0.287*	*<0.292*
Triamterene	396-01-0	68.2	108	42	51
Triclocarban	101-20-2	2.51	2.5	*<0.383*	*<0.39*
Triclosan	3380-34-5	21.4	27.6	12.3	12
Trimethoprim	738-70-5	255	272	242	249
Tylosin	1401-69-0	6.96	*<5.94*	7.78	15.7
Valsartan	137862-53-4	729	968	412	590
Venlafaxine	93413-69-5	337	406	313	392
Verapamil	52-53-9	16	10.6	13.8	13.4
Virginiamycin M1	21411-53-0	*<14*	*<5.35*	*<10.5*	*<6.07*
Warfarin	81-81-2	*<0.423*	*<1.19*	*<0.383*	*<0.39*
Zidovudine	30516-87-1	132	120	21.5	52.1

This table includes all chemicals analyzed by SGS-AXYS. NA = Not Analyzed. All analytes with "<" were below their reporting limit (value shown) for that sample and considered as not quantifiable. Flags denoted by letters following detection values are defined as follows:

B: Analyte found in associated blank and concentration in sample is less than 10X the concentration in the associated blank.

M: Concentration is an estimated maximum value.

K: Peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration.

NQ: Not quantified.

Table A2. Targeted chemistry data from SGS-AXYS for estuarine water samples. All detected concentrations in ng/L.

Analyte	CAS#	Alki	Elliott Bay	Reference Site	West Point North - Deep	West Point North - Shallow	West point South
Alkylphenol							
4-n-Octylphenol	1806-26-4	*<1.63*	*<0.988*	*<1.25*	*<0.868*	*<0.554*	*<0.987*
4-Nonylphenol diethoxylates	20427-84-3	*<5.35*	*<3.85*	*<5.7*	*<3.54*	*<2.65*	*<8.45*
4-Nonylphenol monoethoxylates	104-35-8	*<9.08*	*<7.22*	*<8.9*	*<7.75*	*<5.4*	*<13.9*
4-Nonylphenols	104-40-5	*<2.65*	*<4.83*	*<4.06*	*<1.77*	*<2.11*	*<3.22*
Bisphenol							
Bisphenol AF	1478-61-1	*<1.95*	*<1.96*	*<1.97*	*<1.94*	*<1.93*	*<1.97*
Bisphenol B	77-40-7	*<1.95*	*<1.96*	*<1.97*	*<1.94*	*<1.93*	*<1.97*
Bisphenol E	2081-08-5	*<4.87*	*<4.91*	*<4.92*	*<4.85*	*<4.82*	*<4.92*
Bisphenol F	620-92-8	*<4.87*	*<4.91*	*<4.92*	*<4.85*	*<4.82*	*<4.92*
Bisphenol S	80-09-1	*<12.5 B*	*<12.5 B*	*<12.5 B*	*<12.5 B*	*<12.5 B*	*<12.5 B*
Bisphenol A	80-05-7	*<1.95*	*<1.96*	*<1.97*	*<1.94*	*<1.93*	2.05
Ether carboxylates							
ADONA	958445-44-8	*<1.53*	*<1.54*	*<1.54*	*<1.52*	*<1.52*	*<1.53*
HFPO-DA	13252-13-6	*<1.45*	*<1.46*	*<1.46*	*<1.45*	*<1.45*	*<1.45*
NFDHA	151772-58-6	*<0.765*	*<0.771*	*<0.769*	*<0.762*	*<0.762*	*<0.764*
PFMBA	--	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFMPA	--	*<0.765*	*<0.771*	*<0.769*	*<0.762*	*<0.762*	*<0.764*
Ether sulfonates							
11Cl-PF3OUdS	763051-92-9	*<1.53*	*<1.54*	*<1.54*	*<1.53*	*<1.52*	*<1.53*
9Cl-PF3ONS	--	*<1.53*	*<1.55*	*<1.54*	*<1.53*	*<1.53*	*<1.53*
PFEESA	113507-82-7	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
Fluorotelomer carboxylates							
3:3 FTCA	356-02-5	*<1.53*	*<1.54*	*<1.54*	*<1.52*	*<1.52*	*<1.53*
5:3 FTCA	914637-49-3	*<9.57*	*<9.64*	*<9.61*	*<9.52*	*<9.52*	*<9.55*
7:3 FTCA	812-70-4	*<9.57*	*<9.64*	*<9.61*	*<9.52*	*<9.52*	*<9.55*
Fluorotelomer sulfonates							
4:2 FTS	757124-72-4	*<1.53*	*<1.54*	*<1.54*	*<1.52*	*<1.52*	*<1.53*
6:2 FTS	27619-97-2	*<1.38*	*<1.39*	3.56	*<1.37*	*<1.37*	*<1.38*
8:2 FTS	39108-34-4	*<1.53*	*<1.54*	*<1.54*	*<1.52*	*<1.52*	*<1.53*

Analyte	CAS#	Alki	Elliott Bay	Reference Site	West Point North - Deep	West Point North - Shallow	West point South
Halogenated flame retardant							
1,2,4,5/1,2,3,5-TBB	--	*<0.0281*	*<0.0297*	*<0.0253*	*<0.033*	*<0.0389*	*<0.0301*
1,2,4-TriBB	615-54-3	*<0.448*	*<0.454*	*<0.455*	*<0.655*	*<0.303*	*<0.309*
1,2-DiBB	583-53-9	*<0.0738*	*<0.0992*	*<0.092*	*<0.115*	*<0.0601*	*<0.0916*
1,4-DiBB	106-37-6	*<0.138*	*<0.185*	*<0.172*	*<0.214*	*<0.112*	*<0.171*
ATE	3278-89-5	*<0.0522*	*<0.0468*	*<0.047*	*<0.0579*	*<0.0622*	*<0.0592*
BATE	99717-56-3	*<0.129*	*<0.215*	*<0.175*	*<0.247*	*<0.171*	*<0.261*
BEHTBP	26040-51-7	*<0.37*	*<0.237*	*<0.416*	*<0.233*	*<0.304*	*<0.399*
BTBPE	37853-59-1	*<0.593*	*<0.534*	*<0.941*	*<0.78*	*<0.988*	*<1.22*
Dec 602	31107-44-5	*<0.009*	*<0.0069*	*<0.0067*	*<0.0069*	*<0.0094*	*<0.0077*
Dec 603	13560-92-4	*<0.0063*	*<0.0057*	*<0.0082*	*<0.0055*	*<0.0115*	*<0.0075*
Dec 604	34571-16-9	*<0.525*	*<0.26*	*<0.278*	*<0.278*	*<0.334*	*<0.397*
Dechlorane	2385-85-5	*<0.1 B K*	*<0.1 B*	*<0.1 B*	*<0.1 B K*	*<0.0298*	*<0.1 B*
DP Anti	135821-74-8	*<0.179*	*<0.117*	*<0.176*	*<0.189*	0.108	*<0.092*
DP Syn	135821-03-3	*<0.129*	*<0.0806*	*<0.123*	*<0.133*	*<0.0431*	*<0.0638*
DPTE	35109-60-5	*<0.608*	*<0.666*	*<0.626*	*<0.318*	*<0.302*	*<0.631*
EHTBB	183658-27-7	*<0.239*	*<0.154*	*<0.251*	*<0.143*	*<0.155*	*<0.202*
HBB	87-82-1	*<0.0306*	*<0.0277*	*<0.05 B*	*<0.0252*	*<0.05 B*	*<0.0312*
HCDBCO	51936-55-1	*<0.044*	*<0.0303*	*<0.0453*	*<0.053*	*<0.034*	*<0.0567*
PBBB	38521-51-6	*<0.0644*	*<0.431*	*<0.101*	*<0.348*	*<0.0831*	*<0.148*
PBBZ	608-90-2	*<0.0524*	*<0.0294*	*<0.0358*	*<0.0307*	*<0.0265*	*<0.041*
PBEB	85-22-3	*<0.0195*	*<0.0297*	*<0.0241*	*<0.0286*	*<0.0189*	*<0.0304*
PBT	87-83-2	0.033 B M	0.047 B M	0.046 B M	0.107 B M	*<0.0283*	0.084 B M
pTBX	23488-38-2	*<0.953*	*<1.35*	*<1.18*	*<0.817*	*<0.68*	*<1.29*
TBCT	39569-21-6	*<0.0285*	*<0.0451*	*<0.0314*	*<0.033*	*<0.0276*	*<0.0381*
Total TBECH	3322-93-8	*<0.582*	*<0.482*	*<0.39*	*<0.553*	*<0.43*	*<0.499*
Hormone							
17 alpha-Dihydroequilin	651-55-8	*<1.94*	*<1.88*	*<1.96*	*<1.87*	*<1.92*	*<1.91*
17 alpha-Estradiol	57-91-0	*<7.77*	*<7.5*	*<7.84*	*<7.46*	*<7.68*	*<7.62*
17 alpha-Ethinyl-Estradiol	57-63-6	*<4.86*	*<4.69*	*<4.9*	*<4.66*	*<4.8*	*<4.77*
17 beta-Estradiol	50-28-2	*<3.89*	*<3.75*	*<3.92*	*<3.73*	*<3.84*	*<3.81*

Analyte	CAS#	Alki	Elliott Bay	Reference Site	West Point North - Deep	West Point North - Shallow	West point South
17 beta-Estradiol 3-benzoate	50-50-0	*<0.777*	*<0.75*	*<0.784*	*<0.746*	*<0.768*	*<0.762*
Allyl Trenbolone	850-52-2	*<0.385*	*<0.371*	*<0.388*	*<0.369*	*<0.38*	*<0.377*
Androstenedione	63-05-8	*<0.971*	5.63 K	*<0.98*	*<0.933*	*<0.96*	*<0.953*
Androsterone	53-41-8	*<19.4*	*<23.2*	*<19.6*	*<18.7*	*<20.1*	*<24*
Desogestrel	54024-22-5	*<156*	*<52.1*	*<40.8*	*<108*	*<39.9*	*<89.2*
Equilenin	517-09-9	*<0.389*	*<0.375*	*<0.392*	*<0.373*	*<0.384*	*<0.381*
Equilin	474-86-2	*<1.94*	*<1.88*	*<1.96*	*<1.87*	*<1.92*	*<1.91*
Estriol	50-27-1	*<7.77*	*<7.5*	*<7.84*	*<7.46*	*<7.68*	*<7.62*
Estrone	53-16-7	*<3.11*	*<3*	*<3.14*	*<2.98*	*<3.07*	*<3.05*
Mestranol	72-33-3	*<19.4*	*<18.8*	*<19.6*	*<18.7*	*<19.2*	*<19.1*
Norethindrone	68-22-4	*<0.971*	*<0.938*	*<0.98*	*<0.933*	*<0.96*	*<0.953*
Norgestrel	797-63-7	*<0.971*	*<0.938*	*<0.98*	*<0.933*	*<0.96*	*<0.953*
Progesterone	57-83-0	*<0.389*	*<0.375*	*<0.392*	*<0.373*	*<0.384*	*<0.381*
Testosterone	58-22-0	*<0.389*	*<0.375*	*<0.392*	*<0.373*	*<0.384*	*<0.381*
Perfluoroalkyl carboxylates							
PFBA	375-22-4	*<1.53*	*<1.54*	*<1.54*	*<1.52*	*<1.52*	*<1.53*
PFDA	335-76-2	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFDoA	307-55-1	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFHpA	375-85-9	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFHxA	307-24-4	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFNA	375-95-1	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFOA	335-67-1	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFPeA	2706-90-3	*<0.765*	*<0.771*	*<0.769*	*<0.762*	*<0.762*	*<0.764*
PFTeDA	376-06-7	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFTrDA	72629-94-8	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFUnA	2058-94-8	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFBS	375-73-5	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFDoS	79780-39-5	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFDS	335-77-3	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFHpS	375-92-8	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFHxS	355-46-4	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*

Analyte	CAS#	Alki	Elliott Bay	Reference Site	West Point North - Deep	West Point North - Shallow	West point South
PFNS	68259-12-1	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFOS	1763-23-1	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
PFPeS	2706-91-4	*<0.385*	*<0.387*	*<0.386*	*<0.383*	*<0.383*	*<0.384*
N-EtFOSA	4151-50-2	*<0.957*	*<0.964*	*<0.961*	*<0.952*	*<0.952*	*<0.955*
N-MeFOSA	31506-32-8	*<0.44*	*<0.443*	*<0.442*	*<0.438*	*<0.438*	*<0.439*
PFOSA	754-91-6	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
EtFOSAA	909405-49-8	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
MeFOSAA	2355-31-9	*<0.383*	*<0.385*	*<0.384*	*<0.381*	*<0.381*	*<0.382*
N-EtFOSE	1691-99-2	*<2.86*	*<2.88*	*<2.87*	*<2.85*	*<2.85*	*<2.86*
N-MeFOSE	24448-09-7	*<3.83*	*<3.85*	*<3.84*	*<3.81*	*<3.81*	*<3.82*
Phthalate							
MBP	--	*<298*	*<298*	*<298*	*<298*	*<299*	*<299*
MBzP	2528-16-7	*<99.4*	*<99.2*	*<99.4*	*<99.4*	*<99.8*	*<99.6*
MCHP	7517-36-4	*<99.4*	*<99.2*	*<99.4*	*<99.4*	*<99.8*	*<99.6*
MCPP	66851-46-5	*<99.4*	*<99.2*	*<99.4*	*<99.4*	*<99.8*	*<99.6*
MECPP	40809-41-4	*<99.4*	*<99.2*	*<99.4*	*<99.4*	*<99.8*	*<99.6*
MEHHP	40321-99-1	*<149*	*<149*	*<149*	*<149*	*<150*	*<149*
MEHP	4376-20-9	*<99.4*	*<99.2*	*<99.4*	*<99.4*	*<99.8*	*<99.6*
MEOHP	40321-98-0	*<199*	*<198*	*<199*	*<199*	*<200*	*<199*
MEP	2306-33-4	*<199*	*<198*	*<199*	*<199*	*<200*	*<199*
MiNP	106610-61-1	*<99.4*	*<99.2*	*<99.4*	*<99.4*	*<99.8*	*<99.6*
MMP	4376-18-5	*<199*	*<198*	*<199*	*<199*	*<200*	*<199*
Pharmaceuticals and Personcal Care Products (PPCP)							
1,7-Dimethylxanthine	611-59-6	*<59.5*	*<59.9*	*<59.9*	*<59.6*	*<58.7*	*<59.8*
10-hydroxy-amitriptyline	1159-82-6	*<0.149*	*<0.15*	*<0.15*	*<0.149*	*<0.147*	*<0.149*
2-Hydroxy-ibuprofen	51146-55-5	*<3.97*	*<3.99*	*<3.99*	*<3.98*	*<3.92*	*<3.99*
4-Epianhydrochlortetracycline [EACTC]	81163-11-3	*NQ*	*NQ*	*NQ*	*NQ*	*NQ*	*NQ*
4-Epianhydrotetracycline [EATC]	7518-17-4	*<14.9*	*<15*	*<15.3*	*<14.9*	*<14.7*	*<14.9*
4-Epichlortetracycline [ECTC]	14297-93-9	*<14.9*	*<15*	*<15*	*<14.9*	*<14.7*	*<14.9*
4-Epioxytetracycline [EOTC]	14206-58-7	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*

Analyte	CAS#	Alki	Elliott Bay	Reference Site	West Point North - Deep	West Point North - Shallow	West point South
4-Epitetracycline [ETC]	79-85-6	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Acetaminophen	103-90-2	*<14.9*	*<15*	*<15*	*<14.9*	*<14.7*	*<14.9*
Albuterol	18559-94-9	*<0.298*	*<0.293*	*<0.292*	*<0.295*	*<0.295*	*<0.288*
Alprazolam	28981-97-7	*<0.297*	*<0.3*	*<0.3*	*<0.298*	*<0.294*	*<0.299*
Amitriptyline	50-48-6	*<0.297*	*<0.3*	*<0.3*	*<0.298*	*<0.294*	*<0.299*
Amlodipine	88150-42-9	*<0.998*	*<1*	*<1*	*<1*	*<0.985*	*<1*
Amphetamine	300-62-9	*<0.298*	*<0.293*	*<0.292*	*<0.295*	*<0.295*	*<0.288*
Amsacrine	51264-14-3	*<0.0397*	*<0.0399*	*<0.0399*	*<0.0398*	*<0.0392*	*<0.0399*
Anhydrochlortetracycline [ACTC]	4497-08-9	*NQ*	*NQ*	*NQ*	*NQ*	*NQ*	*NQ*
Anhydrotetracycline [ATC]	4496-85-9	*<14.9*	*<15*	*<15*	*<14.9*	*<14.7*	*<14.9*
Atenolol	29122-68-7	0.332	0.39	0.309	0.552	0.382	0.428
Atorvastatin	134523-00-5	*<1.19*	*<1.17*	*<1.17*	*<1.18*	*<1.18*	*<1.15*
Azathioprine	446-86-6	*<0.992*	*<0.999*	*<0.998*	*<0.994*	*<0.979*	*<0.996*
Azithromycin	83905-01-5	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.47*	*<1.54*
Benzoylecgonine	519-09-5	0.303	0.403	0.326	0.421	0.337	0.377
Benztropine	86-13-5	*<0.694*	*<0.699*	*<0.699*	*<0.696*	*<0.685*	*<0.697*
Betamethasone	378-44-9	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.47*	*<1.49*
Bisphenol A	80-05-7	*<1.95*	*<1.96*	*<1.97*	*<1.94*	*<1.93*	2.05
Busulfan	55-98-1	*<1.98*	*<2*	*<2*	*<1.99*	*<1.96*	*<1.99*
Caffeine	58-08-2	*<14.9*	*<15*	*<15*	*<14.9*	*<14.7*	*<14.9*
Carbadox	6804-07-5	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.47*	*<1.49*
Carbamazepine	298-46-4	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.47*	*<1.49*
Cefotaxime	63527-52-6	*<19.8*	*<20*	*<20*	*<19.9*	*<19.6*	*<19.9*
Chlortetracycline [CTC]	57-62-5	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Cimetidine	51481-61-9	*<0.596*	*<0.586*	*<0.583*	*<0.59*	*<0.59*	*<0.575*
Ciprofloxacin	85721-33-1	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Citalopram	59729-33-8	*<0.397*	*<0.399*	0.449	*<0.398*	*<0.392*	*<0.399*
Clarithromycin	81103-11-9	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.47*	*<1.49*
Clinafloxacin	105956-97-6	*<5.97*	*<5.99*	*<10.2*	*<8.61*	*<5.87*	*<8.78*
Clonidine	4205-90-7	*<1.19*	*<1.17*	*<1.17*	*<1.18*	*<1.18*	*<1.15*

Analyte	CAS#	Alki	Elliott Bay	Reference Site	West Point North - Deep	West Point North - Shallow	West point South
Clotrimazole	23593-75-1	*<0.397*	*<0.399*	*<0.399*	*<0.398*	*<0.392*	*<0.399*
Cloxacillin	61-72-3	*<2.97*	*<3*	*<3*	*<2.98*	*<2.94*	*<2.99*
Cocaine	50-36-2	*<0.149*	*<0.15*	*<0.15*	*<0.149*	*<0.147*	*<0.149*
Codeine	76-57-3	*<1.19*	*<1.17*	*<1.17*	*<1.18*	*<1.18*	*<1.15*
Colchicine	64-86-8	*<0.793*	*<0.799*	*<0.799*	*<0.795*	*<0.783*	*<0.797*
Cotinine	486-56-6	0.655	0.836	0.652	0.792	0.731	0.702
Cyclophosphamide	50-18-0	*<0.397*	*<0.399*	*<0.399*	*<0.398*	*<0.392*	*<0.399*
Daunorubicin	20830-81-3	*<1.98*	*<2*	*<2*	*<1.99*	*<1.96*	*<1.99*
DEET	134-62-3	*<4.8 B*	*<4.8 B*	*<4.8 B*	*<4.8 B*	5.41 B	4.93 B
Dehydronifedipine	67035-22-7	*<0.595*	*<0.599*	*<0.599*	*<0.596*	*<0.587*	*<0.598*
Demeclocycline	127-33-3	*<14.9*	*<15*	*<15*	*<14.9*	*<14.7*	*<14.9*
Desmethyldiltiazem	86408-45-9	*<0.149*	*<0.15*	*<0.15*	*<0.149*	*<0.147*	*<0.149*
Diatrizoic acid	117-96-4	*<11.9*	*<12*	*<12*	*<11.9*	*<11.7*	*<12*
Diazepam	439-14-5	*<0.498*	*<0.501*	*<0.501*	*<0.499*	*<0.491*	*<0.5*
Digoxigenin	1672-46-4	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Digoxin	20830-75-5	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Diltiazem	34933-06-7	*<0.297*	*<0.3*	*<0.3*	*<0.298*	*<0.294*	*<0.299*
Diphenhydramine	58-73-1	*<0.595*	*<0.599*	*<0.599*	0.634	*<0.587*	*<0.598*
Doxorubicin	23214-92-8	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Doxycycline	564-25-0	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Drospirenone	67392-87-4	*<7.93*	*<7.99*	*<7.99*	*<7.95*	*<7.83*	*<7.97*
Enalapril	75847-73-3	*<0.298*	*<0.293*	*<0.292*	*<0.295*	*<0.295*	*<0.288*
Enrofloxacin	93106-60-6	*<2.97*	*<3*	*<3*	*<2.98*	*<2.94*	*<2.99*
Erythromycin-H2O	114-07-8	*<2.28*	*<2.3*	*<2.3*	*<2.29*	*<2.25*	*<2.29*
Etoposide	33419-42-0	*<0.992*	*<0.999*	*<0.998*	*<0.994*	*<0.979*	*<0.996*
Flumequine	42835-25-6	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.51*	*<1.49*
Fluocinonide	356-12-7	*<1.99*	*<2.01*	*<2.01*	*<2*	*<1.97*	*<2*
Fluoxetine	54910-89-3	*<4.96*	*<4.99*	*<4.99*	*<4.97*	*<4.89*	*<4.98*
Fluticasone propionate	80474-14-2	*<1.99*	*<2.01*	*<2.01*	*<2*	*<1.97*	*<2*
Furosemide	54-31-9	*<3.97*	*<3.99*	*<3.99*	*<3.98*	*<3.92*	*<3.99*
Gemfibrozil	25812-30-0	*<0.793*	*<0.799*	*<0.799*	*<0.795*	*<0.783*	*<0.797*

Analyte	CAS#	Alki	Elliott Bay	Reference Site	West Point North - Deep	West Point North - Shallow	West point South
Glipizide	29094-61-9	*<0.793*	*<0.799*	*<0.799*	*<0.795*	*<0.783*	*<0.797*
Glyburide	10238-21-8	*<0.793*	*<0.799*	*<0.799*	*<0.795*	*<0.783*	*<0.797*
Hydrochlorothiazide	58-93-5	*<8.73*	*<8.79*	*<8.79*	*<8.74*	*<8.61*	*<8.77*
Hydrocodone	125-29-1	*<1.19*	*<1.17*	*<1.17*	*<1.18*	*<1.18*	*<1.15*
Hydrocortisone	50-23-7	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Ibuprofen	15687-27-1	*<3.97*	*<3.99*	*<3.99*	*<3.98*	*<3.92*	*<3.99*
Iopamidol	60166-93-0	*<79.3*	*<79.9*	*<79.9*	*<79.5*	*<78.3*	*<79.7*
Isochlortetracycline [ICTC]	514-53-4	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Lincomycin	154-21-2	*<2.97*	*<3*	*<3*	*<2.98*	*<2.94*	*<2.99*
Lomefloxacin	98079-51-7	*<2.97*	*<3*	*<3*	*<2.98*	*<2.94*	*<2.99*
Medroxyprogesterone Acetate	71-58-9	*<3.97*	*<3.99*	*<3.99*	*<3.98*	*<3.92*	*<3.99*
Melphalan	148-82-3	*<23.8*	*<24*	*<24*	*<23.9*	*<23.5*	*<23.9*
Meprobamate	57-53-4	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.47*	*<1.49*
Metformin	657-24-9	31.1	44.3	34.4	60.6	44.2	45.1
Methylprednisolone	83-43-2	*<3.97*	*<3.99*	*<3.99*	*<3.98*	*<3.92*	*<3.99*
Metoprolol	51384-51-1	*<0.498*	*<0.501*	*<0.501*	*<0.499*	*<0.491*	*<0.5*
Metronidazole	443-48-1	*<1.98*	*<2*	*<2*	*<1.99*	*<1.96*	*<1.99*
Miconazole	22916-47-8	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.47*	*<1.49*
Minocycline	10118-90-8	*NQ*	*NQ*	*NQ*	*NQ*	*NQ*	*NQ*
Moxifloxacin	151096-09-2	*<3.97*	*<3.99*	*<3.99*	*<3.98*	*<3.92*	*<3.99*
Naproxen	22204-53-1	*<1.98*	*<2*	*<2*	*<1.99*	*<1.96*	*<1.99*
Norfloxacin	70458-96-7	*<14.9*	*<15*	*<15*	*<18.6*	*<14.7*	*<14.9*
Norfluoxetine	83891-03-6	*<0.498*	*<0.501*	*<0.501*	*<0.499*	*<0.491*	*<0.5*
Norgestimate	35189-28-7	*<2.97*	*<3*	*<3*	*<2.98*	*<3.01*	*<5.35*
Norverapamil	67018-85-3	*<0.149*	*<0.15*	*<0.15*	*<0.149*	*<0.147*	*<0.149*
Ofloxacin	82419-36-1	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.47*	*<1.49*
Ormetoprim	6981-18-6	*<0.595*	*<0.599*	*<0.599*	*<0.596*	*<0.587*	*<0.598*
Oxacillin	66-79-5	*<9.92*	*<9.99*	*<9.98*	*<9.94*	*<9.79*	*<9.96*
Oxazepam	604-75-1	*<3.97*	*<3.99*	*<3.99*	*<3.98*	*<3.92*	*<3.99*
Oxolinic Acid	14698-29-4	*<1.98*	*<2*	*<2*	*<1.99*	*<1.96*	*<1.99*
Oxycodone	76-42-6	*<0.596*	*<0.586*	*<0.583*	*<0.59*	*<0.59*	*<0.575*

Analyte	CAS#	Alki	Elliott Bay	Reference Site	West Point North - Deep	West Point North - Shallow	West point South
Oxytetracycline [OTC]	79-57-2	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Paroxetine	61869-08-7	*<0.998*	*<1*	*<1*	*<1*	*<0.985*	*<1*
Penicillin G	61-33-6	*<2.97*	*<3*	*<3*	*<2.98*	*<2.94*	*<2.99*
Penicillin V	87-08-1	*<2.97*	*<3*	*<3*	*<2.98*	*<2.94*	*<2.99*
Prednisolone	50-24-8	*<3.97*	*<3.99*	*<3.99*	*<3.98*	*<3.92*	*<3.99*
Prednisone	53-03-2	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Promethazine	60-87-7	*<0.297*	*<0.3*	*<0.3*	*<0.298*	*<0.294*	*<0.299*
Propoxyphene	469-62-5	*<0.297*	*<0.3*	*<0.3*	*<0.298*	*<0.294*	*<0.299*
Propranolol	525-66-6	*<0.297*	*<0.3*	*<0.3*	*<0.298*	*<0.294*	*<0.299*
Ranitidine	66357-35-5	*<0.596*	*<0.586*	*<0.583*	*<0.59*	*<0.59*	*<0.575*
Rosuvastatin	287714-41-4	*<3.97*	*<3.99*	*<3.99*	*<3.98*	*<3.92*	*<3.99*
Roxithromycin	80214-83-1	*<0.297*	*<0.3*	*<0.3*	*<0.298*	*<0.294*	*<0.299*
Sarafloxacin	98105-99-8	*<14.9*	*<15*	*<15*	*<14.9*	*<14.7*	*<14.9*
Sertraline	79617-96-2	*<0.297*	*<0.3*	*<0.3*	*<0.298*	*<0.294*	*<0.299*
Simvastatin	79902-63-9	*<1.99*	*<2.01*	*<2.01*	*<2*	*<1.97*	*<2*
Sulfachloropyridazine	80-32-0	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.47*	*<1.49*
Sulfadiazine	68-35-9	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.47*	*<1.49*
Sulfadimethoxine	122-11-2	*<0.297*	*<0.3*	*<0.3*	*<0.298*	*<0.294*	*<0.299*
Sulfamerazine	127-79-7	*<0.595*	*<0.599*	*<0.599*	*<0.596*	*<0.587*	*<0.598*
Sulfamethazine	57-68-1	*<0.595*	*<0.599*	*<0.599*	*<0.596*	*<0.587*	*<0.598*
Sulfamethizole	144-82-1	*<0.595*	*<0.599*	*<0.599*	*<0.596*	*<0.587*	*<0.598*
Sulfamethoxazole	723-46-6	*<0.595*	0.93	*<0.599*	*<0.596*	*<0.587*	0.674
Sulfanilamide	63-74-1	*<14.9*	*<15*	*<15*	*<14.9*	*<14.7*	*<14.9*
Sulfathiazole	72-14-0	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.47*	*<1.49*
Tamoxifen	10540-29-1	*<0.397*	*<0.399*	*<0.399*	*<0.398*	*<0.392*	*<0.399*
Teniposide	29767-20-2	*<3.97*	*<3.99*	*<3.99*	*<3.98*	*<3.92*	*<3.99*
Tetracycline [TC]	60-54-8	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Theophylline	58-55-9	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Thiabendazole	148-79-8	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.47*	*<1.49*
Trenbolone	10161-33-8	*<1.99*	*<2.01*	*<2.01*	*<2*	*<1.97*	*<2*
Trenbolone acetate	10161-34-9	*<0.297*	*<0.3*	*<0.3*	*<0.298*	*<0.294*	*<0.299*

Analyte	CAS#	Alki	Elliott Bay	Reference Site	West Point North - Deep	West Point North - Shallow	West point South
Triamterene	396-01-0	*<0.298*	*<0.293*	*<0.292*	*<0.295*	*<0.295*	*<0.288*
Triclocarban	101-20-2	*<0.397*	*<0.399*	*<0.399*	*<0.398*	*<0.392*	*<0.399*
Triclosan	3380-34-5	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Trimethoprim	738-70-5	*<1.49*	*<1.5*	*<1.5*	*<1.49*	*<1.47*	*<1.49*
Tylosin	1401-69-0	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*
Valsartan	137862-53-4	*<3.97*	*<3.99*	*<3.99*	*<3.98*	*<3.92*	*<3.99*
Venlafaxine	93413-69-5	0.419	0.458	0.65	0.594	0.455	0.519
Verapamil	52-53-9	*<0.149*	*<0.15*	*<0.15*	*<0.149*	*<0.147*	*<0.149*
Virginiamycin M1	21411-53-0	*<2.97*	*<3*	*<3*	*<2.98*	*<2.94*	*<2.99*
Warfarin	81-81-2	*<0.397*	*<0.399*	*<0.399*	*<0.398*	*<0.392*	*<0.399*
Zidovudine	30516-87-1	*<5.95*	*<5.99*	*<5.99*	*<5.96*	*<5.87*	*<5.98*

This table includes all chemicals analyzed by SGS-AXYS. NA = Not Analyzed. All analytes with "<" were below their reporting limit (value shown) for that sample and considered as not quantifiable. Flags denoted by letters following detection values are defined as follows:

B: Analyte found in associated blank and concentration in sample is less than 10X the concentration in the associated blank.

M: Concentration is an estimated maximum value.

K: Peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration.

NQ: Not quantified.

Table A3. Targeted chemistry data from SGS-AXYS for exposure water and exposed fish plasma samples. Treatments are represented via percent wastewater effluent (% WWE). All detected concentrations in ng/L.

		Exposure Water				Exposure Plasma			
Analyte	**CAS#**	**0% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**	**0.4% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**
Alkylphenol									
4-n-Octylphenol	1806-26-4	*<0.733*	*<0.863*	*<0.968*	*<1.82*	*NA*	*NA*	*NA*	*NA*
4-Nonylphenol diethoxylates	20427-84-3	*<6.47*	*<6.75*	*<8.87*	85.7	*NA*	*NA*	*NA*	*NA*
4-Nonylphenol monoethoxylates	104-35-8	*<9.24*	41.1	133	454	*NA*	*NA*	*NA*	*NA*
4-Nonylphenols	104-40-5	17.2	2.55	63.6	158	*NA*	*NA*	*NA*	*NA*
Bisphenol									
Bisphenol AF	1478-61-1	*<1.96*	*<1.96*	*<1.93*	*<1.94*	*NA*	*NA*	*NA*	*NA*
Bisphenol B	77-40-7	*<1.96*	*<1.96*	*<1.93*	*<1.94*	*NA*	*NA*	*NA*	*NA*
Bisphenol E	2081-08-5	*<4.89*	*<4.9*	*<4.83*	*<4.85*	*NA*	*NA*	*NA*	*NA*
Bisphenol F	620-92-8	*<4.89*	7.16	*<4.83*	11.8	*NA*	*NA*	*NA*	*NA*
Bisphenol S	80-09-1	*<12.5*	*<12.5*	15.5	31.6	*NA*	*NA*	*NA*	*NA*
Bisphenol A	80-05-7	20	30.9	58.05	122.1	*NA*	*NA*	*NA*	*NA*
Ether carboxylates									
ADONA	958445-44-8	*<1.63*	*<1.62*	*<1.61*	*<1.66*	*NA*	*NA*	*NA*	*NA*
HFPO-DA	13252-13-6	*<1.55*	*<1.54*	*<1.53*	*<1.58*	*NA*	*NA*	*NA*	*NA*
NFDHA	151772-58-6	*<0.817*	*<0.809*	*<0.806*	*<0.829*	*NA*	*NA*	*NA*	*NA*
PFMBA	--	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*
PFMPA	--	*<0.817*	*<0.809*	*<0.806*	*<0.829*	*NA*	*NA*	*NA*	*NA*
Ether sulfonates									
11Cl-PF3OUdS	763051-92-9	*<1.64*	*<1.62*	*<1.61*	*<1.66*	*NA*	*NA*	*NA*	*NA*
9Cl-PF3ONS	--	*<1.64*	*<1.62*	*<1.62*	*<1.66*	*NA*	*NA*	*NA*	*NA*
PFEESA	113507-82-7	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*
Fluorotelomer carboxylates									
3:3 FTCA	356-02-5	*<1.63*	*<1.62*	*<1.61*	*<1.66*	*NA*	*NA*	*NA*	*NA*
5:3 FTCA	914637-49-3	*<10.2*	*<10.1*	*<10.1*	*<10.4*	*NA*	*NA*	*NA*	*NA*
7:3 FTCA	812-70-4	*<10.2*	*<10.1*	*<10.1*	*<10.4*	*NA*	*NA*	*NA*	*NA*
Fluorotelomer sulfonates									
4:2 FTS	757124-72-4	*<1.63*	*<1.62*	*<1.61*	*<1.66*	*NA*	*NA*	*NA*	*NA*

		Exposure Water				Exposure Plasma			
Analyte	CAS#	0% WWE	1.4% WWE	5.3% WWE	20% WWE	0.4% WWE	1.4% WWE	5.3% WWE	20% WWE
6:2 FTS	27619-97-2	*<1.47*	*<1.46*	*<1.45*	*<1.49*	*NA*	*NA*	*NA*	*NA*
8:2 FTS	39108-34-4	*<1.63*	*<1.62*	*<1.61*	*<1.66*	*NA*	*NA*	*NA*	*NA*
Halogenated flame retardant									
1,2,4,5/1,2,3,5-TBB	--	*<0.0365*	*<0.0221*	*<0.0316*	*<0.0184*	*NA*	*NA*	*NA*	*NA*
1,2,4-TriBB	615-54-3	*<0.344*	*<0.356*	*<0.245*	*<0.664*	*NA*	*NA*	*NA*	*NA*
1,2-DiBB	583-53-9	*<0.0915*	*<0.0804*	*<0.081*	*<0.344*	*NA*	*NA*	*NA*	*NA*
1,4-DiBB	106-37-6	*<0.174*	*<0.153*	*<0.154*	*<0.654*	*NA*	*NA*	*NA*	*NA*
ATE	3278-89-5	*<0.0332*	*<0.0363*	*<0.0365*	*<0.043*	*NA*	*NA*	*NA*	*NA*
BATE	99717-56-3	*<0.159*	*<0.169*	*<0.112*	*<0.0631*	*NA*	*NA*	*NA*	*NA*
BEHTBP	26040-51-7	*<0.298*	*<0.302*	*<0.317*	0.31 K	*NA*	*NA*	*NA*	*NA*
BTBPE	37853-59-1	*<0.824*	*<0.74*	*<0.581*	*<0.943*	*NA*	*NA*	*NA*	*NA*
Dec 602	31107-44-5	*<0.0065*	*<0.0052*	*<0.0084*	*<0.0072*	*NA*	*NA*	*NA*	*NA*
Dec 603	13560-92-4	*<0.0131*	*<0.0089*	*<0.0066*	*<0.0056*	*NA*	*NA*	*NA*	*NA*
Dec 604	34571-16-9	*<0.387*	*<0.292*	*<0.325*	*<0.411*	*NA*	*NA*	*NA*	*NA*
Dechlorane	2385-85-5	*<0.1 B K*	*<0.1 B*	*<0.1 B*	*<0.1 B K*	*NA*	*NA*	*NA*	*NA*
DP Anti	135821-74-8	0.305 X	0.353 X	0.132 X	0.216 X	*NA*	*NA*	*NA*	*NA*
DP Syn	135821-03-3	0.421 X	0.281 X	0.138 X	0.232 X	*NA*	*NA*	*NA*	*NA*
DPTE	35109-60-5	*<0.574*	*<0.297*	*<0.301*	*<0.336*	*NA*	*NA*	*NA*	*NA*
EHTBB	183658-27-7	*<0.233*	*<0.151*	*<0.223*	0.488 B X	*NA*	*NA*	*NA*	*NA*
HBB	87-82-1	0.07 B X	*<0.05 B K*	0.097 B X	*<0.05 B K*	*NA*	*NA*	*NA*	*NA*
HCDBCO	51936-55-1	*<0.0713*	*<0.0399*	*<0.048*	*<0.0406*	*NA*	*NA*	*NA*	*NA*
PBBB	38521-51-6	*<0.115*	*<0.108*	*<0.78*	*<0.15*	*NA*	*NA*	*NA*	*NA*
PBBZ	608-90-2	0.029 K	*<0.0456*	0.024 X	*<0.0224*	*NA*	*NA*	*NA*	*NA*
PBEB	85-22-3	*<0.0292*	*<0.041*	*<0.0241*	*<0.0143*	*NA*	*NA*	*NA*	*NA*
PBT	87-83-2	*<0.0238*	0.032 B M	0.02 B M	*<0.0248*	*NA*	*NA*	*NA*	*NA*
pTBX	23488-38-2	*<1.14*	*<0.807*	*<1.04*	*<1.13*	*NA*	*NA*	*NA*	*NA*
TBCT	39569-21-6	*<0.0525*	*<0.031*	*<0.0321*	*<0.0336*	*NA*	*NA*	*NA*	*NA*
Total TBECH	3322-93-8	*<0.323*	*<0.526*	*<0.351*	*<1.02*	*NA*	*NA*	*NA*	*NA*
Hormone									
17 alpha-Dihydroequilin	651-55-8	*<2.02*	*<2.04*	*<1.95*	*<2.07*	*NA*	*NA*	*NA*	*NA*
17 alpha-Estradiol	57-91-0	*<8.08*	*<8.15*	*<7.79*	*<8.3*	*NA*	*NA*	*NA*	*NA*

		Exposure Water				Exposure Plasma			
Analyte	**CAS#**	**0% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**	**0.4% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**
17 alpha-Ethinyl-Estradiol	57-63-6	*<5.05*	*<5.09*	*<4.87*	*<5.19*	*NA*	*NA*	*NA*	*NA*
17 beta-Estradiol	50-28-2	*<8.08*	*<8.15*	*<7.79*	*<8.3*	*NA*	*NA*	*NA*	*NA*
17 beta-Estradiol 3-benzoate	50-50-0	*<0.808*	*<0.815*	*<0.779*	*<0.83*	*NA*	*NA*	*NA*	*NA*
Allyl Trenbolone	850-52-2	*<0.4*	*<0.403*	*<0.385*	*<0.411*	*NA*	*NA*	*NA*	*NA*
Androstenedione	63-05-8	*<1.01*	*<1.02*	*<0.973*	*<1.04*	*NA*	*NA*	*NA*	*NA*
Androsterone	53-41-8	*<20.2*	*<27.4*	*<26*	*<22.5*	*NA*	*NA*	*NA*	*NA*
Desogestrel	54024-22-5	*<42*	*<42.4*	*<40.5*	*<43.1*	*NA*	*NA*	*NA*	*NA*
Equilenin	517-09-9	*<0.404*	*<0.407*	*<0.389*	*<0.415*	*NA*	*NA*	*NA*	*NA*
Equilin	474-86-2	*<2.02*	*<2.04*	*<1.95*	*<2.07*	*NA*	*NA*	*NA*	*NA*
Estriol	50-27-1	*<8.08*	*<8.15*	*<7.79*	*<8.3*	*NA*	*NA*	*NA*	*NA*
Estrone	53-16-7	*<3.23*	4.09	11.9	36.6	*NA*	*NA*	*NA*	*NA*
Mestranol	72-33-3	*<20.2*	*<20.4*	*<19.5*	*<20.7*	*NA*	*NA*	*NA*	*NA*
Norethindrone	68-22-4	*<1.01*	*<1.02*	*<0.973*	*<1.04*	*NA*	*NA*	*NA*	*NA*
Norgestrel	797-63-7	*<1.01*	*<1.02*	*<0.973*	*<1.04*	*NA*	*NA*	*NA*	*NA*
Progesterone	57-83-0	*<0.404*	*<0.407*	*<0.389*	*<0.415*	*NA*	*NA*	*NA*	*NA*
Testosterone	58-22-0	*<0.404*	*<0.407*	*<0.389*	*<0.415*	*NA*	*NA*	*NA*	*NA*
Perfluoroalkyl carboxylates									
PFBA	375-22-4	*<1.63*	*<1.62*	*<1.61*	*<1.66*	*NA*	*NA*	*NA*	*NA*
PFDA	335-76-2	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*
PFDoA	307-55-1	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*
PFHpA	375-85-9	*<0.409*	*<0.405*	*<0.403*	0.469	*NA*	*NA*	*NA*	*NA*
PFHxA	307-24-4	*<0.409*	*<0.405*	1.04	3.41	*NA*	*NA*	*NA*	*NA*
PFNA	375-95-1	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*
PFOA	335-67-1	*<0.409*	*<0.405*	0.595 K	1.3	*NA*	*NA*	*NA*	*NA*
PFPeA	2706-90-3	*<0.817*	*<0.809*	*<0.806*	1.48	*NA*	*NA*	*NA*	*NA*
PFTeDA	376-06-7	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*
PFTrDA	72629-94-8	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*
PFUnA	2058-94-8	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*
Perfluoroalkyl sulfonates									
PFBS	375-73-5	*<0.409*	*<0.405*	0.465	1.92	*NA*	*NA*	*NA*	*NA*
PFDoS	79780-39-5	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*

		Exposure Water				Exposure Plasma			
Analyte	**CAS#**	**0% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**	**0.4% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**
PFDS	335-77-3	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*
PFHpS	375-92-8	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*
PFHxS	355-46-4	*<0.409*	*<0.405*	*<0.403*	0.938	*NA*	*NA*	*NA*	*NA*
PFNS	68259-12-1	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*
PFOS	1763-23-1	*<0.409*	*<0.405*	0.516	3.07	*NA*	*NA*	*NA*	*NA*
PFPeS	2706-91-4	*<0.411*	*<0.407*	*<0.405*	*<0.417*	*NA*	*NA*	*NA*	*NA*
Perfluorooctane sulfonamides									
N-EtFOSA	4151-50-2	*<1.02*	*<1.01*	*<1.01*	*<1.04*	*NA*	*NA*	*NA*	*NA*
N-MeFOSA	31506-32-8	*<0.47*	*<0.465*	*<0.463*	*<0.477*	*NA*	*NA*	*NA*	*NA*
PFOSA	754-91-6	5.01 X	1.65 X	2.77 X	1.38 X	*NA*	*NA*	*NA*	*NA*
EtFOSAA	909405-49-8	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*
MeFOSAA	2355-31-9	*<0.409*	*<0.405*	*<0.403*	*<0.415*	*NA*	*NA*	*NA*	*NA*
N-EtFOSE	1691-99-2	*<3.06*	*<3.03*	*<3.01*	*<3.1*	*NA*	*NA*	*NA*	*NA*
N-MeFOSE	24448-09-7	*<4.09*	*<4.05*	*<4.03*	*<4.15*	*NA*	*NA*	*NA*	*NA*
Pharmaceuticals and Personcal Care Products (PPCP)									
1,7-Dimethylxanthine	611-59-6	*<60.6*	*<61.1*	*<58.4*	*<62.2*	*<4.27*	*<4.59*	*<4.57*	*<5.42*
10-hydroxy-amitriptyline	1159-82-6	*<0.151*	0.17	0.768	2.58	*<0.0107*	*<0.0115*	*<0.0114*	*<0.0136*
2-Hydroxy-ibuprofen	51146-55-5	*<4.04*	*<4.07*	8.18	29.1	13.1	42.5	35.2	30.6
4-Epianhydrochlortetracycline [EACTC]	81163-11-3	*<60.6*	*<61.1*	*<58.4*	*<62.2*	*<4.27*	*<4.59*	*<4.57*	*<5.42*
4-Epianhydrotetracycline [EATC]	7518-17-4	*<15.1*	*<15.3*	*<14.6*	*<15.6*	*<1.07*	*<1.15*	*<1.14*	*<1.35*
4-Epichlortetracycline [ECTC]	14297-93-9	*<15.1*	*<15.3*	*<14.6*	*<15.6*	*<1.07*	*<1.15*	*<1.14*	*<1.35*
4-Epioxytetracycline [EOTC]	14206-58-7	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<0.427*	*<0.459*	*<0.457*	*<0.542*
4-Epitetracycline [ETC]	79-85-6	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<0.427*	*<0.459*	*<0.457*	*<0.542*
Acetaminophen	103-90-2	*<15.1*	*<15.3*	*<14.6*	*<15.6*	*<1.07*	*<1.15*	*<1.14*	*<1.35*
Albuterol	18559-94-9	*<0.29*	*<0.297*	0.746	2.14	*<0.135*	*<0.0904*	*<0.0908*	*<0.107*
Alprazolam	28981-97-7	*<0.303*	*<0.306*	*<0.292*	0.377	*<0.0214*	*<0.0229*	*<0.0228*	*<0.0271*
Amitriptyline	50-48-6	*<0.303*	0.345	1.35	4.82	*<0.0214*	*<0.0229*	*<0.0228*	*<0.0271*
Amlodipine	88150-42-9	*<1.02*	*<1.02*	1.16	3.88	*<0.0716*	*<0.0769*	*<0.0766*	*<0.0909*

		Exposure Water				Exposure Plasma			
Analyte	**CAS#**	**0% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**	**0.4% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**
Amphetamine	300-62-9	*<0.29*	*<0.297*	*<0.309*	*<0.305*	*<0.087*	*<0.0904*	*<0.0908*	*<0.107*
Amsacrine	51264-14-3	*<0.0404*	*<0.0407*	*<0.0389*	*<0.0415*	*<0.0029*	*<0.0031*	*<0.0031*	*<0.0036*
Anhydrochlortetracycline [ACTC]	4497-08-9	*<15.1*	*<15.3*	*<14.6*	*<15.6*	*<1.07*	*<1.15*	*<1.14*	*<1.35*
Anhydrotetracycline [ATC]	4496-85-9	*<15.1*	*<15.3*	*<14.6*	*<15.6*	*<1.07*	*<1.15*	*<1.14*	*<1.35*
Atenolol	29122-68-7	*<0.29*	0.878	3.56	10.7	*<0.087*	*<0.0904*	*<0.0908*	*<0.107*
Atorvastatin	134523-00-5	*<1.16*	*<1.19*	1.54	7.37	*<0.348*	*<0.361*	*<0.363*	*<0.427*
Azathioprine	446-86-6	*<1.01*	*<1.02*	*<0.973*	*<1.04*	*<0.0712*	*<0.0765*	*<0.0761*	*<0.0903*
Azithromycin	83905-01-5	*<1.51*	2.99	12.9	67.6	*<0.107*	*<0.115*	*<0.114*	*<0.207*
Benzoylecgonine	519-09-5	*<0.151*	0.586	2.38	8.08	*<0.0107*	*<0.0115*	*<0.0114*	*<0.0136*
Benztropine	86-13-5	*<0.707*	*<0.713*	*<0.681*	*<0.726*	0.12 B	0.128 B	0.133 B	0.157 B
Betamethasone	378-44-9	*<1.51*	*<1.53*	*<1.46*	*<1.56*	*<0.107*	*<0.115*	*<0.114*	*<0.135*
Bisphenol A	80-05-7	20	30.9	58.05	122.1	*<3.2 B*	*<0.459 B*	*<3.2 B*	*<3.2 B*
Busulfan	55-98-1	*<2.02*	*<2.04*	*<1.95*	*<2.07*	*<0.142*	*<0.153*	*<0.152*	*<0.181*
Caffeine	58-08-2	*<15.1*	*<15.3*	*<14.6*	16.6	1.33	1.38	1.4	1.43
Carbadox	6804-07-5	*<1.51*	*<1.53*	*<1.46*	*<1.56*	*<0.136*	*<0.26*	*<0.143*	*<0.154*
Carbamazepine	298-46-4	*<1.51*	2.22	9.23	31.1	*<0.107*	*<0.115*	*<0.114*	*<0.135*
Cefotaxime	63527-52-6	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<1.42*	*<1.53*	*<2.74*	*<2.81*
Chlortetracycline [CTC]	57-62-5	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<0.427*	*<0.459*	*<0.457*	*<0.542*
Cimetidine	51481-61-9	*<0.581*	*<0.593*	*<0.618*	*<0.611*	*<0.174*	*<0.181*	*<0.182*	*<0.214*
Ciprofloxacin	85721-33-1	*<6.69*	6.69	9.99	21.9	*<0.427*	*<0.459*	*<0.457*	*<0.558*
Citalopram	59729-33-8	*<0.404*	4.08	14.4	51.8	*<0.0285*	*<0.0306*	0.044	*<0.0361*
Clarithromycin	81103-11-9	*<1.51*	*<1.53*	4.66	14.3	*<0.107*	*<0.115*	*<0.114*	*<0.135*
Clinafloxacin	105956-97-6	*<20.2*	*<21.1*	*<23.1*	*<25.2*	*<0.504*	*<0.89*	*<0.949*	*<1.64*
Clonidine	4205-90-7	*<1.16*	*<1.19*	*<1.24*	*<1.22*	*<0.348*	*<0.361*	*<0.363*	*<0.427*
Clotrimazole	23593-75-1	*<0.404*	*<0.407*	*<0.389*	0.441	*<0.0285*	*<0.0306*	*<0.0305*	*<0.0361*
Cloxacillin	61-72-3	*NQ*	*NQ*	*NQ*	*NQ*	*<0.214*	*<0.229*	*<0.228*	*<0.271*
Cocaine	50-36-2	*<0.151*	*<0.153*	*<0.146*	*<0.156*	*<0.06 B*	*<0.0115*	*<0.06 B*	*<0.06 B*
Codeine	76-57-3	*<1.16*	*<1.19*	2.35	11.4	*<0.348*	*<0.361*	*<0.363*	*<0.427*
Colchicine	64-86-8	*<0.808*	*<0.815*	*<0.779*	*<0.83*	*<0.057*	*<0.0612*	*<0.0609*	*<0.0723*

		Exposure Water				Exposure Plasma			
Analyte	CAS#	0% WWE	1.4% WWE	5.3% WWE	20% WWE	0.4% WWE	1.4% WWE	5.3% WWE	20% WWE
Cotinine	486-56-6	0.741	0.922	2.53	8.02	*<0.087*	*<0.0904*	*<0.0908*	*<0.107*
Cyclophosphamide	50-18-0	*<0.404*	*<0.407*	*<0.389*	*<0.415*	*<0.0285*	*<0.0306*	*<0.0305*	*<0.0361*
Daunorubicin	20830-81-3	*<2.02*	*<2.04*	*<1.95*	*<2.07*	*<0.142*	*<0.153*	*<0.194*	*<0.181*
DEET	134-62-3	5.66 B	19.1 B	53.8 B	159 B	*<3.09 B*	*<3.09 B*	*<3.09 B*	*<3.09 B*
Dehydronifedipine	67035-22-7	*<0.606*	*<0.611*	*<0.584*	0.787	*<0.0427*	*<0.0459*	*<0.0457*	*<0.0542*
Demeclocycline	127-33-3	*<15.1*	*<15.3*	*<14.6*	*<15.6*	*<1.07*	*<1.15*	*<1.14*	*<1.35*
Desmethyldiltiazem	86408-45-9	*<0.151*	0.347	1.4	4.48	*<0.0107*	*<0.0115*	*<0.0114*	*<0.0136*
Diatrizoic acid	117-96-4	*<12.1*	*<12.2*	29.8	110	*<0.854*	*<0.917*	*<0.914*	*<1.08*
Diazepam	439-14-5	*<0.507*	*<0.511*	*<0.489*	*<0.521*	*<0.0357*	*<0.0384*	*<0.0382*	*<0.0453*
Digoxigenin	1672-46-4	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<0.427*	*<0.459*	*<1.27*	*<3.82*
Digoxin	20830-75-5	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<0.427*	*<0.459*	*<0.457*	*<0.542*
Diltiazem	34933-06-7	*<0.303*	1.58	5.74	27.7	*<0.0214*	*<0.0229*	*<0.0228*	*<0.0271*
Diphenhydramine	58-73-1	*<0.606*	8.17	39.2	174	*<0.0427*	*<0.0459*	*<0.0457*	0.062
Doxorubicin	23214-92-8	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<0.427*	*<0.459*	*<0.457*	*<0.542*
Doxycycline	564-25-0	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<0.427*	*<0.459*	*<0.457*	*<0.542*
Drospirenone	67392-87-4	*<8.08*	*<8.15*	*<7.79*	*<8.3*	*<0.569*	*<0.612*	*<0.609*	*<0.723*
Enalapril	75847-73-3	*<0.29*	*<0.297*	*<0.309*	*<0.305*	*<0.0949*	*<0.107*	*<0.194*	*<0.107*
Enrofloxacin	93106-60-6	*<3.03*	*<3.06*	*<2.92*	*<3.11*	*<0.214*	*<0.229*	*<0.228*	*<0.271*
Erythromycin-H2O	114-07-8	*<2.32*	2.69	3.4	7.05	*<1.86 B*	*<1.86 B*	*<1.86 B*	*<1.86 B*
Etoposide	33419-42-0	*<1.01*	*<1.02*	*<0.973*	*<1.04*	*<0.0712*	*<0.0765*	*<0.0761*	*<0.0903*
Flumequine	42835-25-6	*<1.51*	*<1.53*	*<1.46*	*<1.56*	*<0.107*	*<0.115*	*<0.114*	*<0.135*
Fluocinonide	356-12-7	*<2.03*	*<2.05*	*<1.96*	*<2.08*	*<0.143*	*<0.154*	*<0.153*	*<0.182*
Fluoxetine	54910-89-3	*<1.51*	1.58	4.76	8.44	*<0.107*	*<0.115*	*<0.114*	*<0.135*
Fluticasone propionate	80474-14-2	*<2.03*	*<2.05*	*<1.96*	*<2.08*	*<0.143*	*<0.154*	*<0.153*	*<0.182*
Furosemide	54-31-9	*<4.04*	*<4.07*	6.58	5.44	*<1.44 B*	*<1.44 B*	*<1.44 B*	*<1.44 B*
Gemfibrozil	25812-30-0	*<0.808*	9.03	36.4	122	*<0.057*	0.104	0.275	0.664
Glipizide	29094-61-9	*<0.808*	*<0.815*	*<0.779*	3.38	*<0.057*	*<0.0612*	*<0.0609*	*<0.0723*
Glyburide	10238-21-8	*<0.808*	*<0.815*	*<0.779*	*<0.83*	*<0.057*	*<0.0612*	*<0.0609*	*<0.0723*
Hydrochlorothiazide	58-93-5	*<8.89*	21.9	79.1	245	*<3.78 B*	*<3.78 B*	*<3.78 B*	*<3.78 B*
Hydrocodone	125-29-1	*<1.16*	*<1.19*	1.27	4.65	*<0.348*	*<0.361*	*<0.363*	*<0.427*
Hydrocortisone	50-23-7	10.9	8.88	10.1	14.3	69.2	58.2	84.8	56.3

		Exposure Water				Exposure Plasma			
Analyte	**CAS#**	**0% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**	**0.4% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**
Ibuprofen	15687-27-1	*<4.04*	*<4.07*	*<3.89*	4.33	*<1.41 B*	*<1.41 B*	*<1.41 B*	*<1.41 B*
Iopamidol	60166-93-0	*<80.8*	320	1380	5020	*<5.69*	*<6.12*	*<6.09*	*<7.23*
Isochlortetracycline [ICTC]	514-53-4	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<0.427*	*<0.459*	*<0.457*	*<0.542*
Lincomycin	154-21-2	*<3.03*	*<3.06*	*<2.92*	*<3.11*	*<0.214*	*<0.229*	*<0.228*	*<0.271*
Lomefloxacin	98079-51-7	*<3.03*	*<3.06*	*<2.92*	*<3.11*	*<0.214*	*<0.229*	*<0.228*	*<0.271*
Medroxyprogesterone Acetate	71-58-9	*<4.04*	*<4.07*	*<3.89*	*<4.15*	*<0.285*	*<0.306*	*<0.305*	*<0.361*
Melphalan	148-82-3	*<24.2*	*<24.4*	*<23.4*	*<24.9*	*<1.71*	*<1.83*	*<1.83*	*<2.17*
Meprobamate	57-53-4	*<1.51*	*<1.53*	3.16	12.3	*<0.107*	*<0.115*	*<0.114*	*<0.135*
Metformin	657-24-9	*<0.9 B*	38.3 B	160 B	526 B	4.03 B	23.4 B	*<0.66 B*	0.899 B
Methylprednisolone	83-43-2	*<4.04*	*<4.07*	*<3.89*	*<4.15*	*<0.285*	*<0.998*	*<0.778*	*<0.913*
Metoprolol	51384-51-1	*<0.507*	8.11	34.4	118	*<0.0357*	*<0.0384*	*<0.0382*	*<0.0453*
Metronidazole	443-48-1	*<2.02*	*<2.04*	4.54	15	*<0.142*	*<0.153*	*<0.152*	*<0.181*
Miconazole	22916-47-8	*<1.51*	*<1.53*	*<1.46*	*<1.56*	*<0.107*	*<0.115*	*<0.114*	*<0.135*
Minocycline	10118-90-8	*<60.6*	*<61.1*	*<58.4*	*<62.2*	*<4.27*	*<4.59*	*<4.57*	*<5.42*
Moxifloxacin	151096-09-2	*<4.04*	*<4.07*	*<3.89*	*<4.15*	*<0.171*	*<0.183*	*<0.183*	*<0.217*
Naproxen	22204-53-1	*<2.02*	*<2.04*	5.1	19.1	*<0.142*	*<0.153*	*<0.152*	*<0.181*
Norfloxacin	70458-96-7	*<27.7*	*<15.3*	*<14.6*	82	*<1.42*	*<3.3*	*<2.58*	*<2.63*
Norfluoxetine	83891-03-6	*<0.507*	*<0.511*	*<0.489*	*<0.521*	*<0.0357*	*<0.0384*	*<0.0382*	*<0.0453*
Norgestimate	35189-28-7	*<3.03*	*<3.06*	*<2.92*	*<3.11*	*<0.369*	*<0.341*	*<0.386*	*<0.551*
Norverapamil	67018-85-3	*<0.151*	*<0.153*	0.271	1.07	*<0.0107*	*<0.0115*	*<0.0114*	*<0.0136*
Ofloxacin	82419-36-1	*<1.51*	2.8	9.02	24.2	*<0.107*	*<0.115*	*<0.114*	*<0.135*
Ormetoprim	6981-18-6	218	141	135	122	2.32	2.54	2.72	1.76
Oxacillin	66-79-5	*NQ*	*NQ*	*NQ*	*NQ*	*<0.214*	*<0.229*	*<0.228*	*<0.271*
Oxazepam	604-75-1	*<4.04*	*<4.07*	*<3.89*	*<4.15*	*<0.285*	*<0.306*	*<0.305*	*<0.361*
Oxolinic Acid	14698-29-4	*<0.606*	*<0.611*	*<0.584*	*<0.924*	*<0.0482*	*<0.0475*	*<0.0457*	*<0.0562*
Oxycodone	76-42-6	*<0.581*	0.699	2.58	9.22	*<0.174*	*<0.181*	*<0.182*	*<0.214*
Oxytetracycline [OTC]	79-57-2	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<0.427*	*<0.459*	*<0.457*	*<0.542*
Paroxetine	61869-08-7	*<1.02*	*<1.02*	*<0.979*	*<1.04*	*<0.0716*	*<0.0769*	*<0.0766*	*<0.0909*
Penicillin G	61-33-6	*<3.03*	*<3.06*	*<2.92*	*<3.11*	*<0.712*	*<0.765*	*<0.761*	*<0.903*
Penicillin V	87-08-1	*<3.03*	*<3.06*	*<2.92*	*<3.11*	*<0.214*	*<0.229*	*<0.228*	*<0.271*

		Exposure Water				Exposure Plasma			
Analyte	**CAS#**	**0% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**	**0.4% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**
Prednisolone	50-24-8	*<4.04*	*<4.07*	*<3.89*	*<4.15*	*<0.285*	*<0.306*	*<0.305*	*<0.361*
Prednisone	53-03-2	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<0.427*	*<0.459*	*<0.457*	*<0.542*
Promethazine	60-87-7	*<0.303*	*<0.306*	*<0.292*	*<0.311*	*<0.0214*	*<0.0229*	*<0.0228*	*<0.0271*
Propoxyphene	469-62-5	*<0.303*	*<0.306*	*<0.292*	*<0.311*	*<0.0214*	*<0.0229*	*<0.0228*	*<0.0271*
Propranolol	525-66-6	*<0.303*	0.892	3.43	11.7	*<0.0214*	*<0.0229*	*<0.0228*	*<0.0271*
Ranitidine	66357-35-5	*<0.581*	*<0.593*	*<0.618*	*<0.611*	*<0.174*	*<0.181*	*<0.182*	*<0.214*
Rosuvastatin	287714-41-4	*<4.04*	7.74	31.4	100	*<0.285*	*<0.306*	*<0.305*	*<0.361*
Roxithromycin	80214-83-1	*<0.303*	*<0.306*	*<0.292*	*<0.311*	*<0.0214*	*<0.0229*	*<0.0228*	*<0.0271*
Sarafloxacin	98105-99-8	*<15.1*	*<15.3*	*<14.6*	*<15.6*	*<1.07*	*<1.15*	*<1.14*	*<1.35*
Sertraline	79617-96-2	*<0.303*	1.12	4.66	15	*<0.0214*	*<0.0229*	0.073	0.193
Simvastatin	79902-63-9	*<2.03*	*<2.05*	*<1.96*	*<2.08*	*<0.143*	*<0.154*	*<0.153*	*<0.182*
Sulfachloropyridazine	80-32-0	*<1.51*	*<1.53*	*<1.46*	*<1.56*	*<0.107*	*<0.115*	*<0.114*	*<0.135*
Sulfadiazine	68-35-9	*<1.51*	*<1.53*	*<1.46*	*<1.56*	*<0.107*	*<0.115*	*<0.114*	*<0.135*
Sulfadimethoxine	122-11-2	183	86.1	96.6	72.1	1.28	0.883	0.816	1.46
Sulfamerazine	127-79-7	*<0.606*	*<0.611*	*<0.584*	*<0.622*	*<0.0427*	*<0.0459*	*<0.0457*	*<0.0542*
Sulfamethazine	57-68-1	*<0.606*	*<0.611*	*<0.584*	*<0.622*	*<0.0427*	*<0.0459*	*<0.0457*	*<0.0542*
Sulfamethizole	144-82-1	*<0.606*	*<0.611*	*<0.584*	*<0.622*	*<0.0427*	*<0.0459*	*<0.0457*	*<0.0592*
Sulfamethoxazole	723-46-6	*<0.606*	5.7	19.7	48.6	*<0.0427*	*<0.0459*	*<0.0457*	*<0.0542*
Sulfanilamide	63-74-1	*<15.1*	*<15.3*	*<14.6*	*<15.6*	*<10.7*	*<11.5*	*<11.4*	*<13.5*
Sulfathiazole	72-14-0	*<1.51*	*<1.53*	*<1.46*	*<1.56*	*<0.107*	*<0.115*	*<0.114*	*<0.135*
Tamoxifen	10540-29-1	*<0.404*	*<0.407*	*<0.389*	*<0.415*	*<0.0285*	*<0.0306*	*<0.0305*	*<0.0361*
Teniposide	29767-20-2	*<4.04*	*<4.07*	*<3.89*	*<4.15*	*<0.285*	*<0.306*	*<0.305*	*<0.361*
Tetracycline [TC]	60-54-8	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<0.427*	*<0.459*	*<0.457*	*<0.542*
Theophylline	58-55-9	7.1	*<6.11*	14.9	47.9	0.539	0.62	0.536	0.704
Thiabendazole	148-79-8	*<1.51*	*<1.53*	2.49	6.69	0.136	*<0.115*	0.149	*<0.135*
Trenbolone	10161-33-8	*<2.03*	*<2.05*	*<1.96*	*<2.08*	*<0.143*	*<0.154*	*<0.153*	*<0.182*
Trenbolone acetate	10161-34-9	*<0.303*	*<0.306*	*<0.292*	*<0.311*	*<0.0214*	*<0.0229*	*<0.0228*	*<0.0271*
Triamterene	396-01-0	*<0.29*	1.32	5.47	18.4	*<0.087*	*<0.0904*	*<0.0908*	*<0.107*
Triclocarban	101-20-2	*<0.404*	*<0.407*	*<0.389*	0.555	*<0.0285*	*<0.0306*	*<0.0305*	*<0.0361*
Triclosan	3380-34-5	*<6.06*	*<6.11*	*<5.84*	10.2	*<0.427*	*<0.459*	*<0.457*	*<0.542*
Trimethoprim	738-70-5	*<1.51*	3.66	16	62.3	*<0.107*	*<0.115*	*<0.114*	*<0.135*

		Exposure Water				Exposure Plasma			
Analyte	**CAS#**	**0% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**	**0.4% WWE**	**1.4% WWE**	**5.3% WWE**	**20% WWE**
Tylosin	1401-69-0	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<0.427*	*<0.459*	*<0.457*	*<0.542*
Valsartan	137862-53-4	*<4.04*	14.4	55	182	*<0.285*	*<0.306*	*<0.305*	*<0.361*
Venlafaxine	93413-69-5	*<0.404*	5.76	22.7	79.7	*<0.0285*	*<0.0306*	*<0.0305*	*<0.0361*
Verapamil	52-53-9	*<0.151*	0.258	1.05	3.76	*<0.0107*	*<0.0115*	*<0.0114*	*<0.0136*
Virginiamycin M1	21411-53-0	*<3.03*	*<3.06*	*<2.92*	*<3.11*	0.631	1.82	*<2.08*	1.2
Warfarin	81-81-2	*<0.404*	*<0.407*	*<0.389*	*<0.415*	*<0.0285*	*<0.0306*	*<0.0305*	*<0.0361*
Zidovudine	30516-87-1	*<6.06*	*<6.11*	*<5.84*	*<6.22*	*<0.427*	*<0.459*	*<0.457*	*<0.542*

This table includes all chemicals analyzed by SGS-AXYS. NA = Not Analyzed. All analytes with "<" were below their reporting limit (value shown) for that sample and considered as not quantifiable. Flags denoted by letters following detection values are defined as follows:

B: Analyte found in associated blank and concentration in sample is less than 10X the concentration in the associated blank.

M: Concentration is an estimated maximum value.

K: Peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration.

NQ: Not quantified.

X: Analyte detected in 0% WWE treatment.

Table A4. Targeted chemistry data from SGS-AXYS for exposed fish tissue samples. Treatments are represented via percent wastewater effluent (% WWE). All detected concentrations in ng/L.

Analyte	CAS#	0% WWE	0.4% WWE	1.4% WWE	5.3% WWE	20% WWE
% Lipid	--	3.83	*NA*	3.85	2.97	2.26
Alkylphenol						
4-n-Octylphenol	1806-26-4	*<0.481*	*NA*	*<0.483*	*<0.478*	*<0.433*
4-Nonylphenol diethoxylates	20427-84-3	0.637	*NA*	3.4	10.6	21.2
4-Nonylphenol monoethoxylates	104-35-8	*<5.95 B*	*NA*	6.73 B	15.3 B	32.1 B
4-Nonylphenols	104-40-5	*<36.3 B*	*NA*	*<36.3 B*	*<36.3 B*	59.1 B
Halogenated flame retardant						
1,2,4,5/1,2,3,5-TBB	--	*<0.132*	*NA*	*<0.152*	*<0.289*	*<0.0736*
1,2,4-TriBB	615-54-3	*<0.173*	*NA*	*<0.546*	*<0.421*	*<0.212*
1,2-DiBB	583-53-9	*<0.0367*	*NA*	*<0.0447*	*<0.0509*	*<0.0527*
1,4-DiBB	106-37-6	*<0.0701*	*NA*	*<0.0852*	*<0.0971*	*<0.1*
ATE	3278-89-5	*<0.13*	*NA*	*<0.119*	*<0.204*	*<0.12*
BATE	99717-56-3	*<0.243*	*NA*	*<0.383*	*<0.513*	*<0.376*
BTBPE	37853-59-1	*<0.43*	*NA*	*<0.727*	*<0.802*	*<0.922*
Dec 602	31107-44-5	*<0.0177*	*NA*	*<0.0206*	*<0.0299*	0.012 K
Dec 603	13560-92-4	*<0.0214*	*NA*	*<0.0117*	*<0.0255*	*<0.0106*
Dec 604	34571-16-9	*<0.529*	*NA*	*<0.388*	*<0.575*	*<0.473*
Dechlorane	2385-85-5	0.102 B K	*NA*	*<0.104*	*<0.174*	*<0.0529*
DP Anti	135821-74-8	*<0.123*	*NA*	*<0.0873*	*<0.211*	*<0.0943*
DP Syn	135821-03-3	*<0.0831*	*NA*	*<0.0578*	*<0.137*	*<0.0607*
DPTE	35109-60-5	*<1.44*	*NA*	*<1.85*	*<1.1*	*<0.974*
EHTBB	183658-27-7	*<1.08*	*NA*	*<1.37*	*<1.03*	*<0.969*
HBB	87-82-1	*<0.0498*	*NA*	*<0.0522*	*<0.253*	*<0.0487*
HCDBCO	51936-55-1	*<0.134*	*NA*	*<0.127*	*<0.231*	*<0.0414*
PBBB	38521-51-6	*<0.833*	*NA*	*<0.758*	*<0.789*	*<0.54*
PBBZ	608-90-2	*<0.282*	*NA*	*<0.408*	*<0.0917*	*<0.0631*
PBEB	85-22-3	*<0.0923*	*NA*	*<0.124*	*<0.404*	*<0.0307*
PBT	87-83-2	*<0.0643*	*NA*	*<0.0518*	*<0.294*	0.058 B M
pTBX	23488-38-2	*<1.73*	*NA*	*<1.3*	*<2.51*	*<1.5*
TBCT	39569-21-6	0.966 K	*NA*	1.19 K	0.942 K	1.18 K
Total TBECH	3322-93-8	*<0.245*	*NA*	*<0.275*	*<0.78*	*<0.335*

Analyte	CAS#	0% WWE	0.4% WWE	1.4% WWE	5.3% WWE	20% WWE
Pharmaceuticals and Personal Care Products (PPCP)						
1,7-Dimethylxanthine	611-59-6	*<22.9*	*<23.3*	*NA*	*<23.2*	*<23.9*
10-hydroxy-amitriptyline	1159-82-6	*<0.0955*	*<0.0583*	*<0.0574*	*<0.13*	*<0.263*
2-Hydroxy-ibuprofen	51146-55-5	*<1.53*	*<1.55*	*<1.54*	*<1.55*	*<1.6*
4-Epianhydrochlortetracycline [EACTC]	81163-11-3	*<22.9*	*<23.3*	*NA*	*<23.2*	*<23.9*
4-Epianhydrotetracycline [EATC]	7518-17-4	*<5.73*	*<5.83*	*<5.76*	*<5.8*	*<5.99*
4-Epichlortetracycline [ECTC]	14297-93-9	*<5.73*	*<5.83*	*<5.76*	*<5.8*	*<5.99*
4-Epioxytetracycline [EOTC]	14206-58-7	*<2.29*	*<2.33*	*NA*	*<2.32*	*<2.39*
4-Epitetracycline [ETC]	79-85-6	*<2.29*	*<2.33*	*NA*	*<2.32*	*<2.39*
Acetaminophen	103-90-2	*<5.73*	*<5.83*	*<5.76*	*<5.8*	*<5.99*
Albuterol	18559-94-9	*<0.269*	*<0.256*	*<0.254*	*<0.243*	*<0.257*
Alprazolam	28981-97-7	*<0.115*	*<0.117*	*NA*	*<0.116*	*<0.12*
Amitriptyline	50-48-6	*<0.115*	*<0.117*	*NA*	*<0.116*	0.561
Amlodipine	88150-42-9	*<0.385*	*<0.391*	*<0.386*	*<0.389*	*<0.401*
Amphetamine	300-62-9	*<0.269*	*<0.256*	*<0.254*	*<1.75*	*<0.257*
Amsacrine	51264-14-3	*<0.0153*	*<0.0155*	*<0.0153*	*<0.0159*	*<0.0154*
Anhydrochlortetracycline [ACTC]	4497-08-9	*<5.73*	*<5.83*	*<5.76*	*<5.8*	*<5.99*
Anhydrotetracycline [ATC]	4496-85-9	*<5.73*	*<5.83*	*<5.76*	*<5.8*	*<5.99*
Atenolol	29122-68-7	*<0.269*	*<0.256*	*<0.254*	*<0.243*	*<0.257*
Atorvastatin	134523-00-5	*<1.08*	*<1.02*	*<1.02*	*<0.97*	*<1.03*
Azathioprine	446-86-6	*<0.382*	*<0.389*	*<0.384*	*<0.387*	*<0.399*
Azithromycin	83905-01-5	*<8.5*	*<11.6*	*<4.19*	*<6.93*	*<8.54*
Benzoylecgonine	519-09-5	*<0.0573*	0.06	*<0.0574*	*<0.0595*	*<0.0579*
Benztropine	86-13-5	*<0.268*	*<0.272*	*<0.269*	*<0.271*	*<0.279*
Betamethasone	378-44-9	*<0.573*	*<0.583*	*<0.576*	*<0.58*	*<0.599*
Bisphenol A	80-05-7	3.59	4.03	4.795	6.215	11.07
Busulfan	55-98-1	*<0.765*	*<0.777*	*<0.768*	*<0.773*	*<0.798*
Caffeine	58-08-2	*<5.73*	*<5.83*	*<5.76*	*<5.8*	*<5.99*
Carbadox	6804-07-5	*<1.13*	*<0.583*	*<0.687*	*<1.06*	*<0.936*
Carbamazepine	298-46-4	*<0.573*	*<0.583*	*<0.576*	*<0.58*	*<0.599*
Cefotaxime	63527-52-6	*<8.54*	*<30.4*	*<2.3*	*<2.3*	*<29.6*

Analyte	CAS#	0% WWE	0.4% WWE	1.4% WWE	5.3% WWE	20% WWE
Chlortetracycline [CTC]	57-62-5	*<2.29*	*<2.33*	*NA*	*<2.32*	*<2.39*
Cimetidine	51481-61-9	*<0.538*	*<0.512*	*<0.508*	*<0.486*	*<0.514*
Ciprofloxacin	85721-33-1	*<7.34*	*<8.38*	*<9.53*	*<9.53*	*<4.44*
Citalopram	59729-33-8	*<0.153*	*<0.155*	*<0.154*	0.265	1.24
Clarithromycin	81103-11-9	*<0.573*	*<0.583*	*<0.576*	*<0.58*	*<0.599*
Clinafloxacin	105956-97-6	*<6.09*	*<10.7*	*<28.6*	*<17.6*	*<9.89*
Clonidine	4205-90-7	*<1.08*	*<1.02*	*<1.02*	*<0.97*	*<1.03*
Clotrimazole	23593-75-1	*<0.153*	*<0.155*	*<0.154*	*<0.155*	*<0.16*
Cloxacillin	61-72-3	*<2.43*	*<1.17*	*<3.34*	6.09	4.53
Cocaine	50-36-2	*<0.0573*	*<0.0583*	*<0.0574*	*<0.0595*	*<0.0579*
Codeine	76-57-3	*<1.08*	*<1.02*	*<1.02*	*<0.97*	*<1.03*
Colchicine	64-86-8	*<0.306*	*<0.311*	*<0.307*	*<0.309*	*<0.319*
Cotinine	486-56-6	*<0.269*	*<0.256*	*<0.254*	*<0.243*	*<0.257*
Cyclophosphamide	50-18-0	*<0.153*	*<0.155*	*<0.154*	*<0.155*	*<0.16*
Daunorubicin	20830-81-3	*<0.765*	*<0.777*	*<0.768*	*<0.773*	*<0.798*
DEET	134-62-3	*<3.09 B*	*<3.09 B*	*<3.09 B*	*<3.09 B*	*<3.09 B*
Dehydronifedipine	67035-22-7	*<0.229*	*<0.233*	*NA*	*<0.232*	*<0.23*
Demeclocycline	127-33-3	*<5.73*	*<5.83*	*<5.76*	*<5.8*	*<5.99*
Desmethyldiltiazem	86408-45-9	*<0.0573*	*<0.0583*	*<0.0574*	0.09	0.3075
Diatrizoic acid	117-96-4	*<4.59*	*<4.66*	*<4.61*	*<4.64*	*<4.79*
Diazepam	439-14-5	*<0.192*	*<0.195*	*<0.193*	*<0.194*	*<0.2*
Digoxigenin	1672-46-4	*<2.29*	*<2.33*	*<2.3*	*<2.3*	*<5.1*
Digoxin	20830-75-5	*<2.29*	*<2.33*	*<2.3*	*<2.3*	*<2.39*
Diltiazem	34933-06-7	*<0.123*	*<0.127*	0.217	0.409	2.335
Diphenhydramine	58-73-1	*<0.229*	0.522	1.805	5.5	29.6
Doxorubicin	23214-92-8	*<2.29*	*<2.33*	*NA*	*<2.32*	*<2.39*
Doxycycline	564-25-0	*<2.29*	*<2.33*	*NA*	*<2.32*	*<2.39*
Drospirenone	67392-87-4	*<3.06*	*<3.11*	*<3.07*	*<3.09*	*<3.19*
Enalapril	75847-73-3	*<0.269*	*<0.256*	*<0.254*	*<0.243*	*<0.257*
Enrofloxacin	93106-60-6	*<1.15*	*<1.17*	*NA*	*<1.16*	*<1.2*
Erythromycin-H2O	114-07-8	*<0.879*	*<0.894*	*<0.883*	*<0.889*	*<0.918*
Etoposide	33419-42-0	*<0.382*	*<0.389*	*<0.384*	*<0.387*	*<0.399*
Flumequine	42835-25-6	*<0.699*	*<0.613*	1.05 K	1.21	2.2

Analyte	CAS#	0% WWE	0.4% WWE	1.4% WWE	5.3% WWE	20% WWE
Fluocinonide	356-12-7	*<0.768*	*<0.781*	*<0.772*	*<0.777*	*<0.802*
Fluoxetine	54910-89-3	*<0.573*	*<0.583*	*<0.576*	*<0.58*	0.8895
Fluticasone propionate	80474-14-2	*<0.768*	*<0.781*	*<0.772*	*<0.777*	*<0.802*
Furosemide	54-31-9	*<1.53*	*<1.55*	*<1.54*	*<1.55*	*<1.6*
Gemfibrozil	25812-30-0	*<0.306*	*<0.311*	*<0.307*	0.418	2.05
Glipizide	29094-61-9	*<0.306*	*<0.311*	*<0.307*	*<0.309*	*<0.319*
Glyburide	10238-21-8	*<0.306*	*<0.311*	*<0.307*	*<0.309*	*<0.319*
Hydrochlorothiazide	58-93-5	*<3.36*	*<3.42*	*<3.38*	*<3.4*	*<3.51*
Hydrocodone	125-29-1	*<1.08*	*<1.02*	*<1.02*	*<0.97*	*<1.03*
Hydrocortisone	50-23-7	16.9	16.9	15.9	26.95	13.6
Ibuprofen	15687-27-1	*<1.53*	*<1.55*	*<1.54*	*<1.55*	*<1.6*
Iopamidol	60166-93-0	*<30.6*	*<31.1*	*<30.7*	*<30.9*	*<31.9*
Isochlortetracycline [ICTC]	514-53-4	*<2.29*	*<2.33*	*NA*	*<2.32*	*<2.39*
Lincomycin	154-21-2	*<1.15*	*<1.17*	*NA*	*<1.16*	*<1.2*
Lomefloxacin	98079-51-7	*<1.15*	*<1.27*	*<1.15*	*<1.16*	*<1.2*
Medroxyprogesterone Acetate	71-58-9	*<1.53*	*<1.55*	*<1.54*	*<1.55*	*<1.6*
Melphalan	148-82-3	*<9.17*	*<9.32*	*<9.22*	*<9.28*	*<9.58*
Meprobamate	57-53-4	*<0.573*	*<0.583*	*<0.576*	*<0.58*	*<0.599*
Metformin	657-24-9	*<0.269*	*<0.256*	*<0.254*	*<0.243*	*<0.257*
Methyl Triclosan	--	*<0.0184*	*NA*	*<0.0194*	0.049	0.18
Methylprednisolone	83-43-2	*<2.49*	*<1.69*	*<1.72*	*<1.55*	*<2.94*
Metoprolol	51384-51-1	*<0.192*	*<0.195*	*<0.193*	*<0.194*	0.4685
Metronidazole	443-48-1	*<0.765*	*<0.777*	*<0.768*	*<0.773*	*<0.798*
Miconazole	22916-47-8	0.681	0.77	0.9475	1.635	1.775
Minocycline	10118-90-8	*<22.9*	*<23.3*	*NA*	*<23.2*	*<23.9*
Moxifloxacin	151096-09-2	*<1.53*	*<1.55*	*<1.54*	*<1.55*	*<1.6*
Naproxen	22204-53-1	*<0.765*	*<0.777*	*<0.768*	*<0.773*	*<0.798*
Norfloxacin	70458-96-7	*<8.49*	*<9.45*	*<18.5*	*<9.55*	*<15.6*
Norfluoxetine	83891-03-6	*<0.192*	*<0.195*	*<0.193*	0.203	0.798
Norgestimate	35189-28-7	*<2.45*	*<4.1*	*<4.25*	*<2.98*	*<3.31*
Norverapamil	67018-85-3	*<0.0573*	*<0.0583*	*<0.0574*	*<0.0595*	*<0.0579*
Ofloxacin	82419-36-1	*<0.573*	*<1.13*	*<0.58*	*<0.58*	*<0.0599*
Ormetoprim	6981-18-6	1960	1750	1760	2405	1570

Analyte	CAS#	0% WWE	0.4% WWE	1.4% WWE	5.3% WWE	20% WWE
Oxacillin	66-79-5	*<1.15*	1.21	*<1.41*	2.94	3.79
Oxazepam	604-75-1	*<1.53*	*<1.55*	*<1.54*	*<1.55*	*<1.6*
Oxolinic Acid	14698-29-4	*<0.229*	*<0.233*	*NA*	0.321	*<0.23*
Oxycodone	76-42-6	*<0.538*	*<0.512*	*<0.508*	*<0.486*	*<0.514*
Oxytetracycline [OTC]	79-57-2	*<2.29*	*<2.33*	*NA*	*<2.32*	*<2.39*
Paroxetine	61869-08-7	*<0.385*	*<0.391*	*<0.386*	*<0.389*	*<0.401*
Penicillin G	61-33-6	*<11.5*	*<11.7*	*NA*	*<11.6*	*<12*
Penicillin V	87-08-1	*<1.21*	1.48 K	*<1.53*	2.01	2.69
Prednisolone	50-24-8	*<1.53*	*<1.55*	*<1.54*	*<1.55*	*<1.6*
Prednisone	53-03-2	*<2.29*	*<2.33*	*NA*	*<2.32*	*<2.39*
Promethazine	60-87-7	*<0.115*	*<0.117*	*NA*	*<0.116*	*<0.12*
Propoxyphene	469-62-5	*<0.115*	*<0.117*	*NA*	*<0.116*	*<0.12*
Propranolol	525-66-6	*<0.115*	*<0.117*	*NA*	*<0.116*	0.276
Ranitidine	66357-35-5	*<0.538*	*<0.512*	*<0.508*	*<0.486*	*<0.514*
Rosuvastatin	287714-41-4	*<1.53*	*<1.55*	*<1.54*	*<1.55*	*<1.6*
Roxithromycin	80214-83-1	*<0.115*	*<0.117*	*NA*	*<0.116*	*<0.12*
Sarafloxacin	98105-99-8	*<5.73*	*<5.83*	*<5.76*	*<5.8*	*<5.99*
Sertraline	79617-96-2	*<0.115*	0.13	0.58	1.85	8.73
Simvastatin	79902-63-9	*<0.768*	*<0.781*	*<0.772*	*<0.777*	*<0.802*
Sulfachloropyridazine	80-32-0	*<5.73*	*<5.83*	*<5.76*	*<5.8*	*<5.99*
Sulfadiazine	68-35-9	*<0.573*	*<0.583*	*<0.576*	*<0.58*	*<0.599*
Sulfadimethoxine	122-11-2	93.4	69.8	68.8	60.5	67.55
Sulfamerazine	127-79-7	*<0.577*	*<0.233*	*<0.346*	*<0.232*	*<0.277*
Sulfamethazine	57-68-1	*<0.725*	*<1.19*	*<0.832*	*<0.58*	*<0.67*
Sulfamethizole	144-82-1	*<0.229*	*<0.233*	*NA*	*<0.232*	*<0.239*
Sulfamethoxazole	723-46-6	*<0.229*	*<0.233*	*NA*	*<0.232*	*<0.239*
Sulfanilamide	63-74-1	*<5.73*	*<5.83*	*<5.76*	*<5.8*	*<5.99*
Sulfathiazole	72-14-0	*<0.573*	*<0.583*	*<0.576*	*<0.58*	*<0.599*
Tamoxifen	10540-29-1	*<0.153*	*<0.155*	*<0.154*	*<0.155*	*<0.16*
Teniposide	29767-20-2	*<1.53*	*<1.55*	*<1.54*	*<1.55*	*<1.6*
Tetracycline [TC]	60-54-8	*<2.29*	*<2.33*	*NA*	*<2.32*	*<2.39*
Theophylline	58-55-9	*<2.29*	*<2.33*	*NA*	*<2.32*	*<2.39*
Thiabendazole	148-79-8	*<0.573*	*<0.583*	*<0.576*	*<0.58*	*<0.599*

Analyte	CAS#	0% WWE	0.4% WWE	1.4% WWE	5.3% WWE	20% WWE
Trenbolone	10161-33-8	*<0.768*	*<0.781*	*<0.772*	*<0.777*	*<0.802*
Trenbolone acetate	10161-34-9	*<0.116*	*<0.117*	*<0.127*	*<0.116*	*<0.12*
Triamterene	396-01-0	*<0.269*	*<0.256*	*<0.254*	*<0.243*	*<0.257*
Triclocarban	101-20-2	*<0.153*	*<0.155*	*<0.154*	*<0.155*	*<0.16*
Triclosan	3380-34-5	*<2.29, <0.183*	*<2.33*	*<0.345*	1.08	4.65
Trimethoprim	738-70-5	*<0.573*	*<0.583*	*<0.576*	*<0.58*	*<0.599*
Tylosin	1401-69-0	*<2.57*	*<2.7*	*<2.39*	*<2.64*	*<2.82*
Valsartan	137862-53-4	*<1.53*	*<1.55*	*<1.54*	*<1.55*	*<1.6*
Venlafaxine	93413-69-5	*<0.153*	*<0.155*	*<0.154*	*<0.155*	0.4385
Verapamil	52-53-9	*<0.0573*	*<0.0583*	*<0.0574*	*<0.0595*	0.38
Virginiamycin M1	21411-53-0	*<2.37*	*<2.29*	*<2.21*	*<2.42*	*<2.01*
Warfarin	81-81-2	*<0.306*	*<0.311*	*<0.307*	*<0.309*	*<0.319*
Zidovudine	30516-87-1	*<2.29*	*<2.33*	*NA*	*<2.32*	*<2.39*

This table includes all chemicals analyzed by SGS-AXYS. NA = Not Analyzed. All analytes with "<" were below their reporting limit (value shown) for that sample and considered as not quantifiable. Flags denoted by letters following detection values are defined as follows:

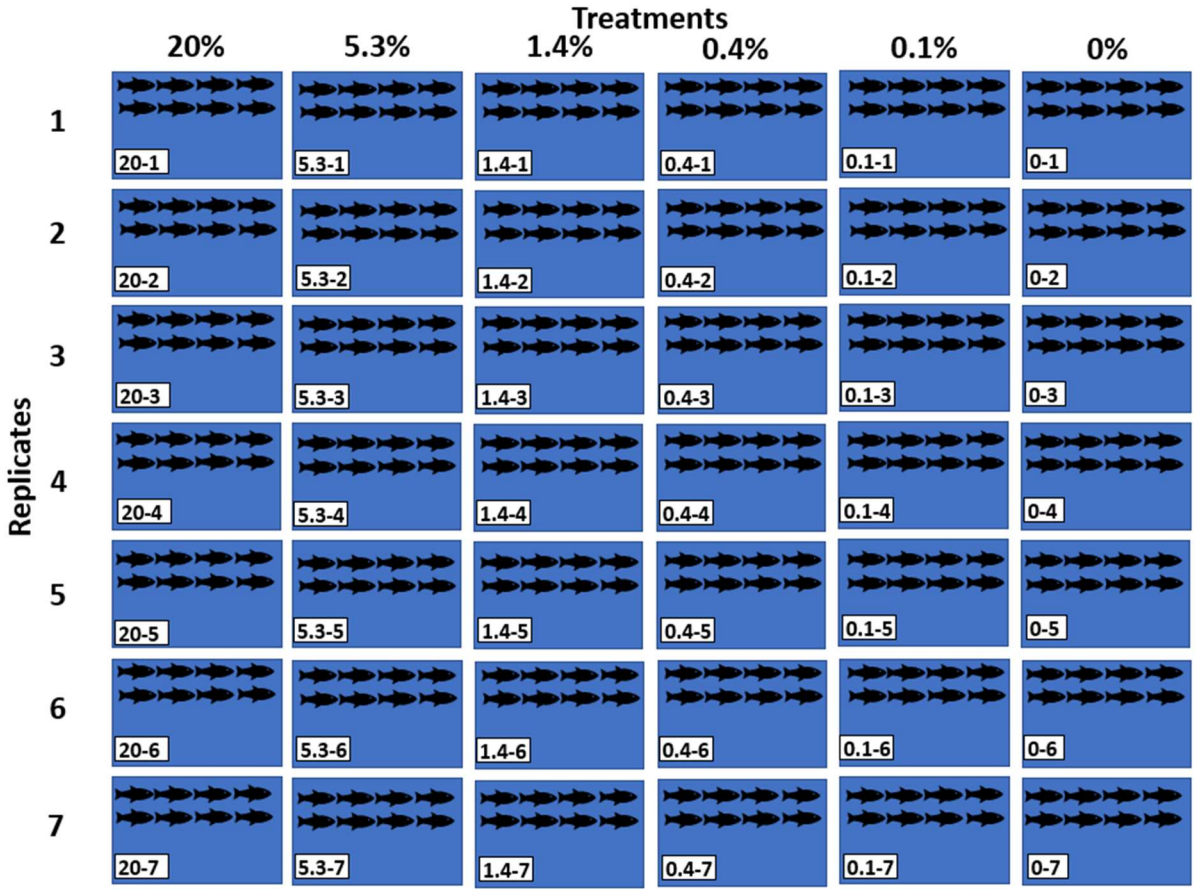
B: Analyte found in associated blank and concentration in sample is less than 10X the concentration in the associated blank.

M: Concentration is an estimated maximum value.

K: Peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration.

NQ: Not quantified.

APPENDIX B: LABORATORY EXPOSURE DESIGN



This figure represents the treatments, number of tank replicates, and total number of fish. Each blue rectangle represents one tank, with its respective tank identifier in the bottom left corner. Each fish represents one fish, and all tanks contained the same number of fish (eight). Tank order was randomized in the experiment, so tanks were not laid out as they appear here.

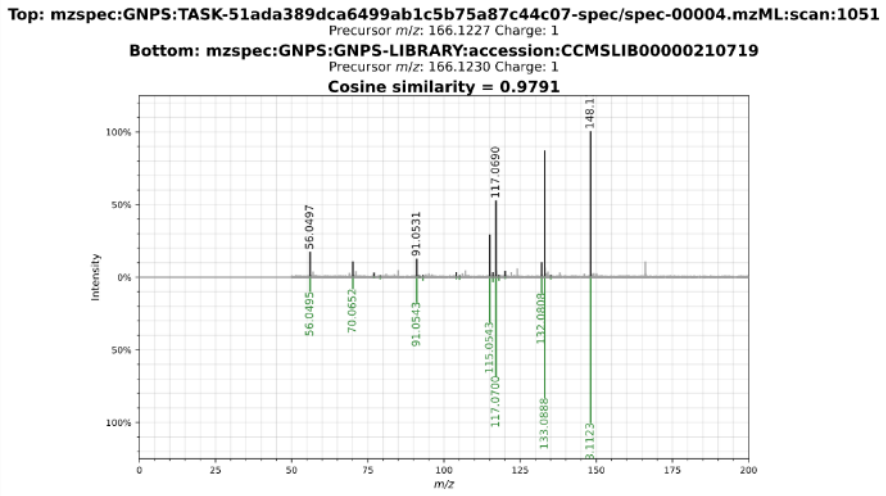
Compound identification results of King County wastewater effluent utilizing LC-HRMS analytical results.

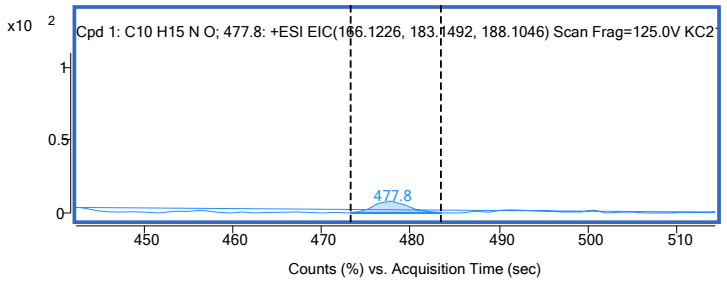
Each subsequent sheet provides:

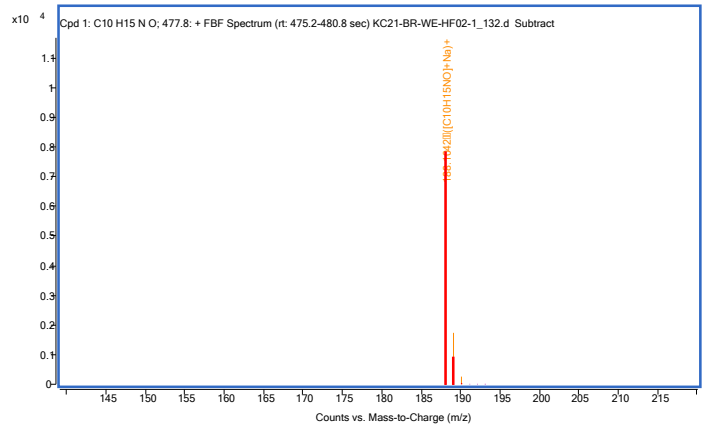
- MS/MS spectra mirror plot for measured and library spectra
- MS peak
- MS isotopic abundance patterns for measured and library (predicted)
- Compound structure diagram
- Compound name

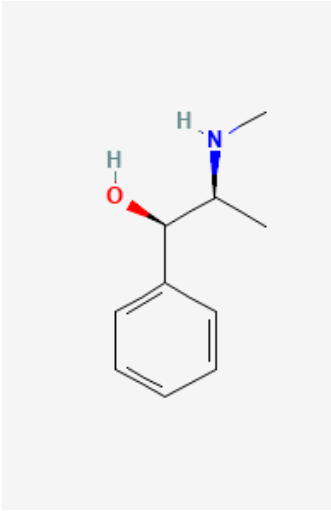
MS/MS data analysis by Dhruvi Joshi (UW) and C. Andrew James (UW)

King County Orca Proviso



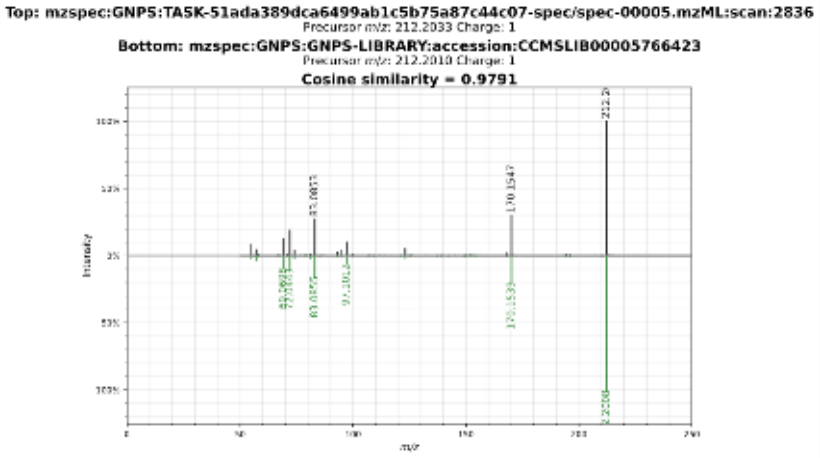


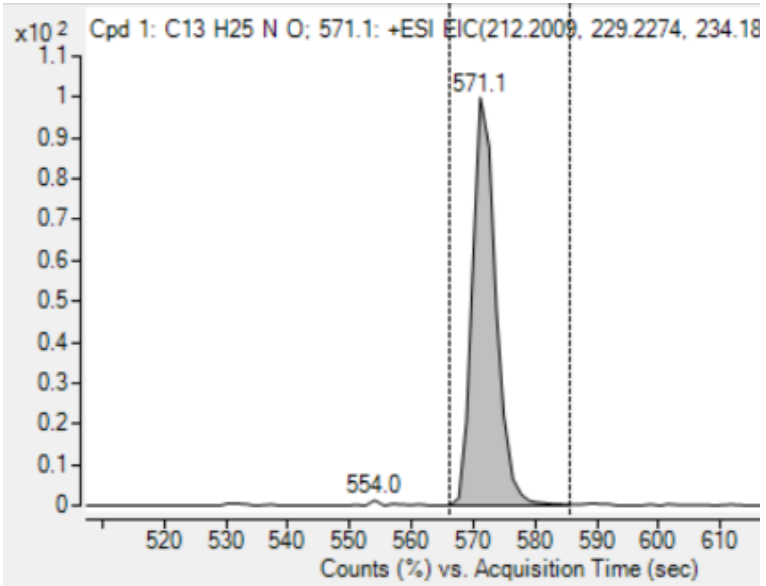


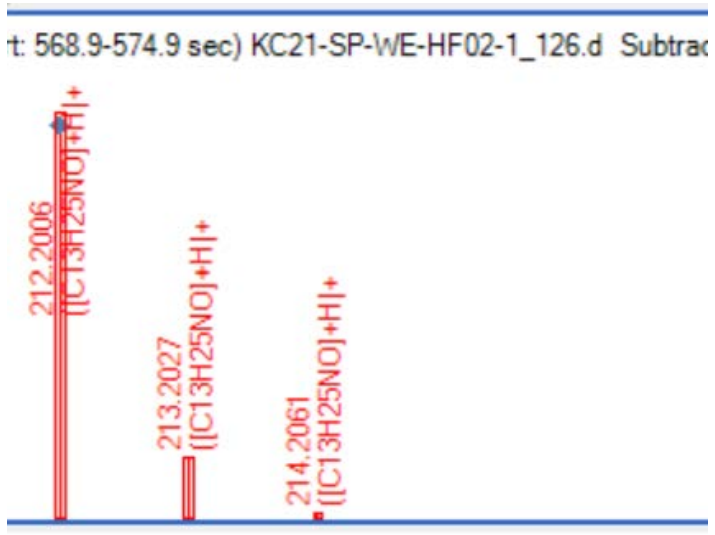


EPHEDRINE

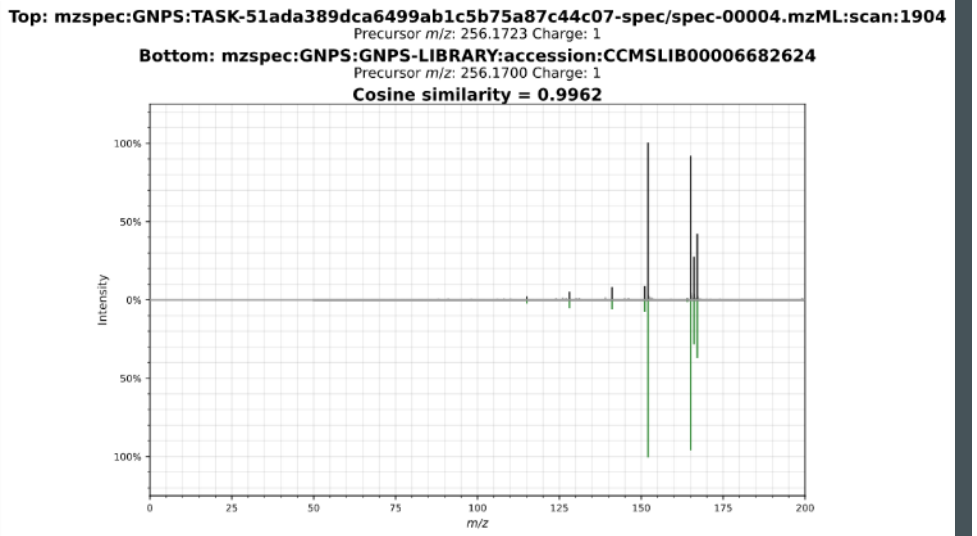
A PRESCRIPTION MEDICATION USED TO TREAT HYPOTENSION.
CLASS: NONSELECTIVE ADRENERGIC AGONIST

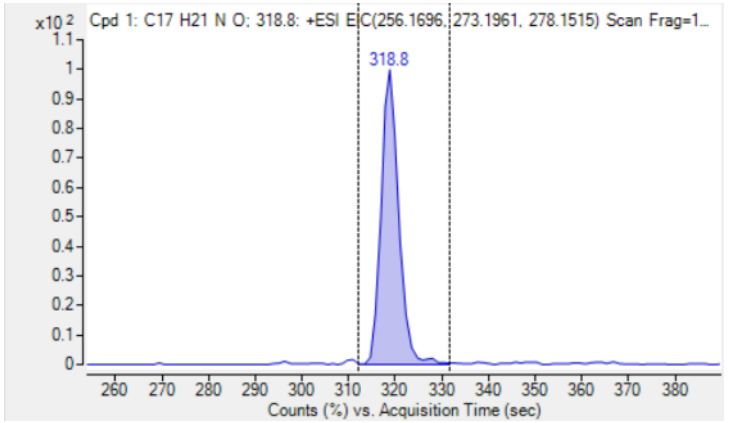


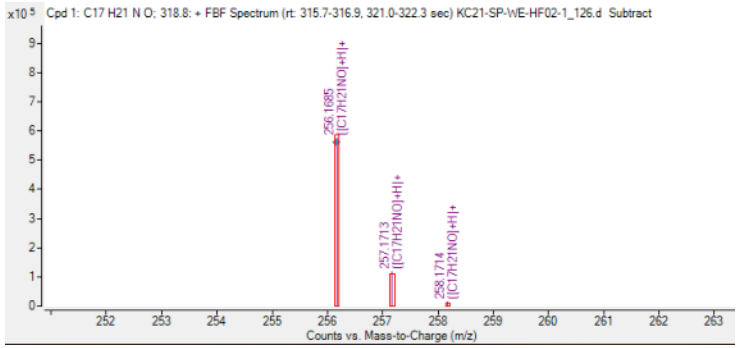


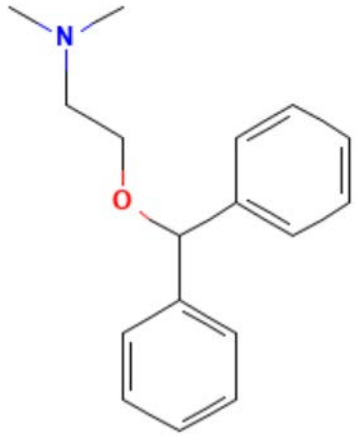


N-ETHYL-4-MENTHANE-3-CARBOXAMIDE
COMPOUND INFO: FOOD ADDITIVE AND FLAVORING AGENT
DRUG CLASS: N/A



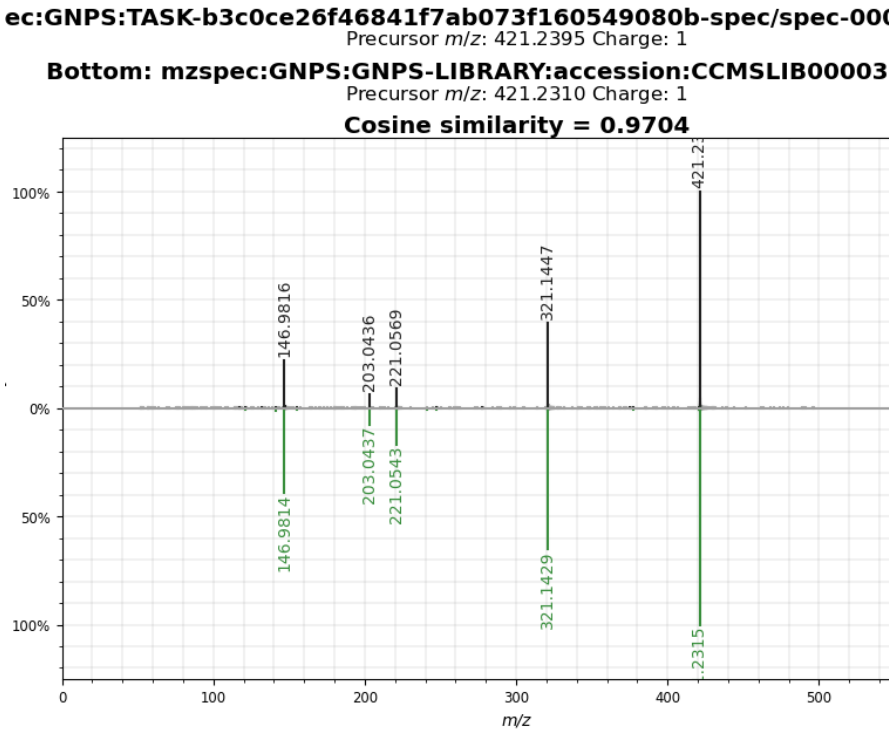


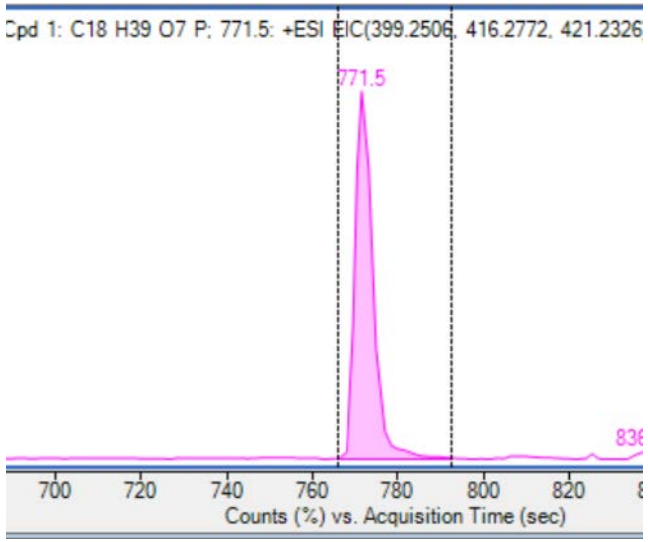


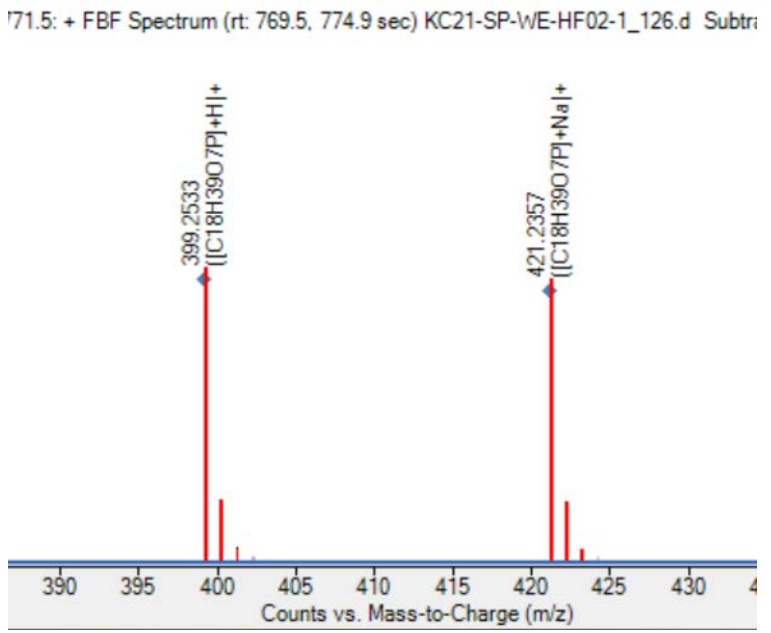


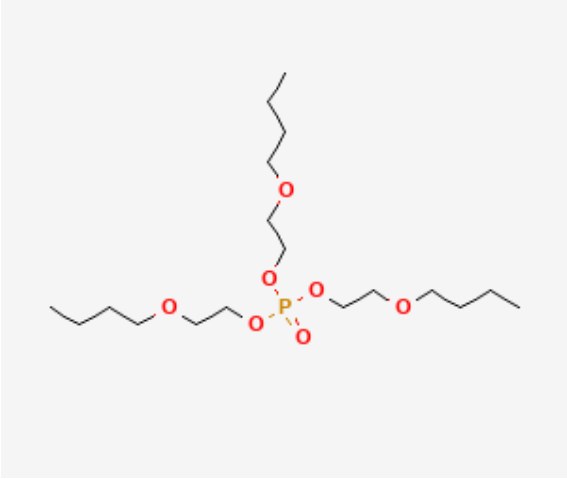
DIPHENHYDRAMINE

COMPOUND INFO: USED TO RELIEVE ALLERGY SYMPTOMS SUCH AS ITCHING, WATERY EYES, RUNNING NOSE, AND MORE.
DRUG CLASS: ANTIHISTAMINE



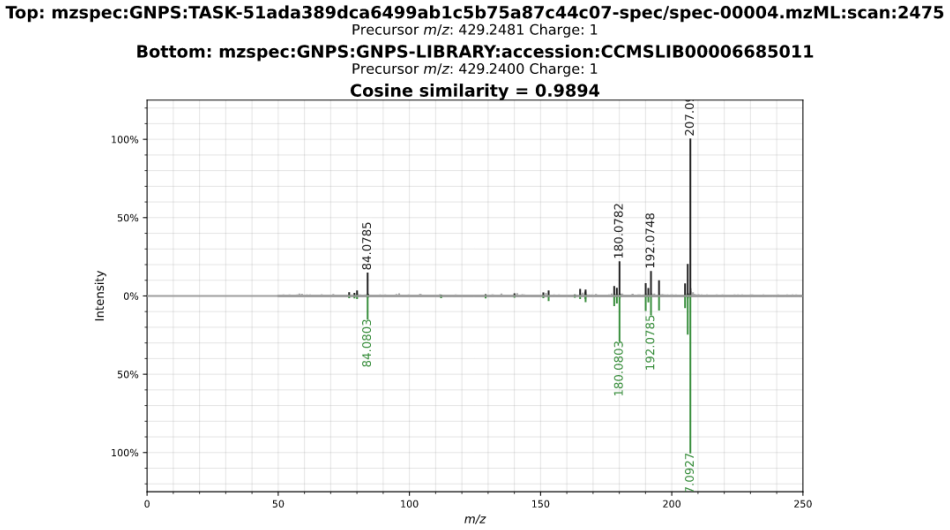


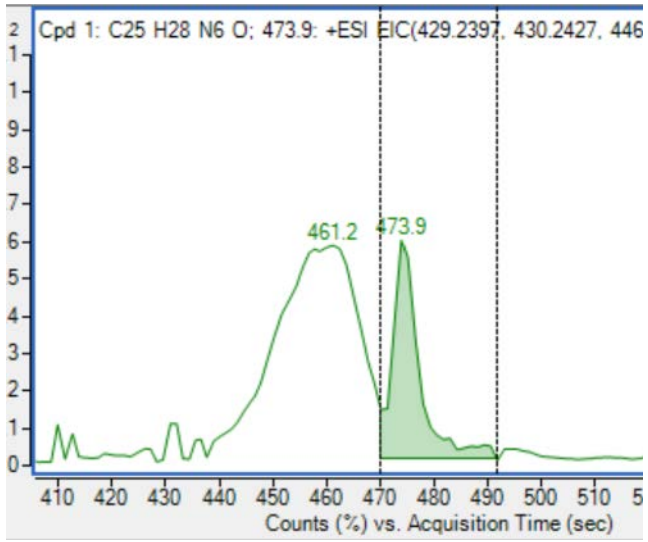


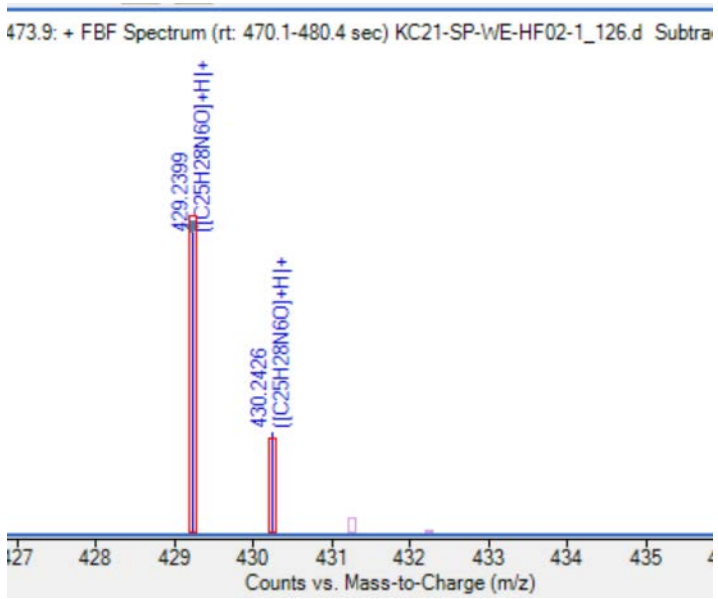


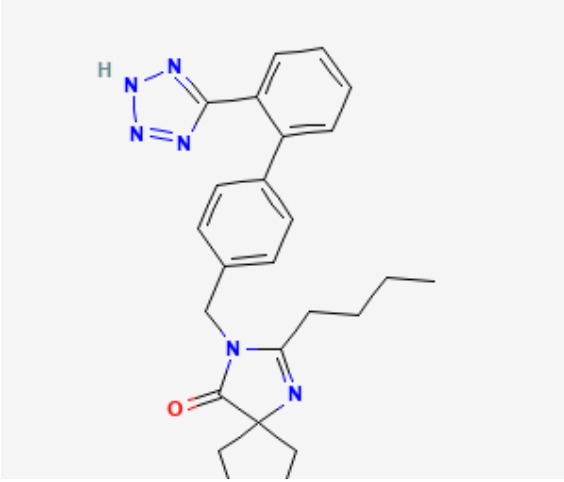
TRIS(2-BUTOXYETHYL) PHOSPHATE

- Compound info: Environmental contaminant and a flame retardant
- Drug Class: n/a



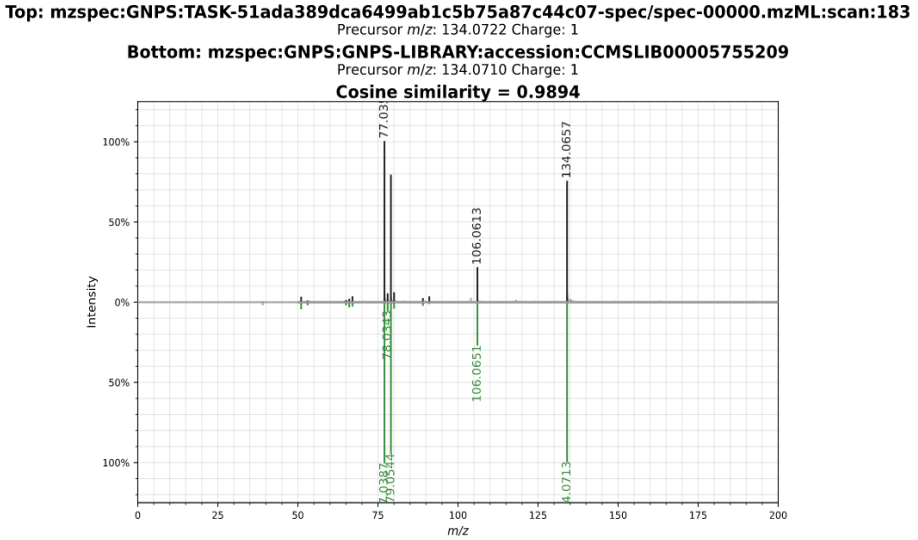


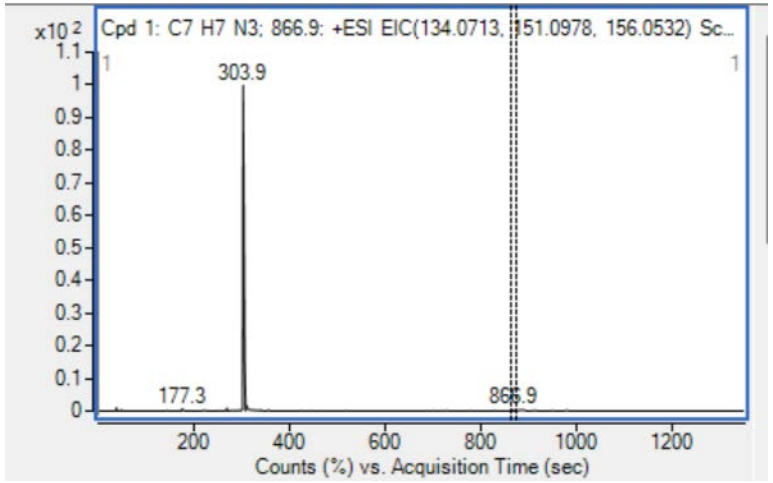


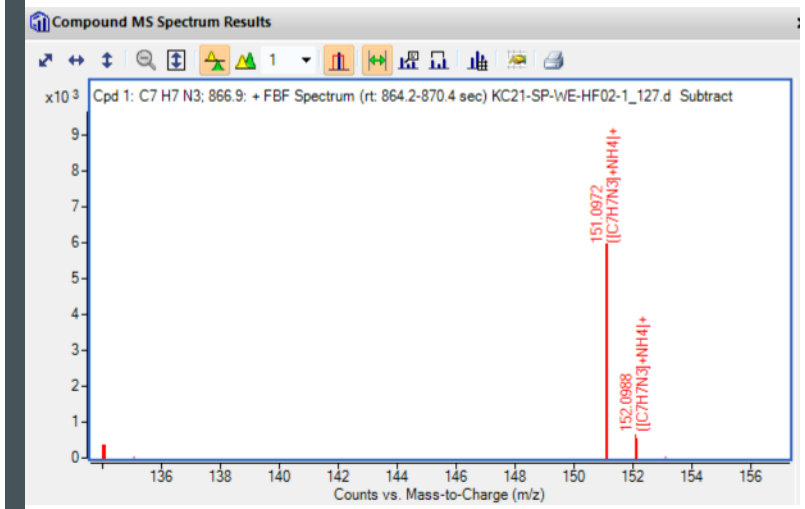


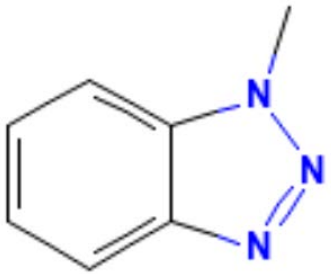
IRBESARTAN

- Compound info: used to treat high blood pressure and protect kidneys due to diabetes
- Drug Class: Angiotensin receptor blockers



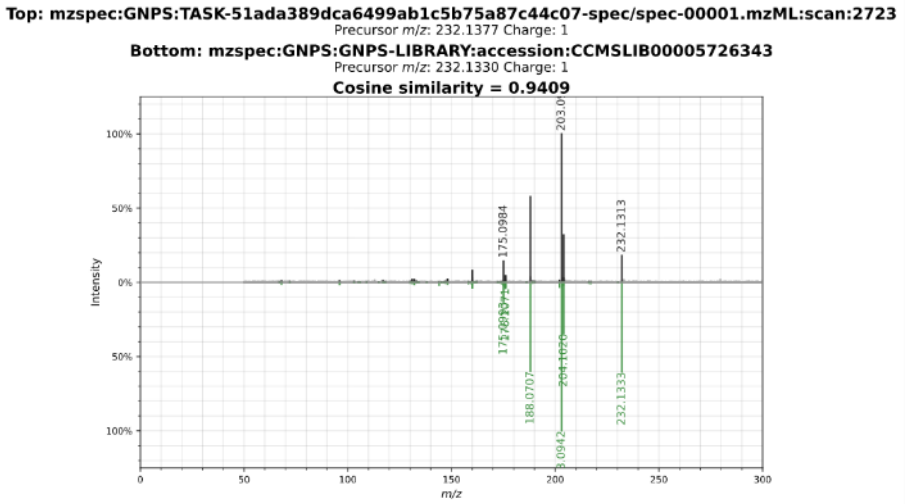


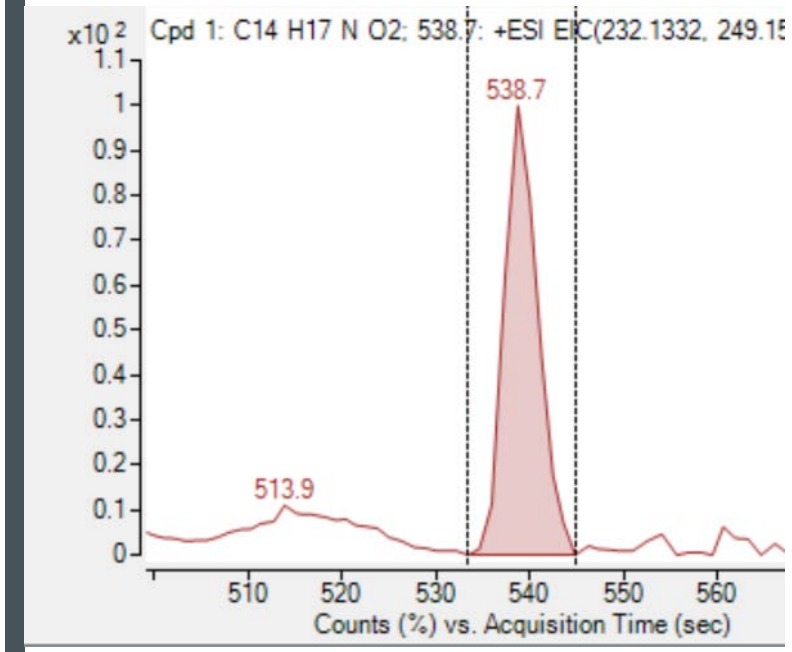


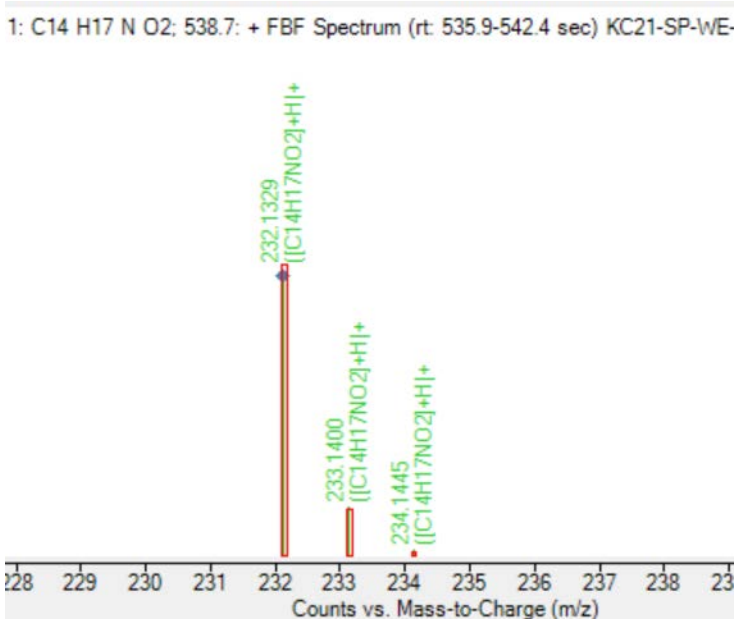


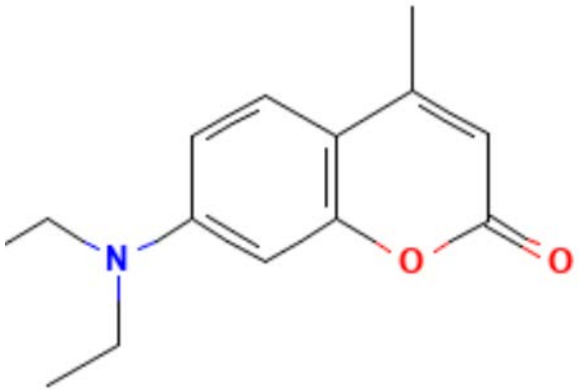
4-METHYLBENZOTRIAZOLE

- Compound info: N/a- is an irritant
- Drug Class: N/a



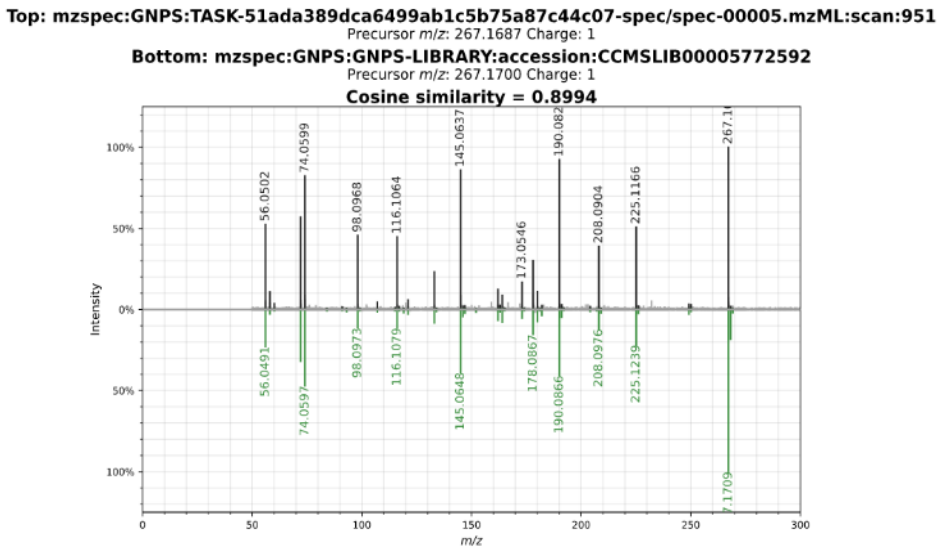


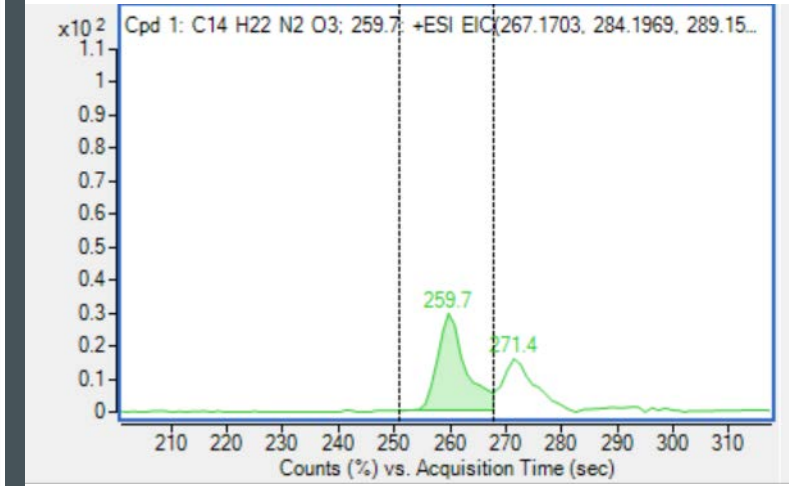


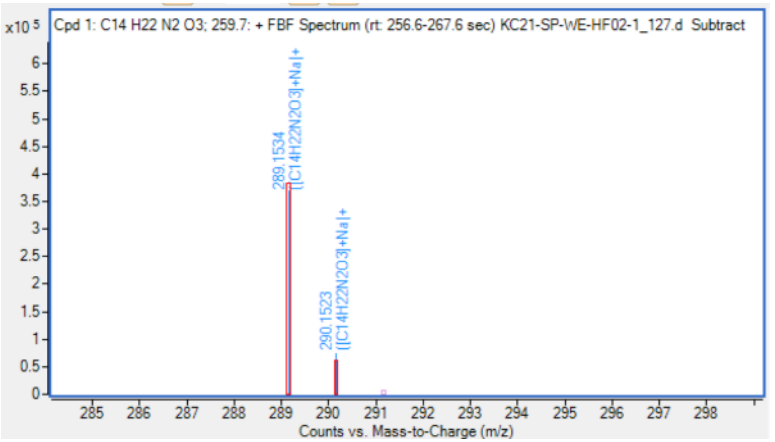


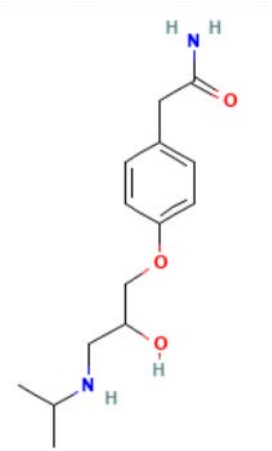
4-METHYL-7-DIETHYLAMINOCOUMARIN

- Compound info: environmental hazard and irritant
- Drug Class: used in cosmetics to stabilize and in dyes for the industry



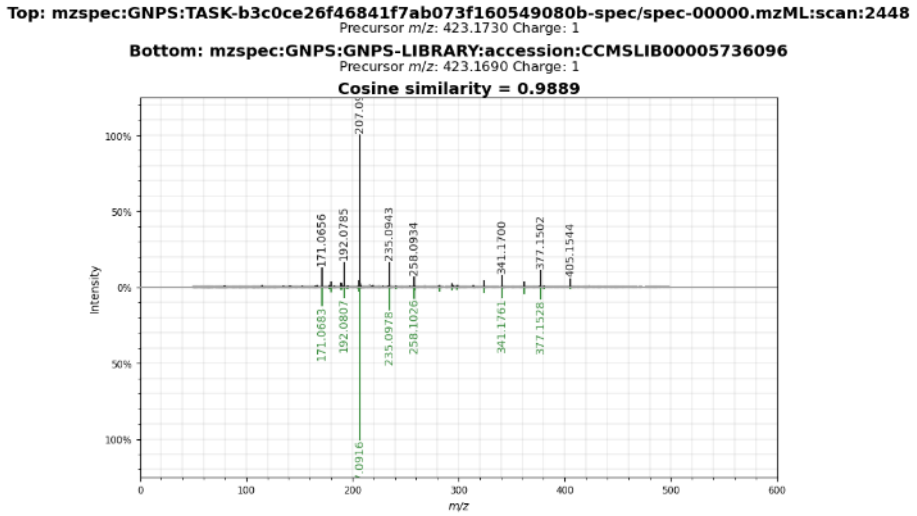


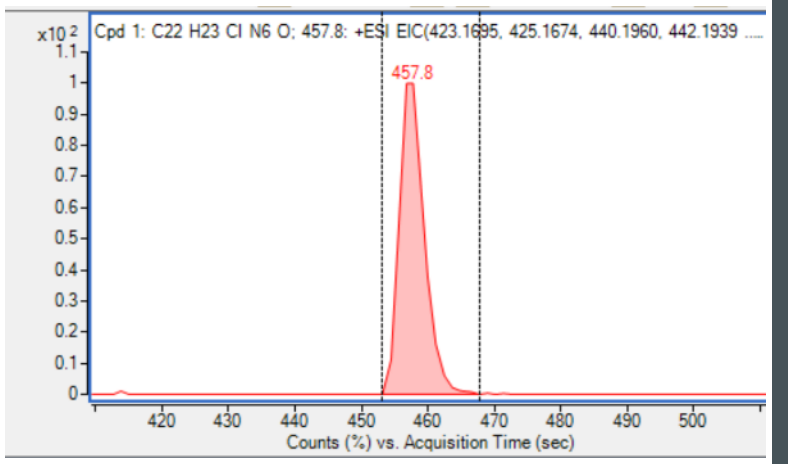


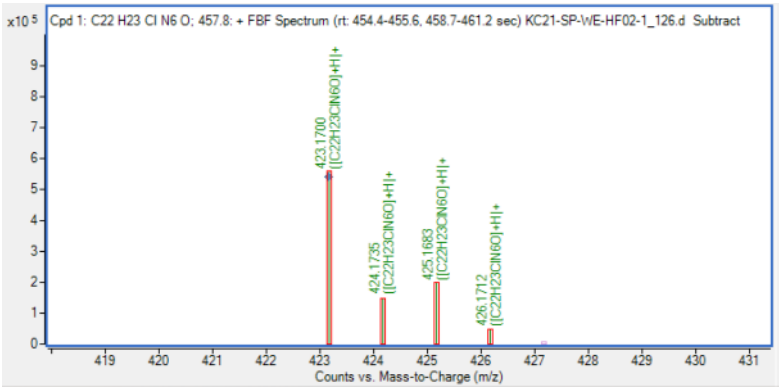


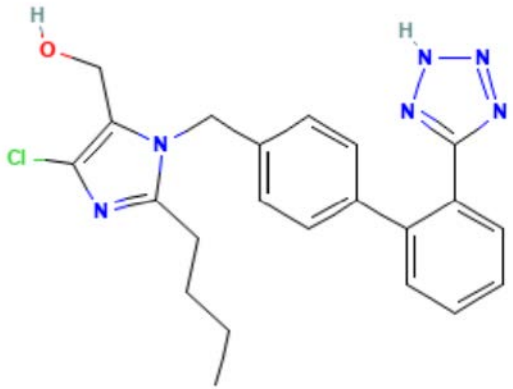
ATENOLOL

- Compound info: used to treat high blood pressure – hypertension
- Drug Class: beta blockers



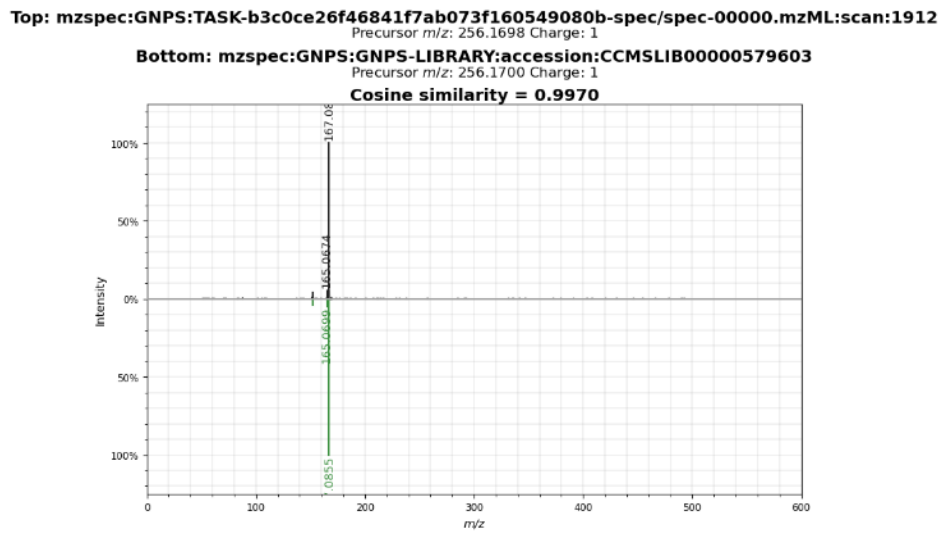


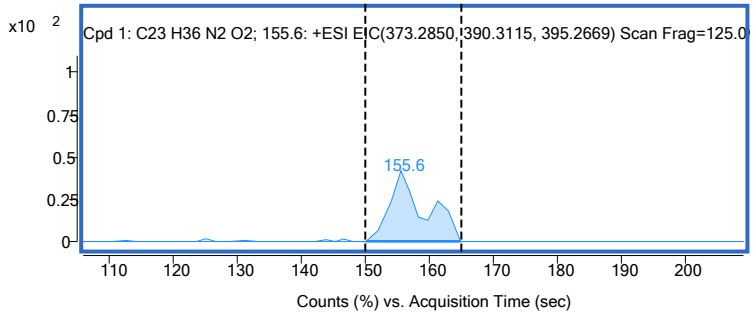


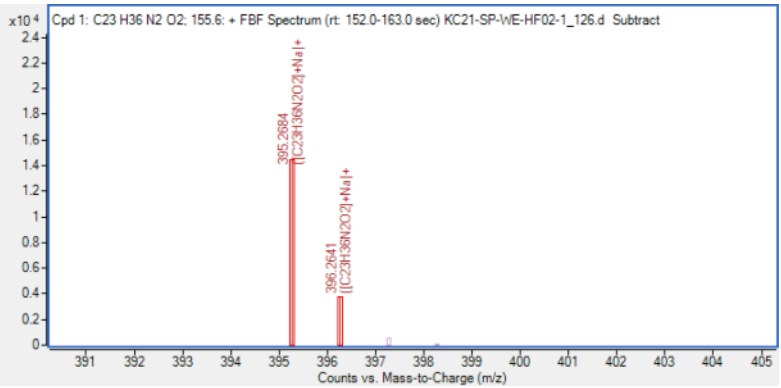


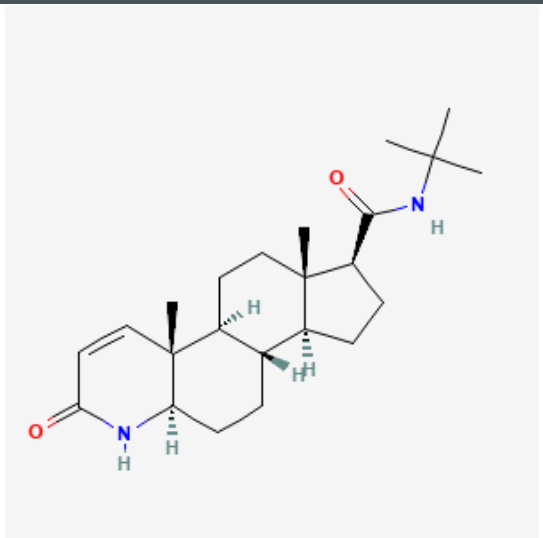
LOSARTAN –

- Compound info: Used to treat high blood pressure as well as diabetes and kidney disease
- Drug Class: Angiotensin II blocker (ARB)



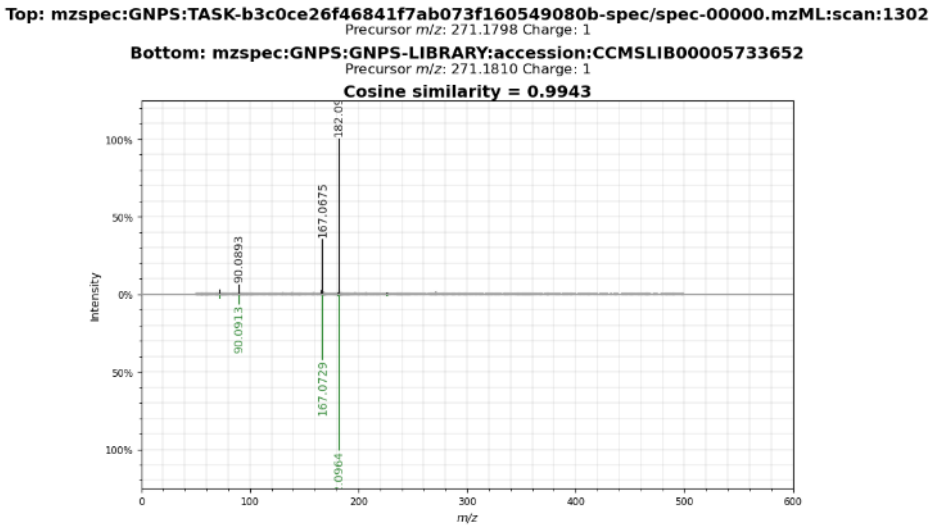


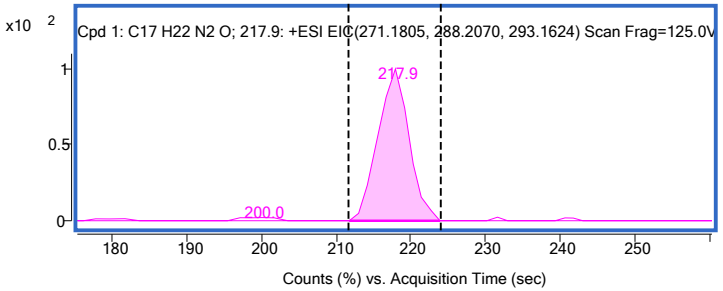


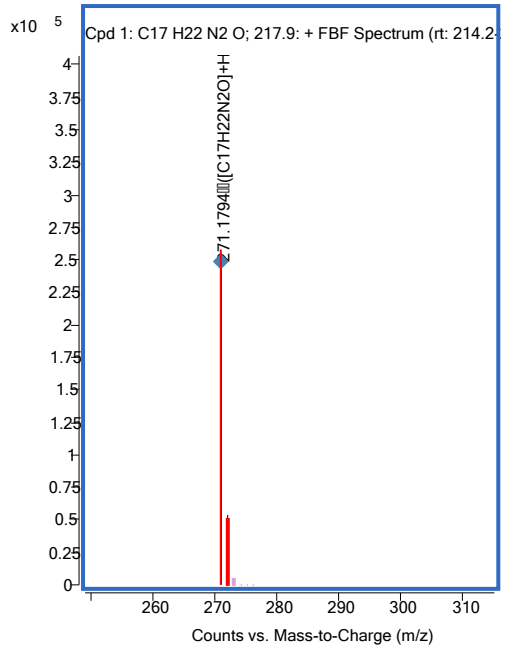


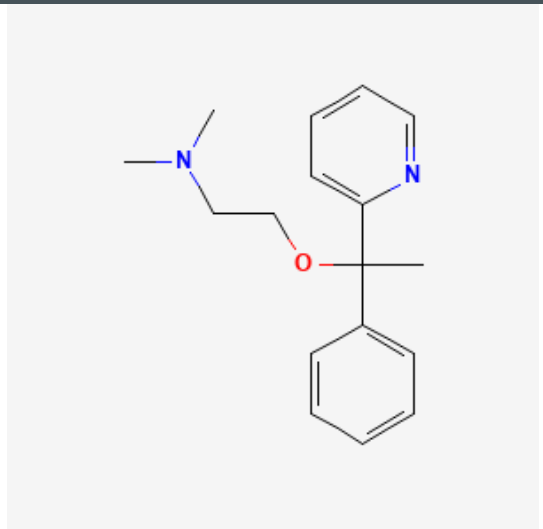
FINASTERIDE

- Compound info: used to treat male pattern hair loss and benign prostatic hyperplasia
- Drug Class: 5-alpha reductase inhibitors



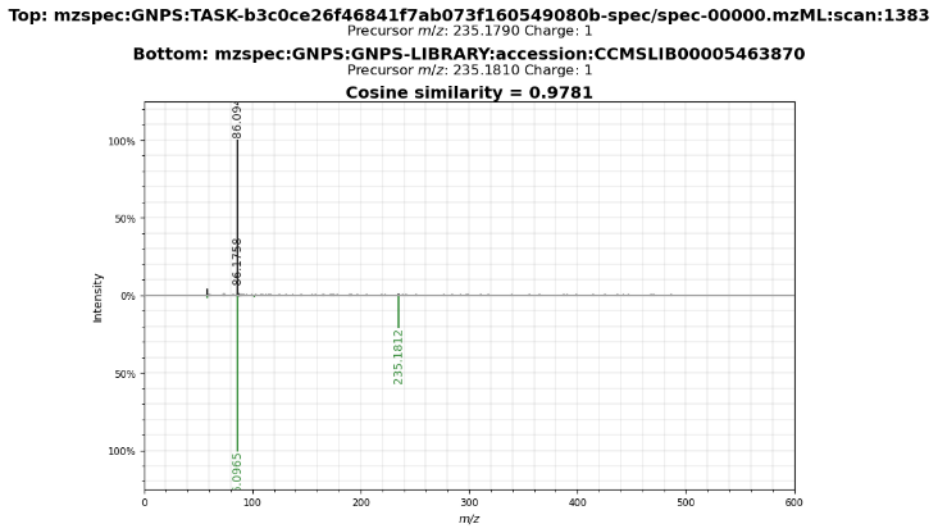


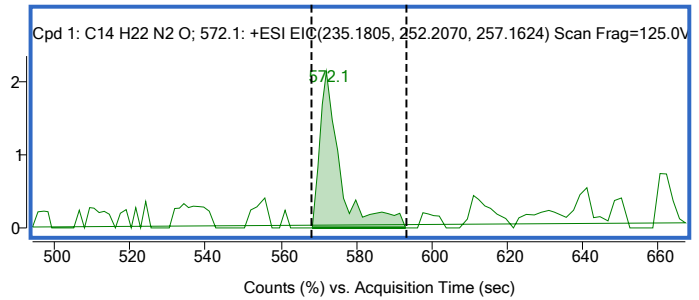


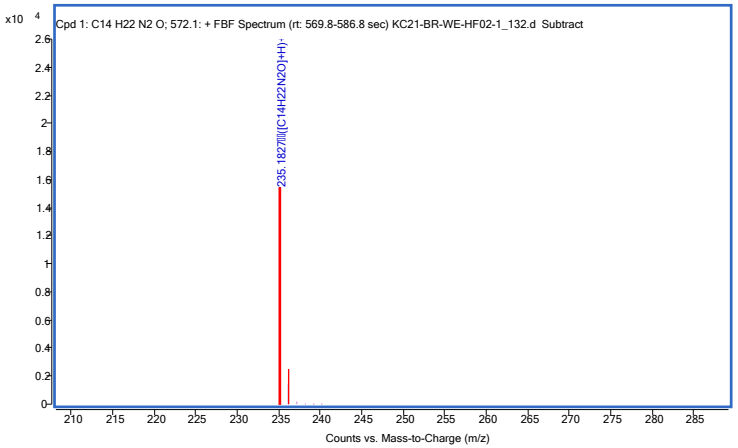


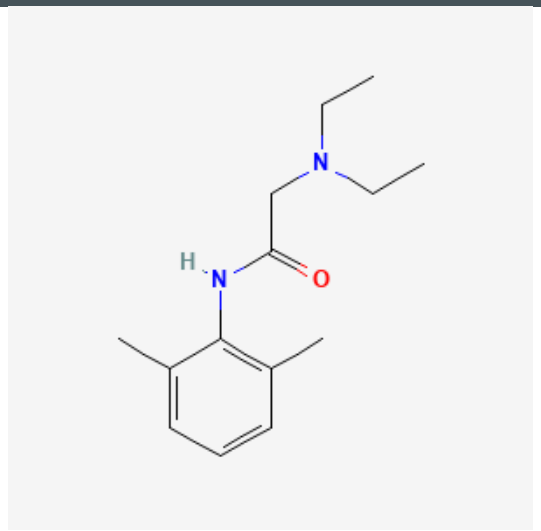
DOXYLAMINE

- Compound info: used for short-term treatment of insomnia as well as to help relieve allergy symptoms
- Drug Class: Antihistamines



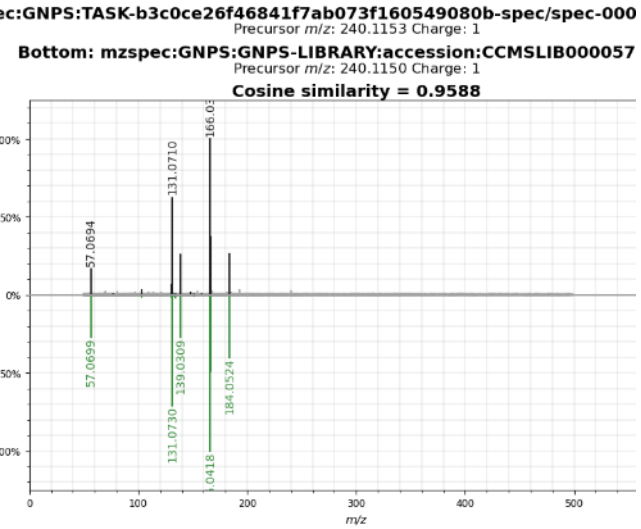


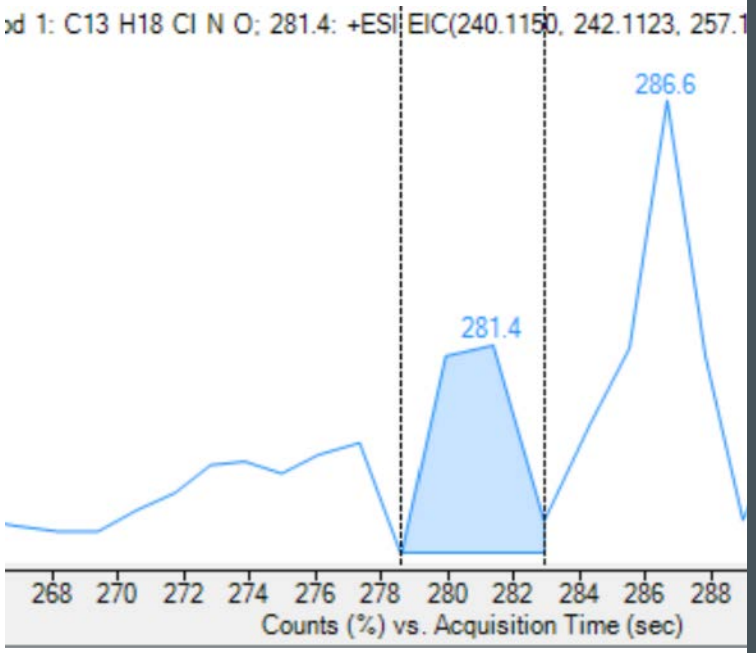


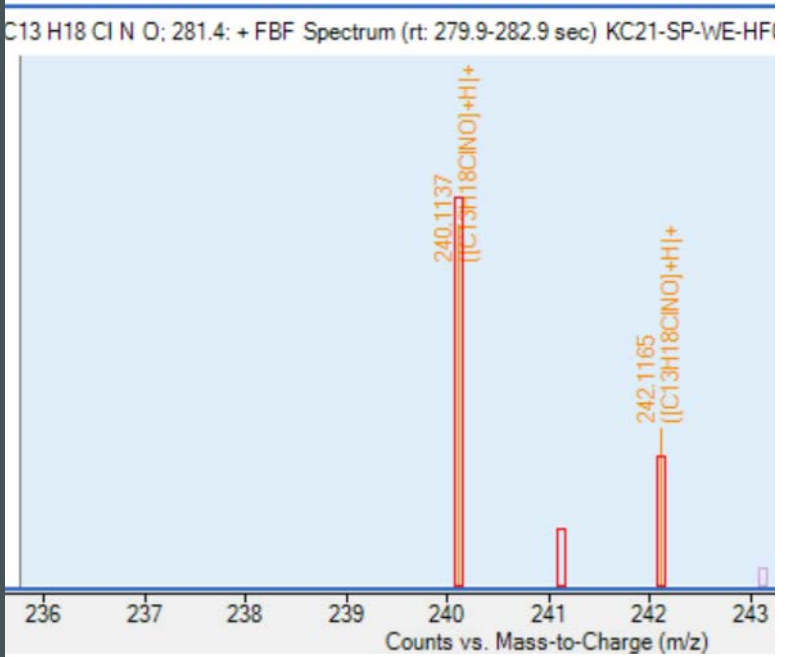


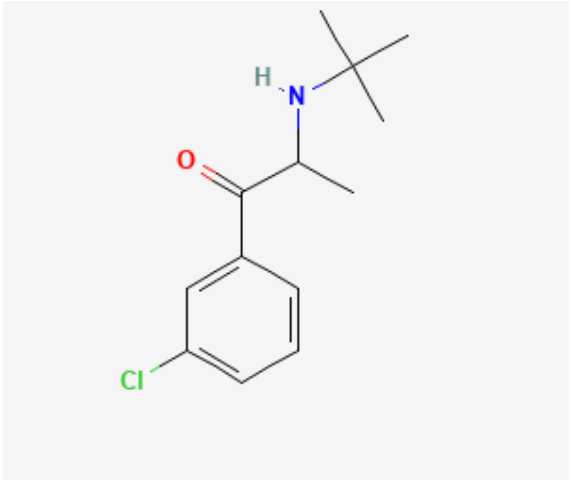
LIDOCAINE

- Compound info: used for pain relief, can be OTC or prescription
- Drug Class: local anesthetics



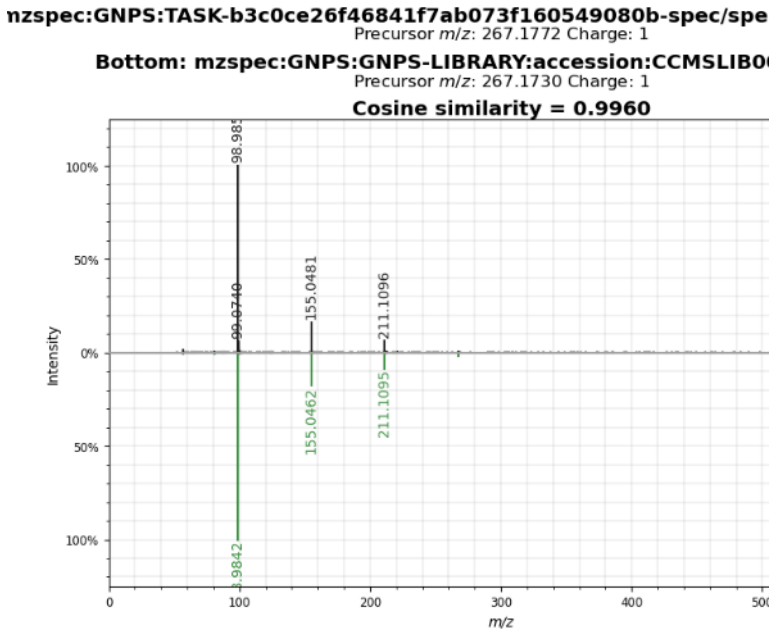


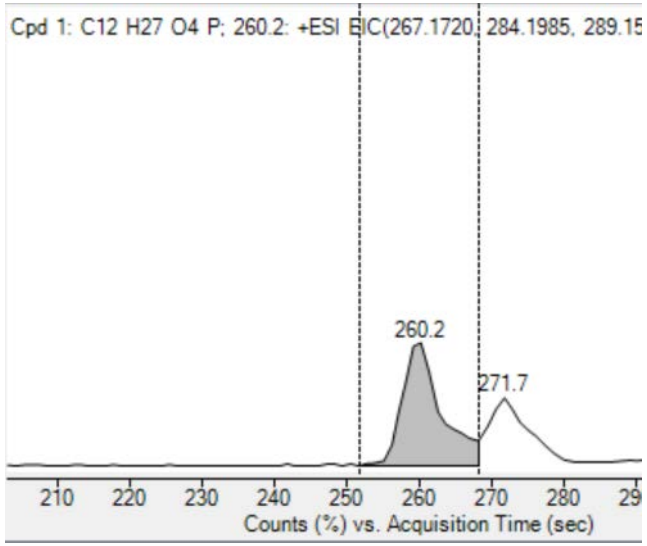


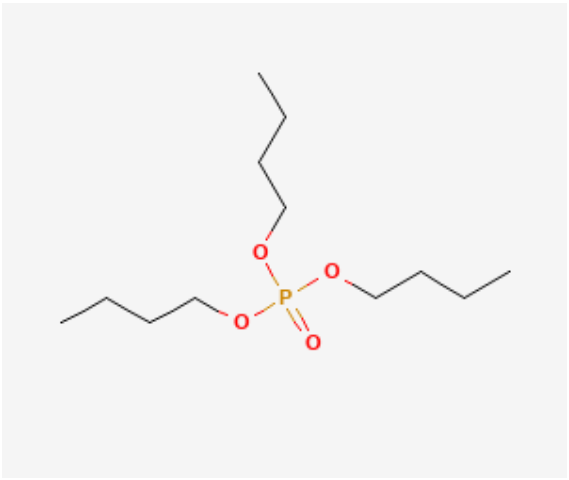


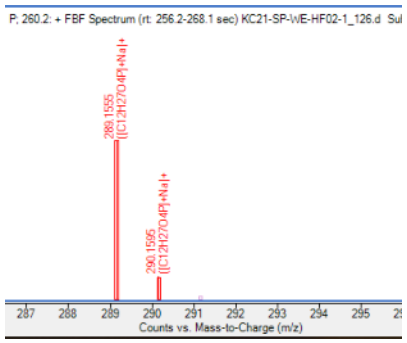
BUPROPION

- Compound info: Used to treat and prevent depression in patients with Seasonal Depressive Disorder
- Drug Class: Dopamine reuptake inhibitors



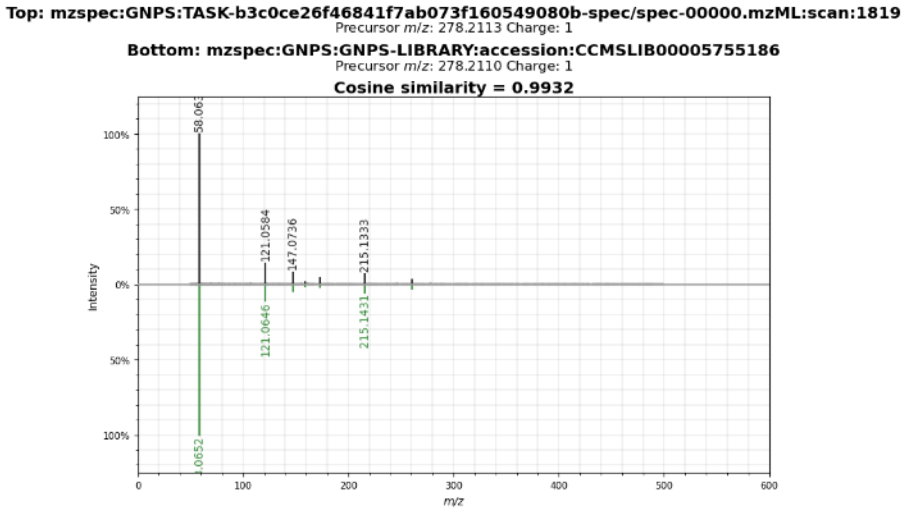


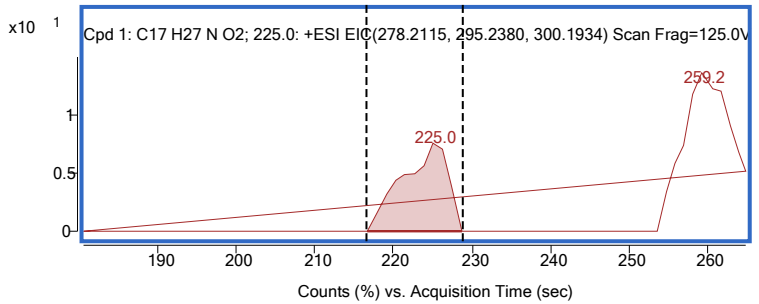


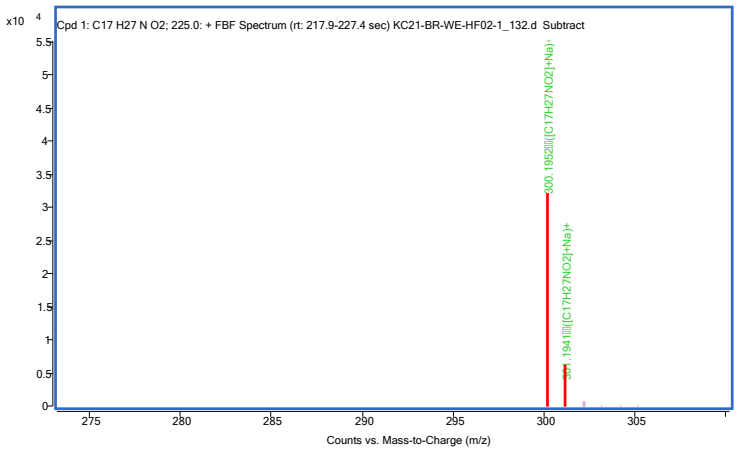


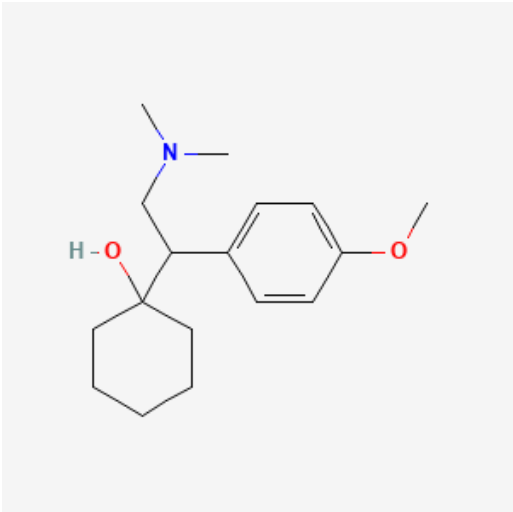
TRIBUTYL PHOSPHATE

- Compound info: used as plasticizer, a solvent, in hydraulic fluid, extractant, and heat extractant agent
- Drug Class: Irritant and Health Hazard



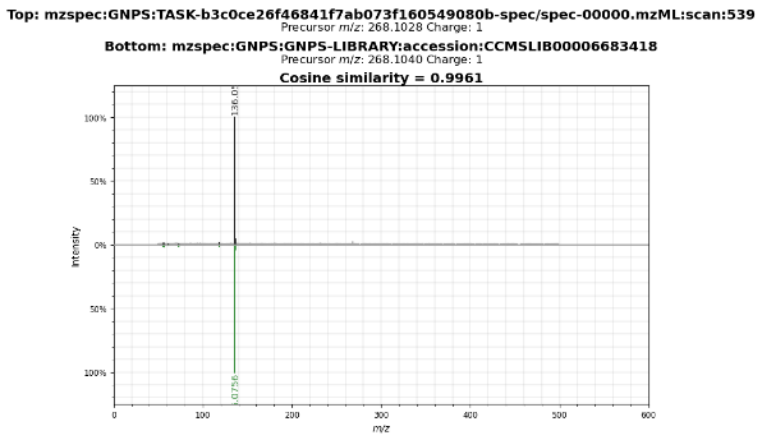


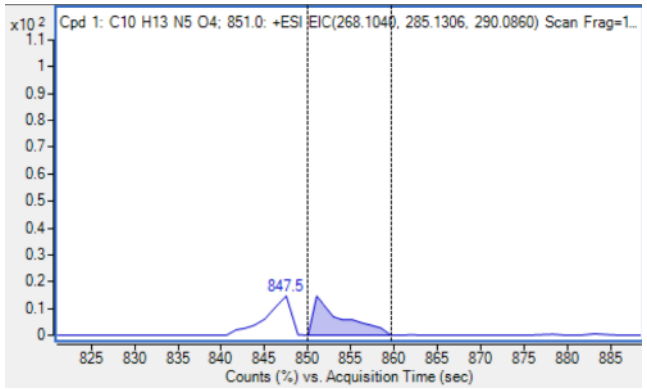


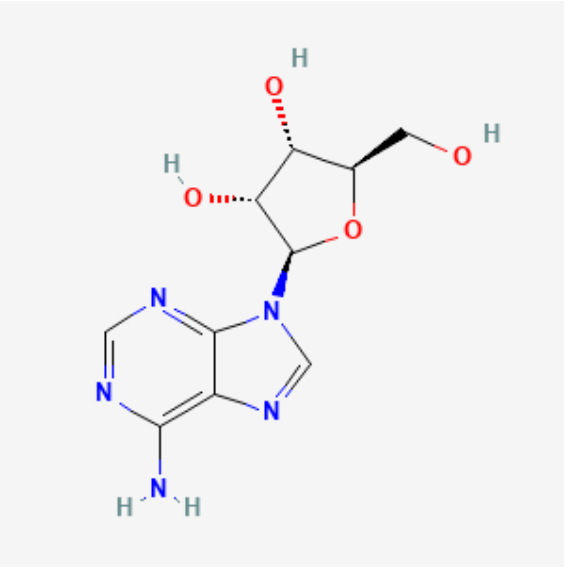


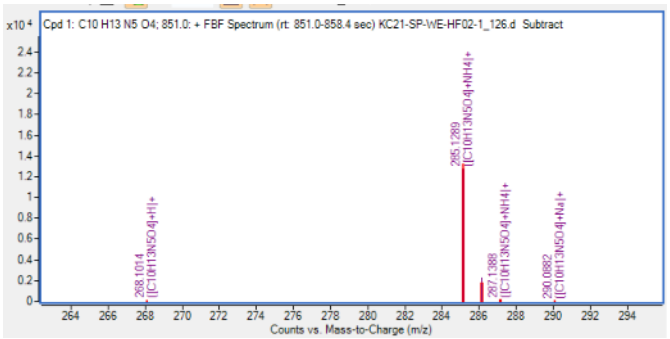
VENLAFAXINE

- Compound info: Used to treat depression, General anxiety disorder, panic disorder, and social anxiety
- Drug Class: Norepinephrine reuptake inhibitors (SNRI)



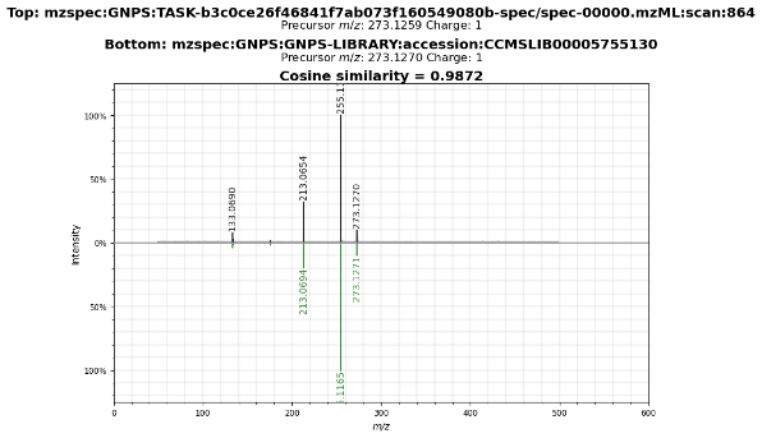


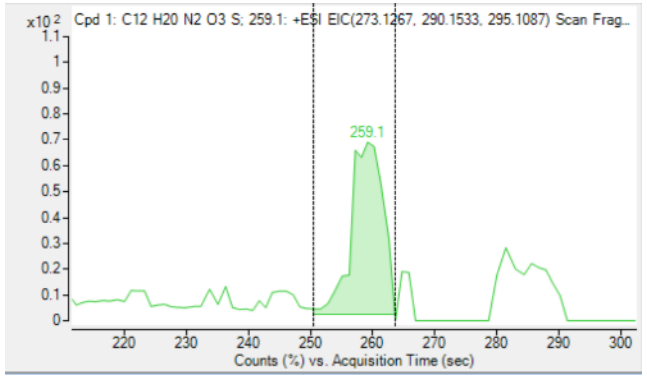


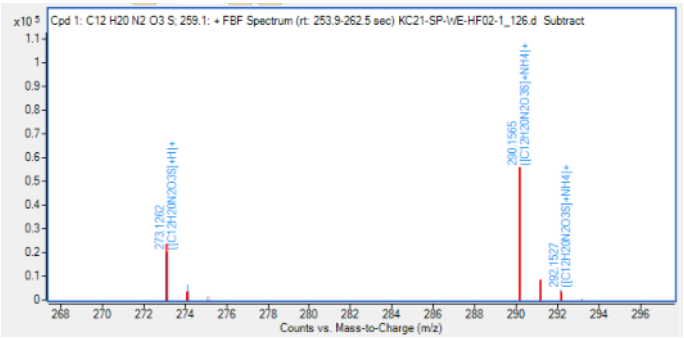


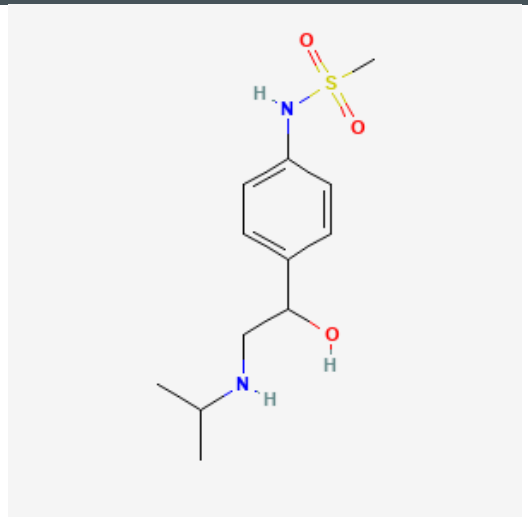
ADENOSINE

- Compound info: Used as diagnostic and therapeutic agent used to treath antiarrhythmic
- Drug Class: Class V antiarrhythmic agent



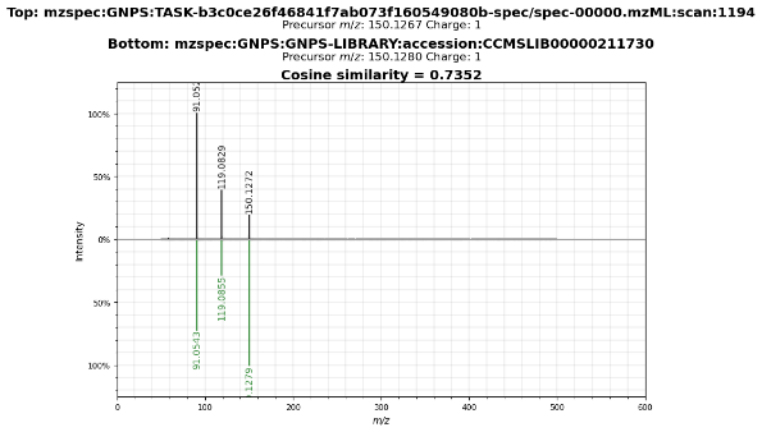


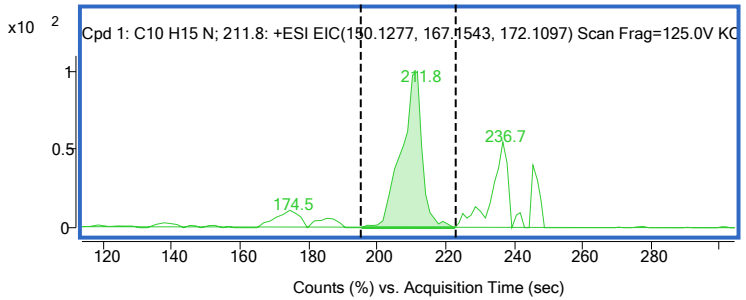


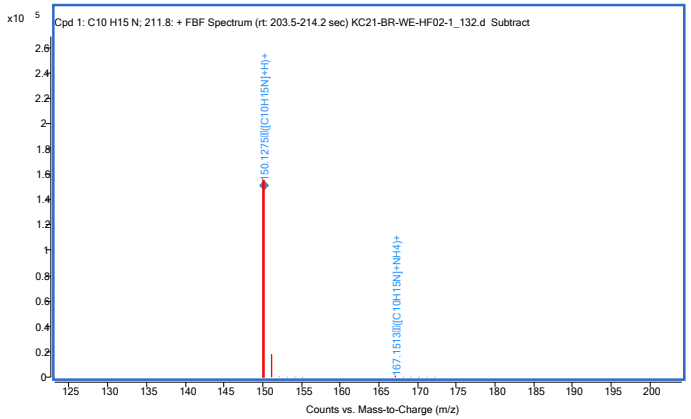


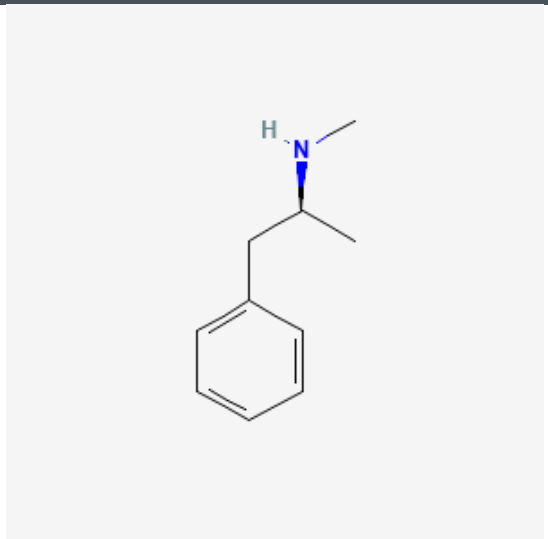
SOTALOL

- Compound info: used to treat irregular heartbeats
- Drug Class: antiarrhythmics



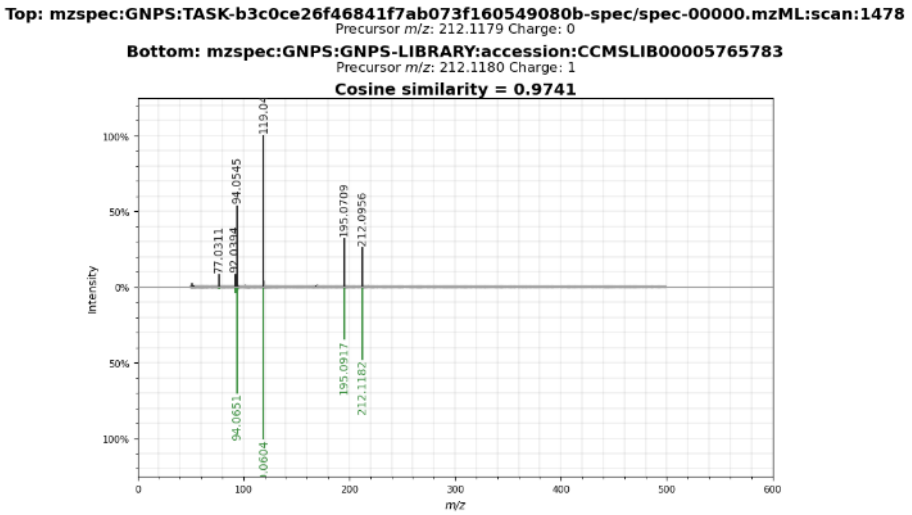


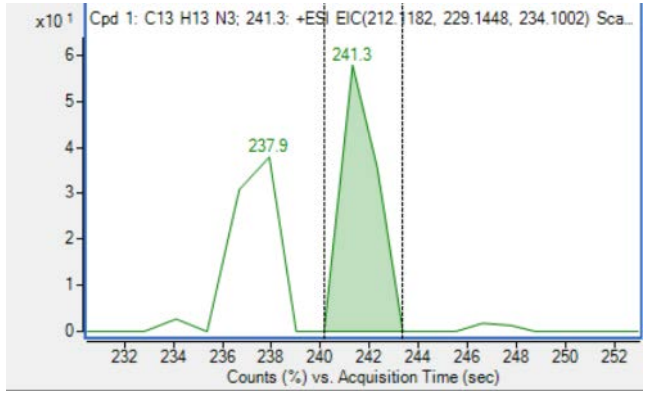


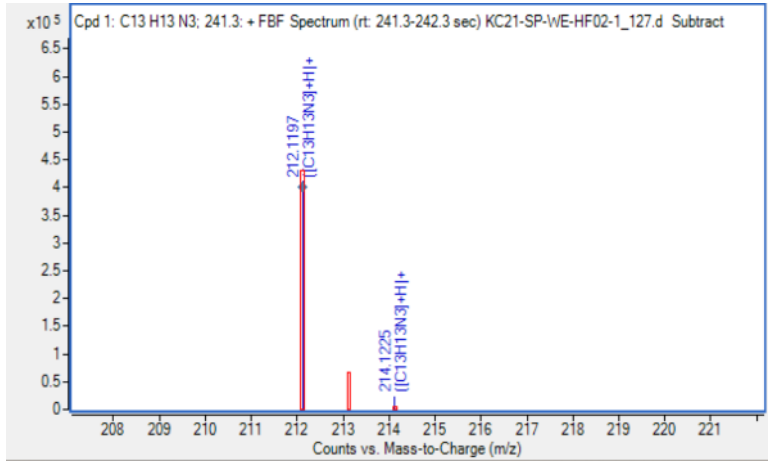


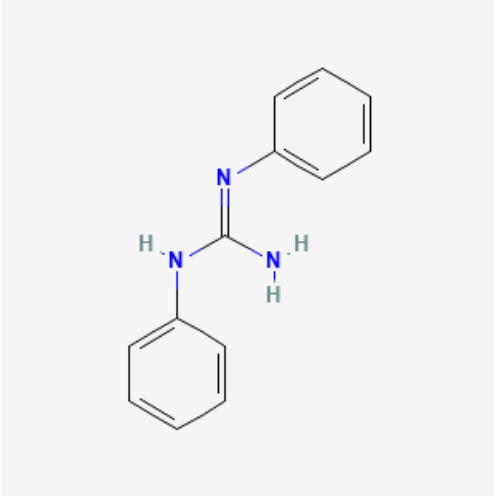
METHAMPHETAMINE

- Compound info: schedule 2 controlled substance
- Drug Class: amphetamine



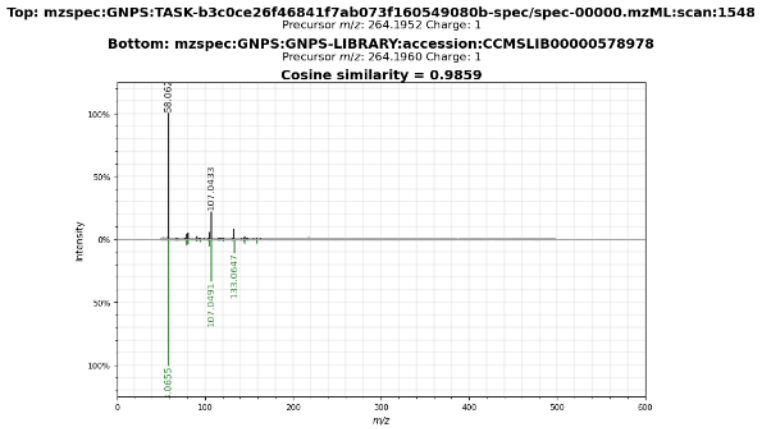


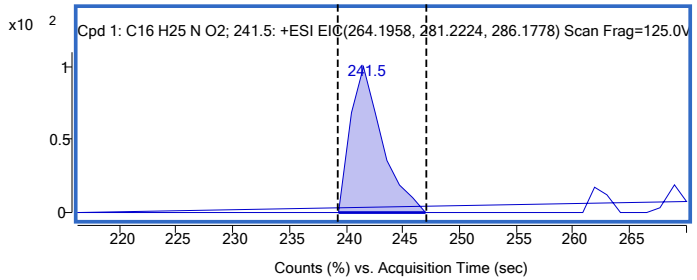


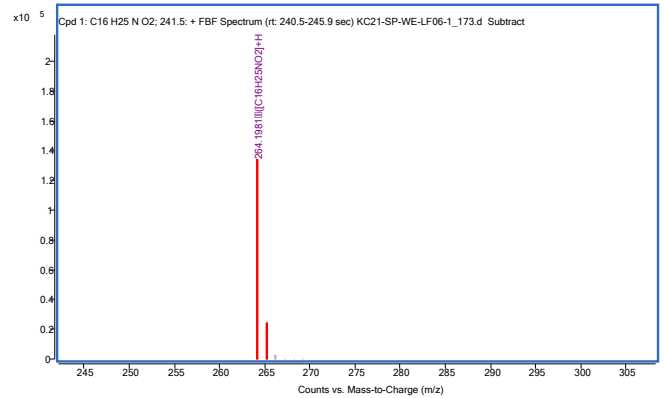


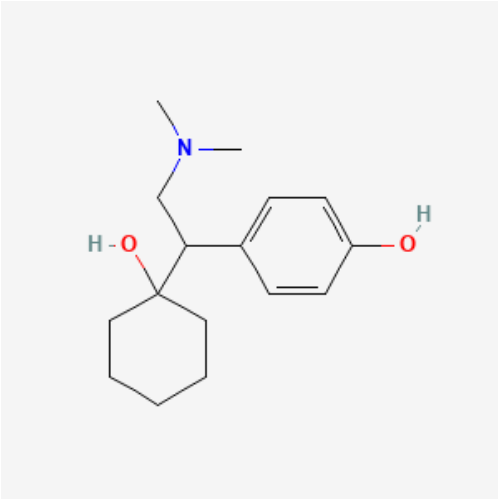
1,3-DIPHENYLGUANIDINE

- Compound info: complexing agent, used to detect metal and organic bases as well as an accelerator in vulcanization of rubber
- Drug Class: irritant, Health Hazard, Environmental hazard



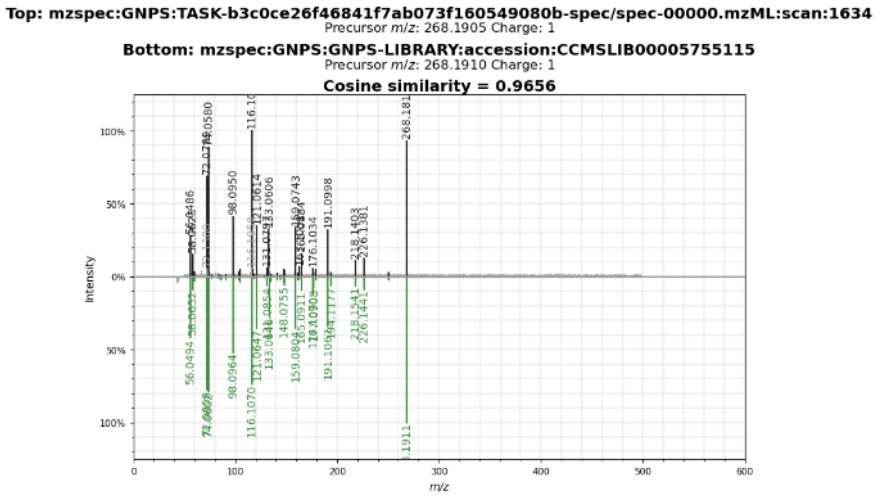


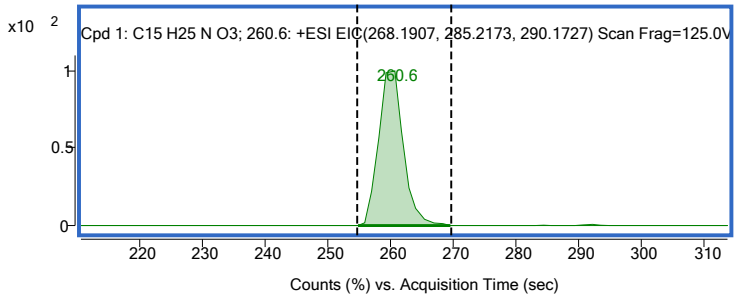


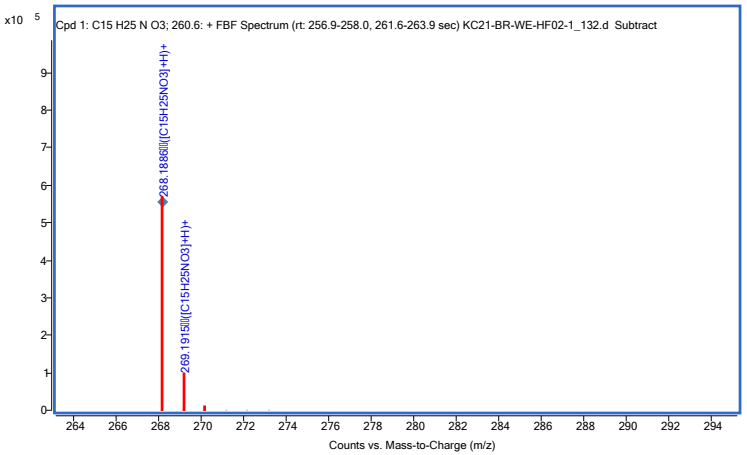


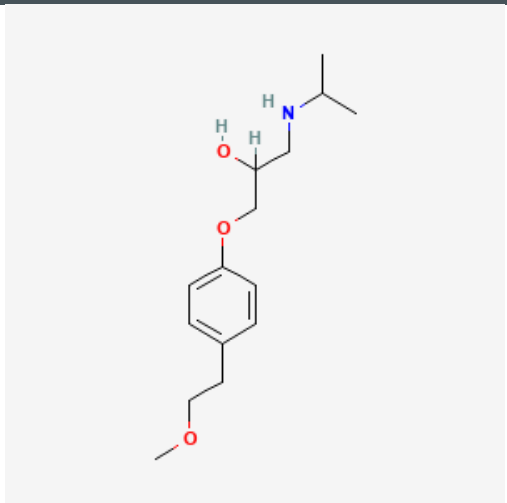
DESVENLAFAXINE

- Compound info: Used to treat depression
- Drug Class: SNRI



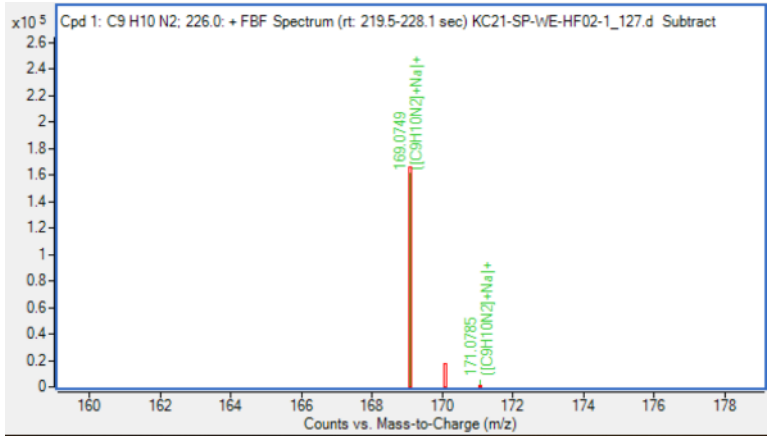


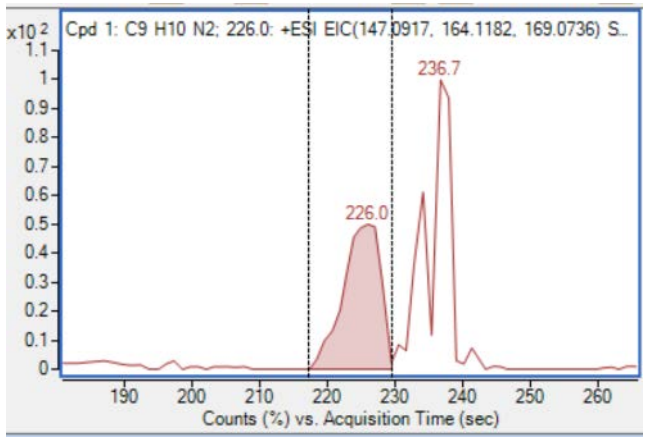


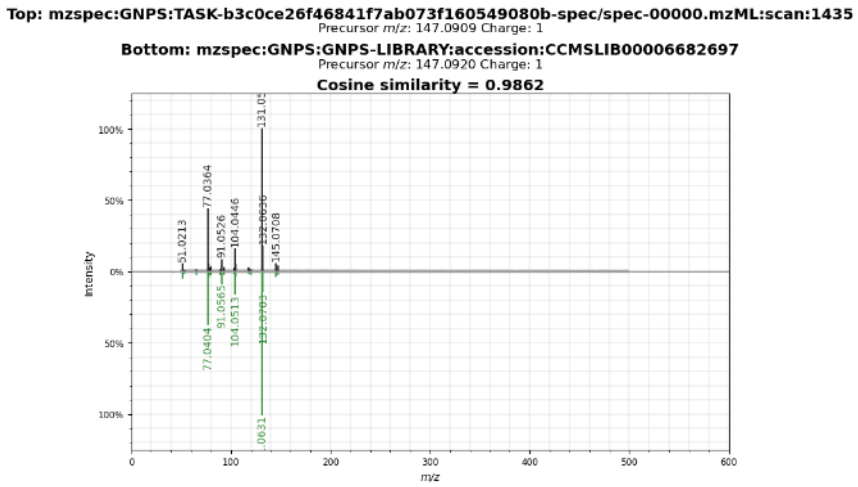


METOPROLOL

- Compound info: used to help treat high blood pressure, after heart attacks, and treat
- Drug Class: Beta Blocker

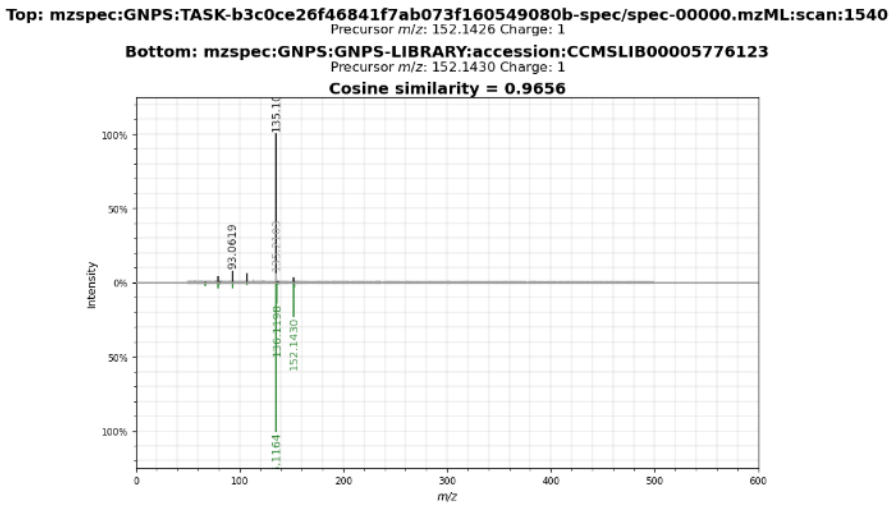


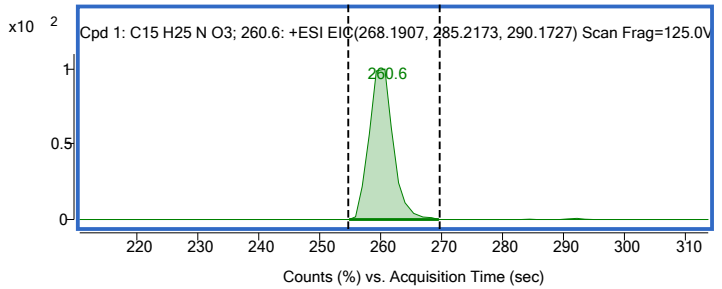


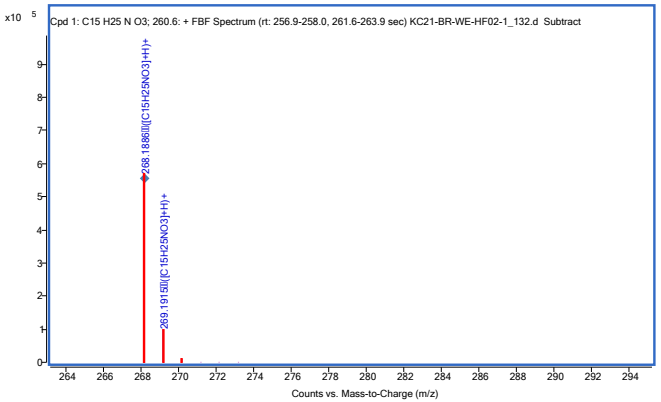


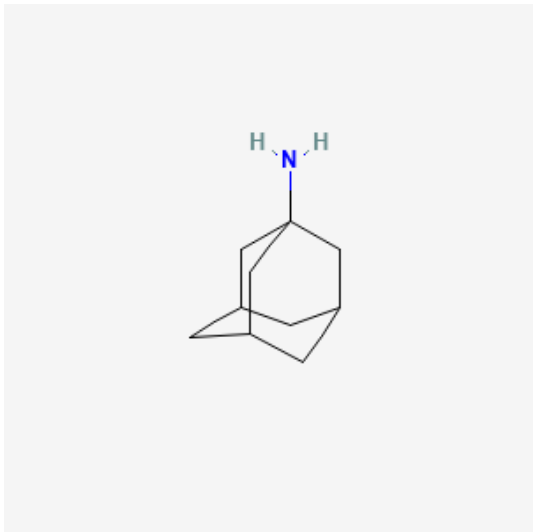
DIMETHYLBENZIMIDAZOLE

- Compound info: metabolite- disregard
- Drug Class: n/a



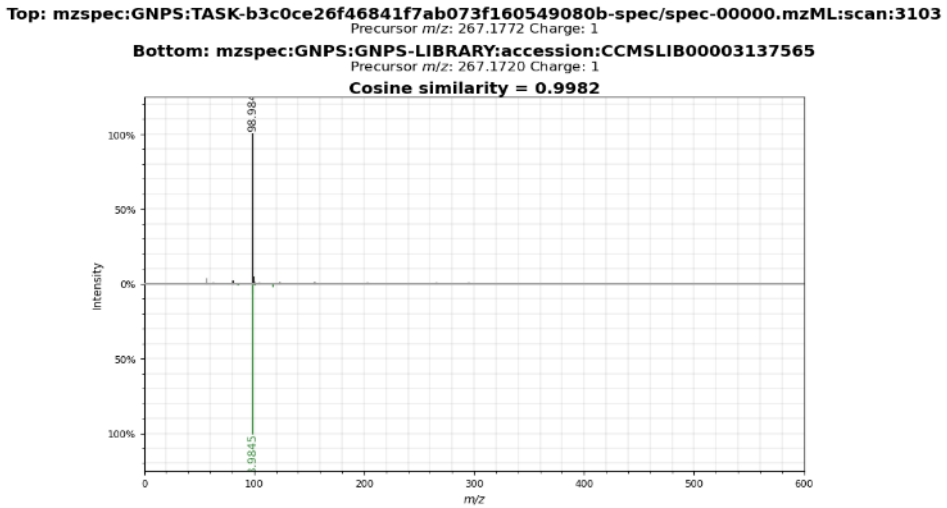


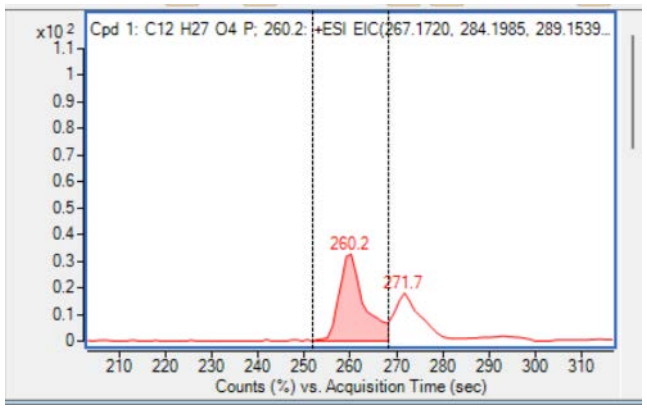


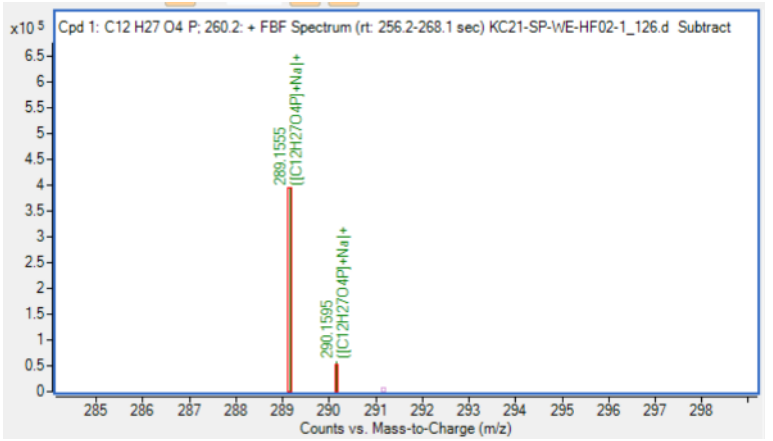


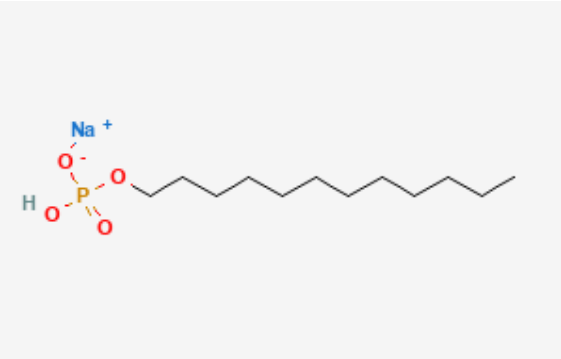
AMANTADINE

- Compound info: Used to treat Parkinson's disease and other similar conditions
- Drug Class: Adamantanes



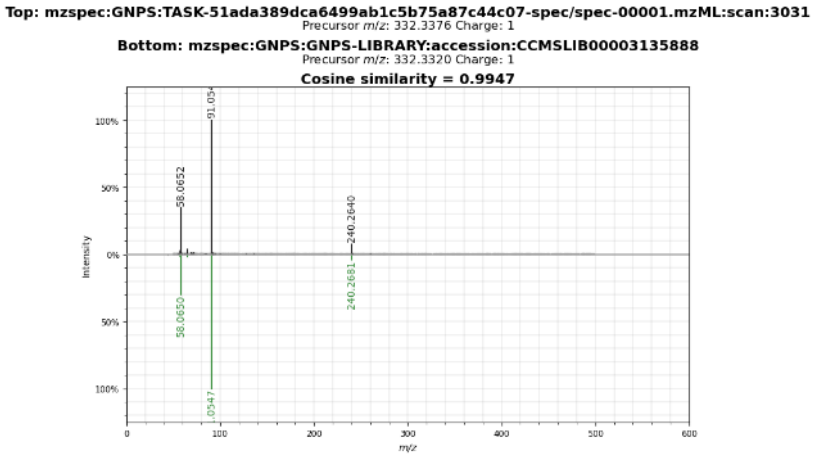


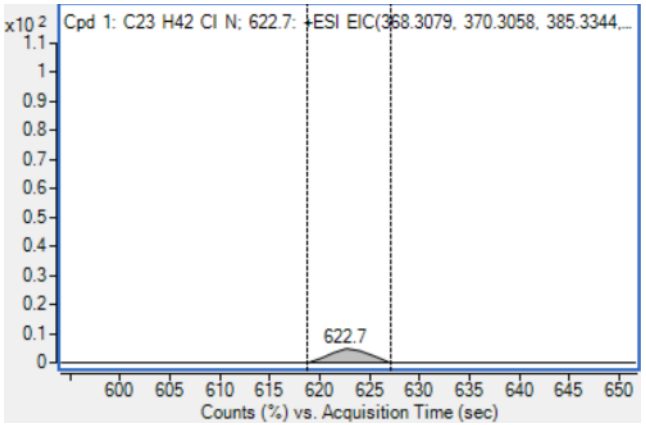


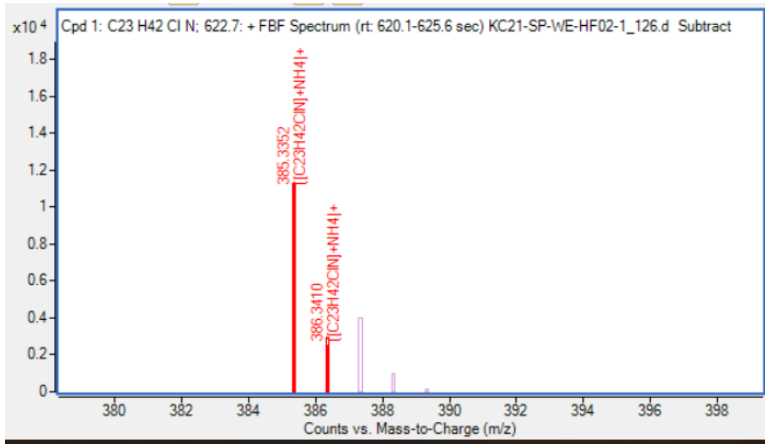


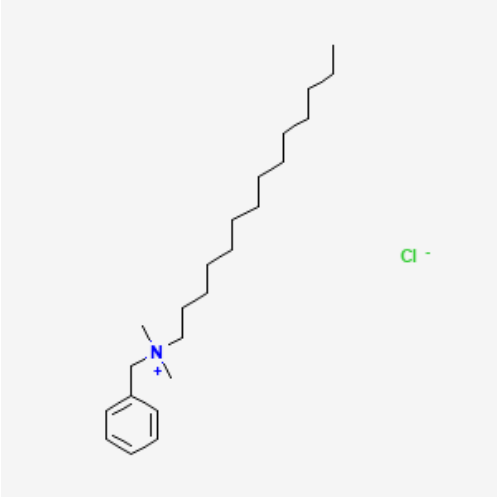
MONOLAURYL PHOSPHATE

- Used as Emulsifier



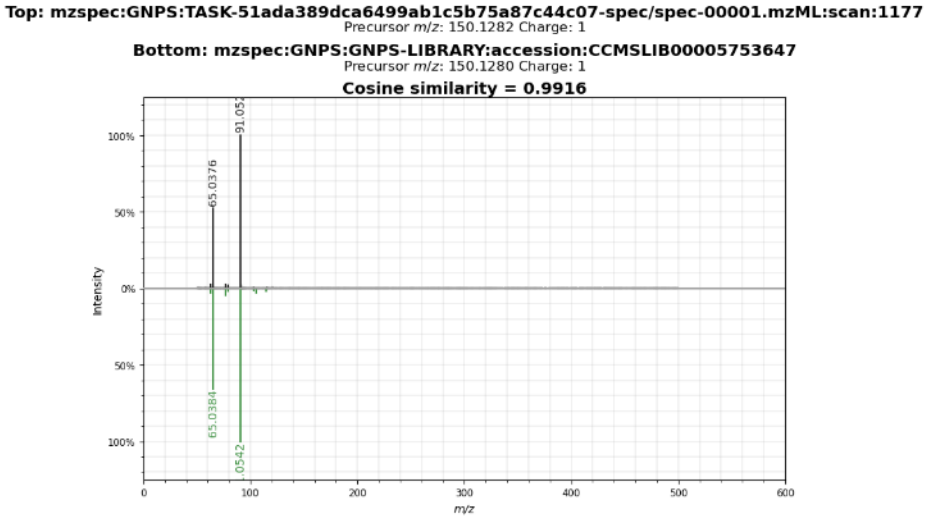


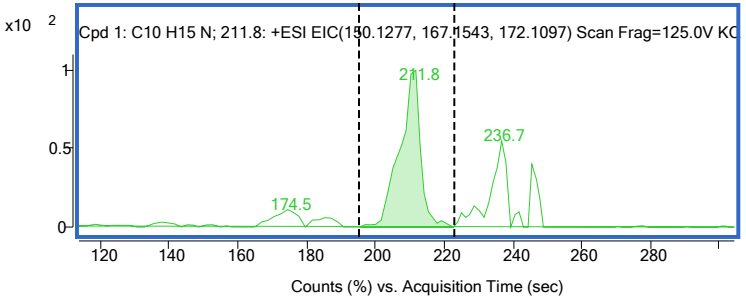


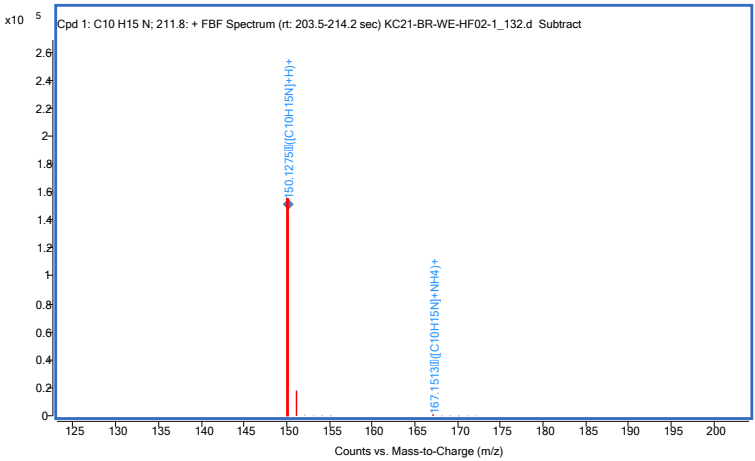


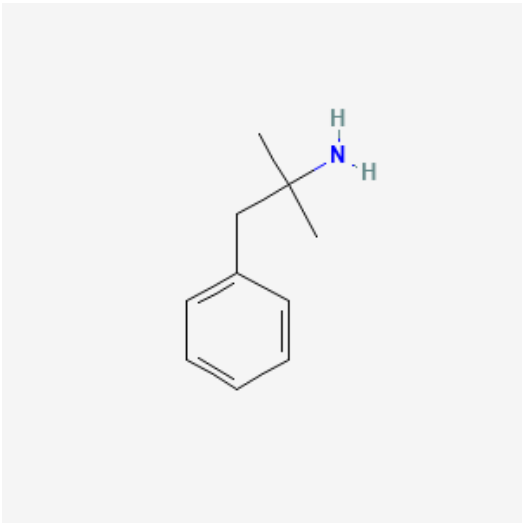
BENZYLTETRADECYLDIMETHYLAMMONIUM

- Used in household cleaners



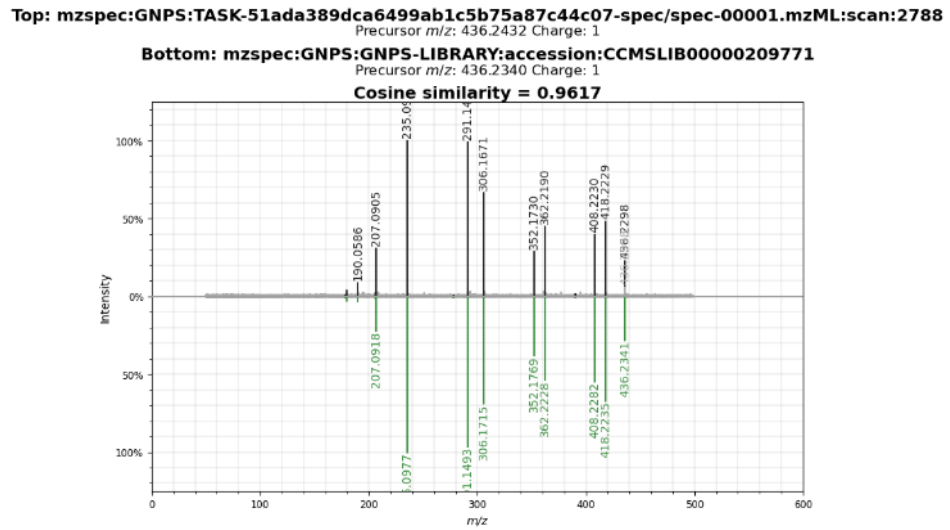


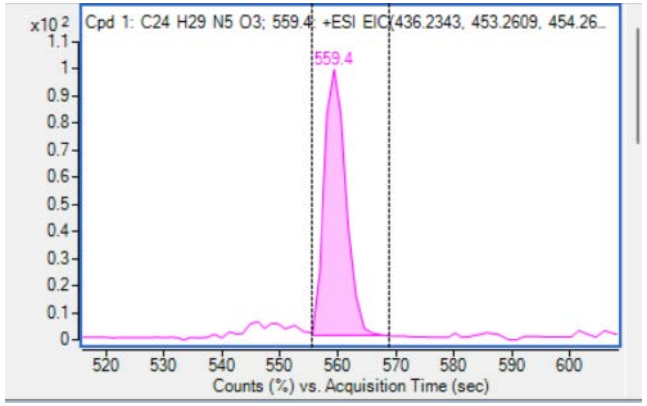


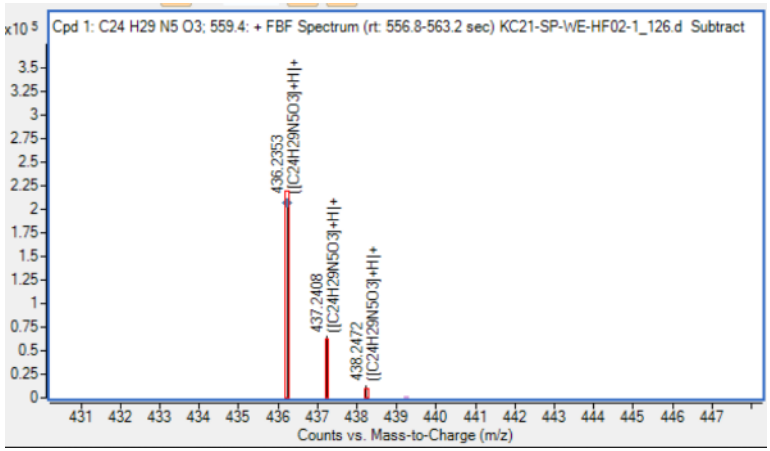


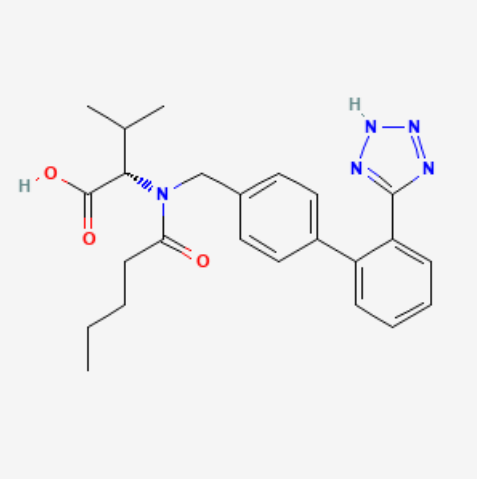
PHENTERMINE

- Appetite suppressant
- DEA Schedule IV controlled substance



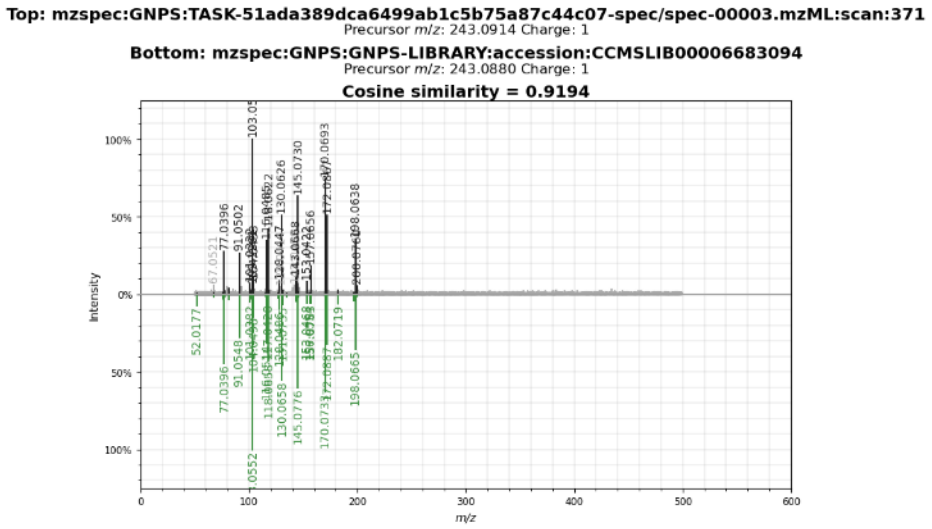


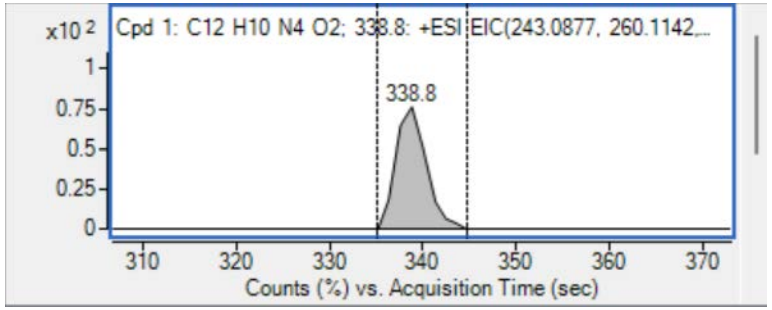


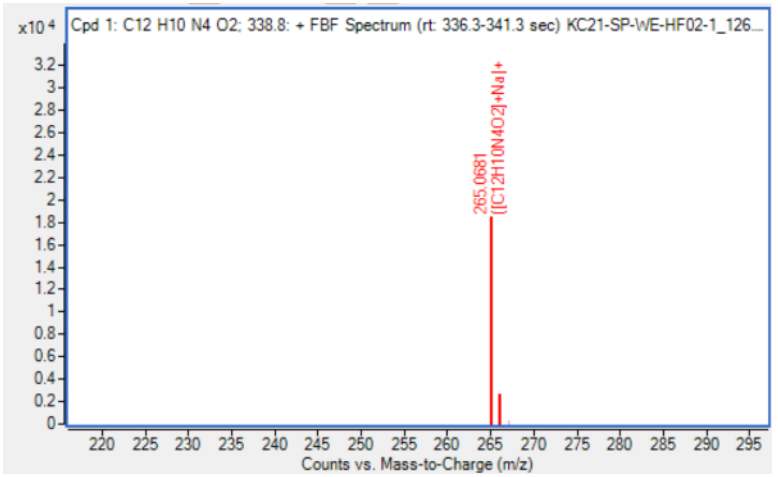


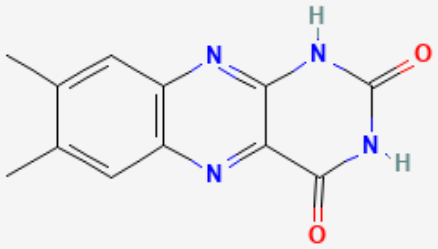
VALSARTAN

- Antihypertensive drug
- Angiotensin II Type 1 Receptor Blockers; Antihypertensive Agents



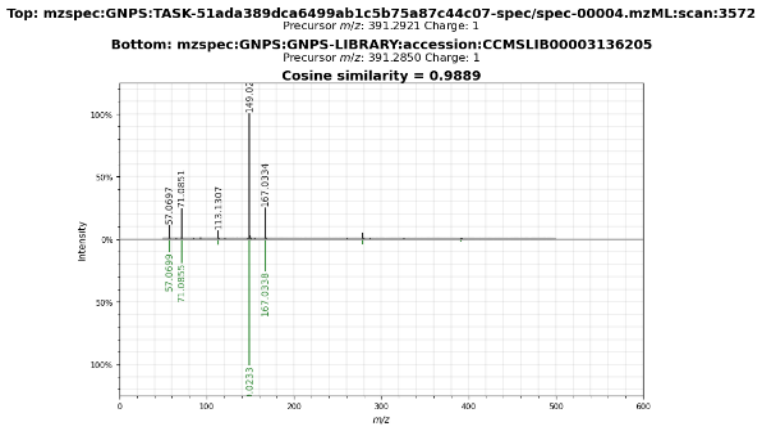


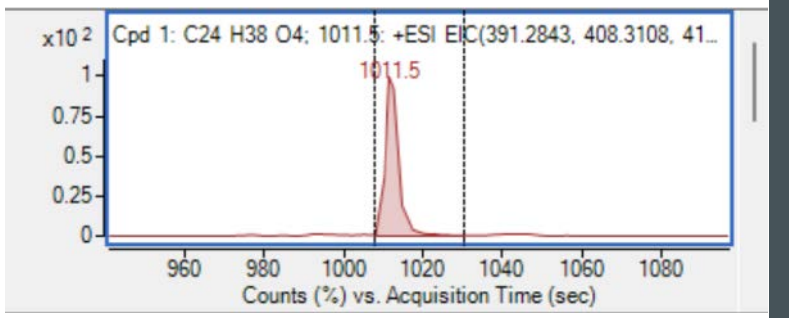


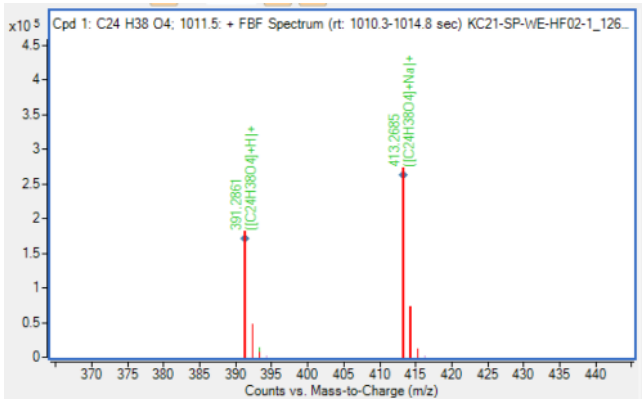


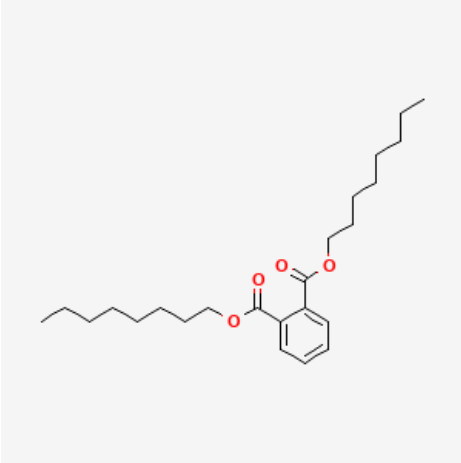
LUMICHROME

- Plant metabolite



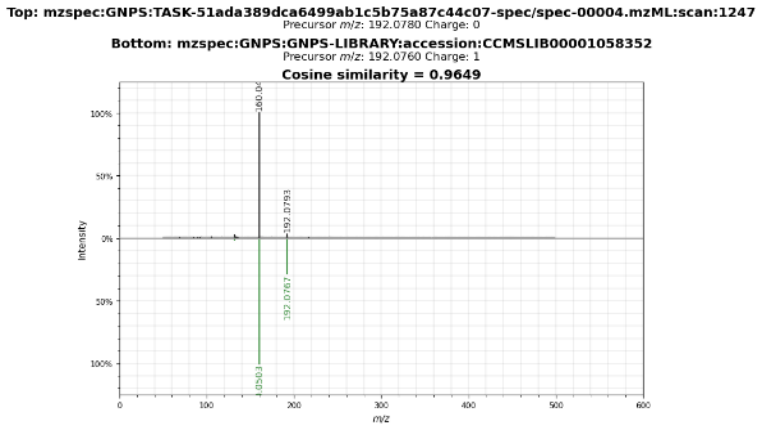


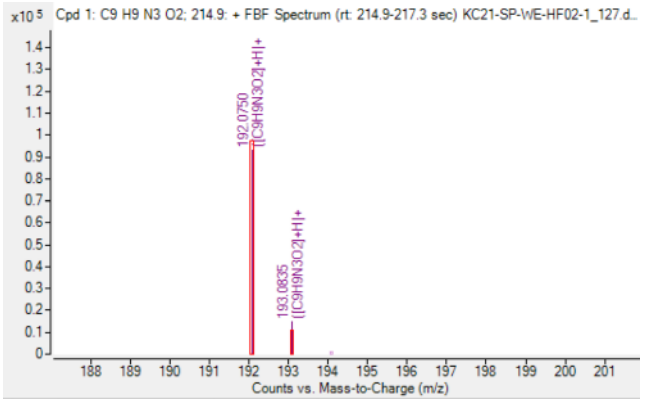


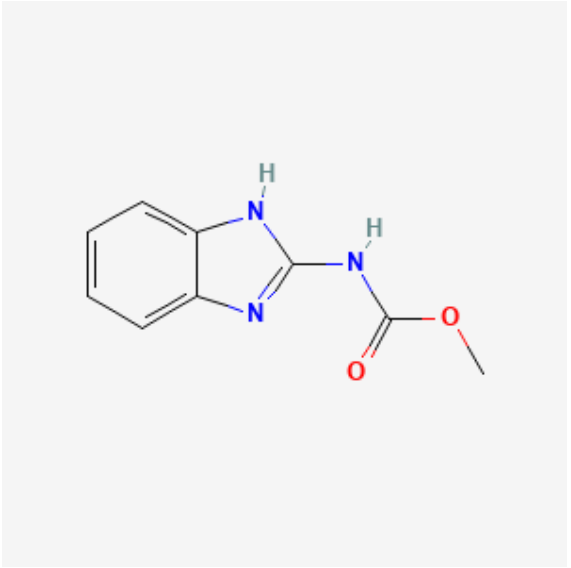


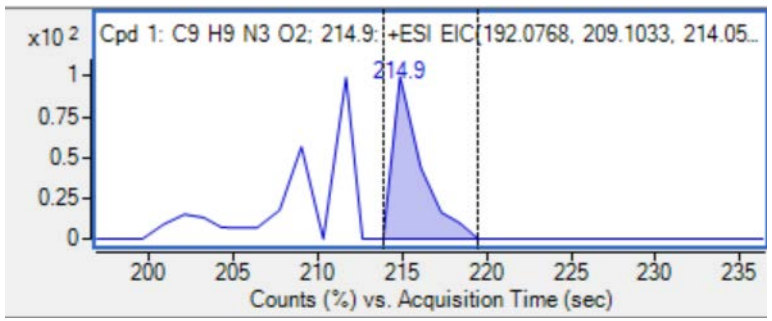
DIOCTYL PHTHALATE

- used in manufacturing of plastic varieties and coatings- primary hazard to environment



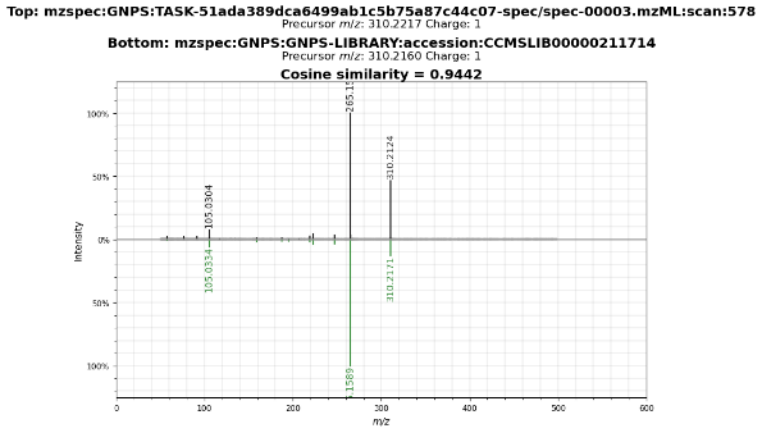


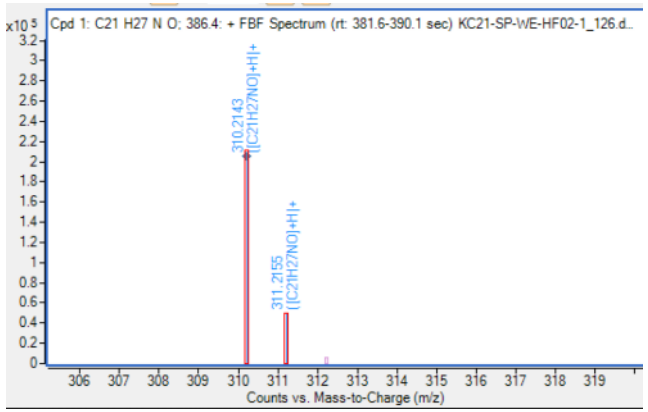


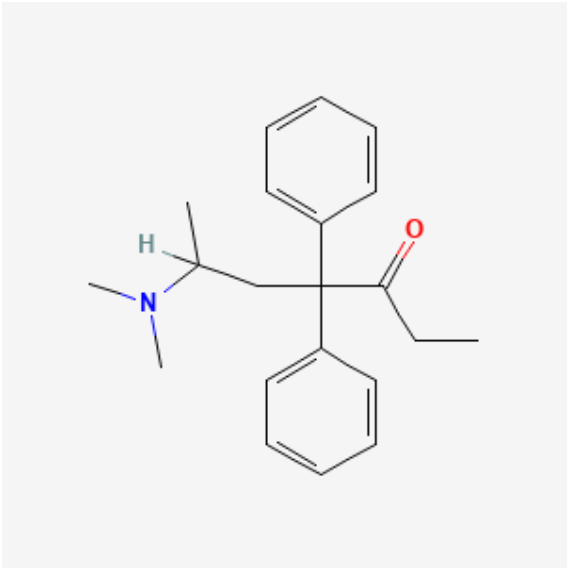


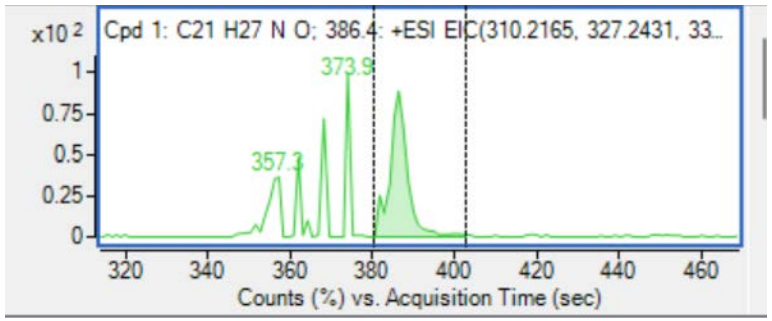
CARBENDAZIM

- fungicide



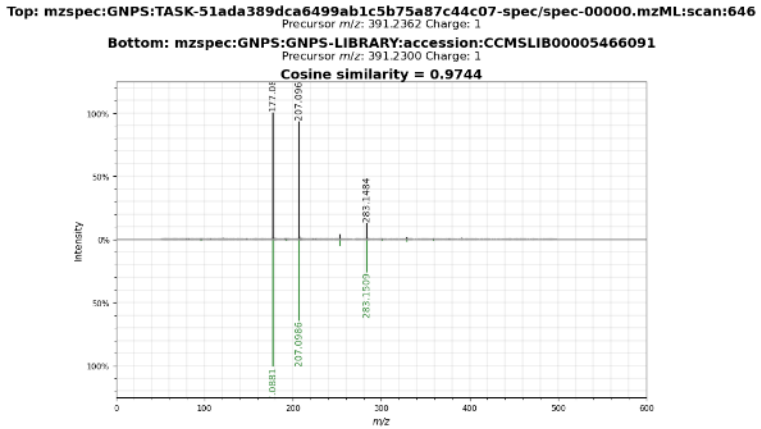


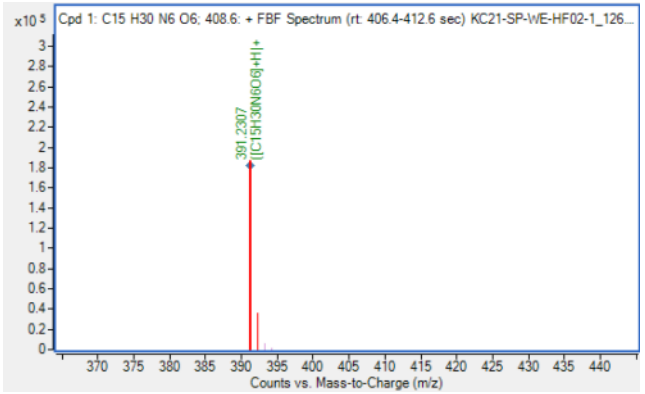


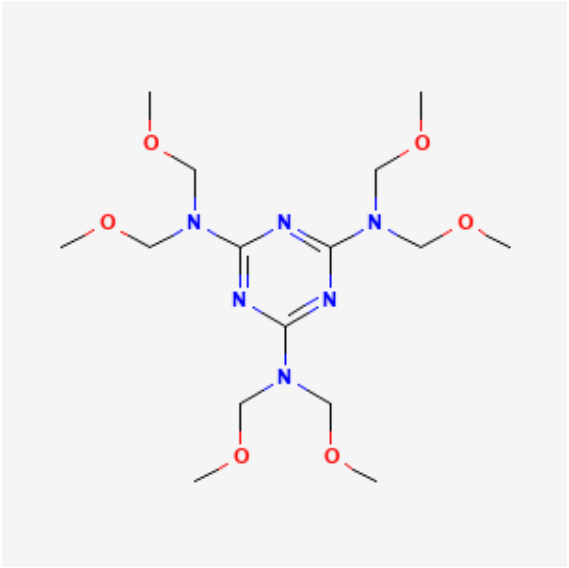


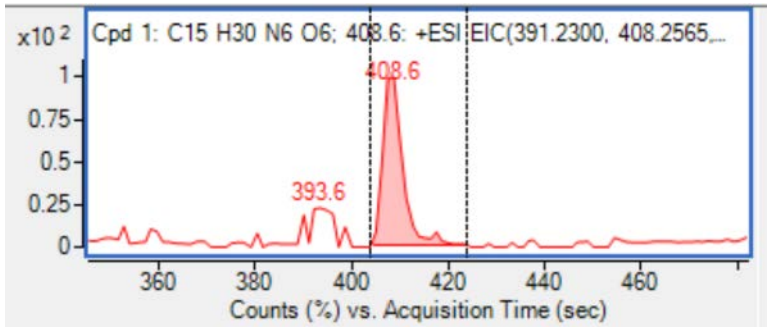
METHADONE

- Synthetic opioid used as analgesic and maintenance therapy for those w/ opioid dependence- DEA schedule 2 controlled substance



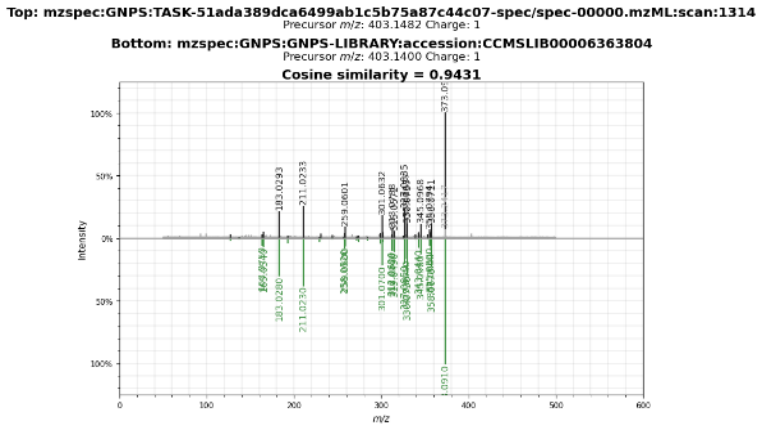


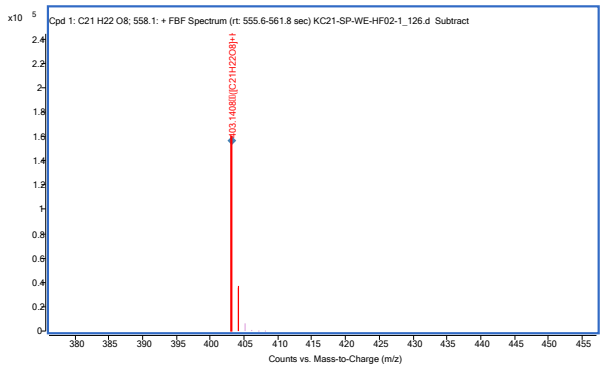


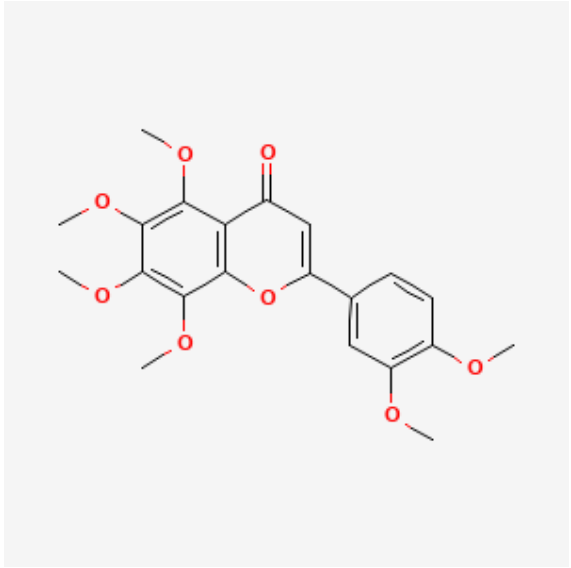


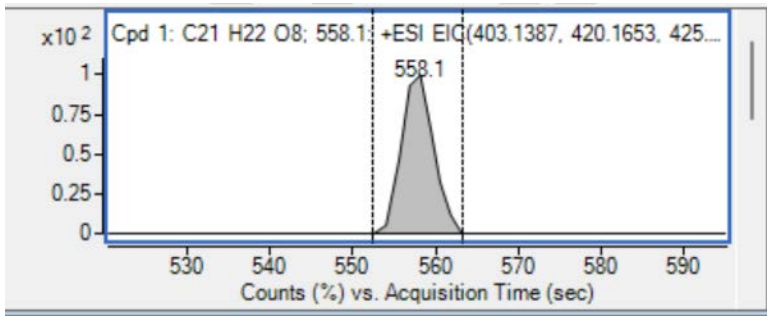
HEXA-METHOXYMETHYL-MELAMINE

- Paint topcoat ingredient



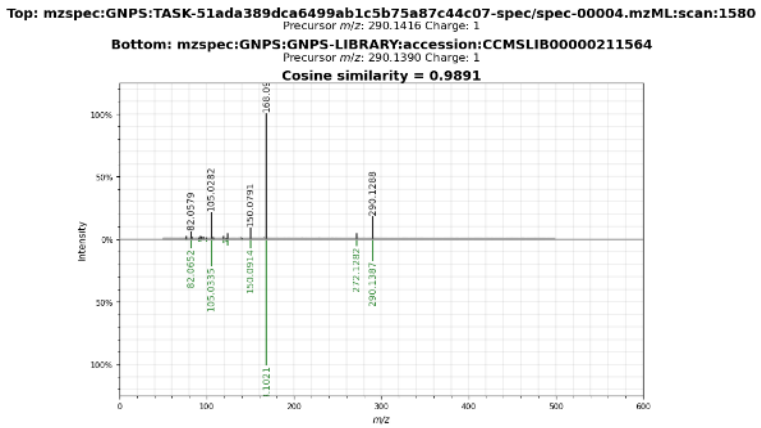


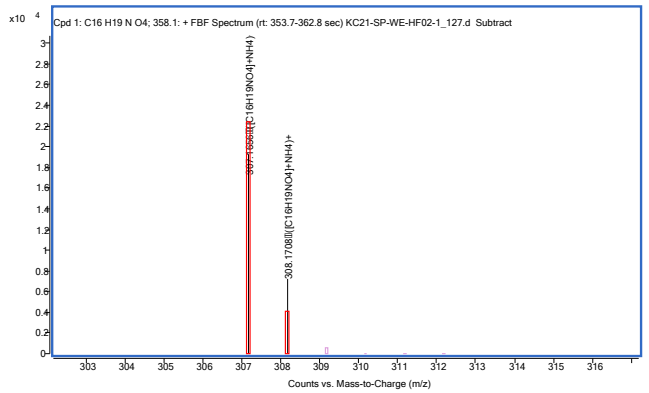


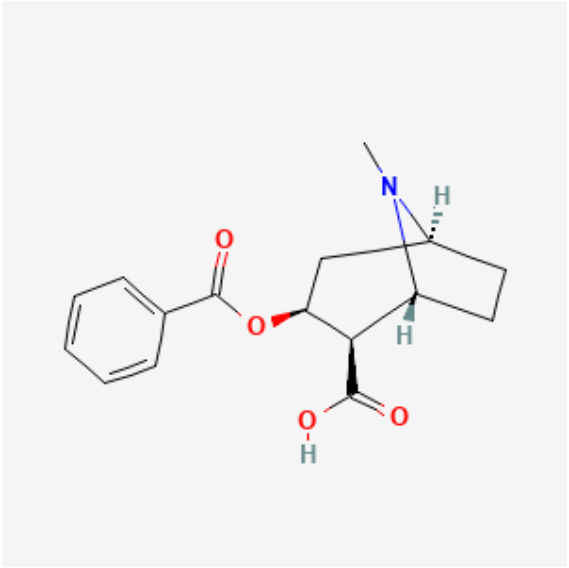


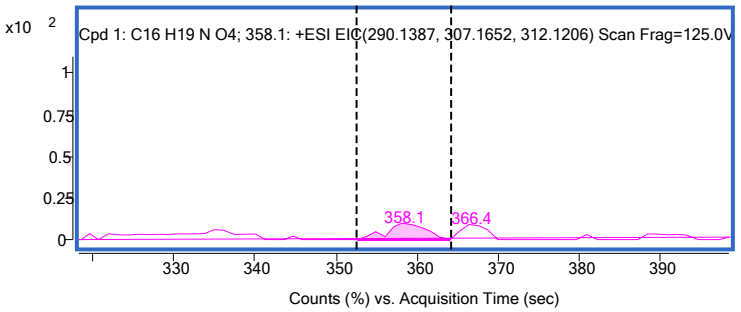
NOBILETIN

- Plant metabolite as well as an antineoplastic agent



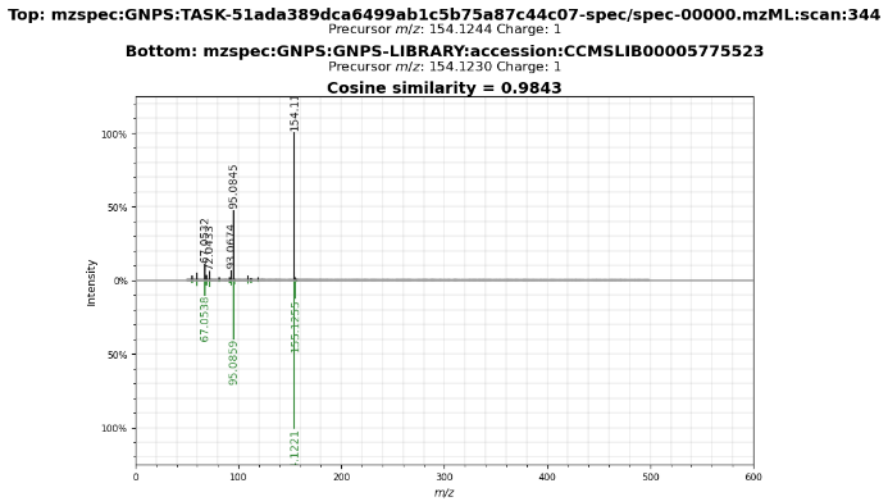


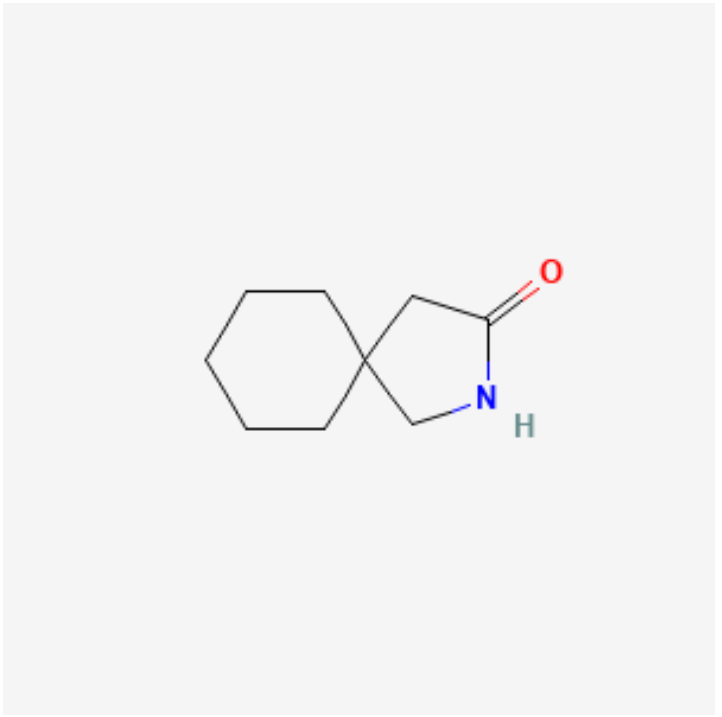


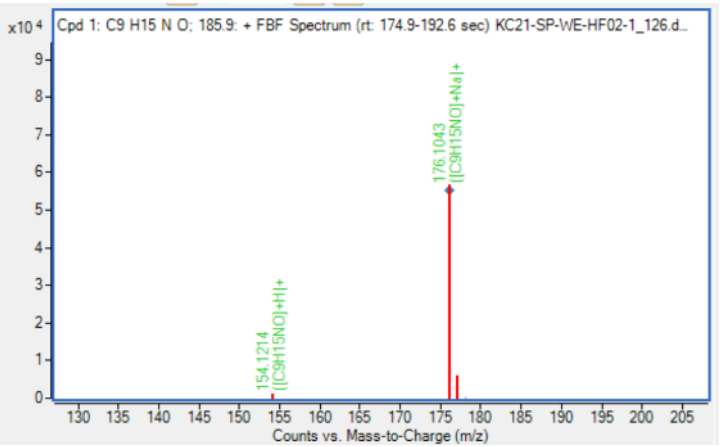


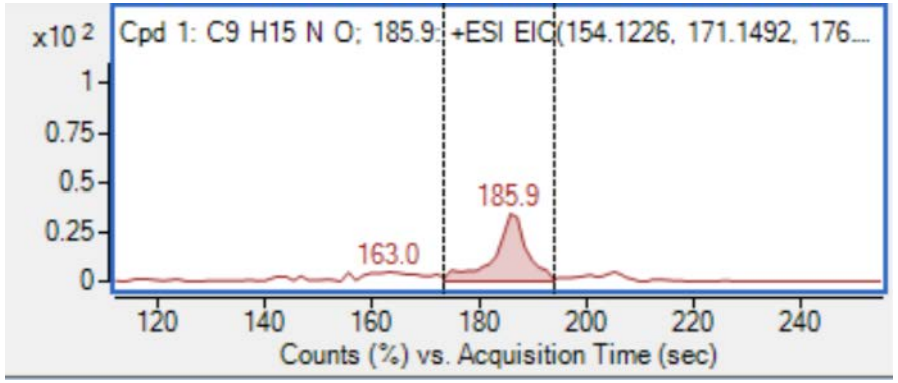
BENZOYLECGONINE

- Main metabolite of cocaine

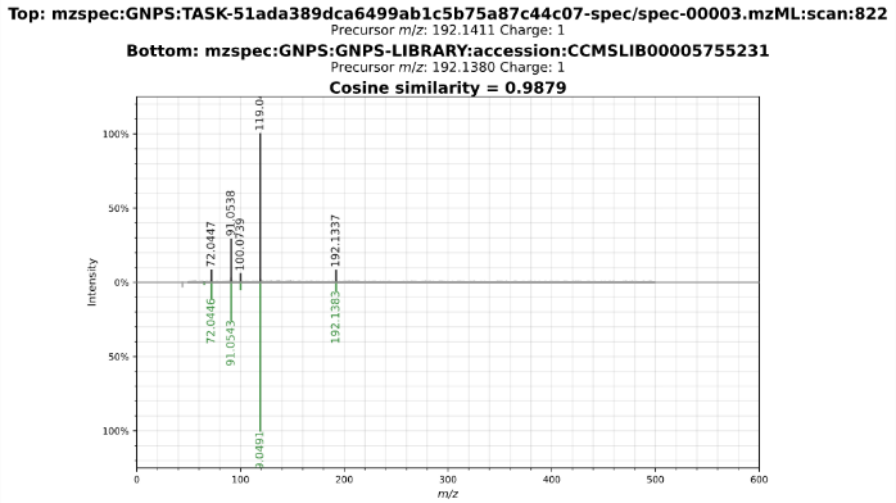


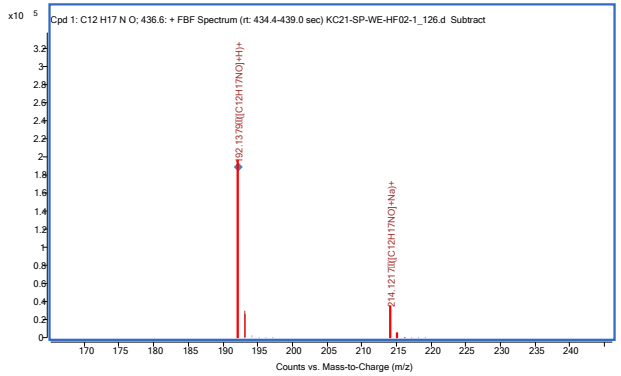


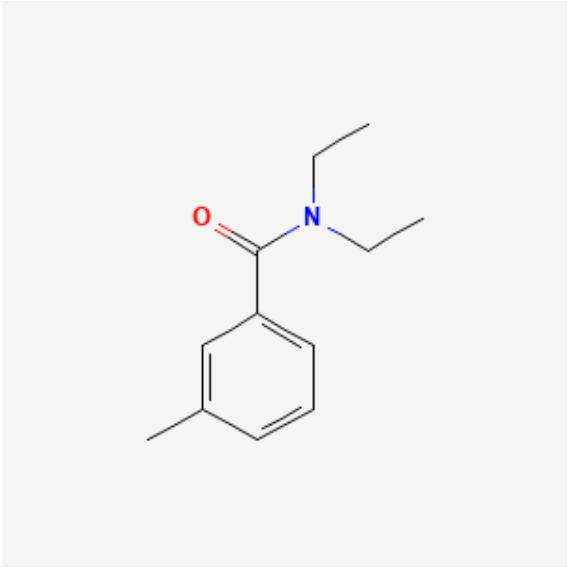


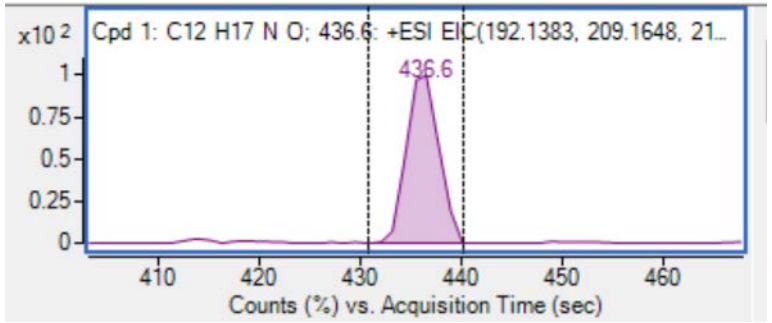


2-AZASPIRO[4.5]DECAN-3-ONE, 3,3-PENTAMETHYLENE-4-BUTYROLACTAM



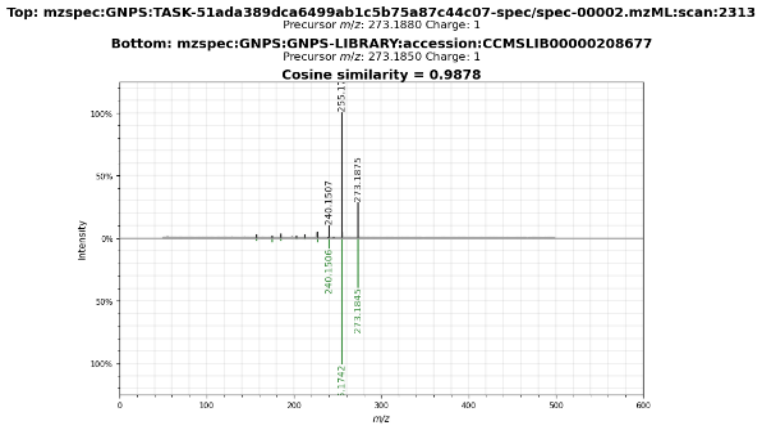


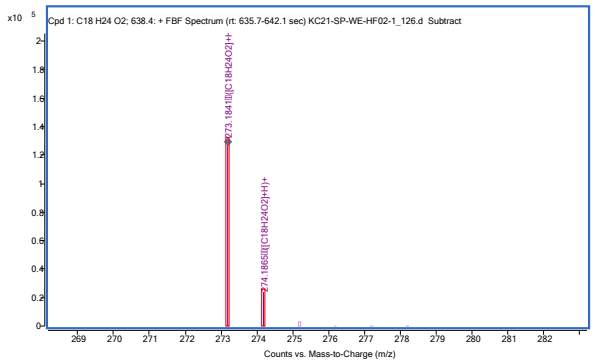


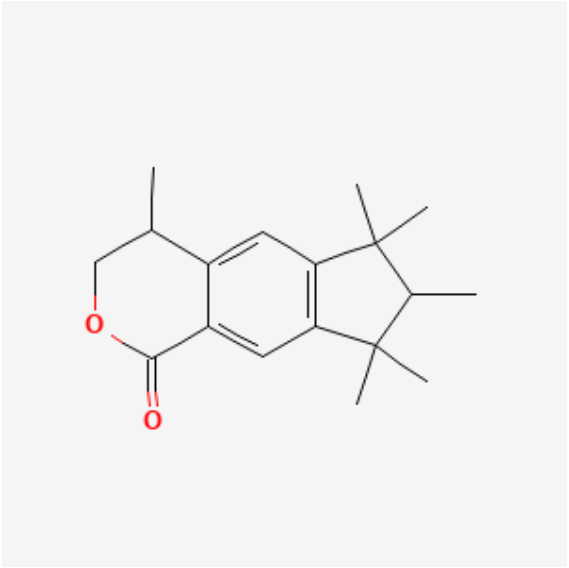


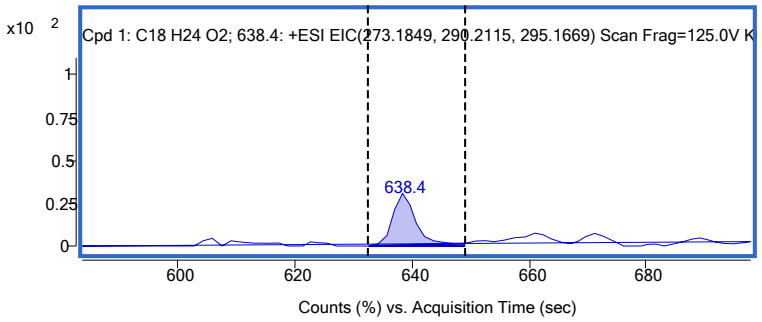
DIETHYLTOLUAMIDE

- Used as active ingredient in many insect repellents



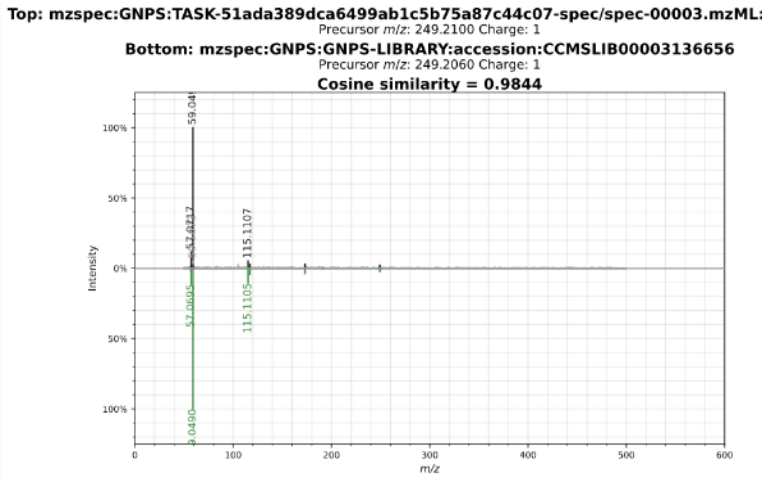


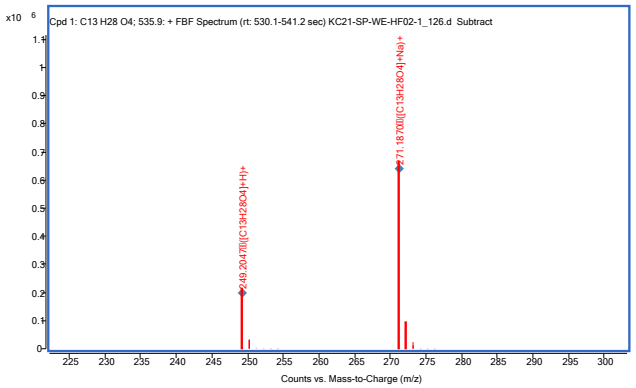


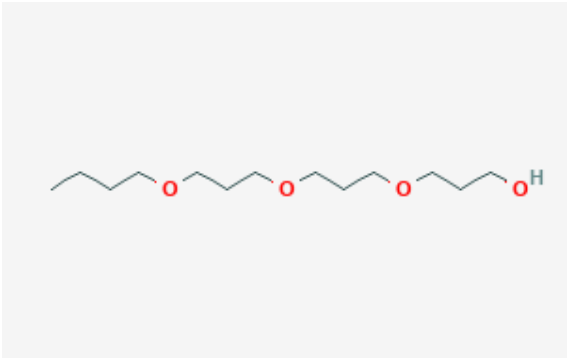


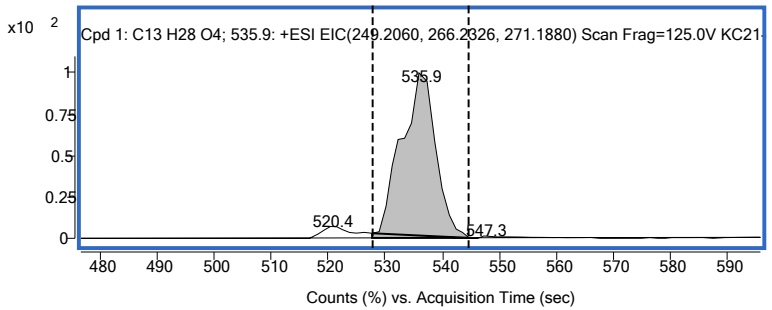
GALAXOLIDONE

- Marine xenobiotic (substance foreign to body or ecosystem)

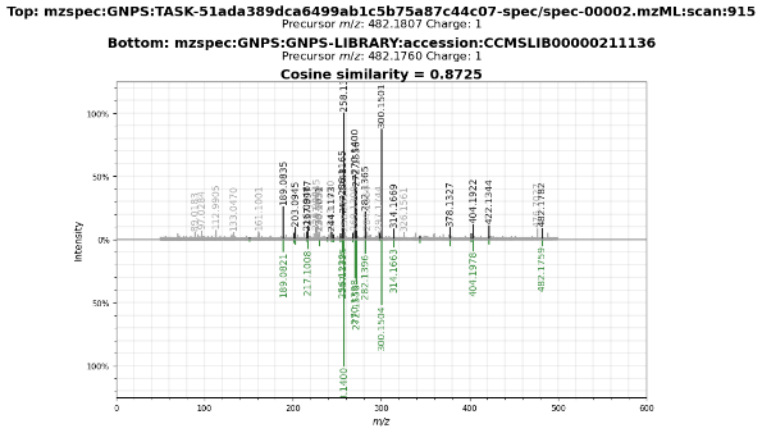


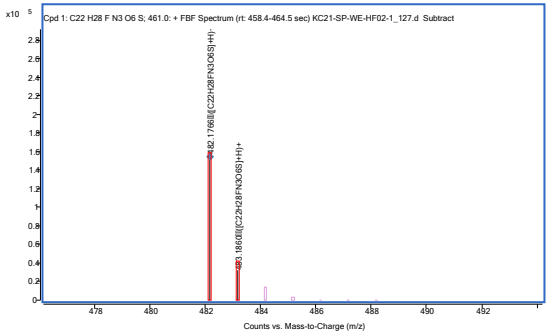


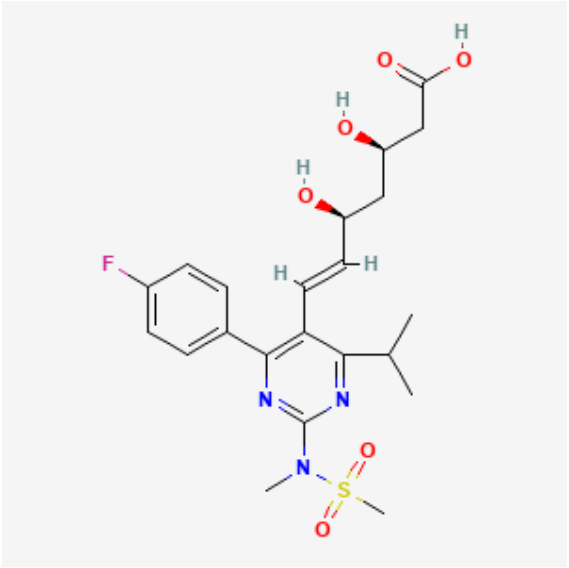


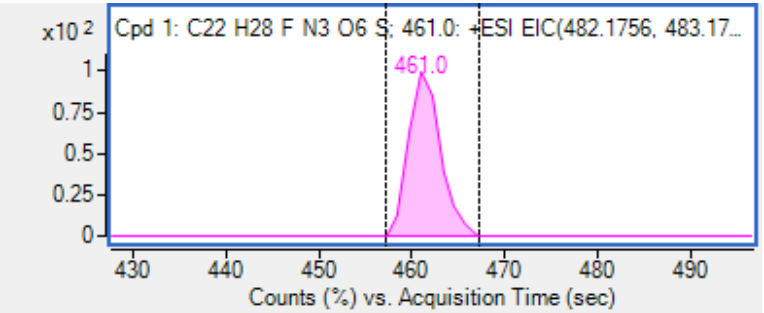


TRI(PROPYLENE GLYCOL) BUTYL ETHER



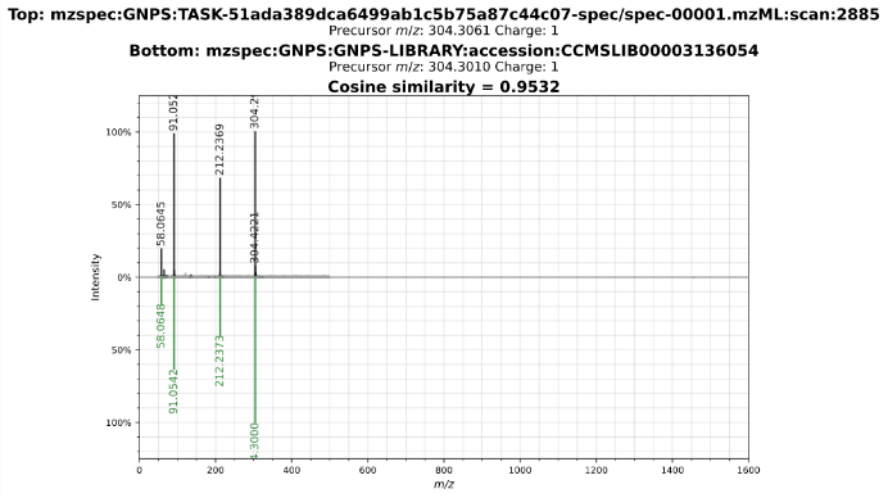


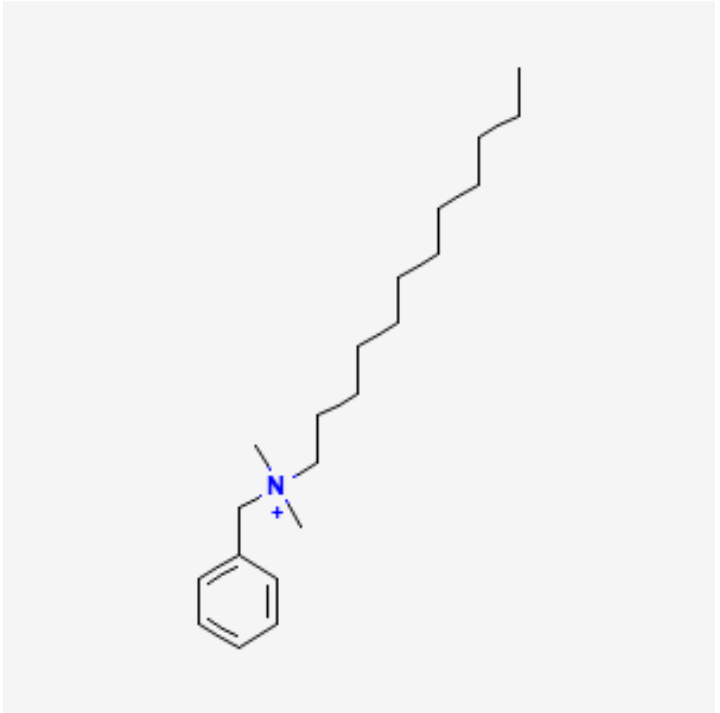


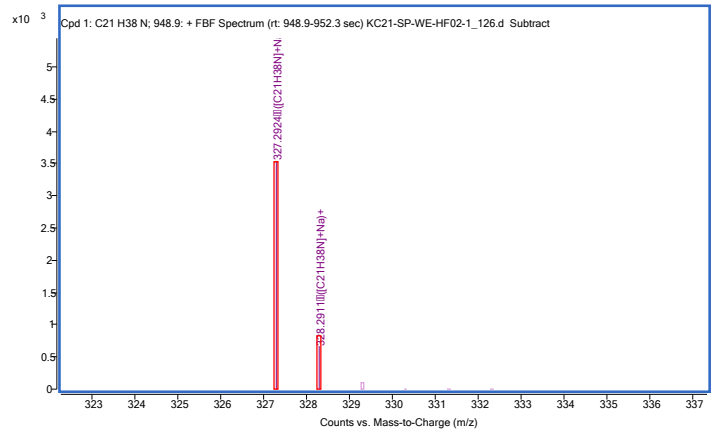


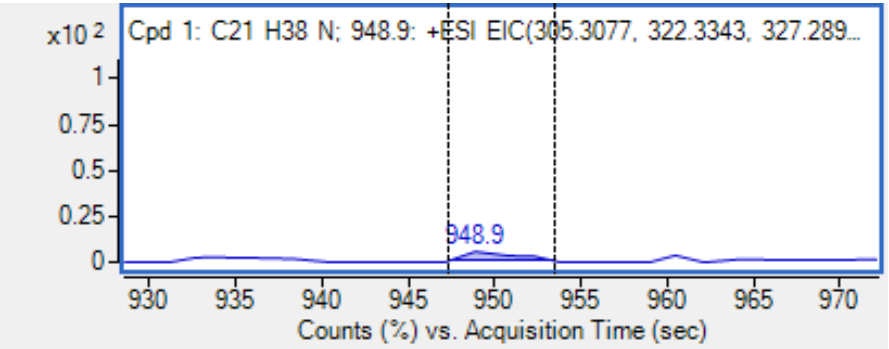
ROSUVASTATIN

- Common cholesterol lowering agent



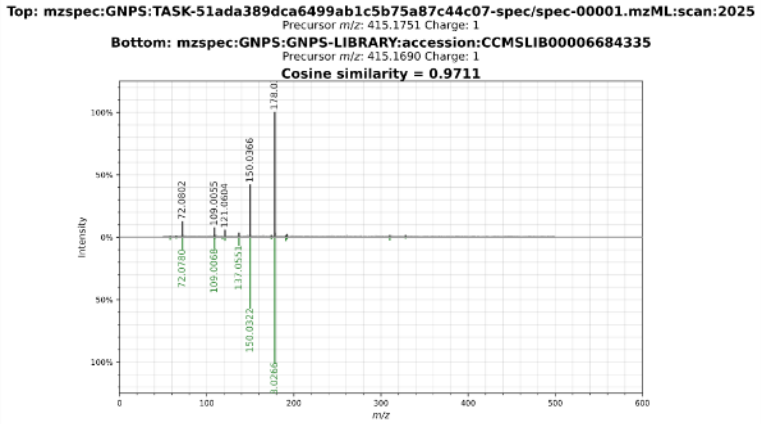


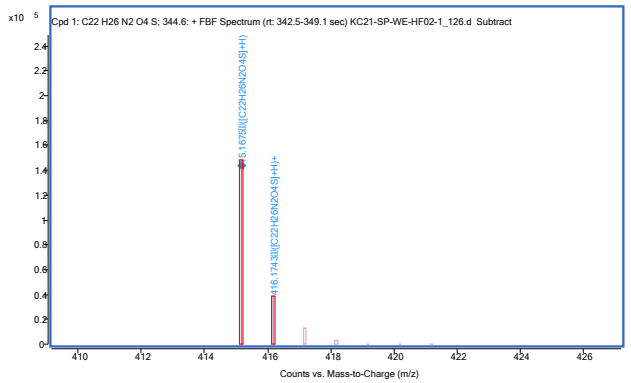


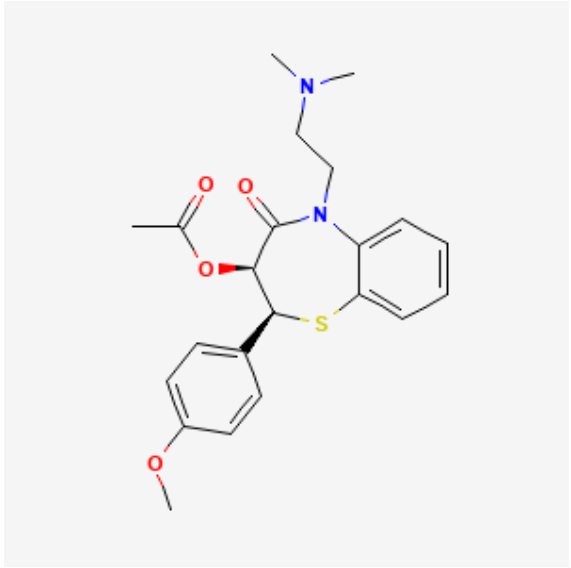


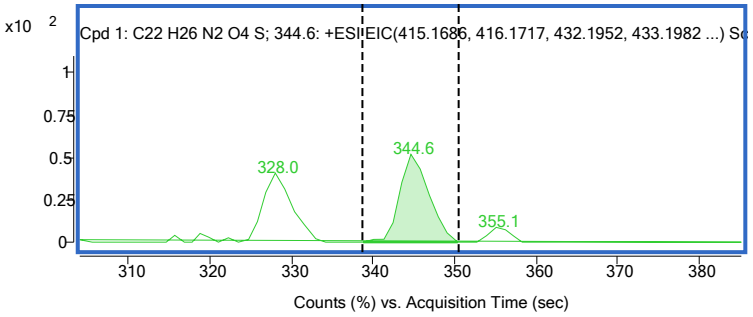
BENZODODECINIUM

Used as an antiseptic/disinfectant



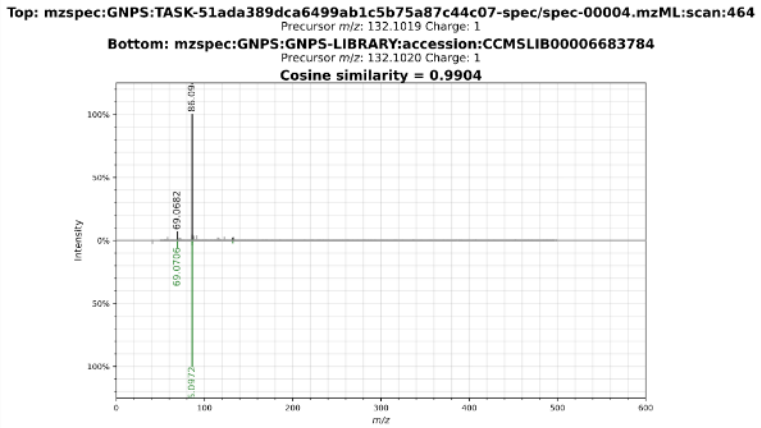


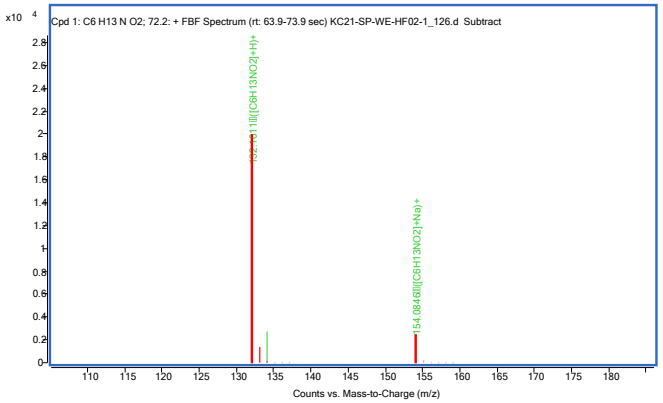


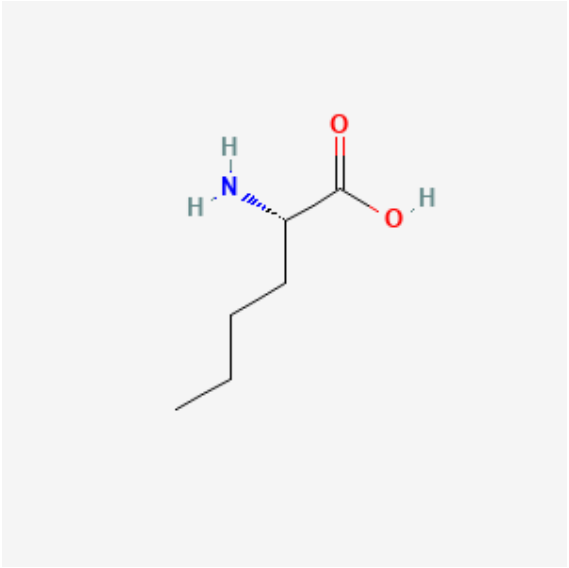


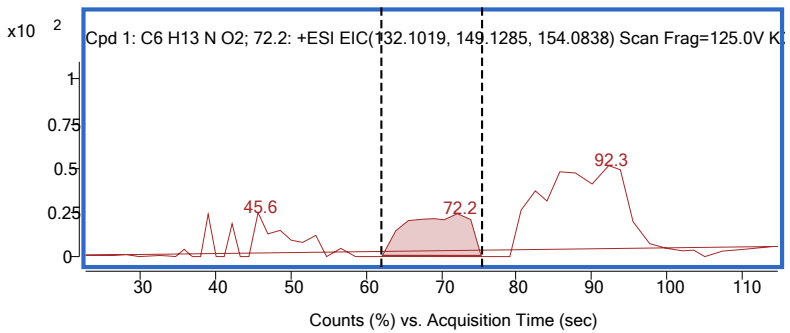
DILTIAZEM

- anti-hypertensive, antiarrhythmic



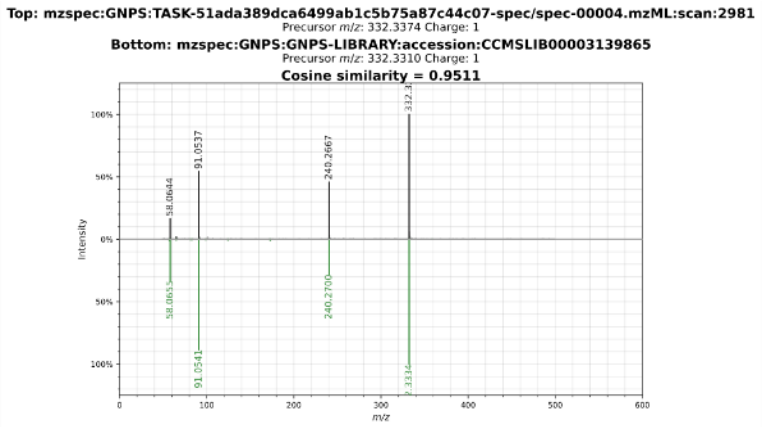


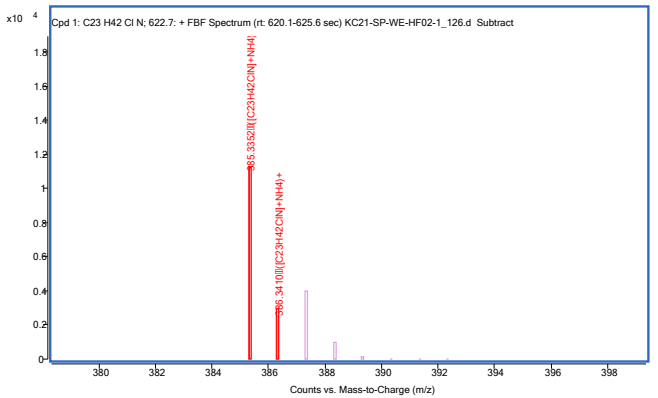


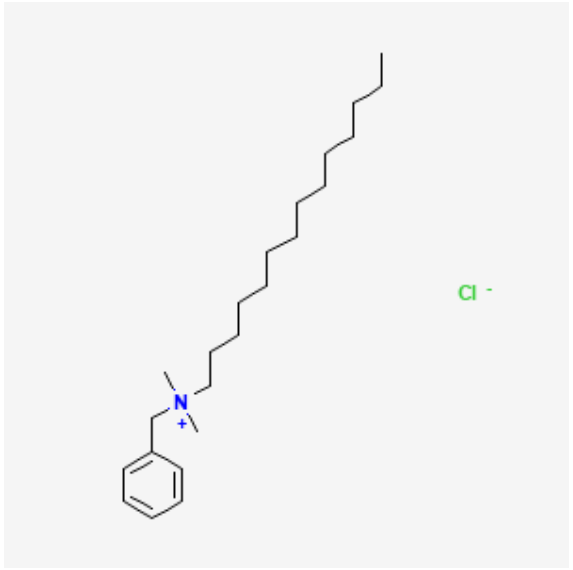


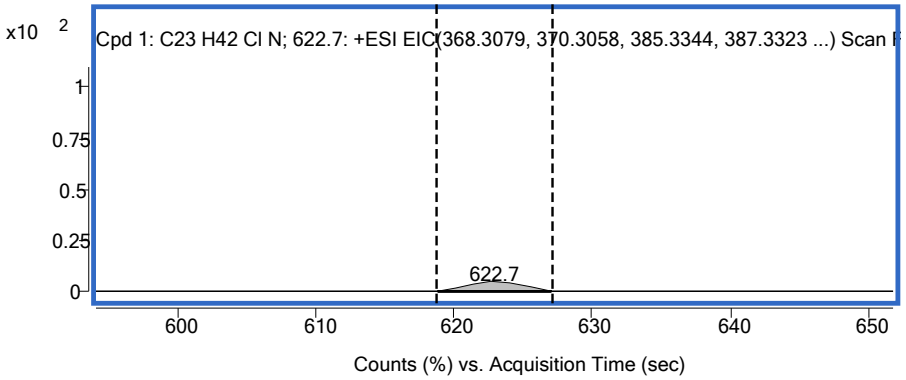
NORLEUCINE

- Synthetic
- amino acid



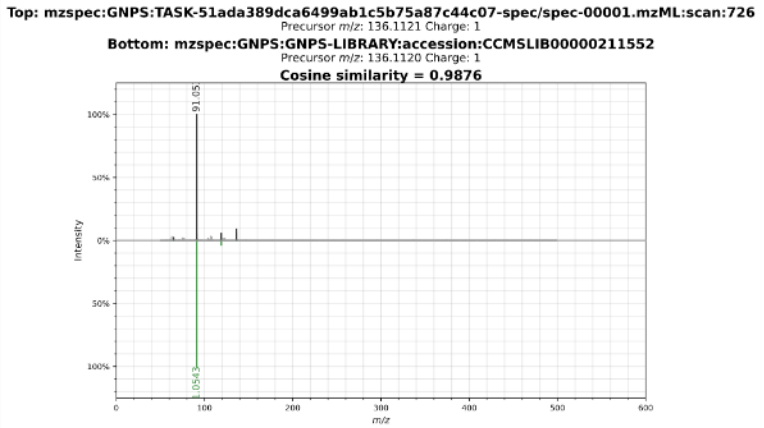


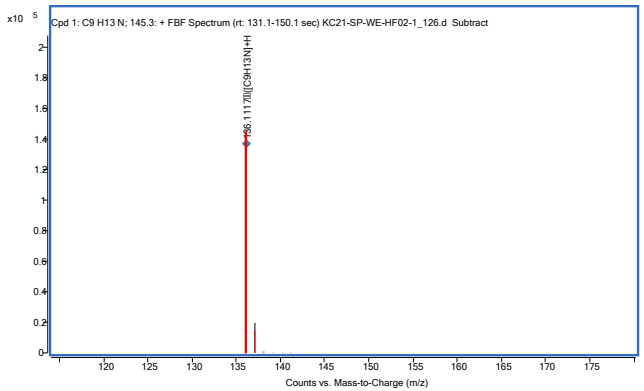


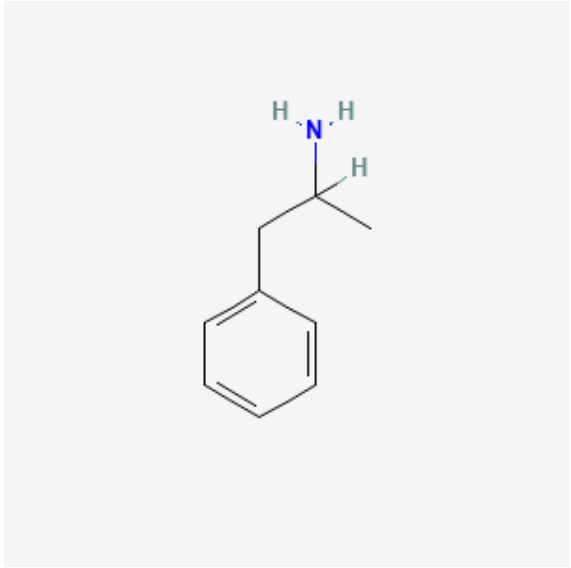


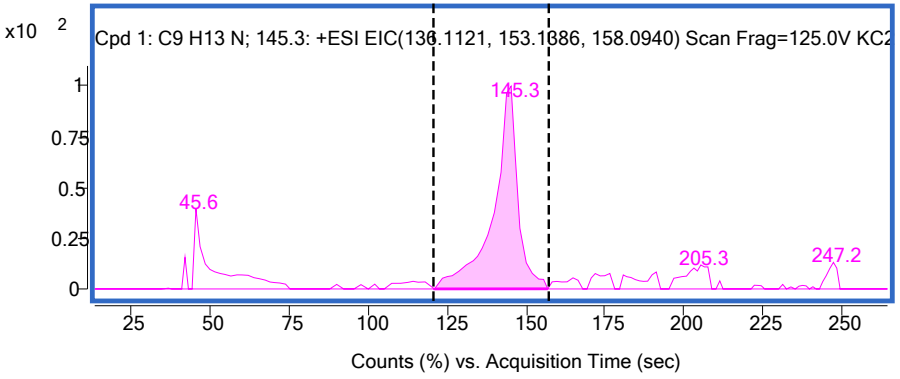
BENZYLTETRADECYLDIMETHYLAMMONIUM

- cleaning product & household care



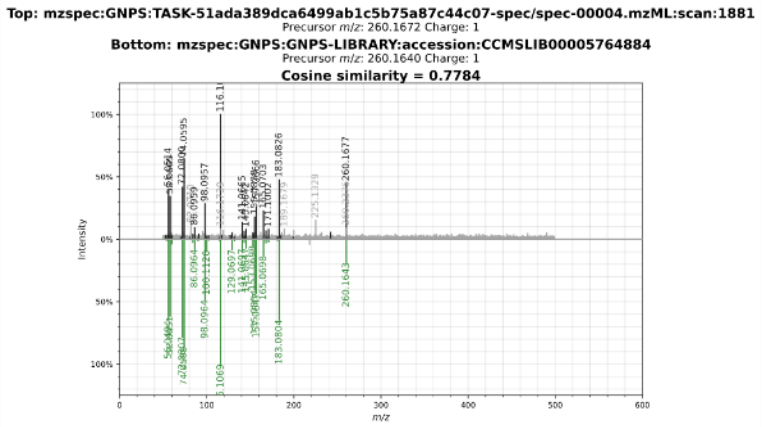


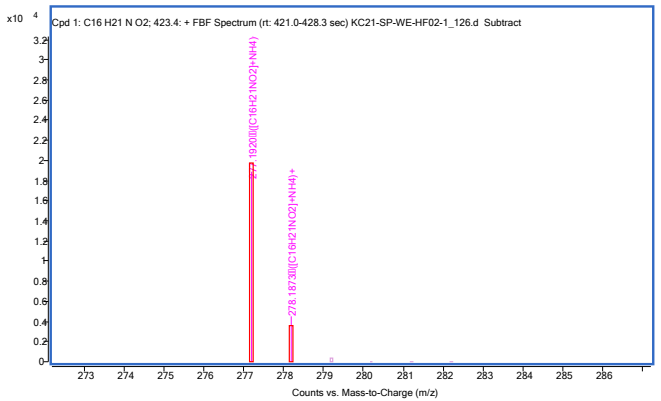


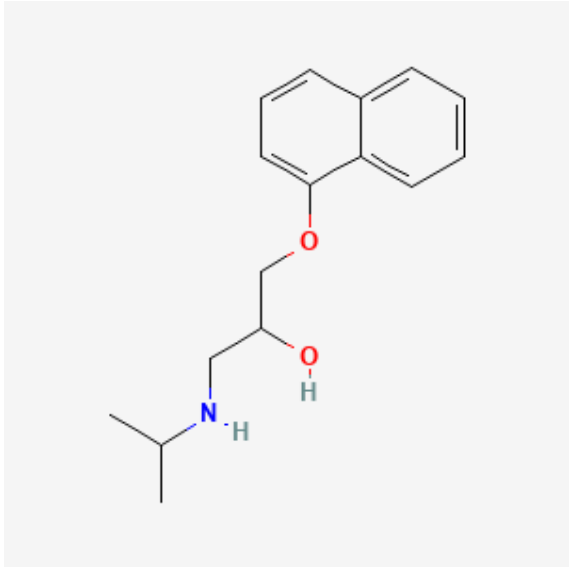


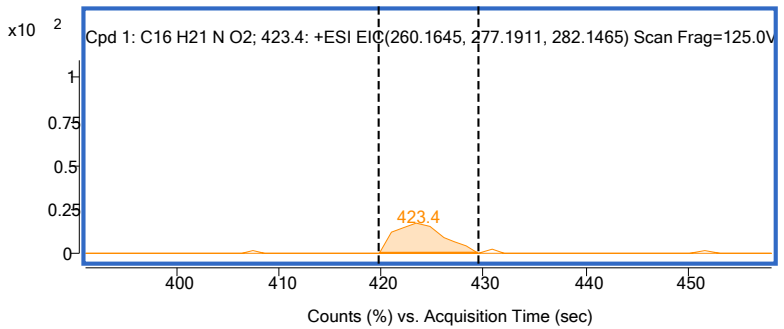
AMPHETAMINE

- DEA schedule 2 controlled sunstance, also used to treat ADHD and narcolepsy



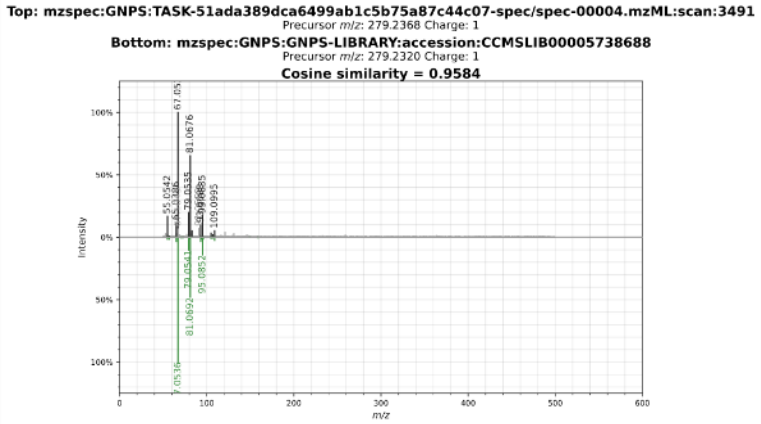


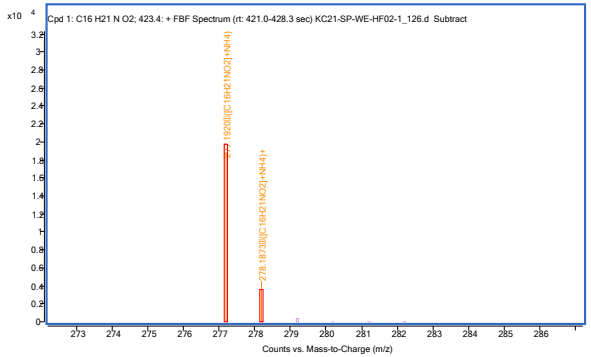


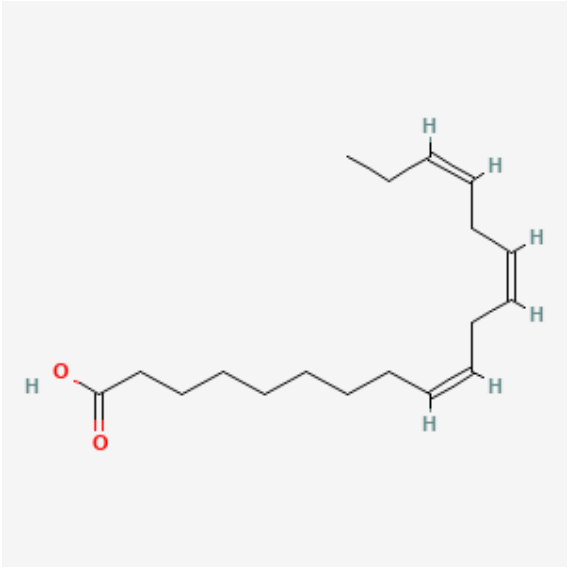


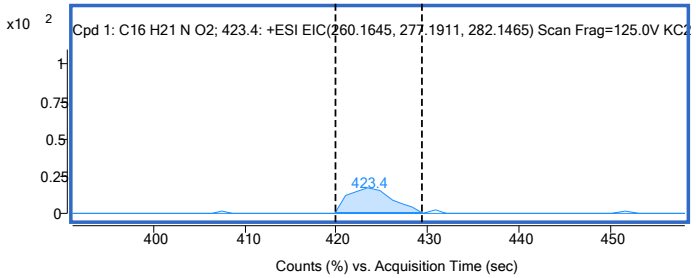
PROPRANOLOL

- used for many conditions such as hypertension, cardiac arrythmias angina pectoris, and hyperthyoridsm



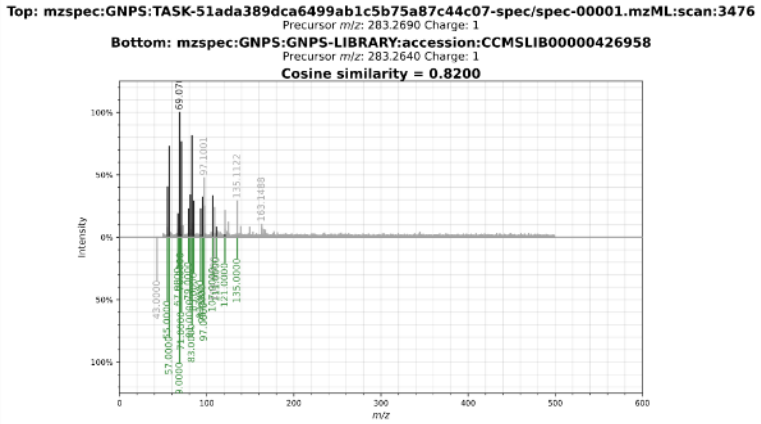


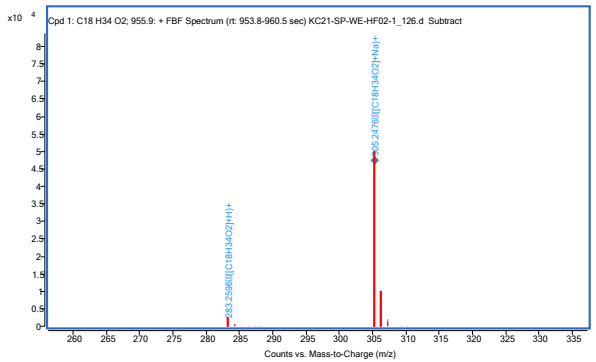


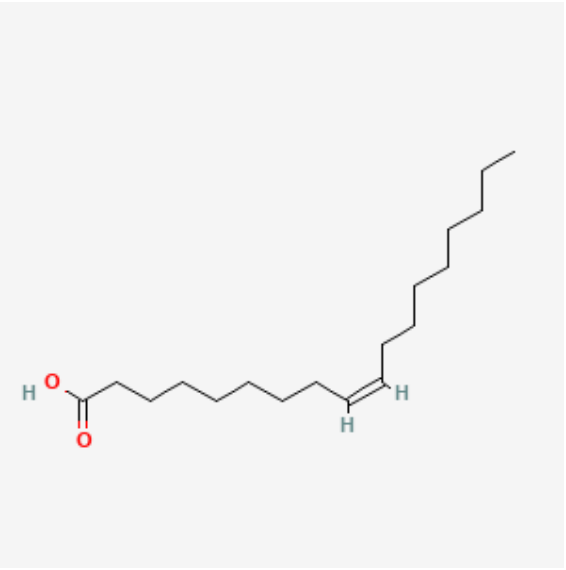


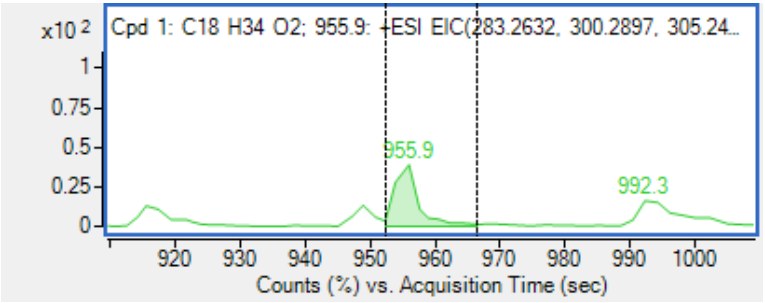
LINOLENIC ACID

- fatty acid, part if omega-3 fatty acids



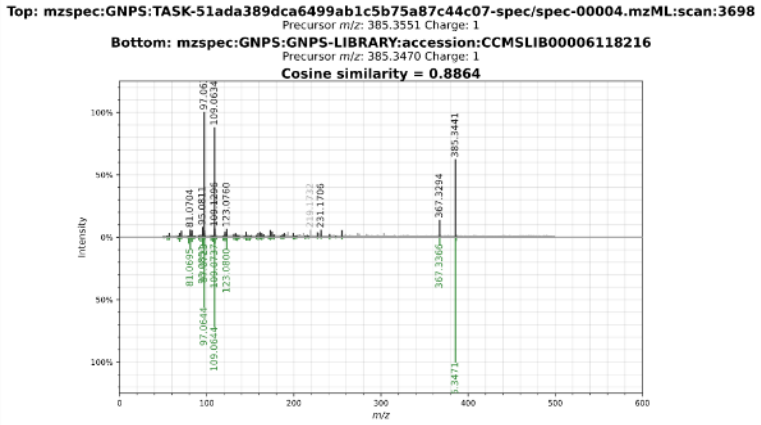


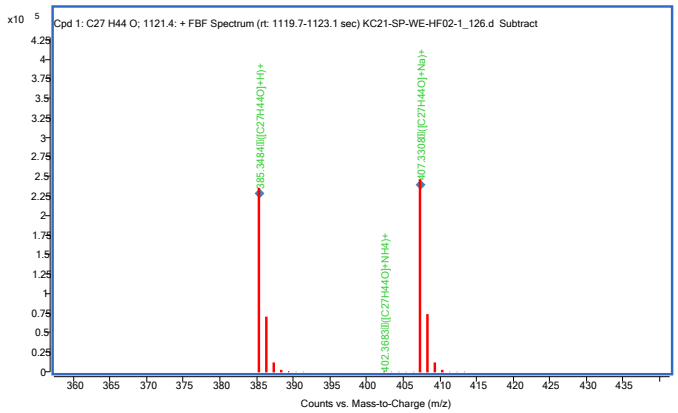


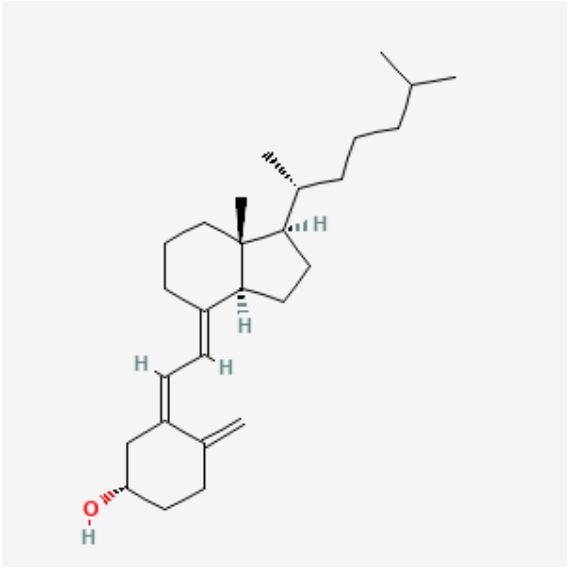


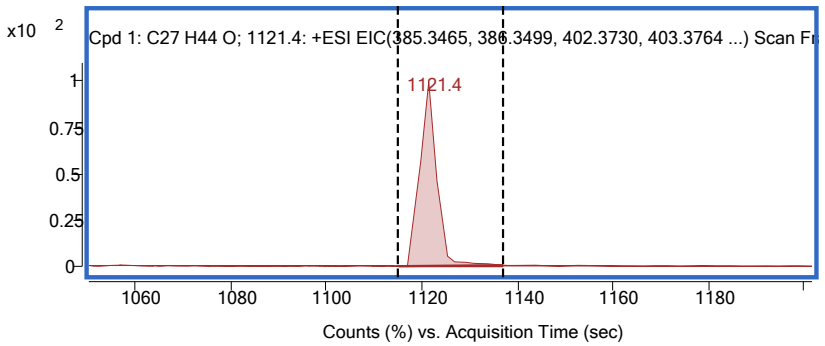
OLEIC ACID

- Paint/sealers for fabrics, crafts, and writing utensils



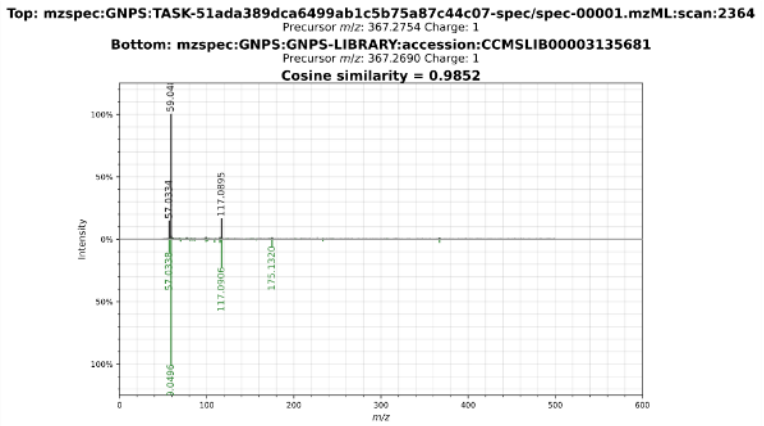


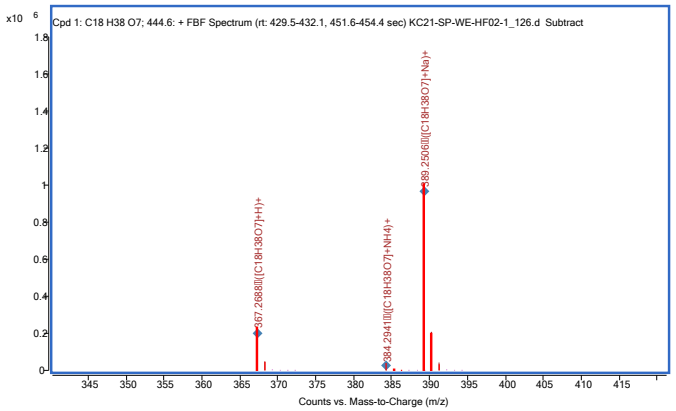


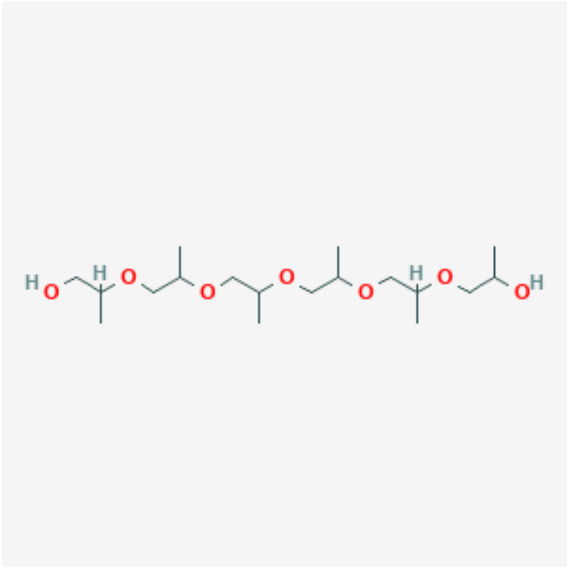


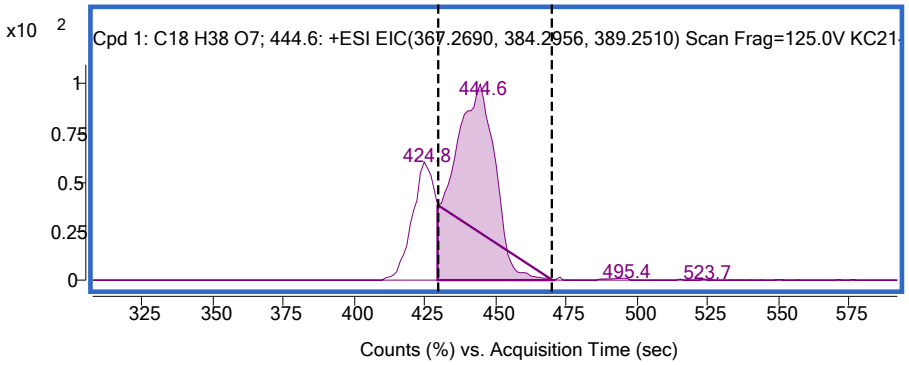
CHOLECALCIFEROL

- Bar and other soaps



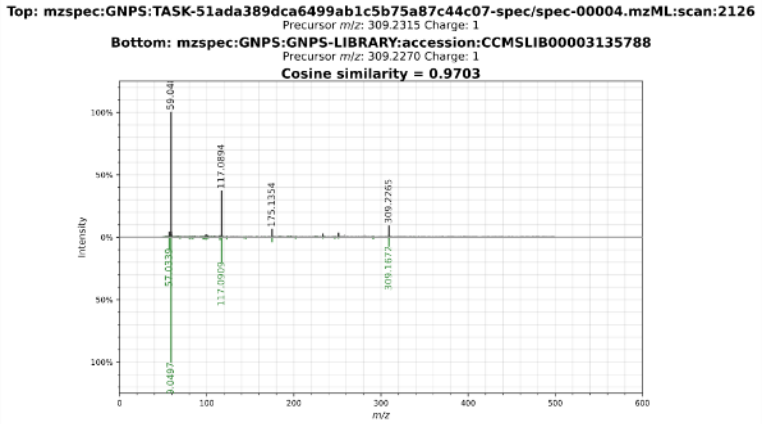


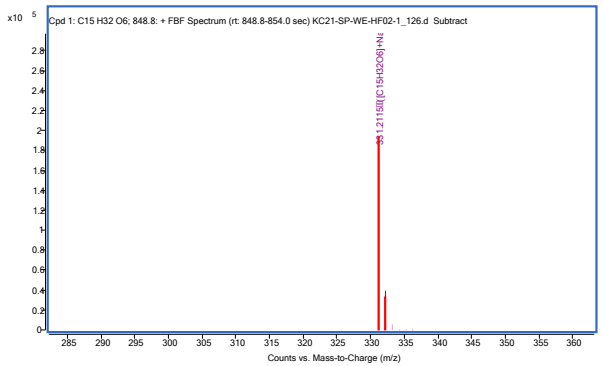


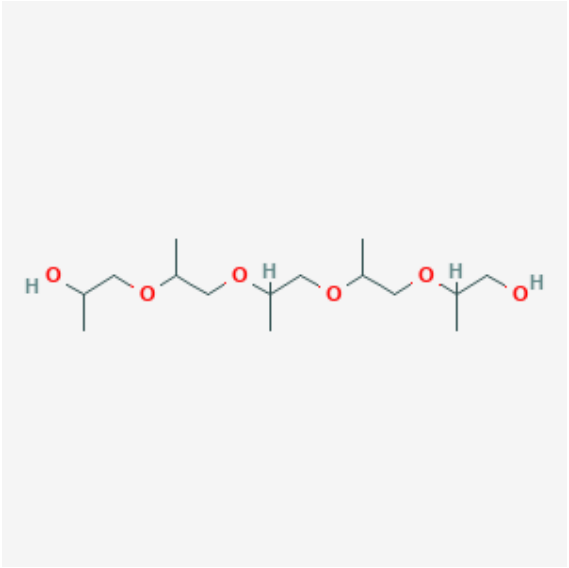


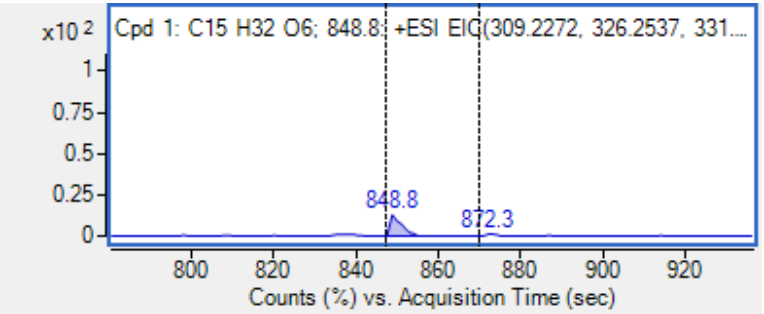
HEXAPROPYLENE GLYCOL

- patents for manufacturing agent



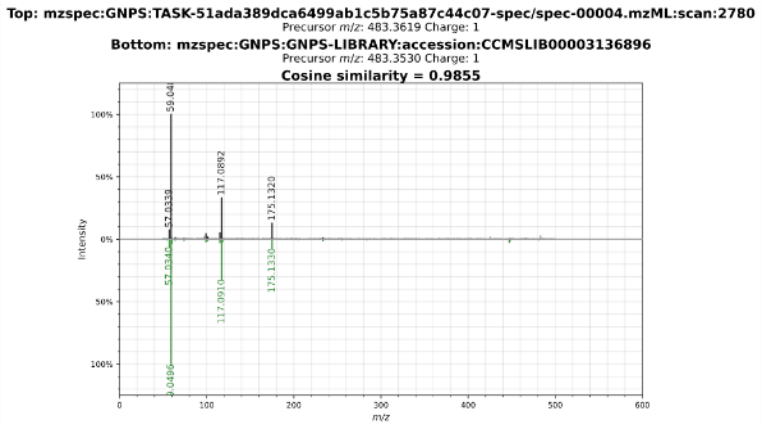


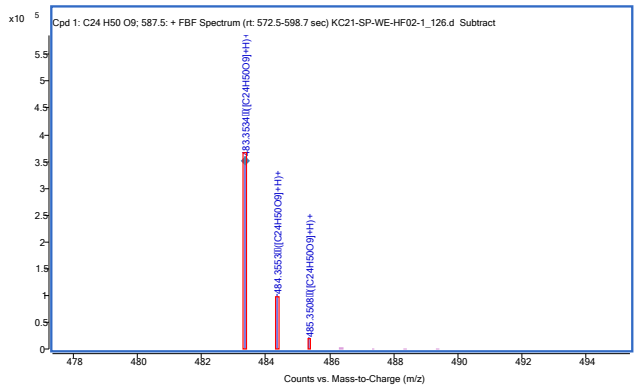


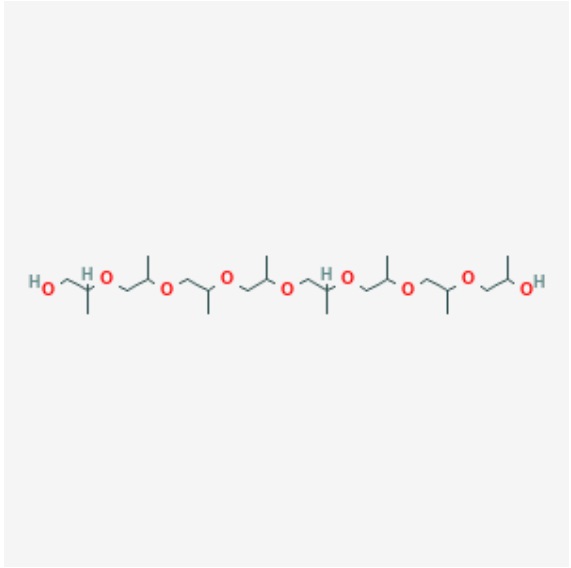


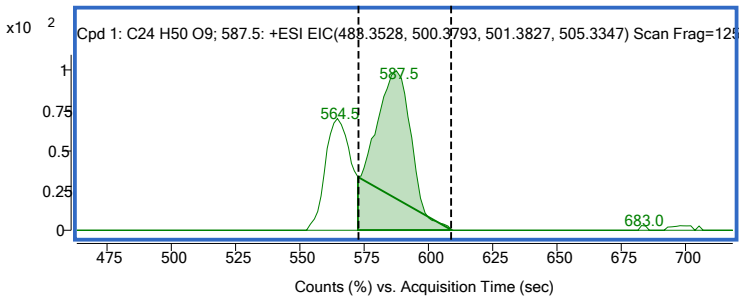
PENTAPROPYLENE GLYCOL

- patents for manufacturing agent



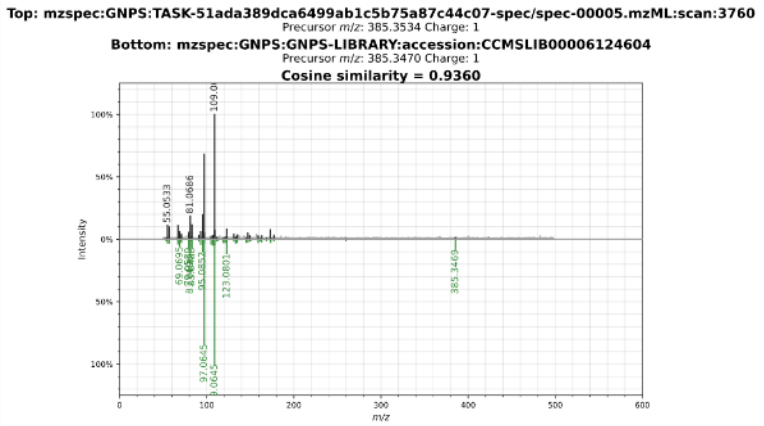


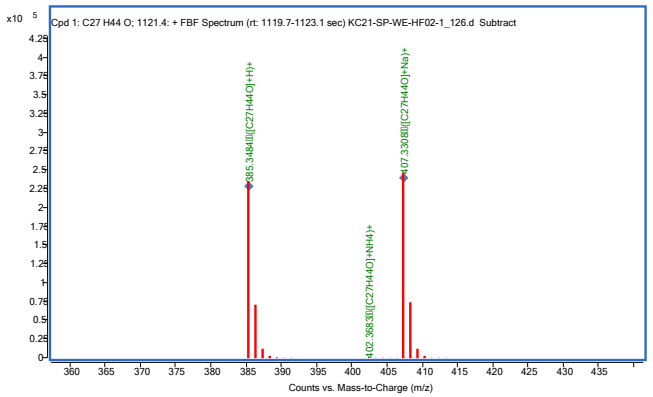


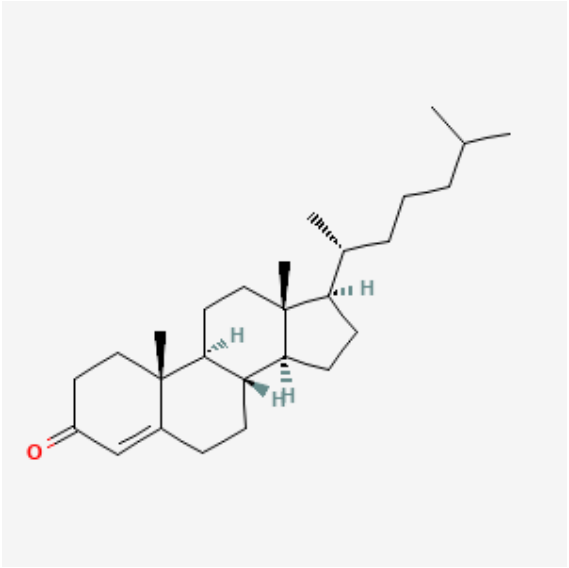


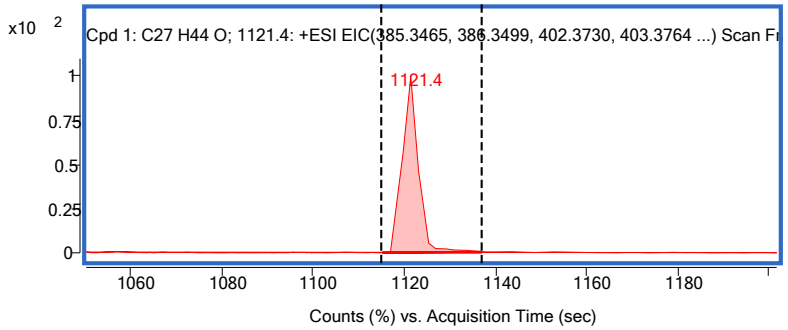
OCTAPROPYLENE GLYCOL

- patents for manufacturing agent



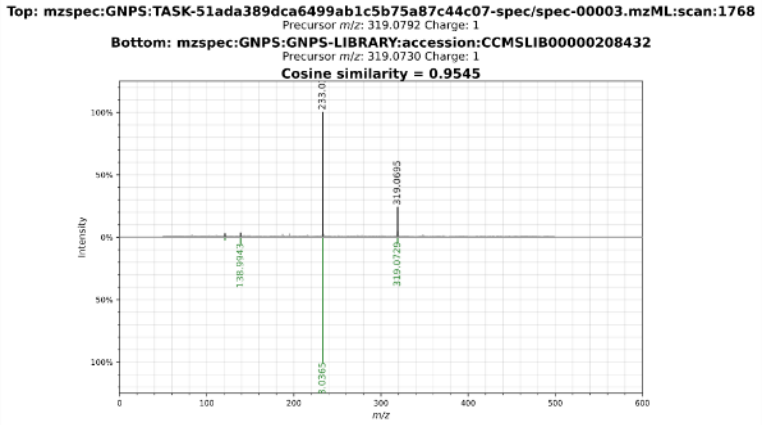


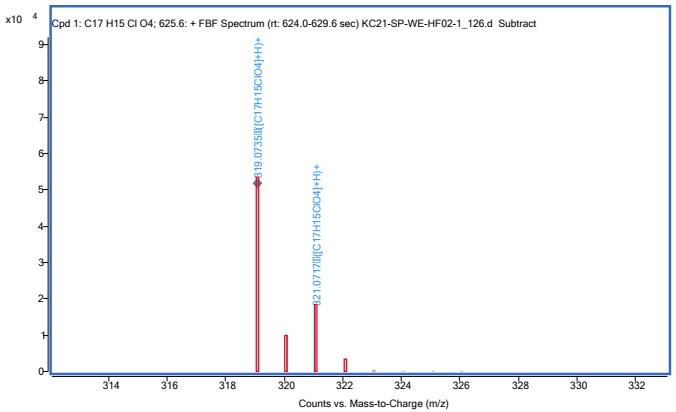


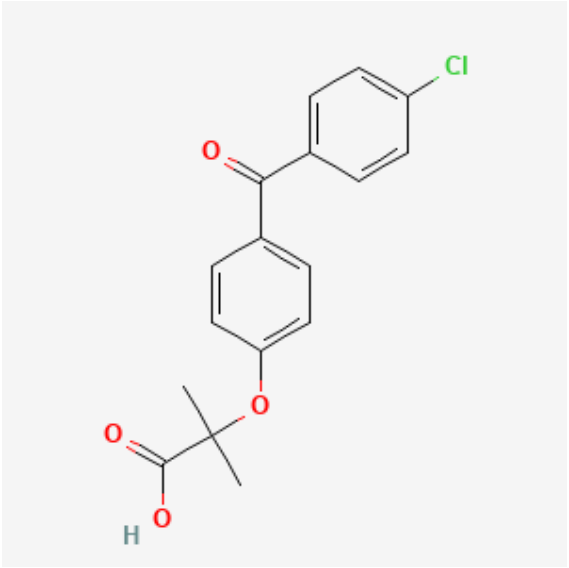


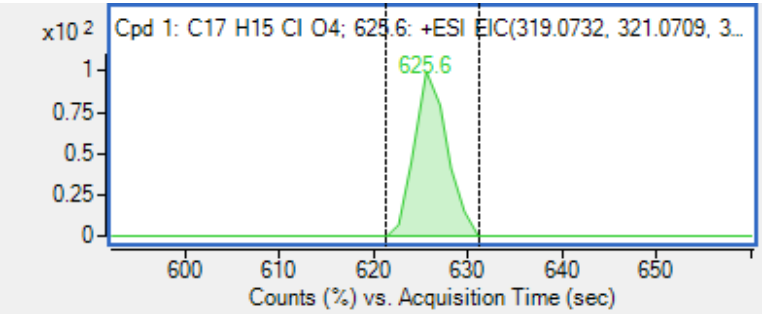
CHOLEST-4-EN-3-ONE
IDENTIFIED FROM SMILES IN GNPS

- human and plant metabolite, also used to treat colorectal cancer



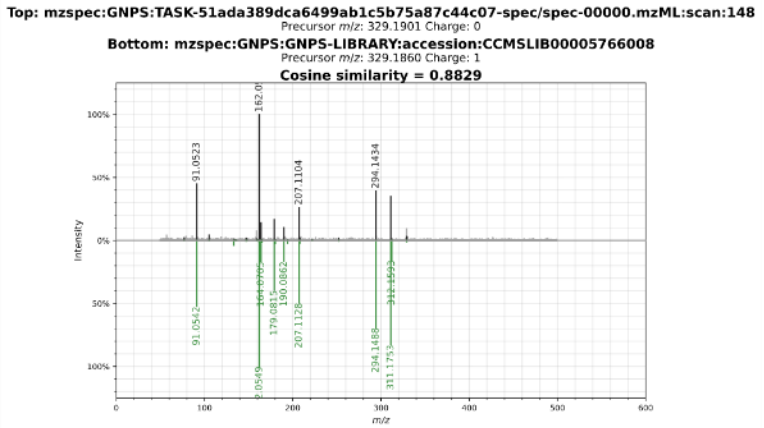


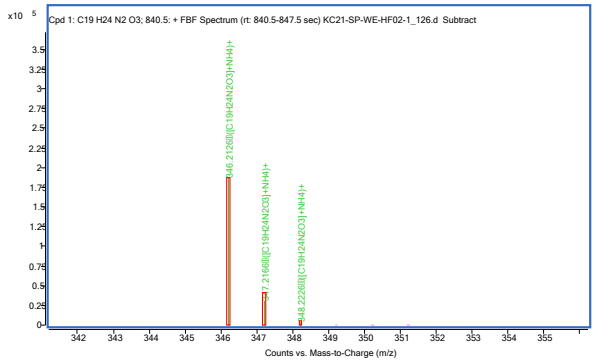


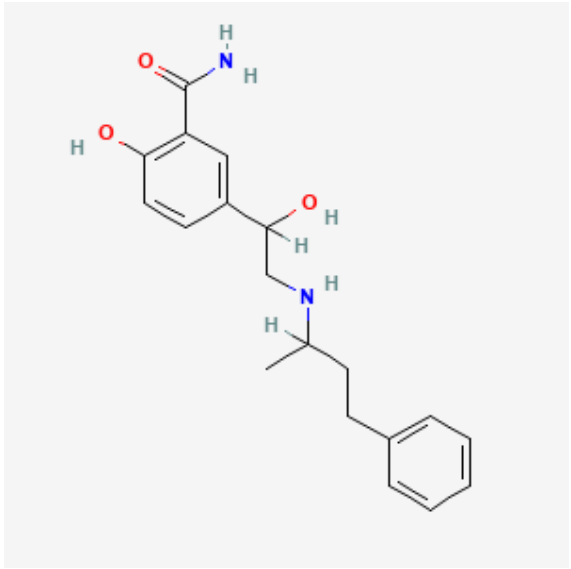


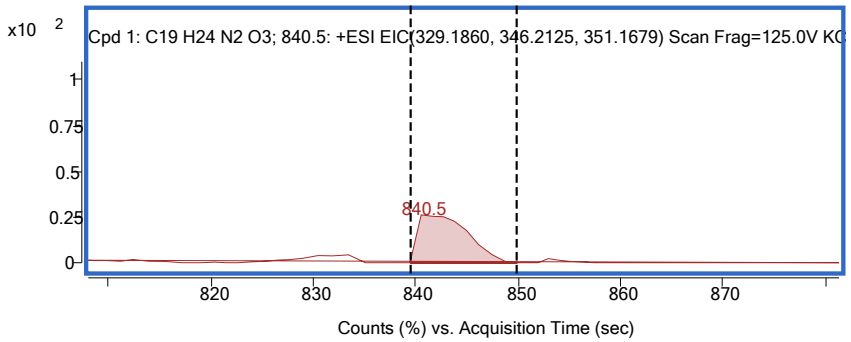
FENOFIBRIC ACID

- Used as a lipid modifying agent



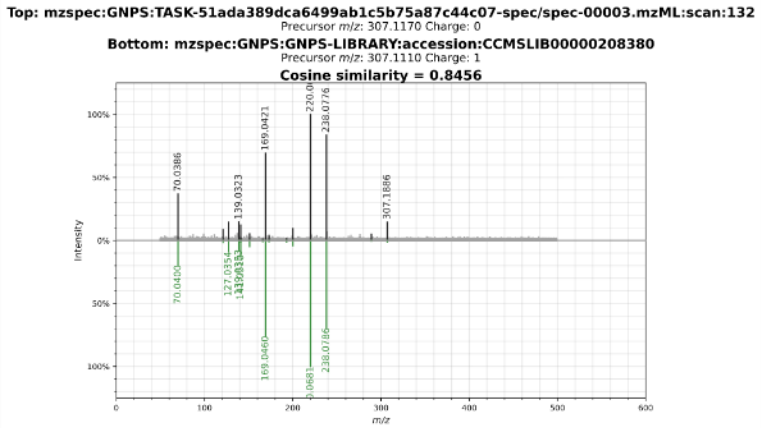


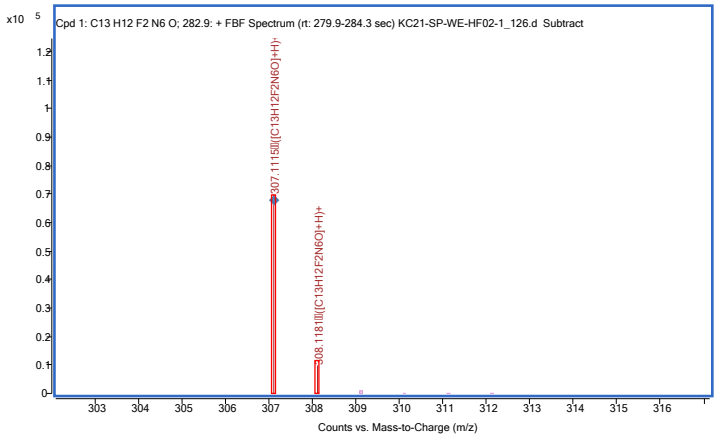


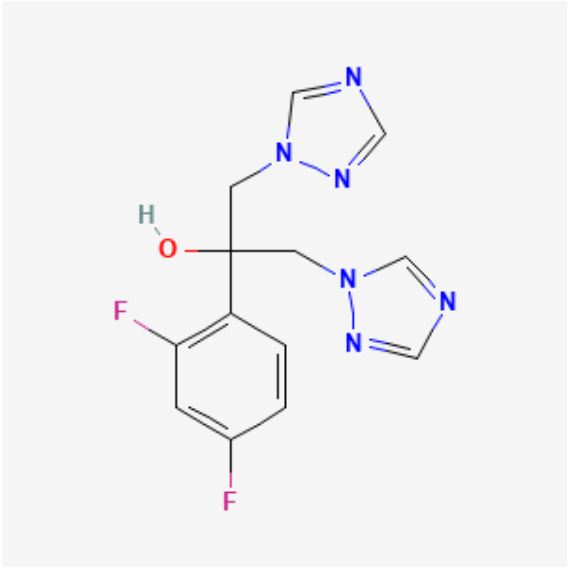


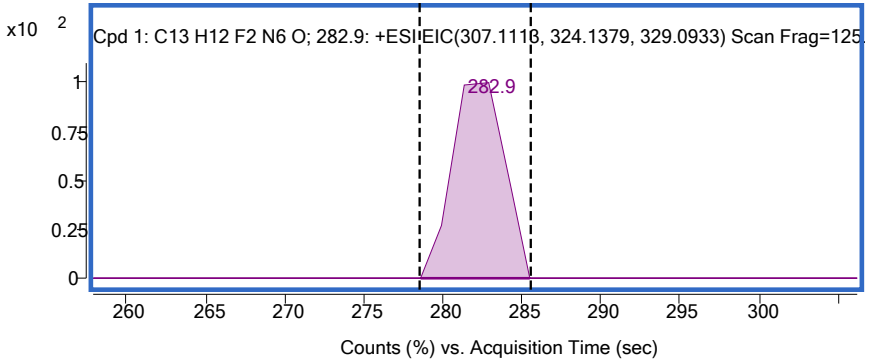
LABETALOL

- Antihypertensive agent



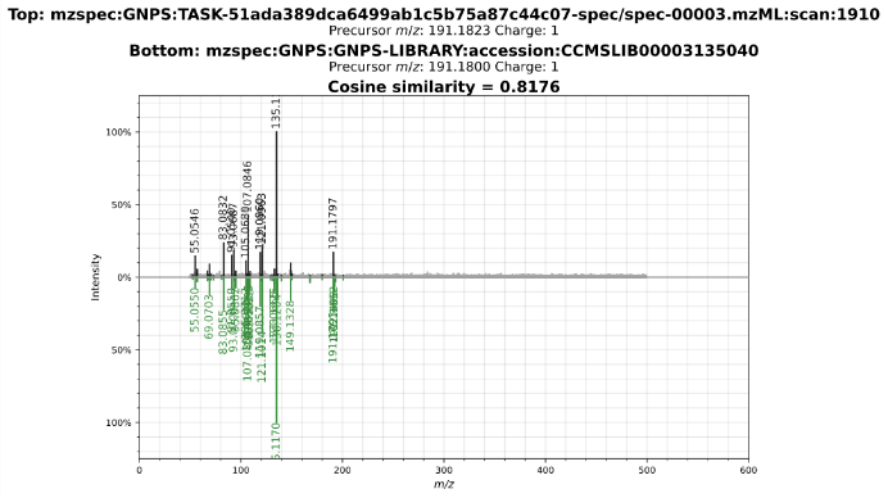


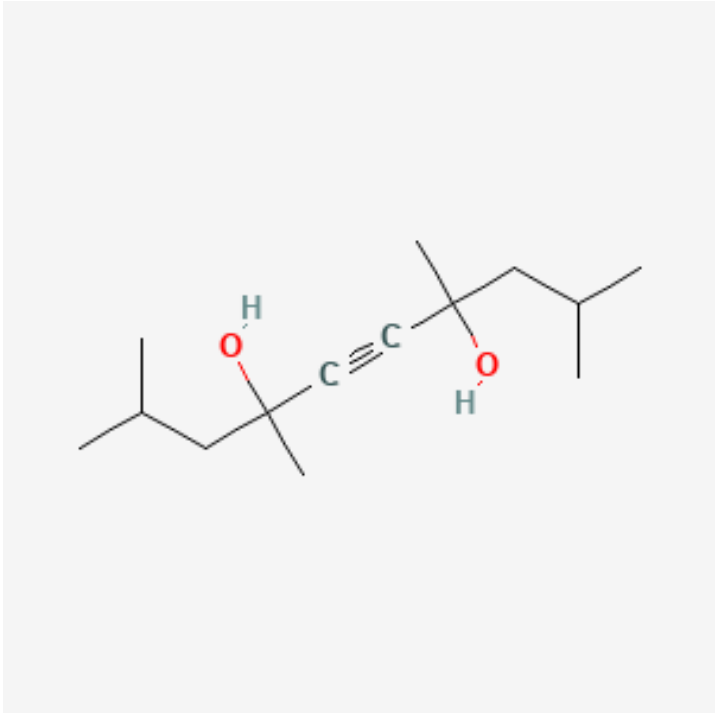


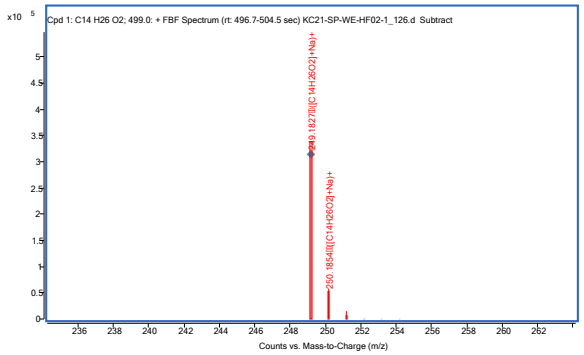


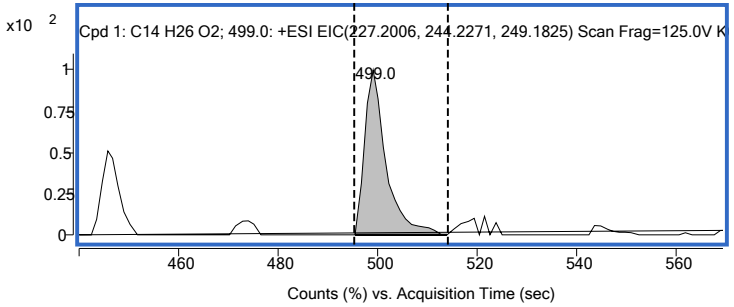
FLUCONAZOLE

- Prescribed as antifungal



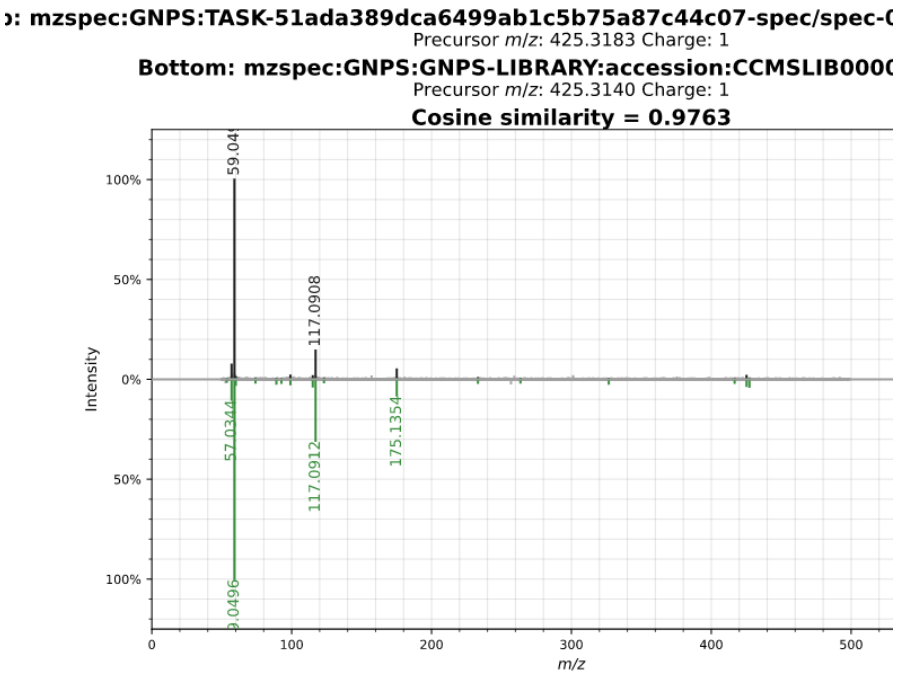


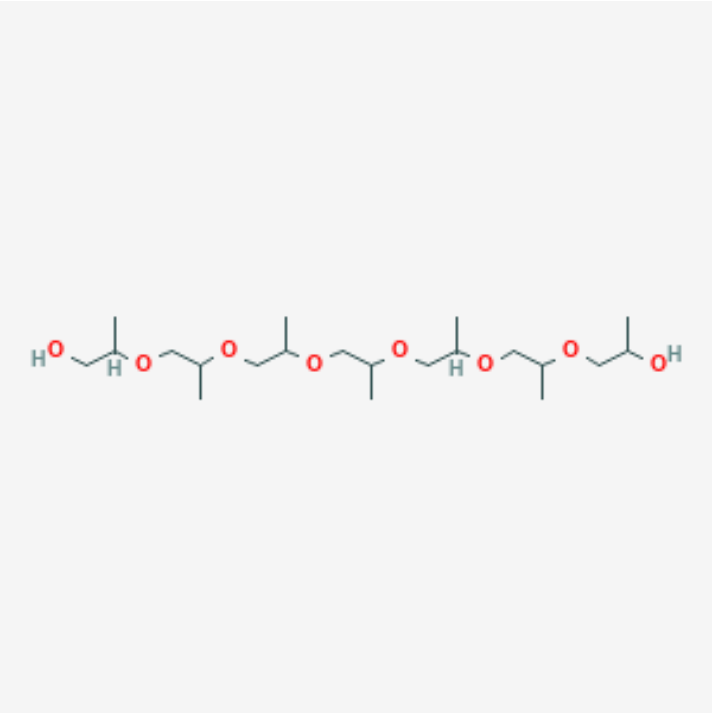


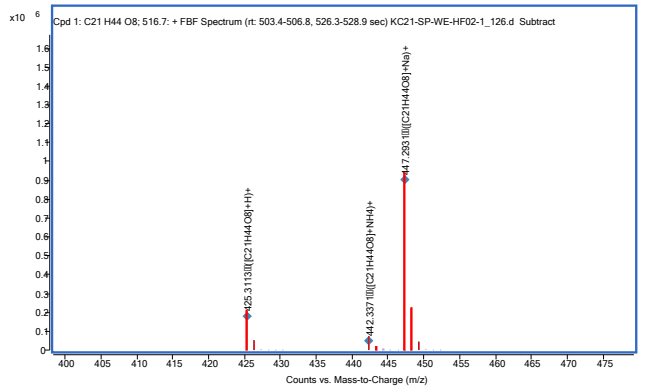


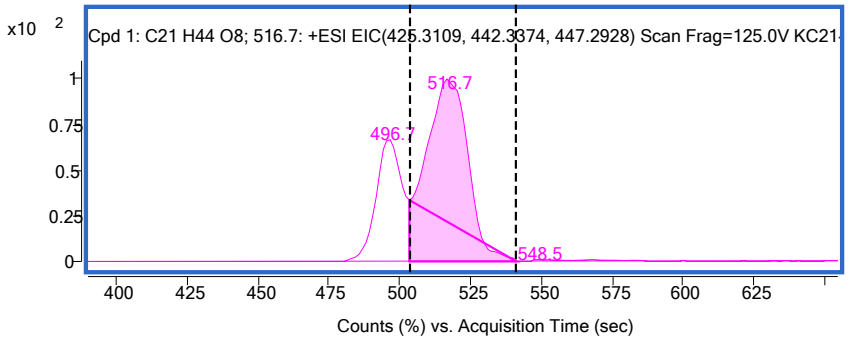
2,4,7,9-TETRAMETHYL-5-DECYNE-4,7-DIOL

CLEANING/SAFETY IN INDUSTRIAL/OCCUPATIONAL SETTINGS, HARD FLOOR CLEANER



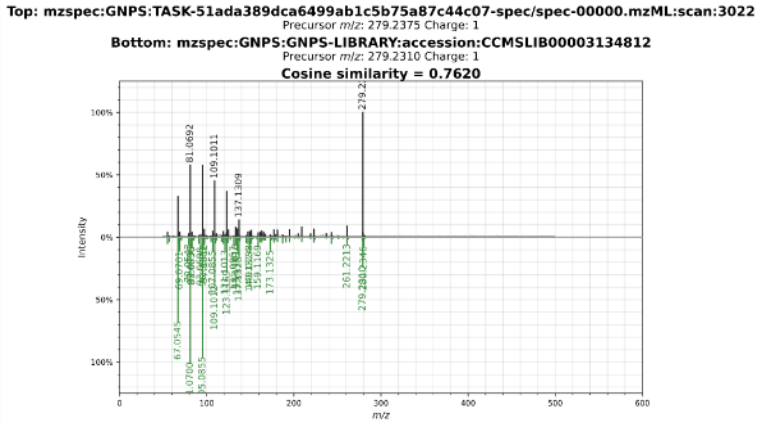


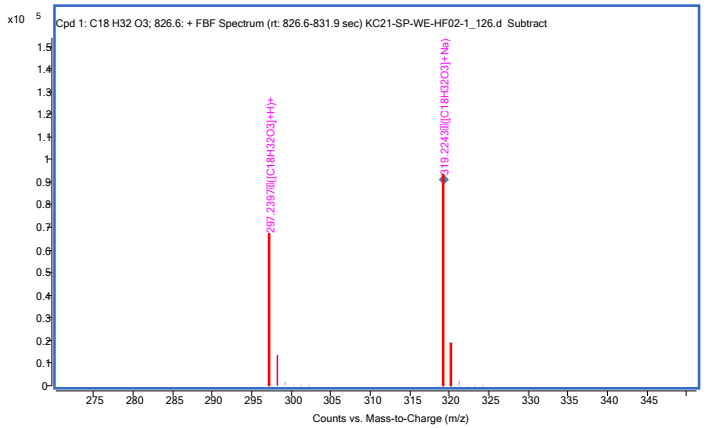


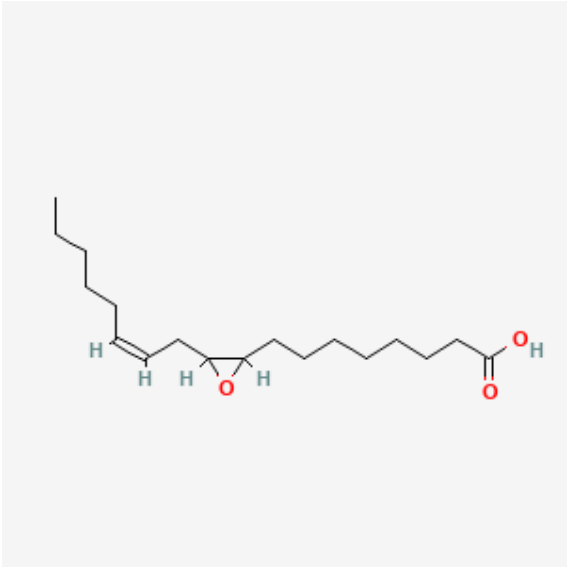


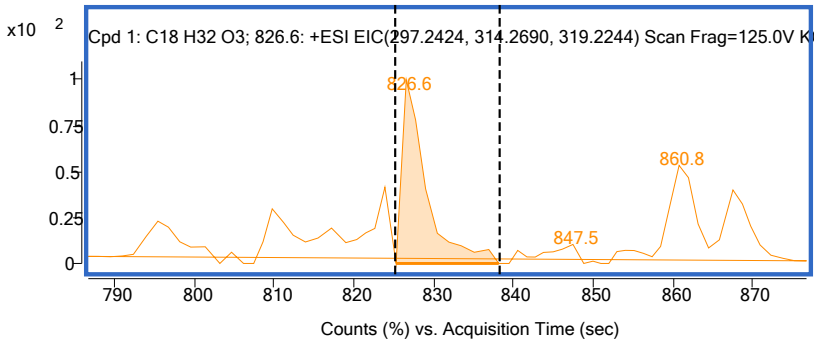
HEPTAPROPYLENE GLYCOL

USED IN MOLDS, EG- DENTAL MOLDS



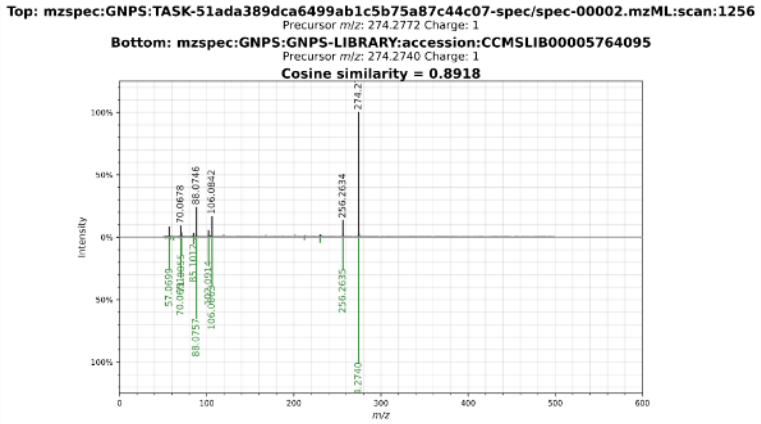


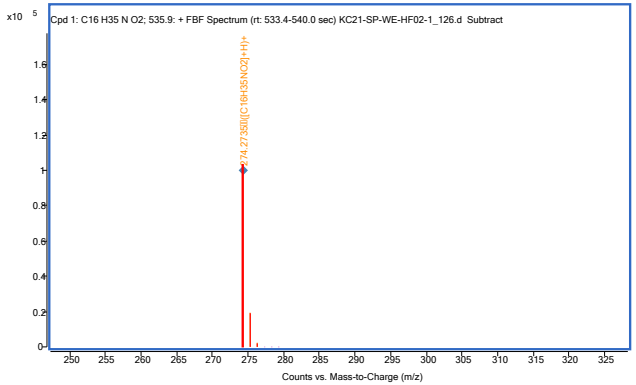


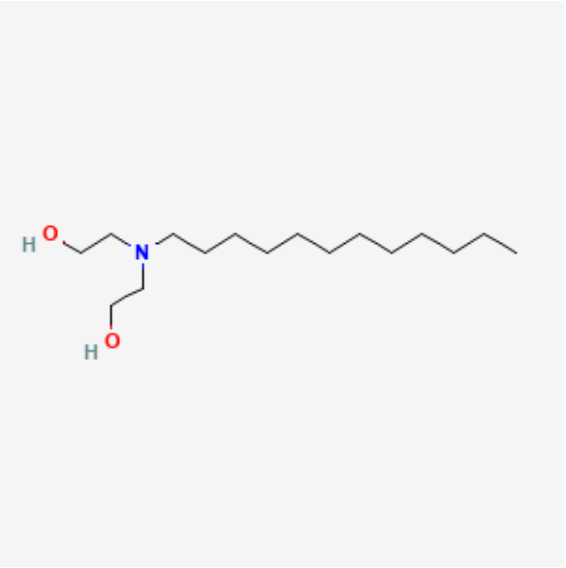


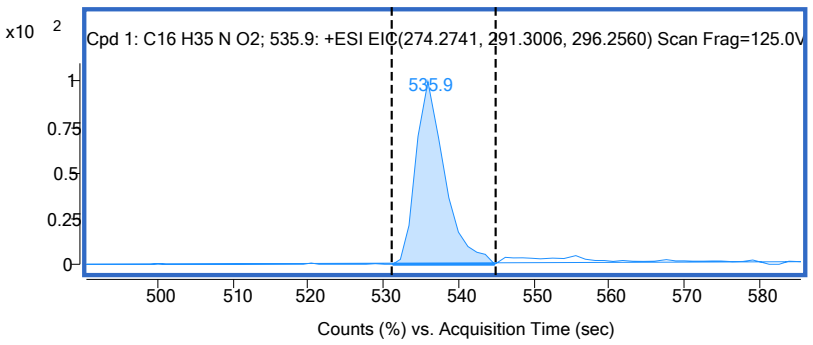
CORONAMIC ACID

- Human metabolite of linoleic acid



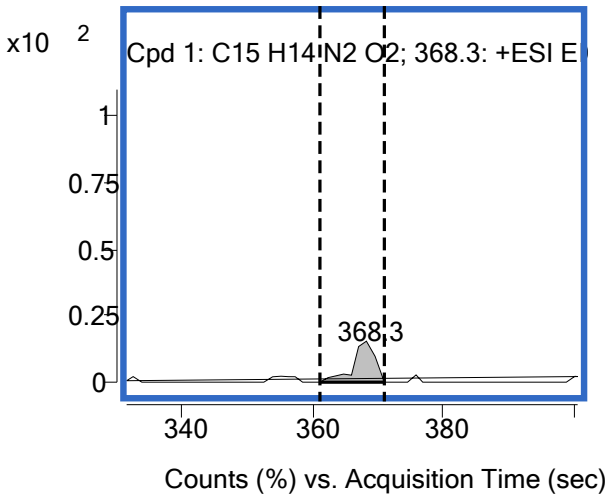


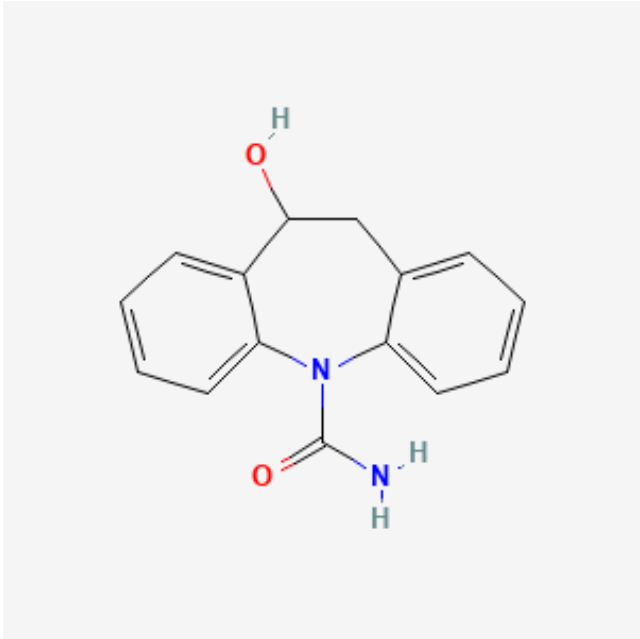


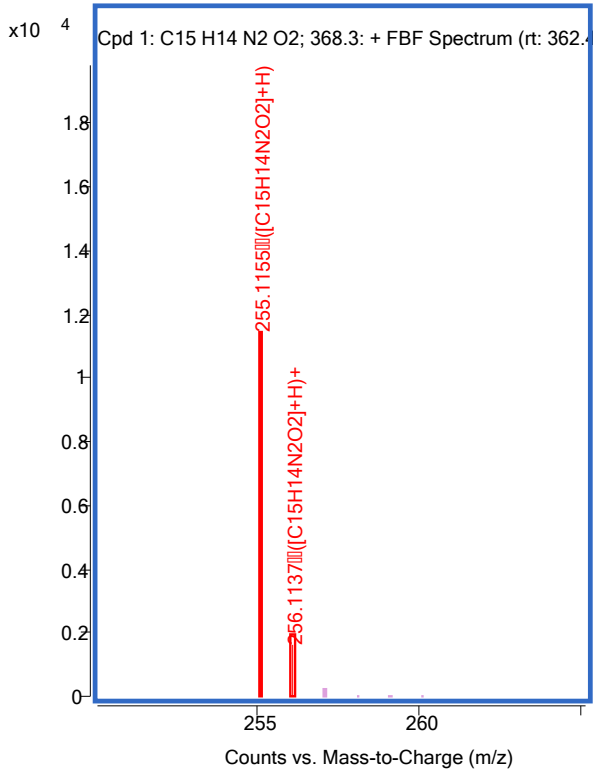


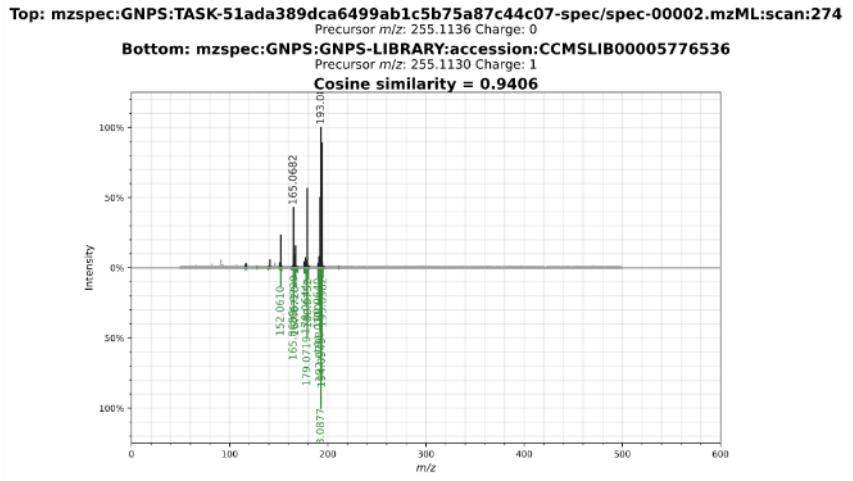
LAURYLDIETHANOLAMINE

- Used in hair conditioner



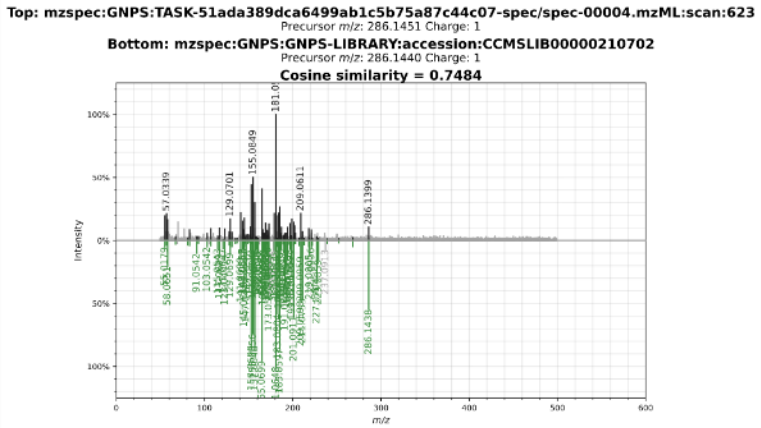


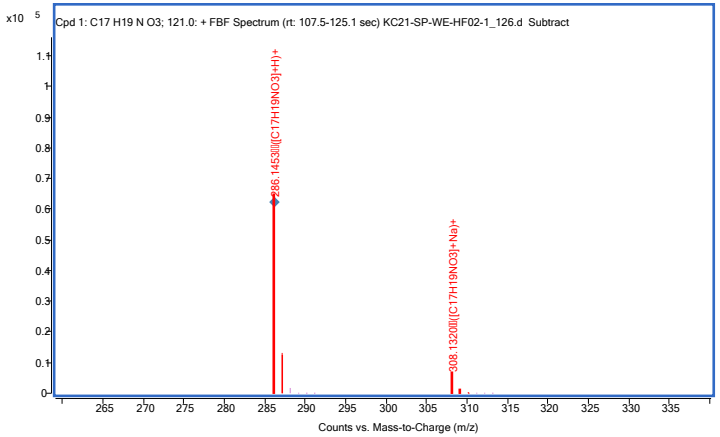


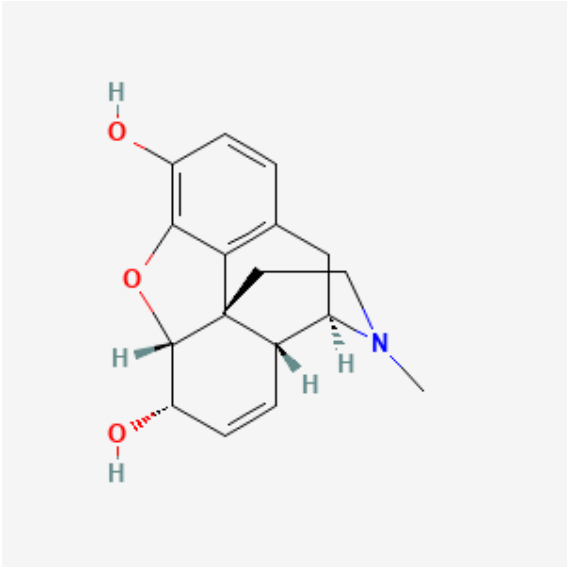


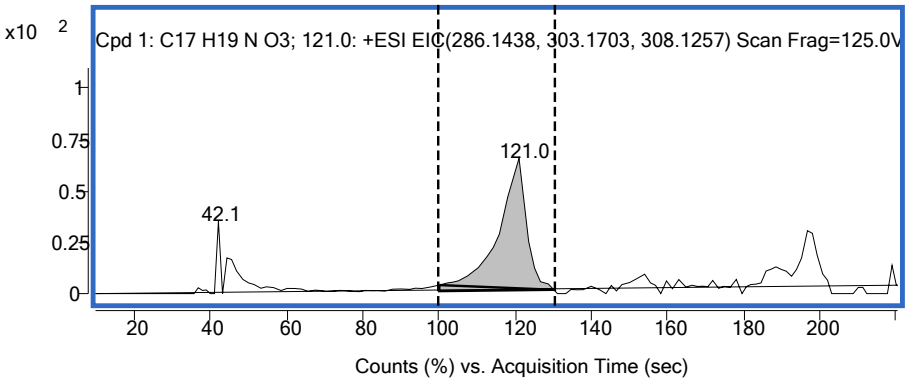
LICARBAZEPINE

- active metabolite of oxcarbazepine



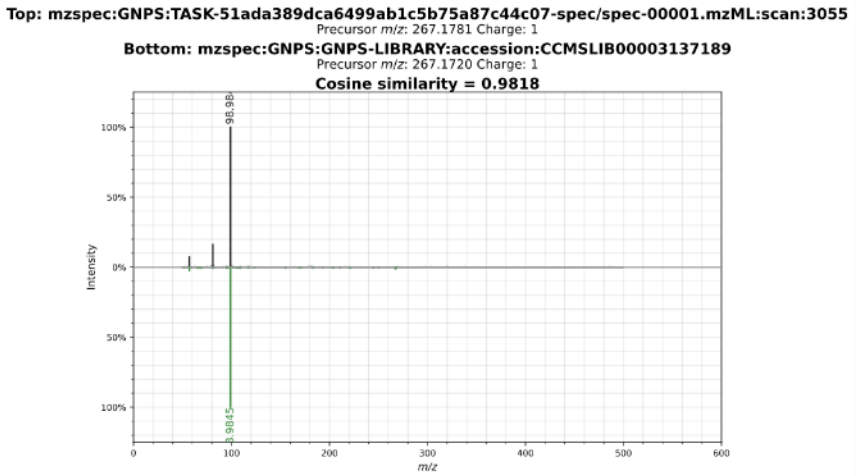


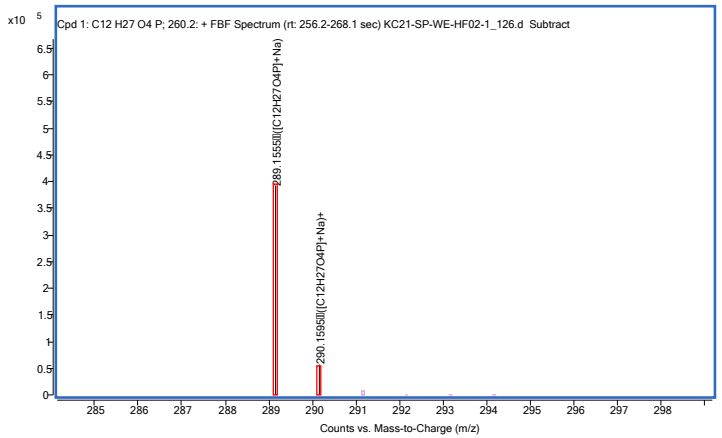


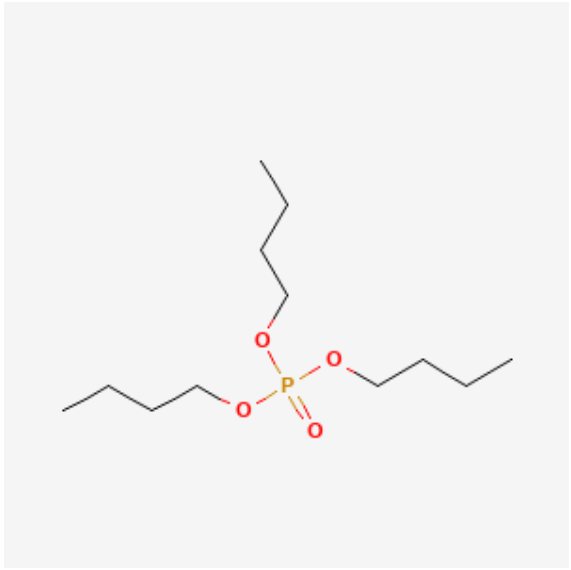


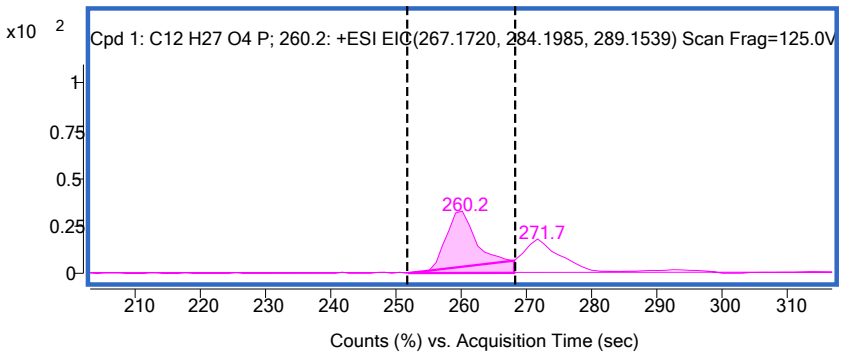
MORPHINE

- Used as pain relief



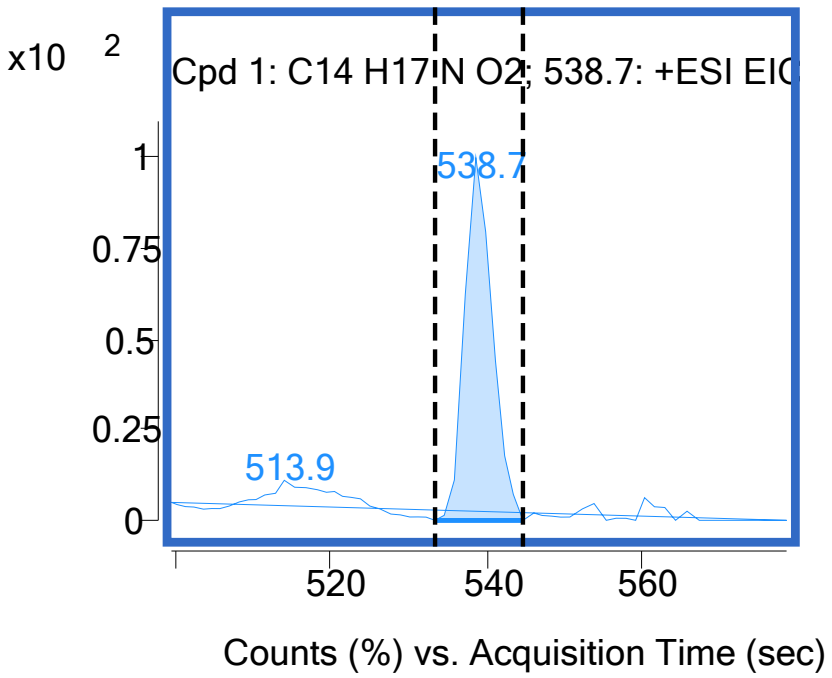


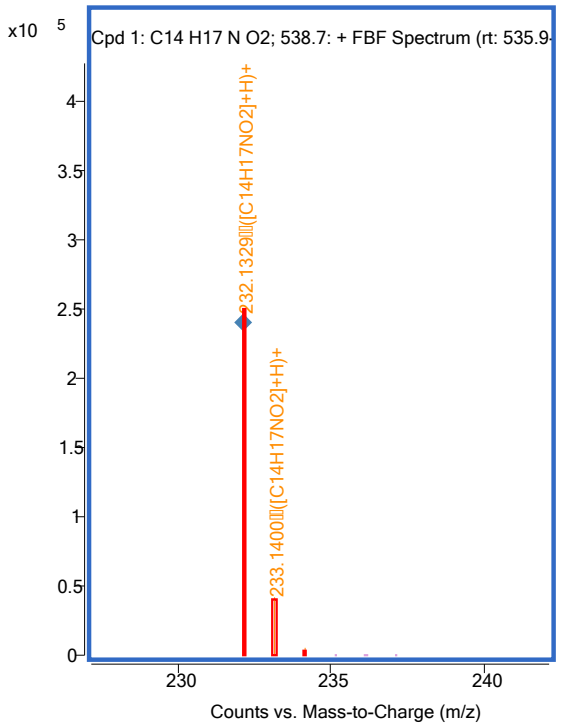


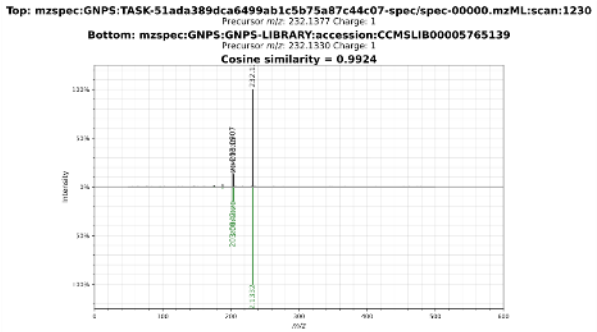


TRIBUTYL PHOSPHATE

- Product use: specifically paints, colorants, and pigments

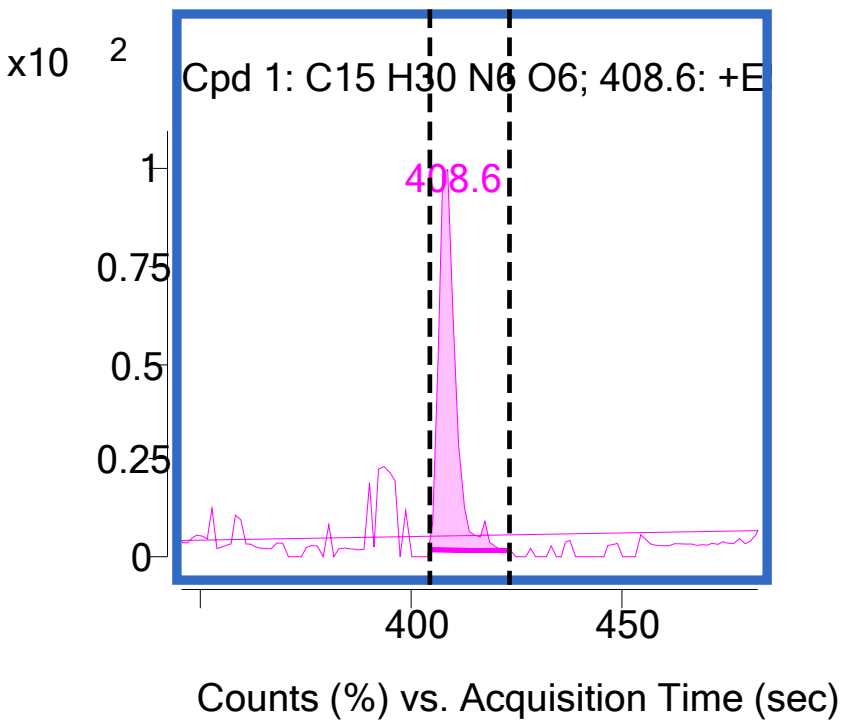


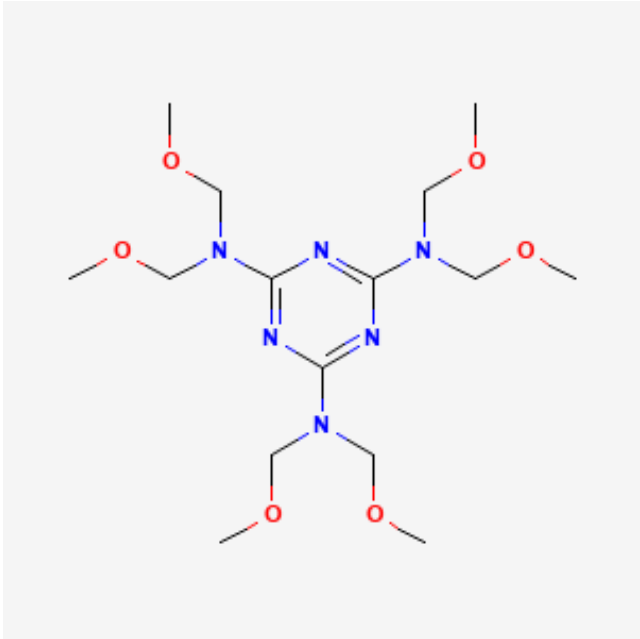


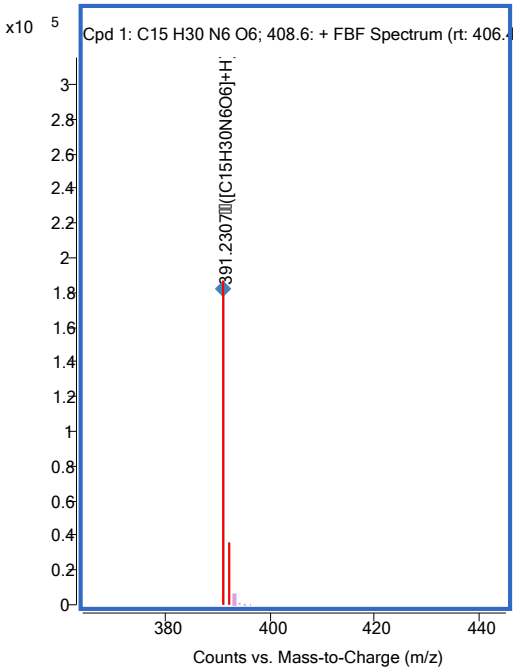


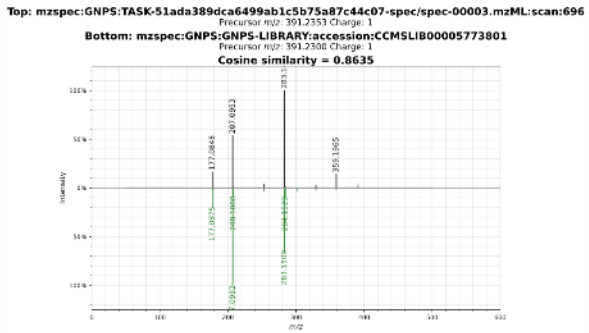
7-DIETHYLAMINO-4-METHYLCOUMARIN

- carpet and furniture cleaner for pet hair



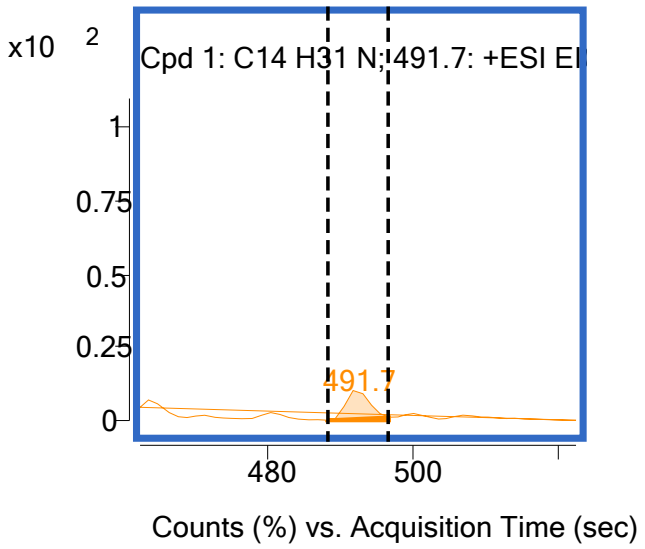


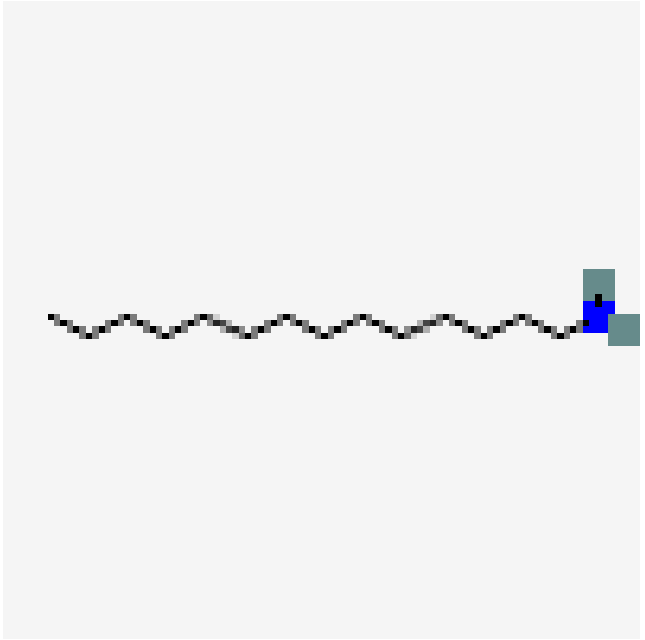


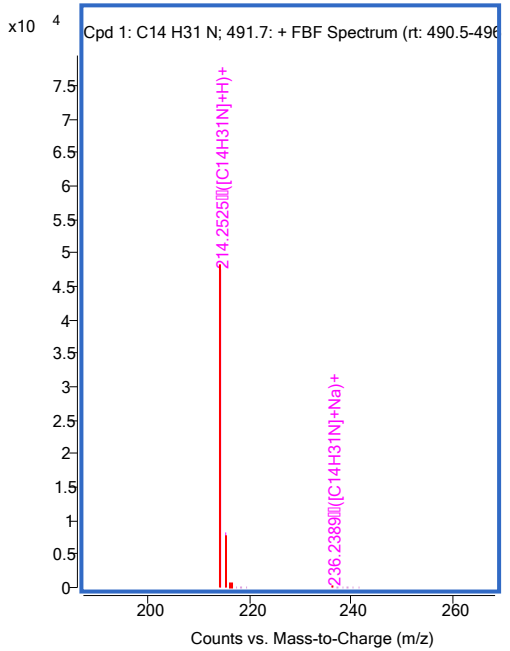


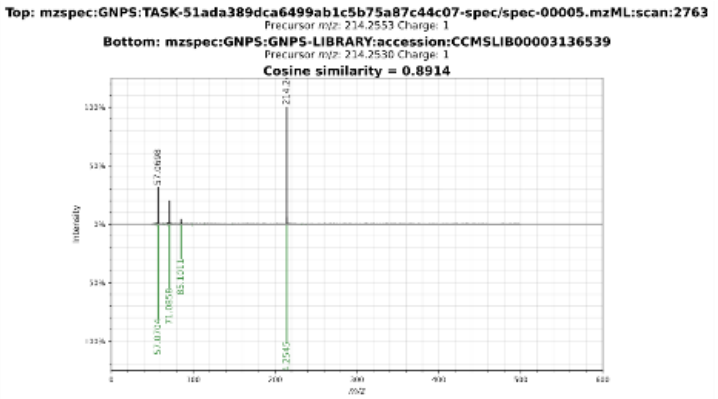
HEXA(METHOXYMETHYL)MELAMINE

- Paint topcoat and additive



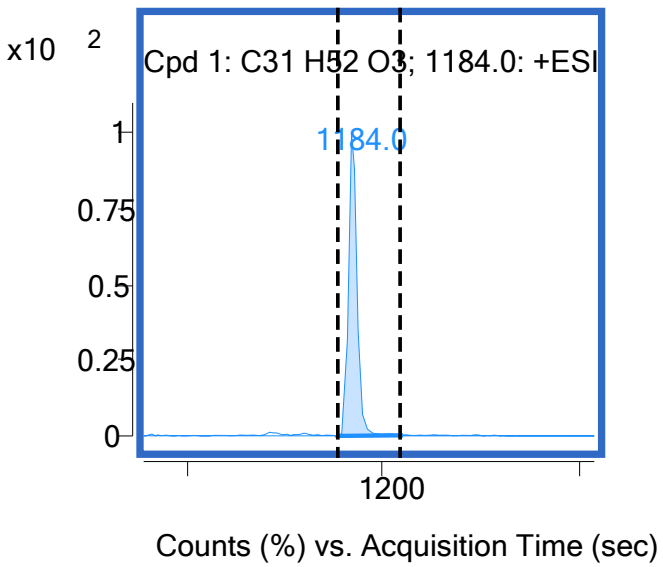


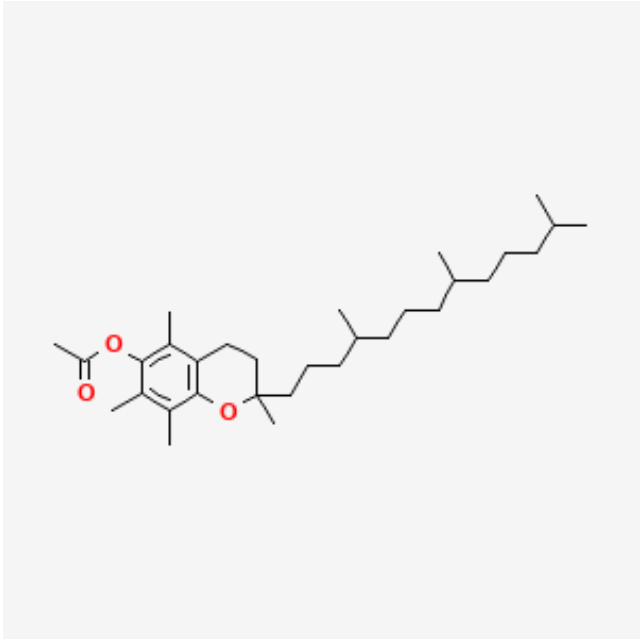


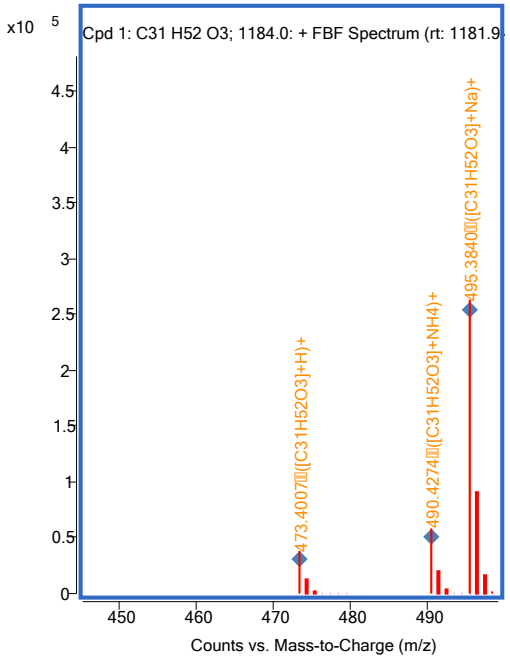


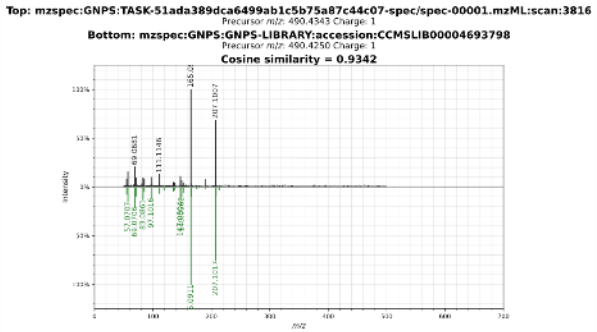
TETRADECYLAMINE

- Natural product



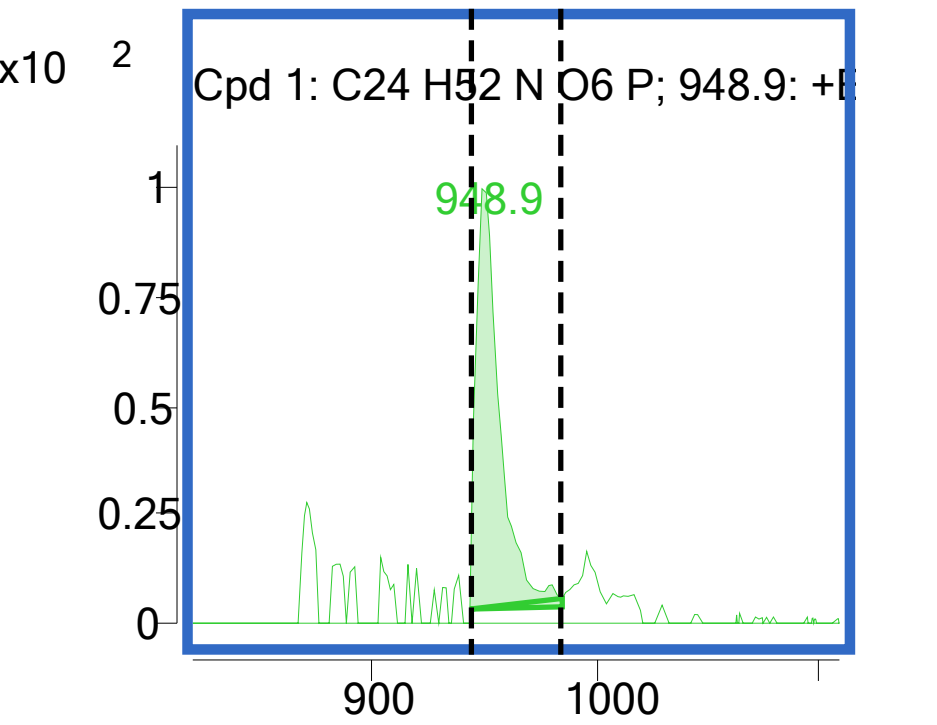


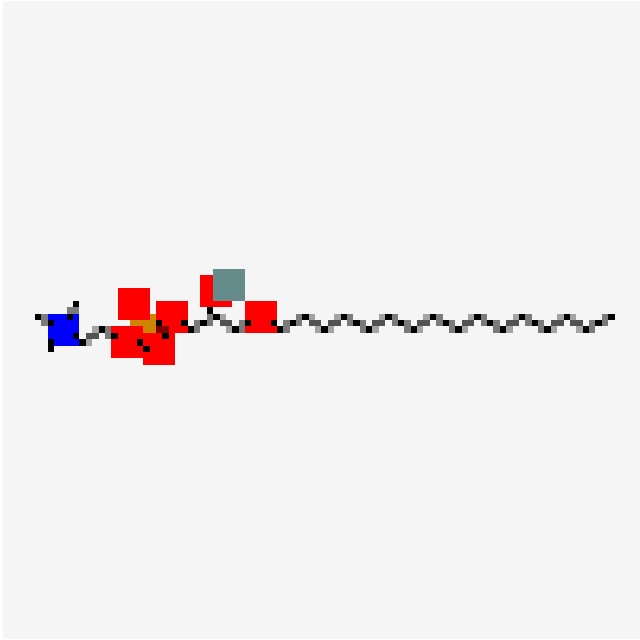


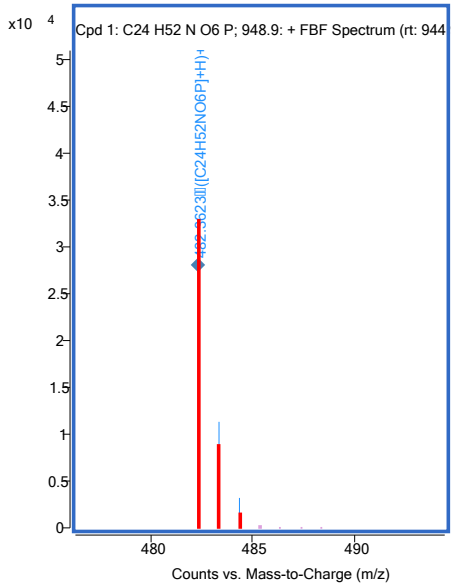


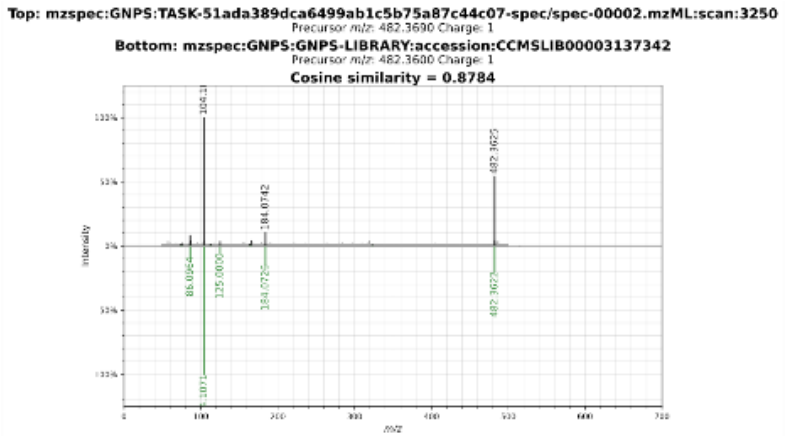
DL-ALPHA-TOCOPHEROL ACETATE

- antioxidant



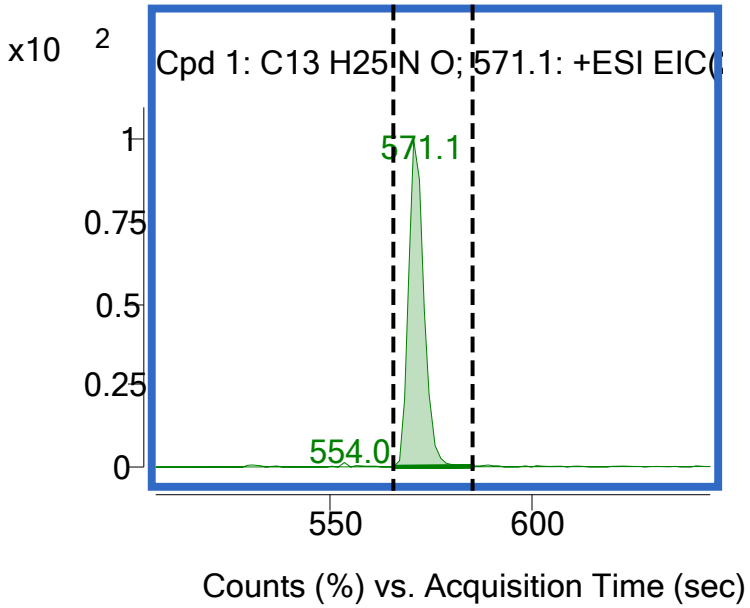


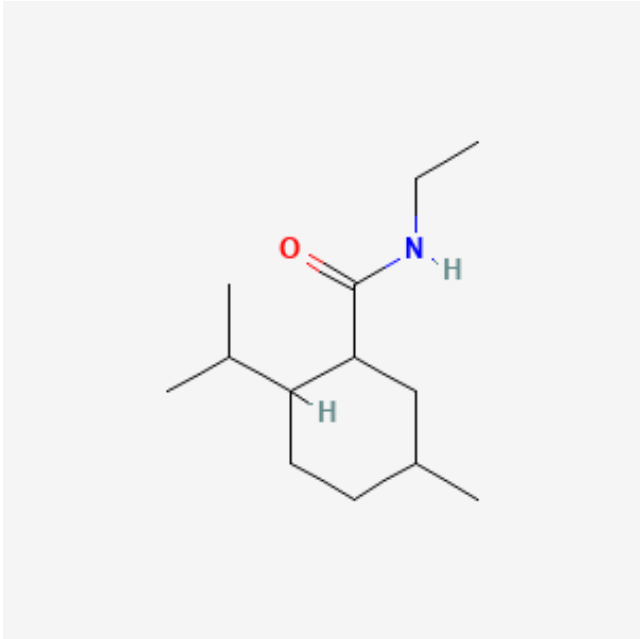


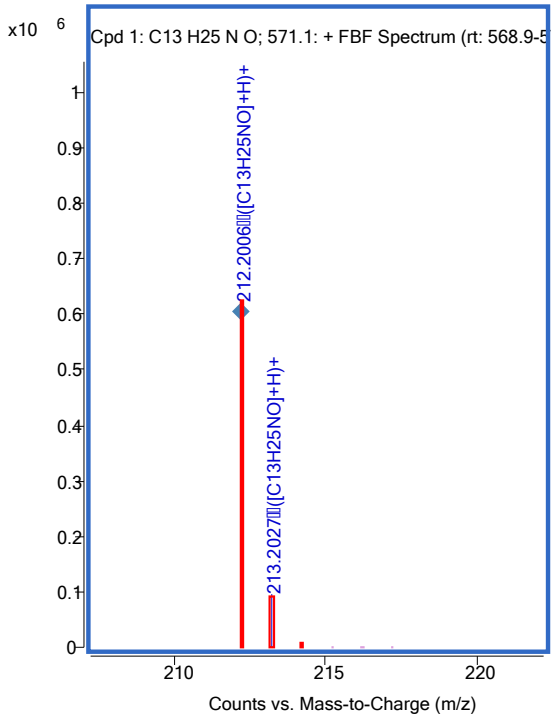


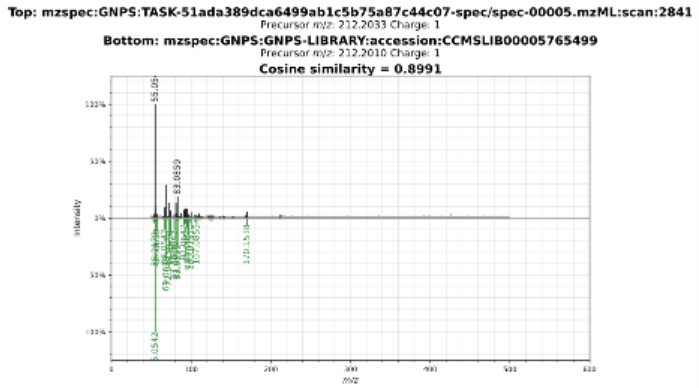
1-O-HEXADECYL-SN-GLYCERO-3-PHOSPHOCHOLINE

- Fatty acid



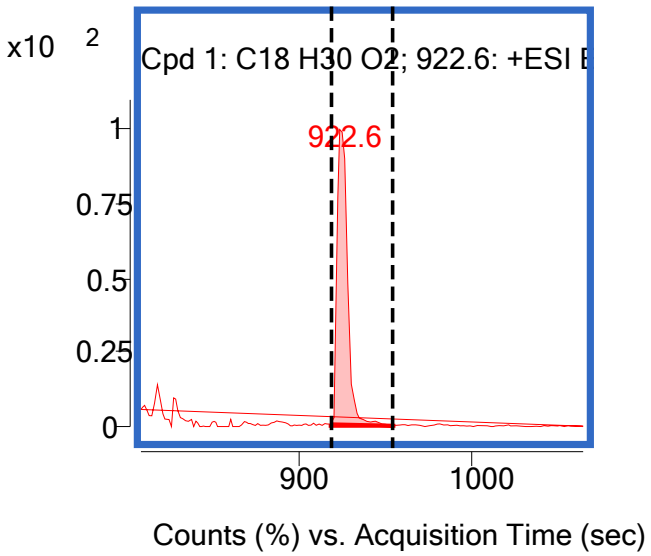


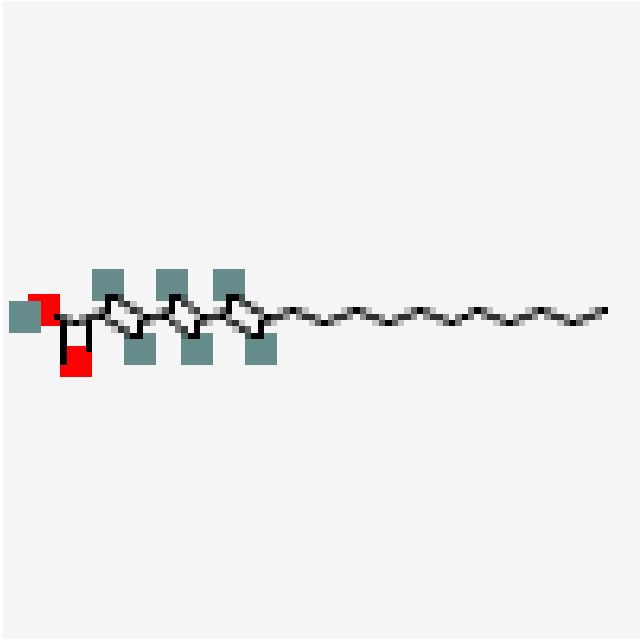


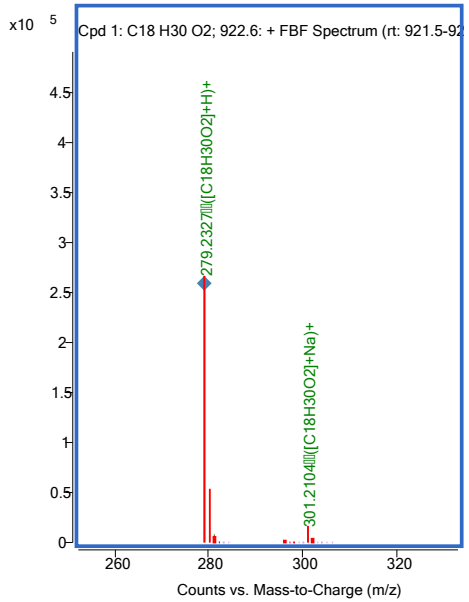


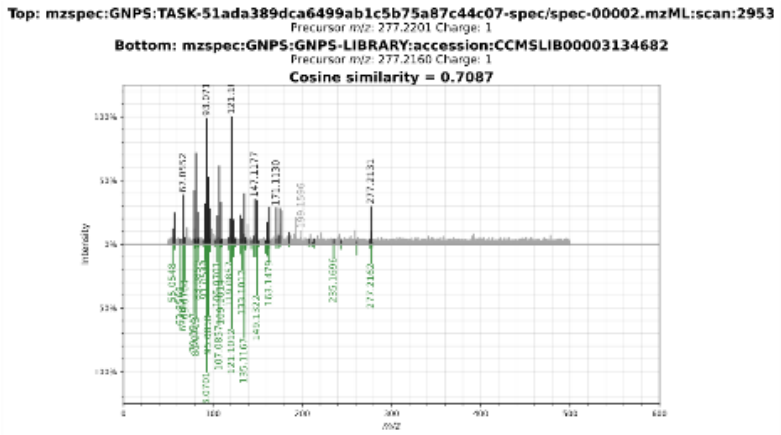
N-ETHYL-P-MENTHANE-3-CARBOXAMIDE

- Flavoring agent



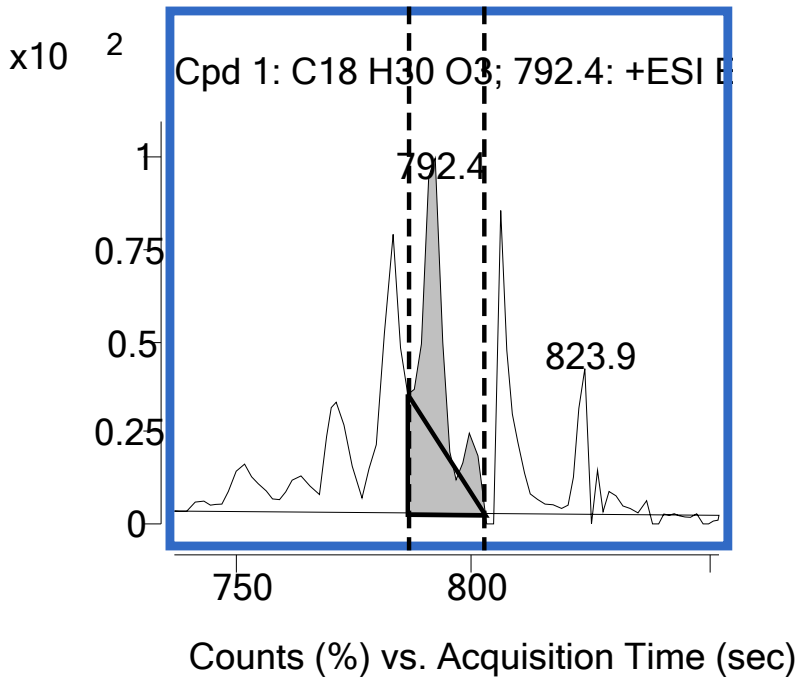


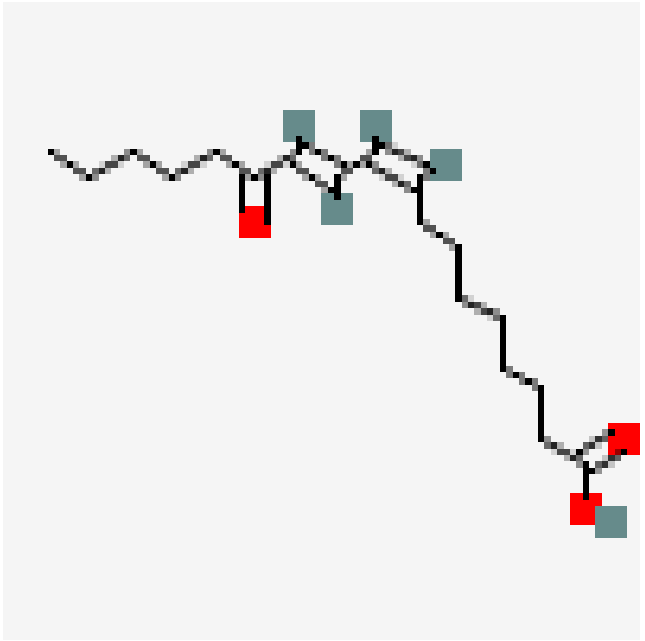


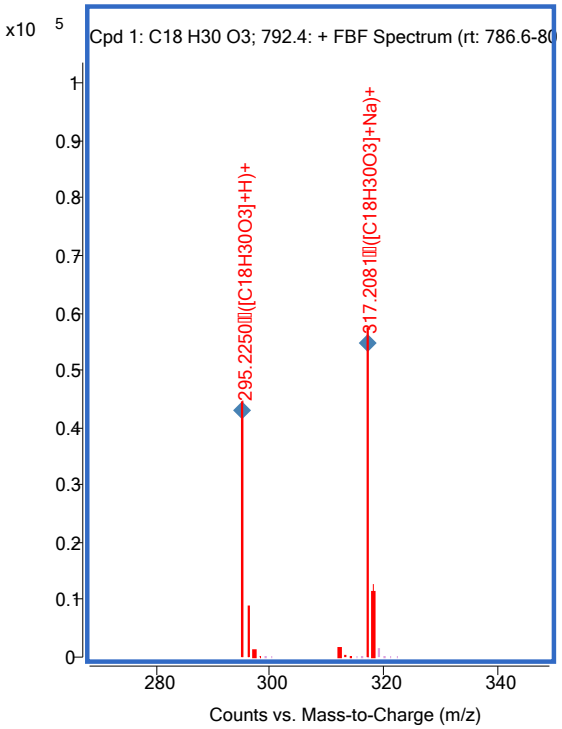


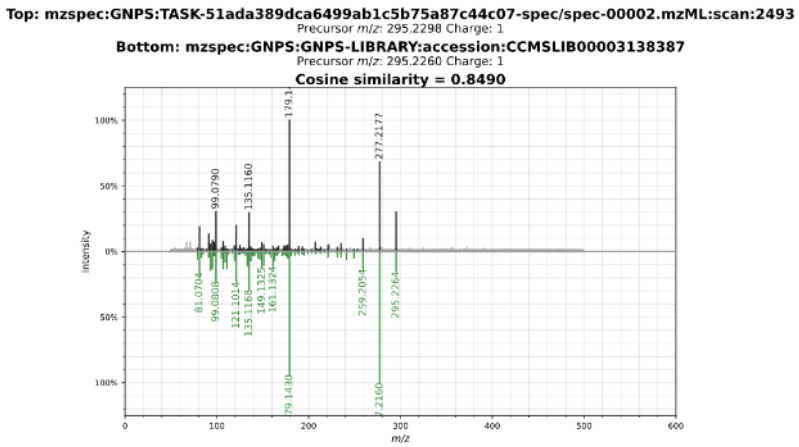
OCTADECATRIENOIC ACID

- natural product found in galeopsis tetrahit, and other organisms



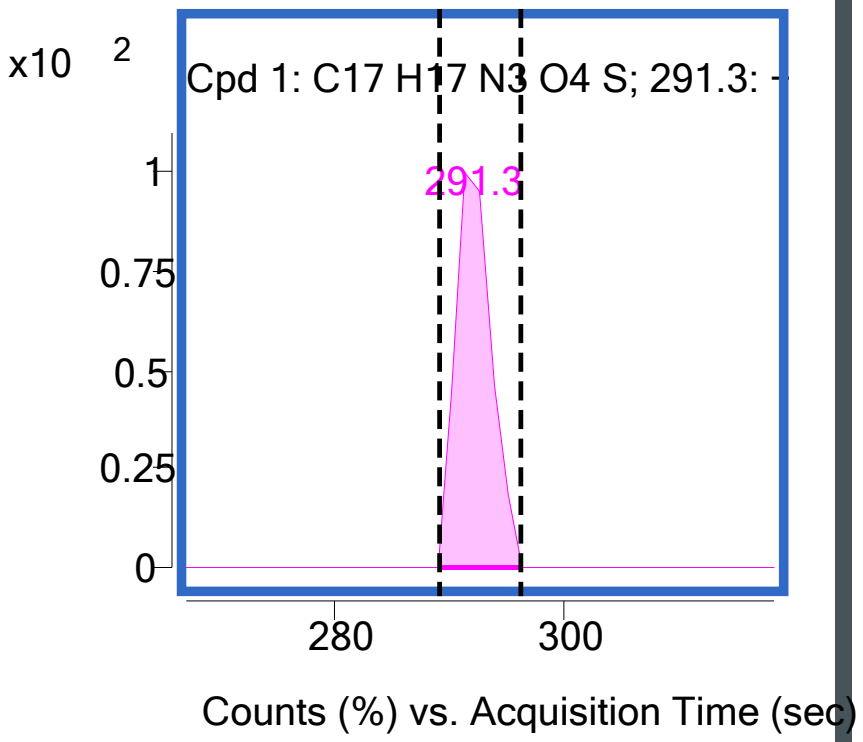


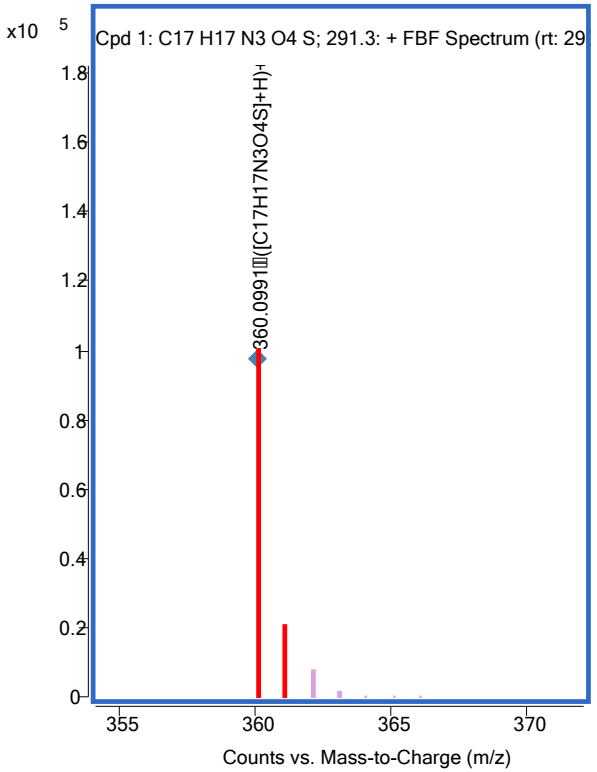


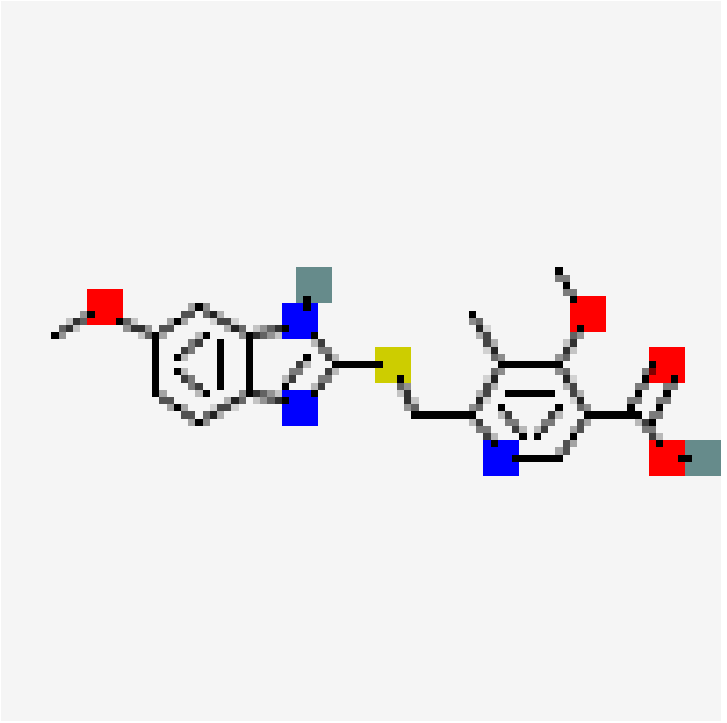


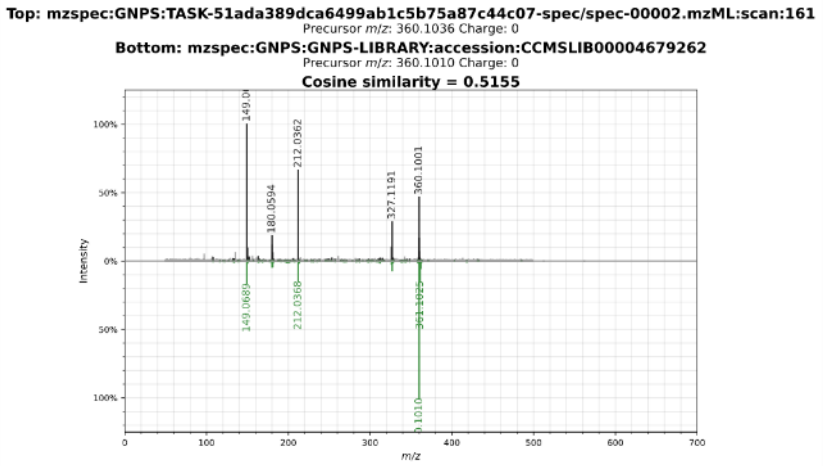
13-KETO-9Z,11E-OCTADECADIENOIC ACID

- Metabolite and mouse metabolite



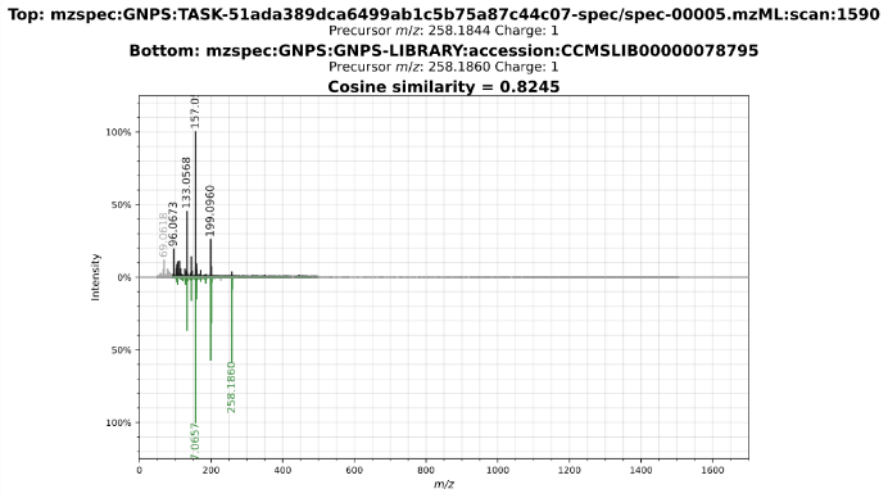


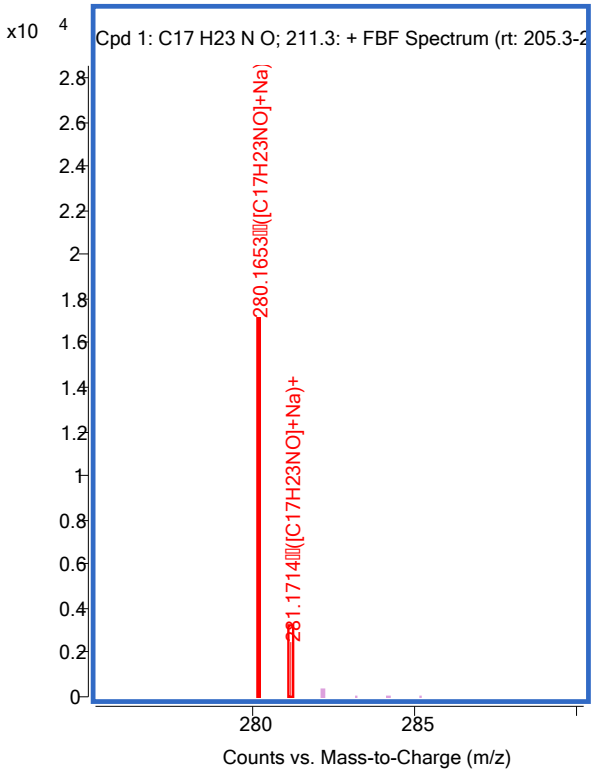


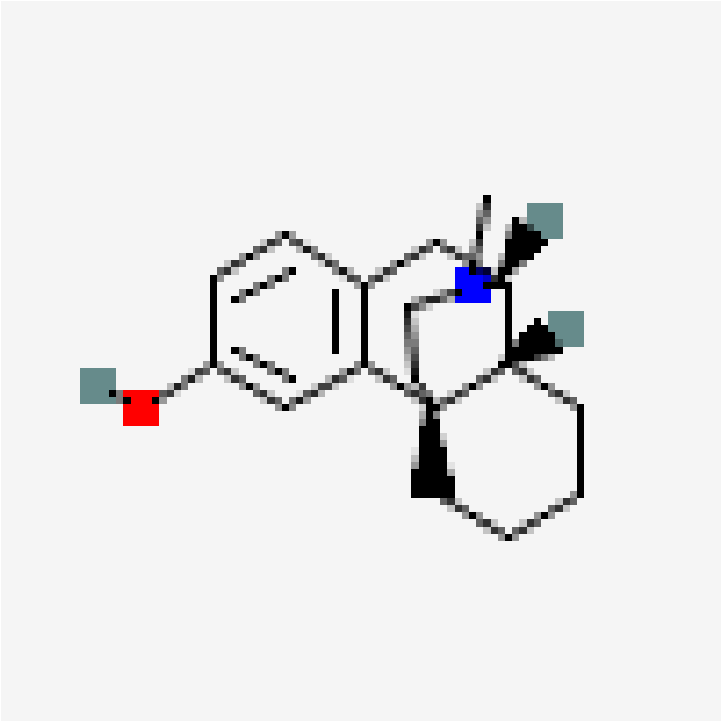


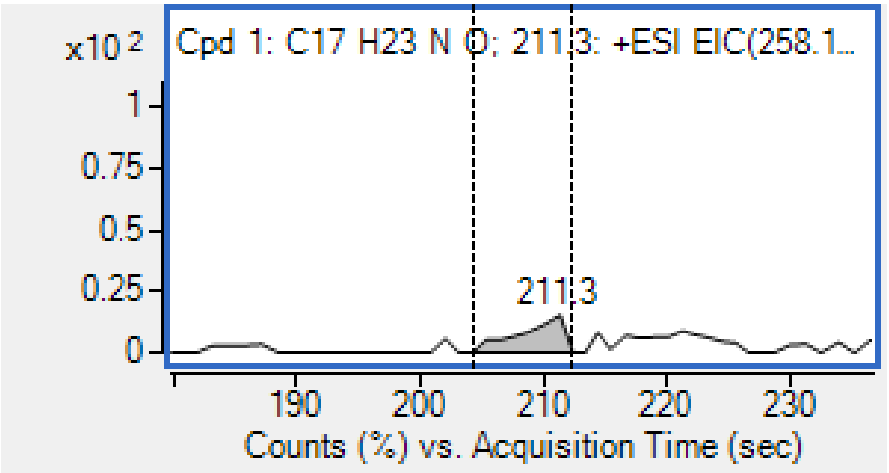
5-CARBOXYLIC ACID

?



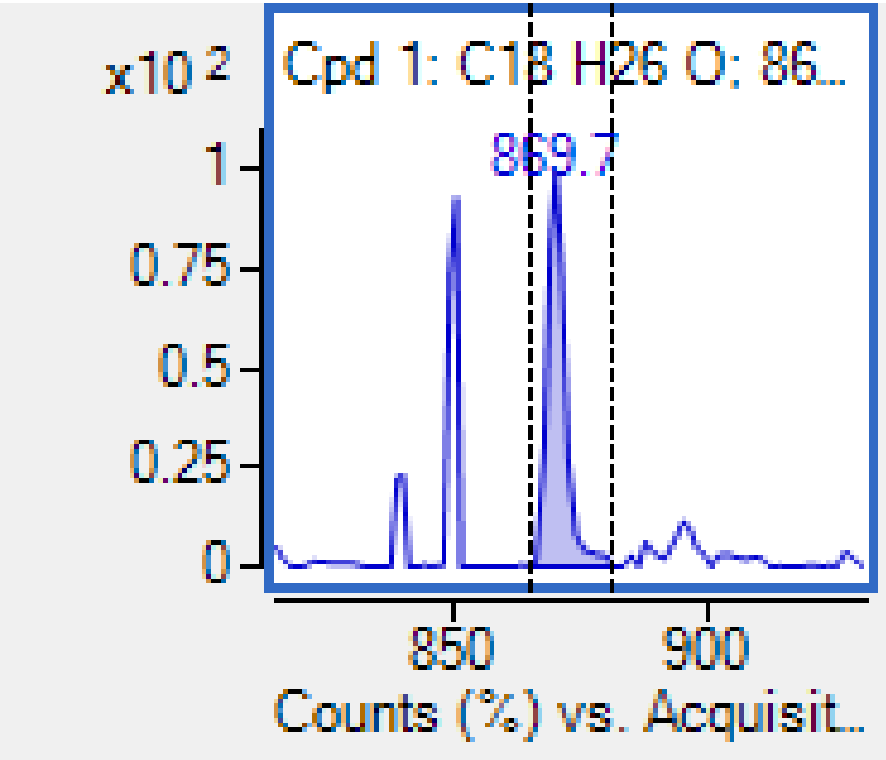


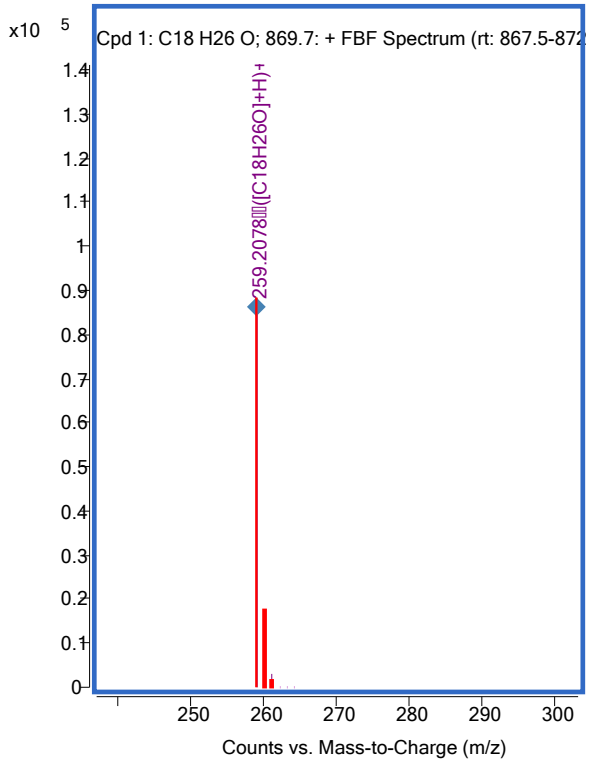


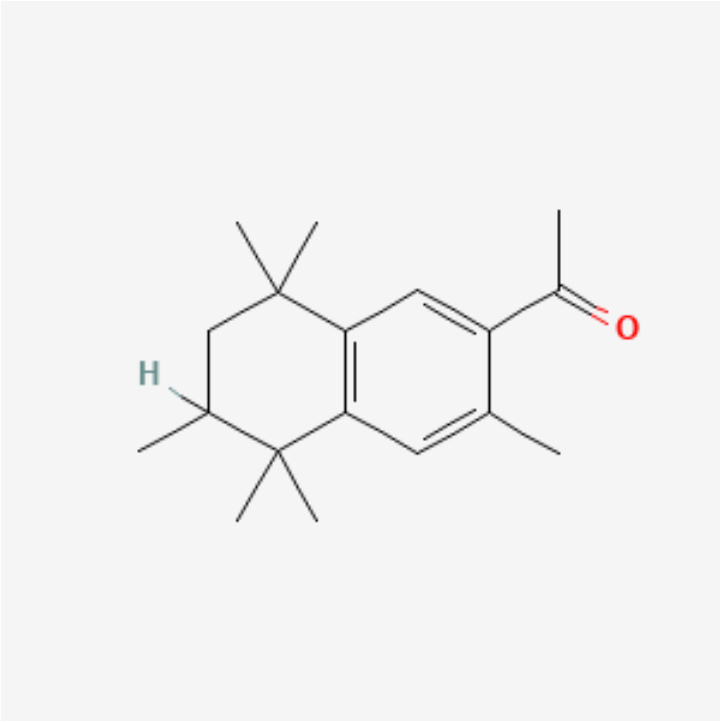


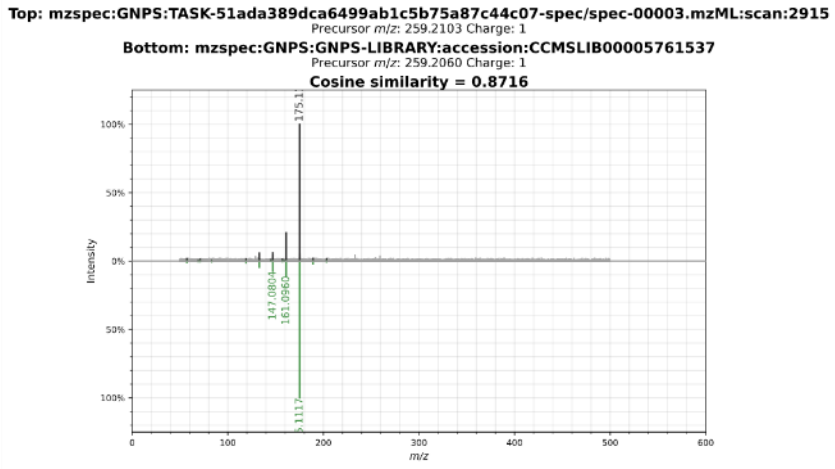
LEVORPHANOL

PAINKILLER, DEA SCHEDULE 2



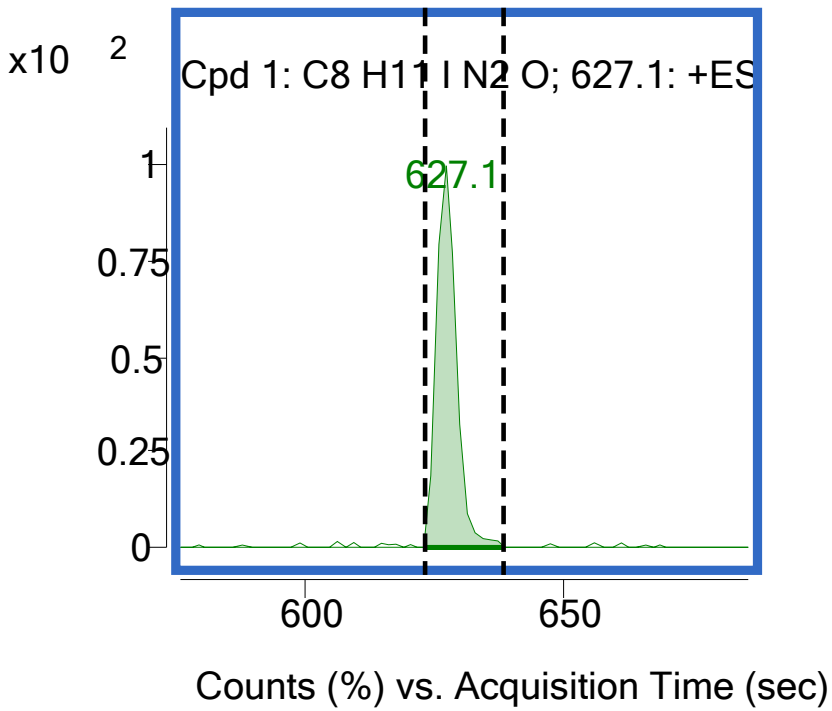


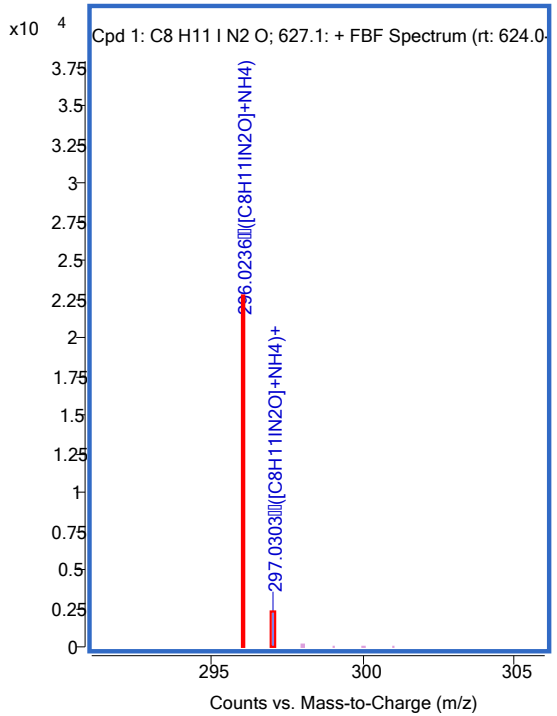


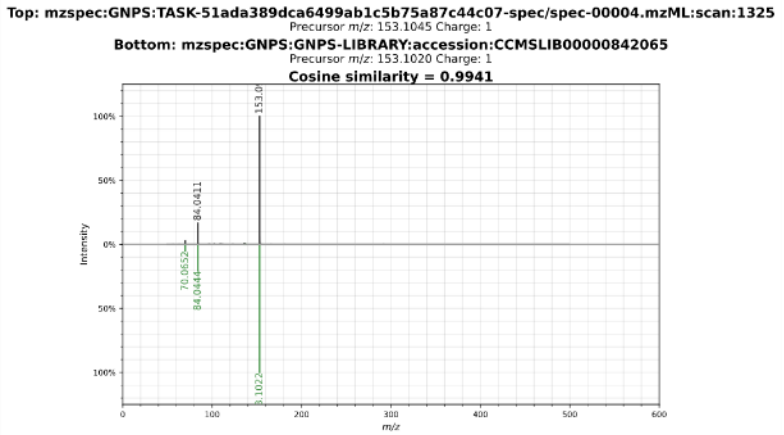


TONALID

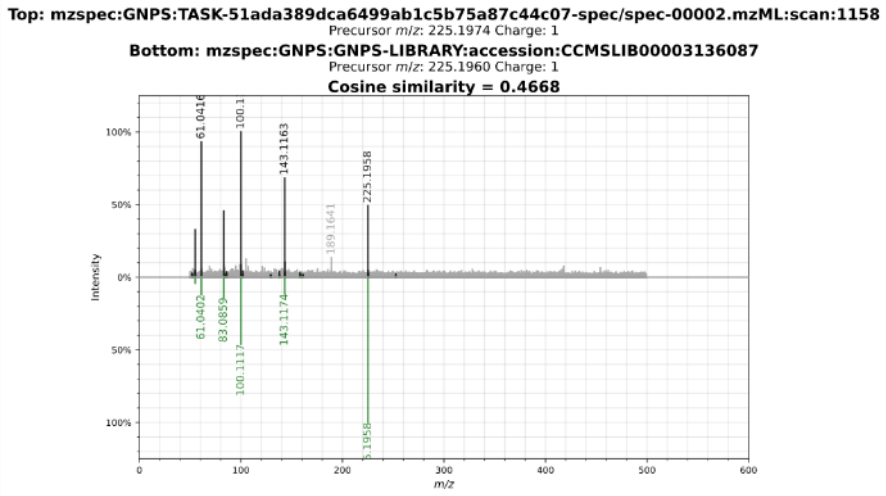
FRAGRANCE IN AIR FRESHNERS AND LAUNDRY DETERGENTS

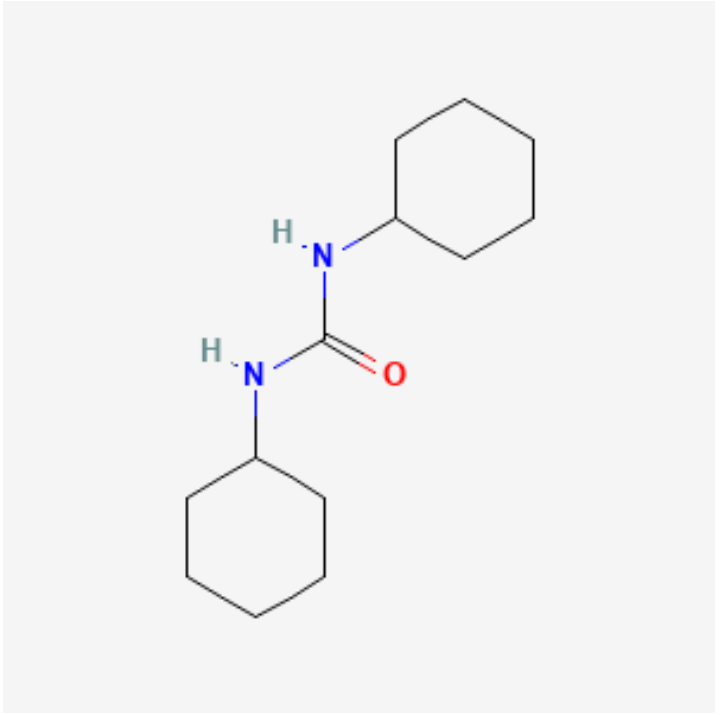


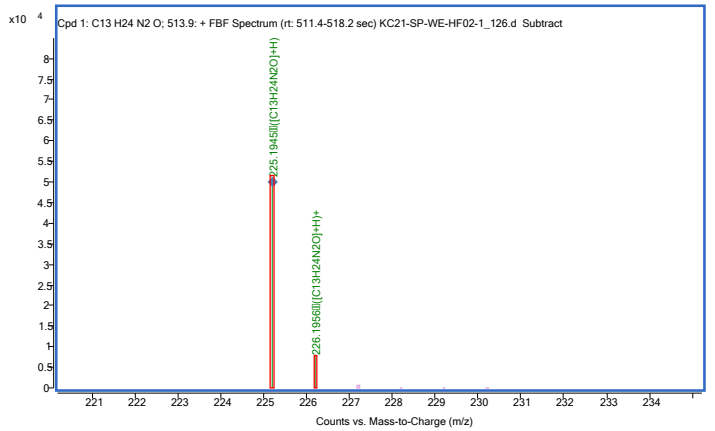


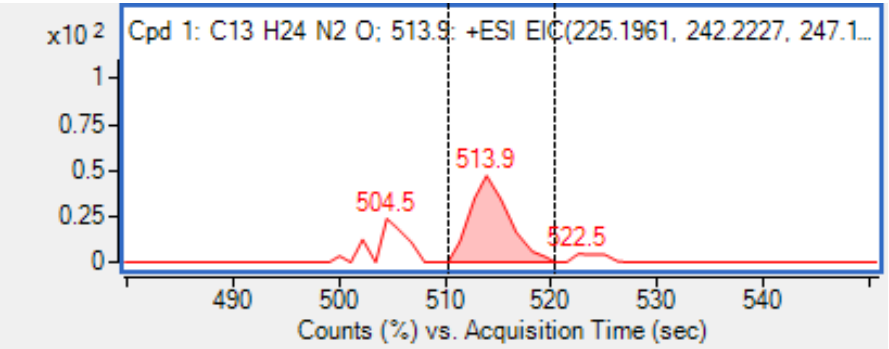


5-IODO-6-METHYL-2-(PROPAN-2-YL)PYRIMIDIN-4(1H)-ONE



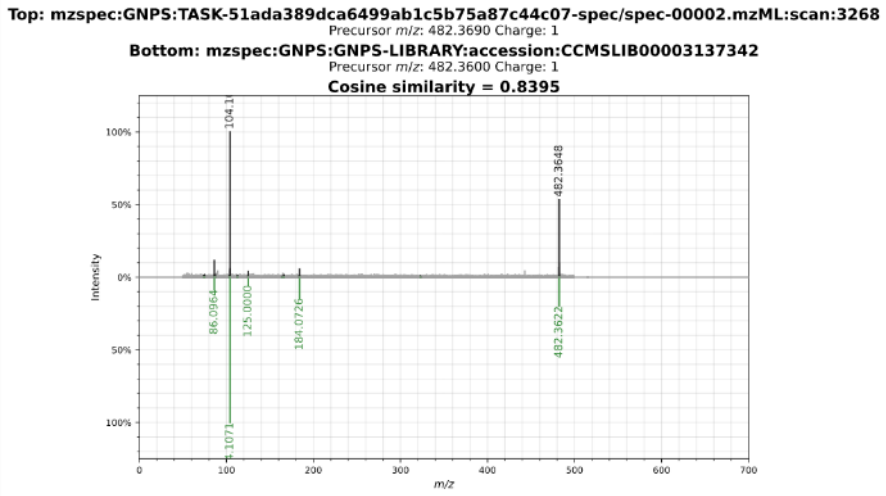


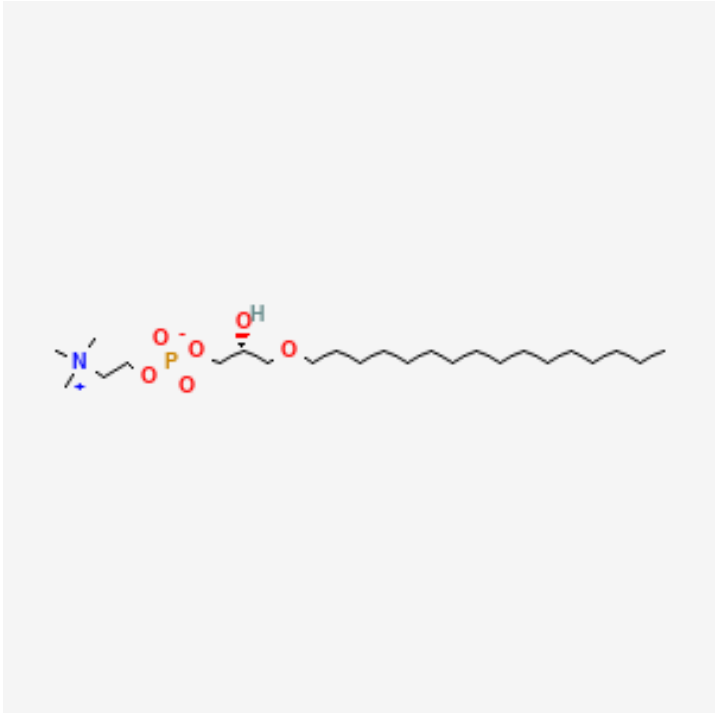


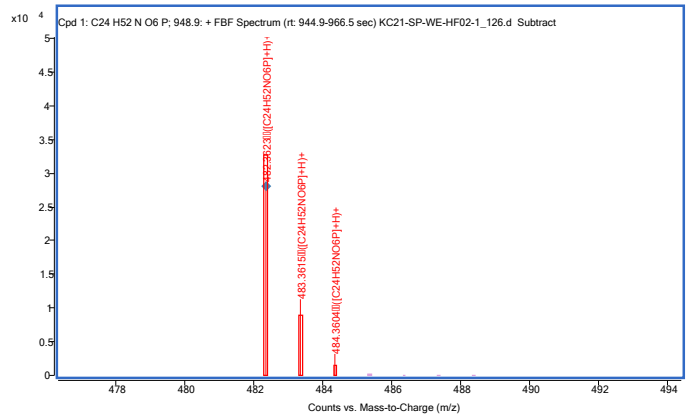


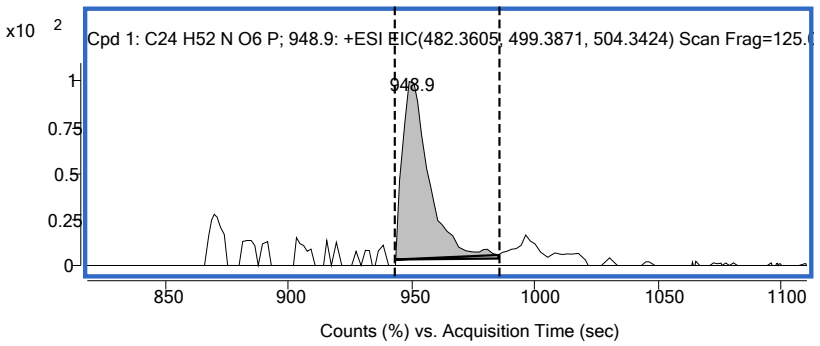
1,3-DICYCLOHEXYLUREA

a urea, a potent soluble epoxide hydrolase (sEH) inihibiors



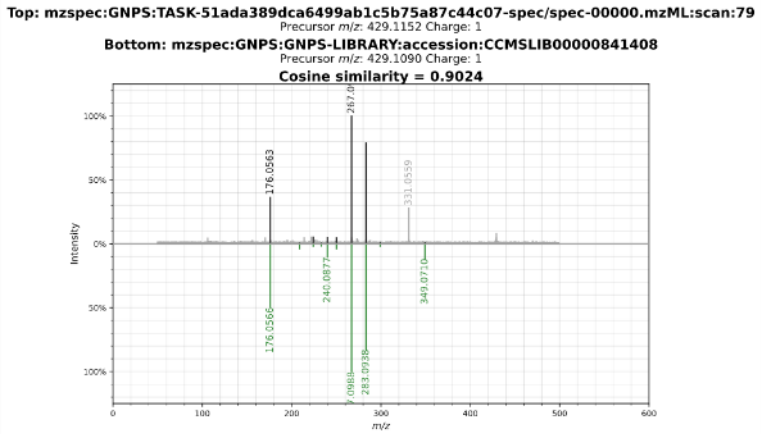






1-O-HEXADECYL-SN-GLYCERO-3-PHOSPHOCHOLINE

Metabolite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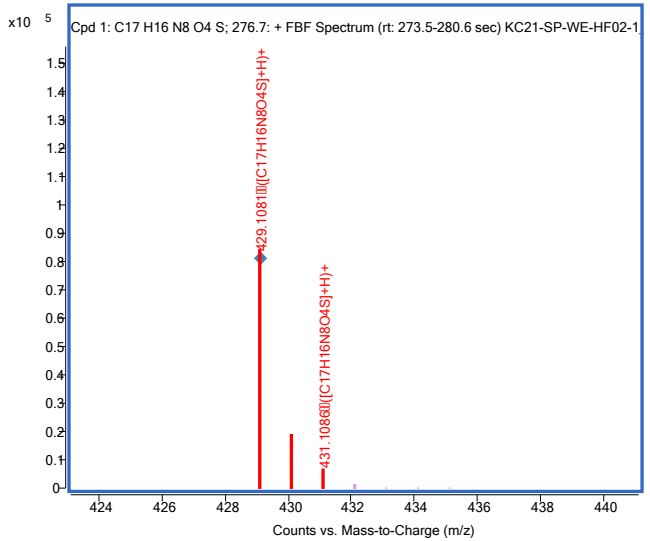
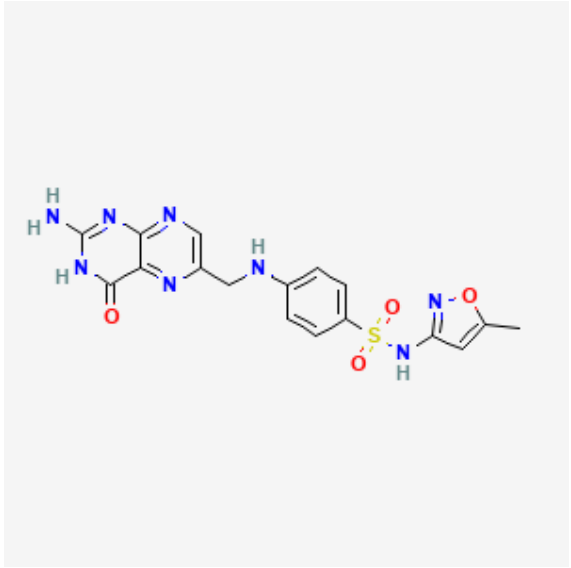
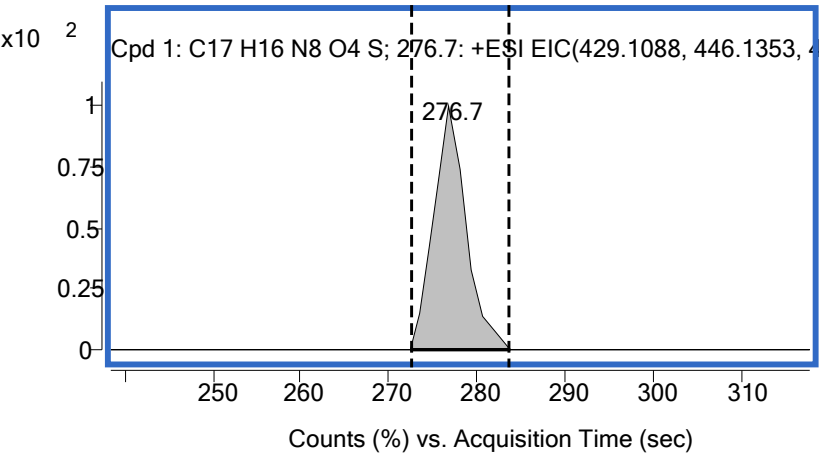
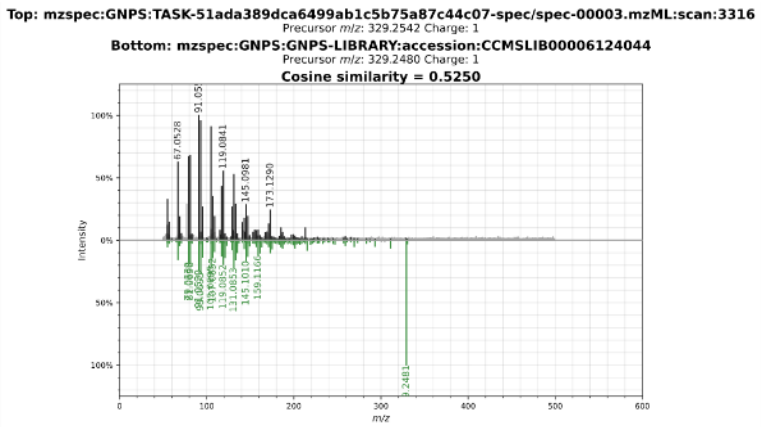
Precursor m/z: 429.1152 Charge: 1
Bottom: mzspec:GNPS:GNPS-LIBRARY:accession:CCMSLIB00000841408
Precursor m/z: 429.1090 Charge: 1
Cosine similarity = 0.9024
Intensity
m/z

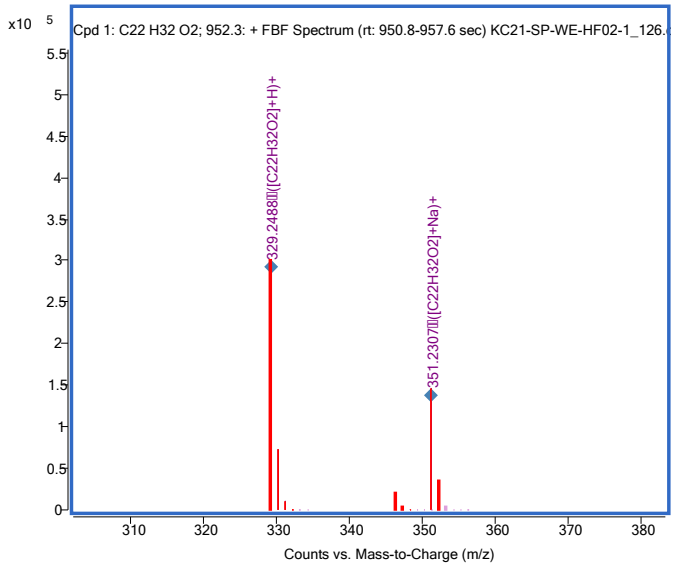

x10 5
Cpd 1: C17 H16 N8 O4 S; 276.7: + FBF Spectrum (rt: 273.5-280.6 sec) KC21-SP-WE-HF02-1
[M+H]+
[C17H16N8O4S]+H)+
[C17H16N8O4S]+H)+
Counts vs. Mass-to-Charge (m/z)

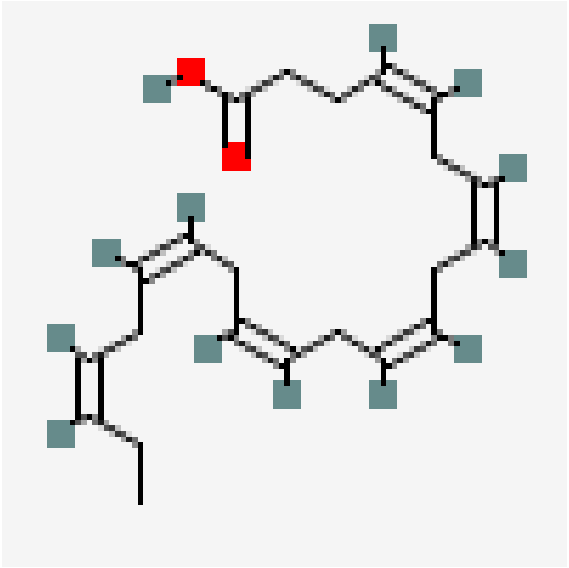


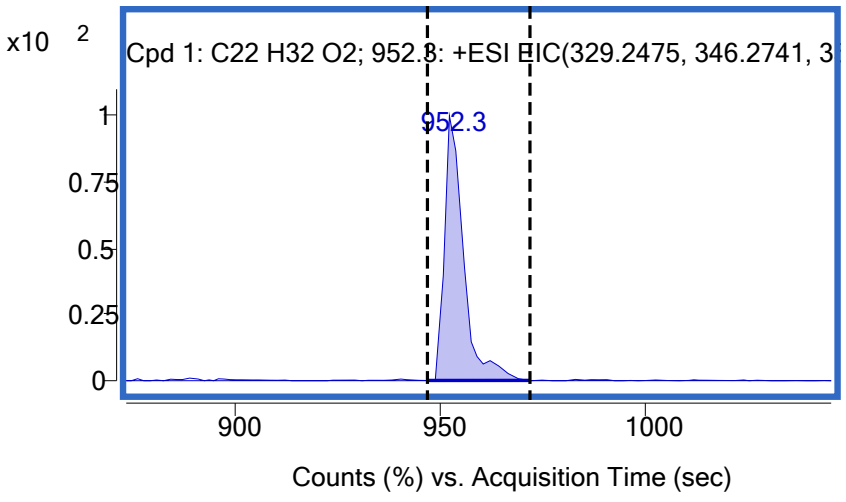

x10 2
Cpd 1: C17 H16 N8 O4 S; 276.7: +ESI EIC(429.1088, 446.1353,
276.7
Counts (%) vs. Acquisition Time (sec)

SMZ-PT

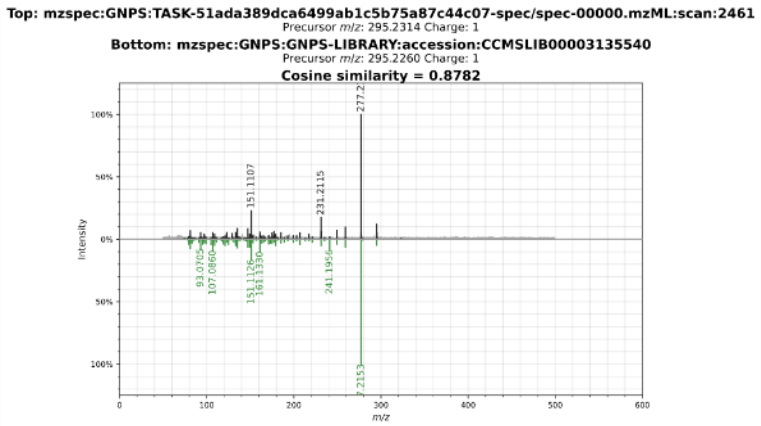


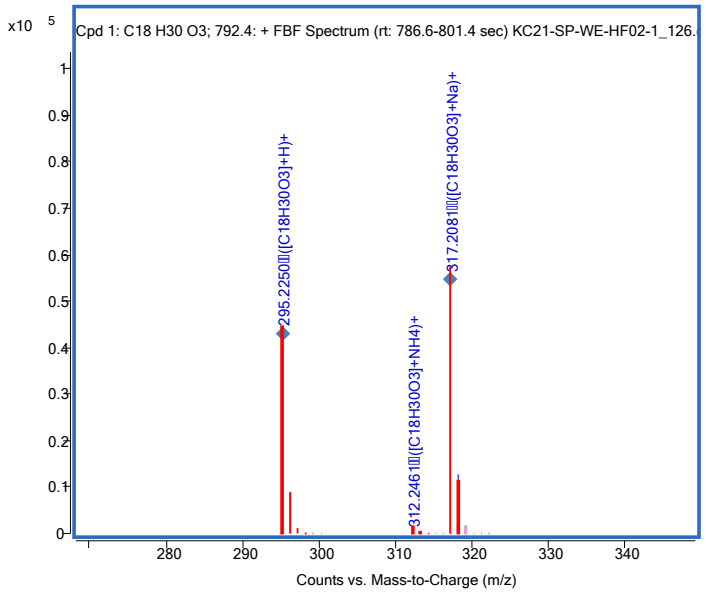


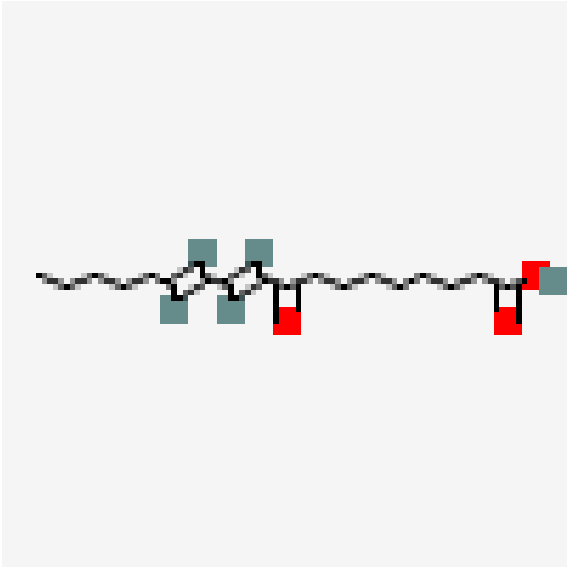


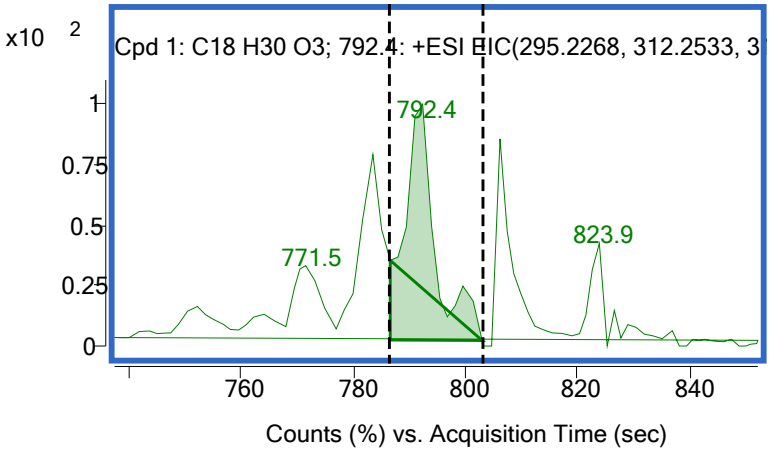


- Metabolite (human, mouse, and more)



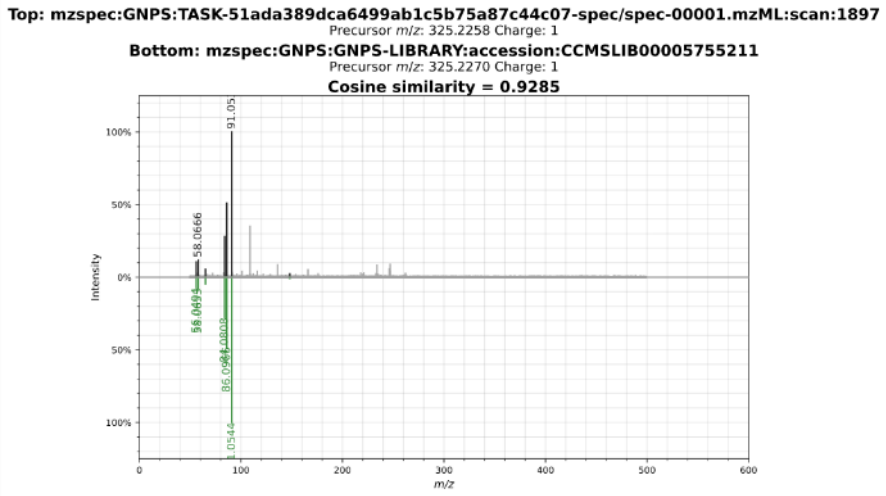


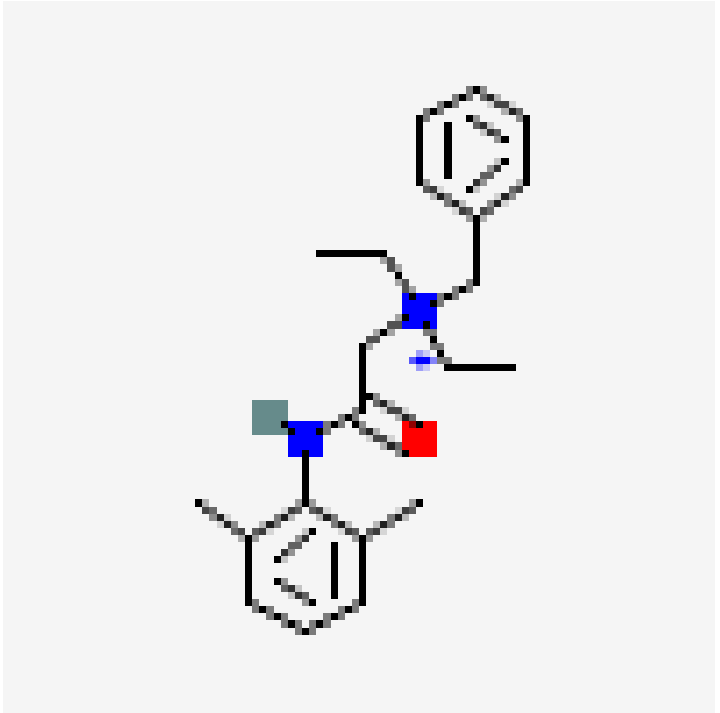


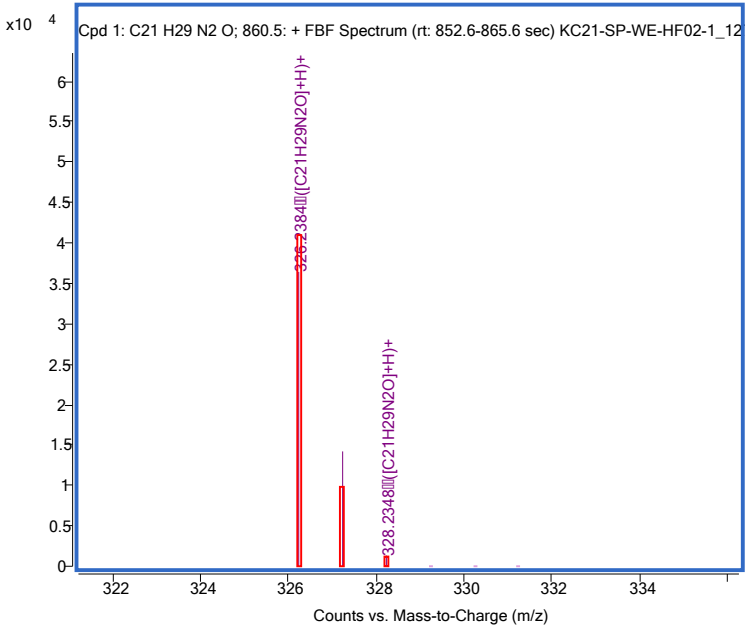


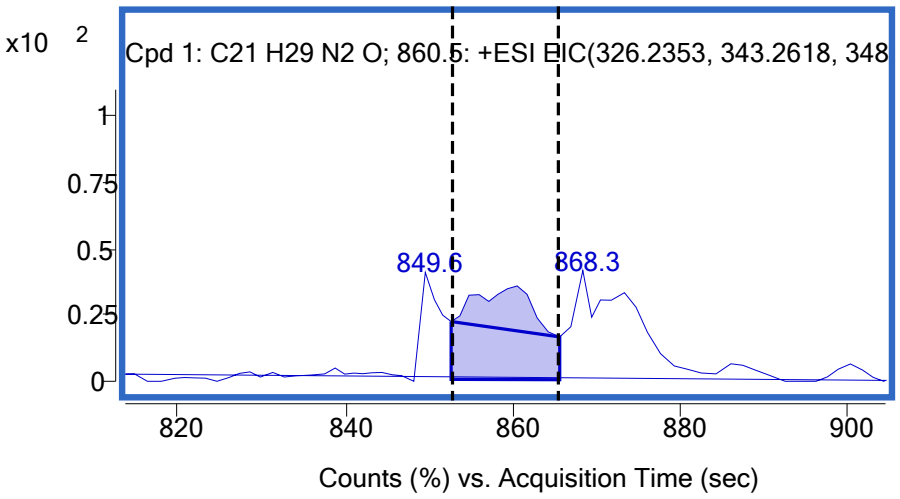
9-OXO-10

- Natural product



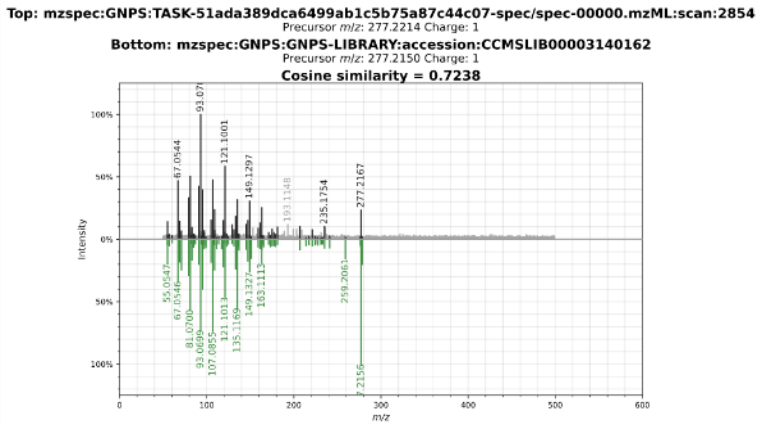


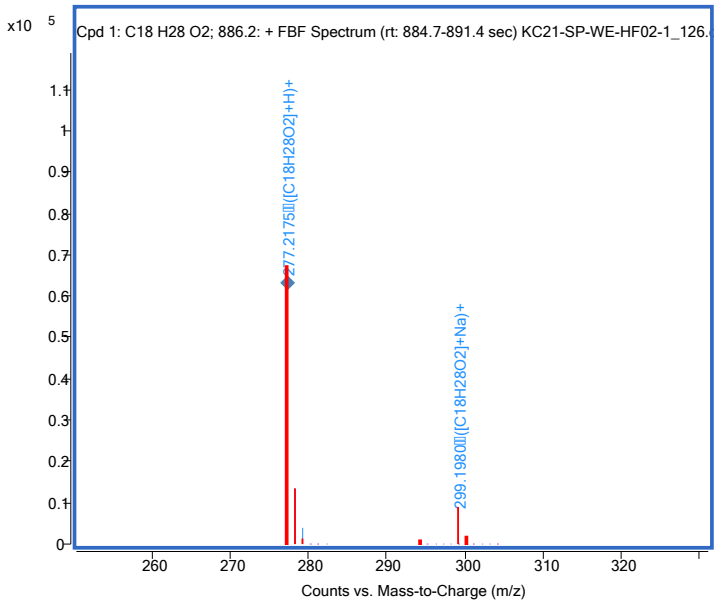


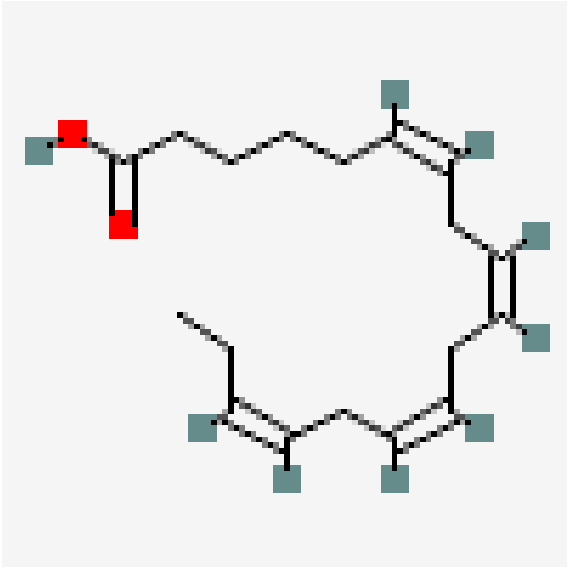


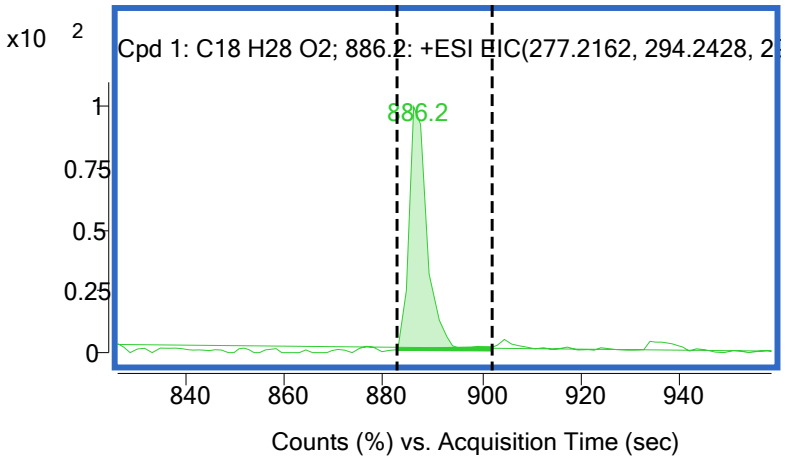
DENATONIUM

AMINO ACID AMIDE



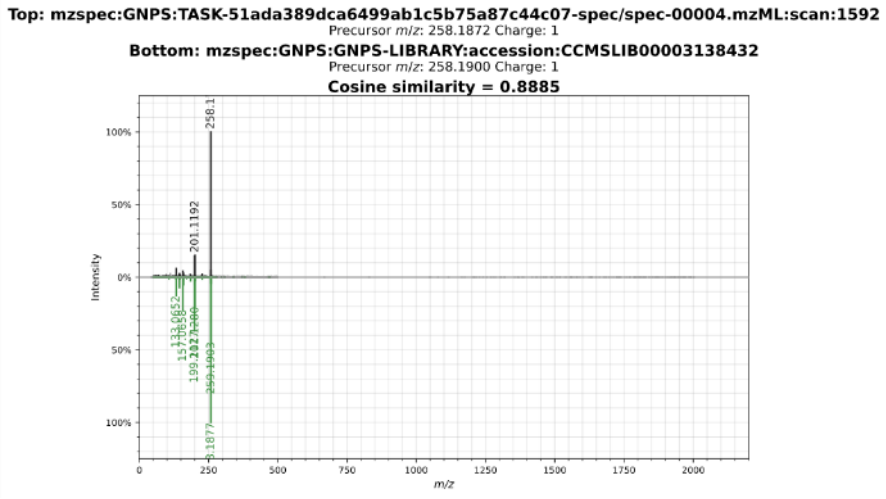


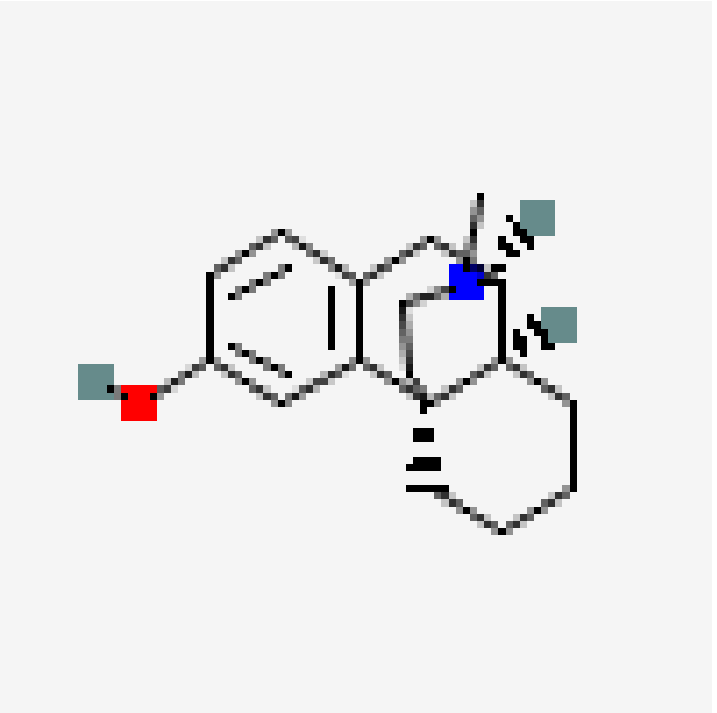


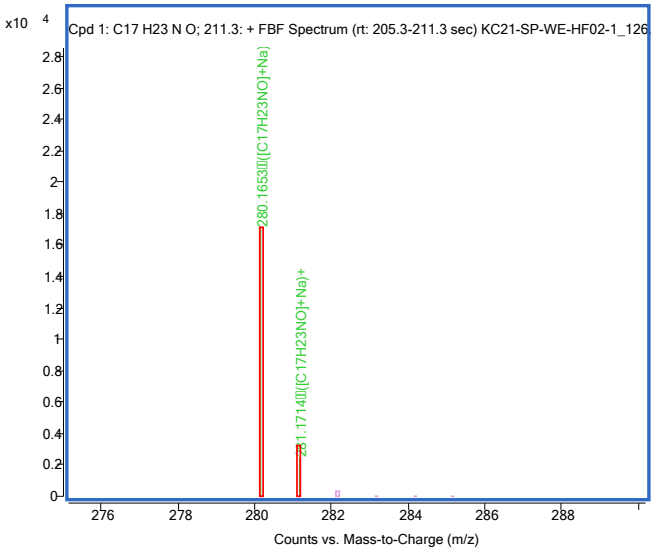


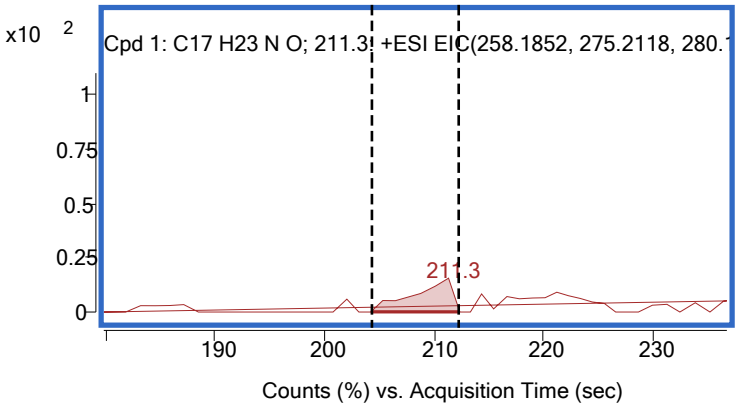
STEARIDONIC ACID

- Natural product- fatty acid



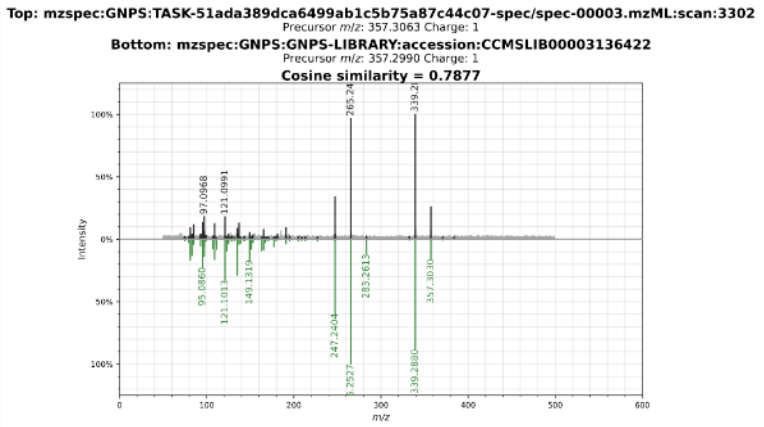


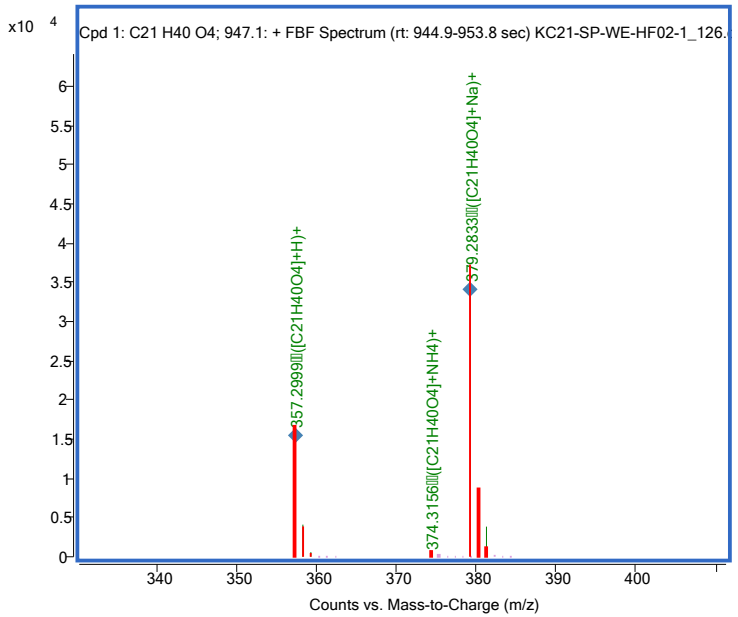


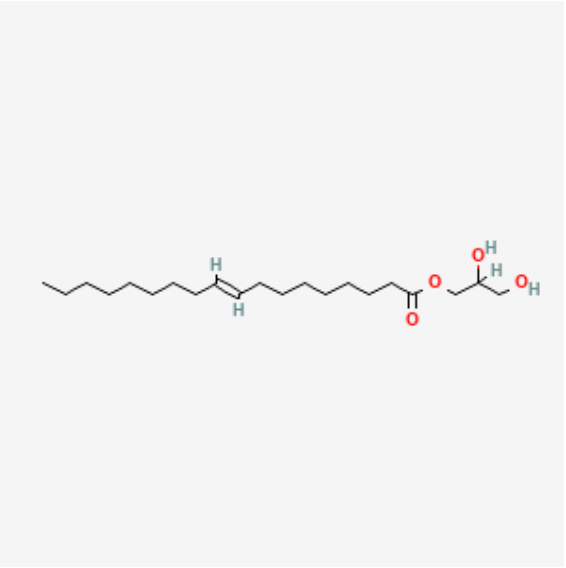


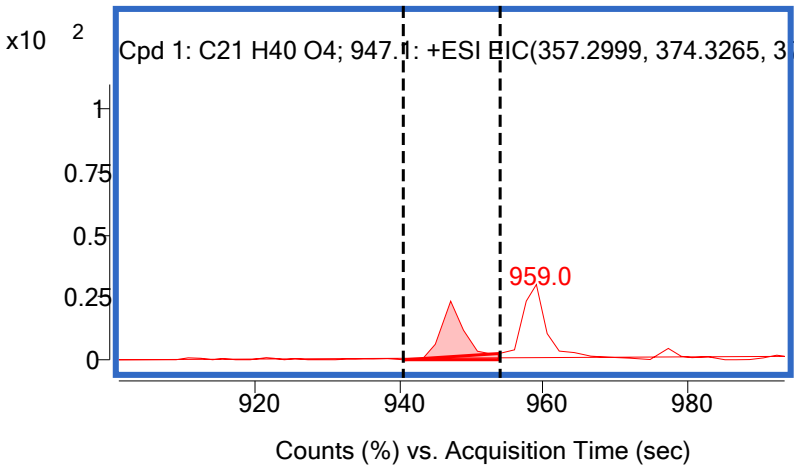
DEXTRORPHAN

NEUROPROTECTIVE AGENT, FORM OF LEVORPHANOL AND METABOLITE OF DEXTROMETHORPHAN



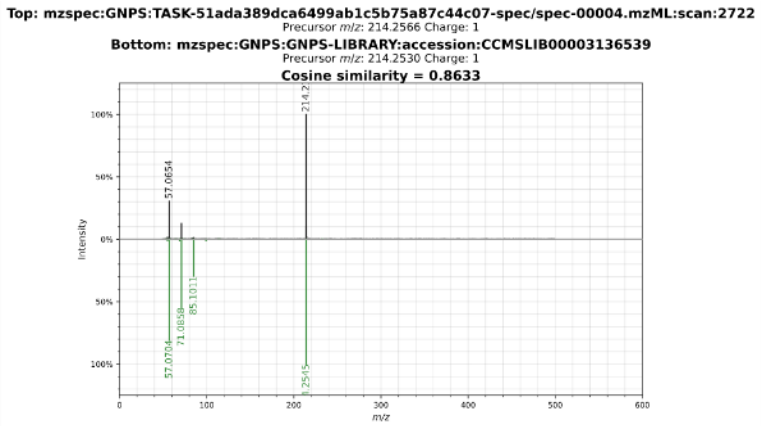


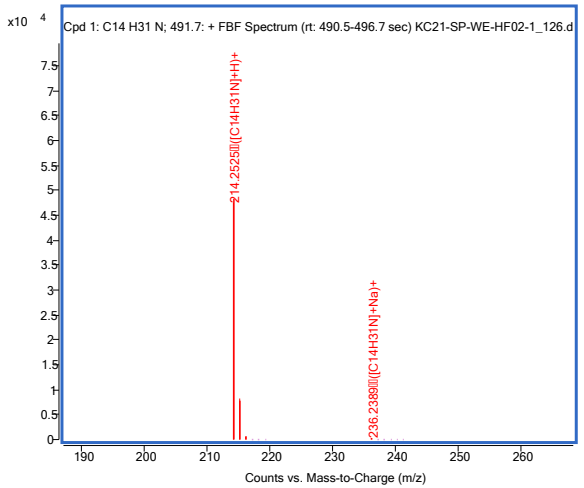


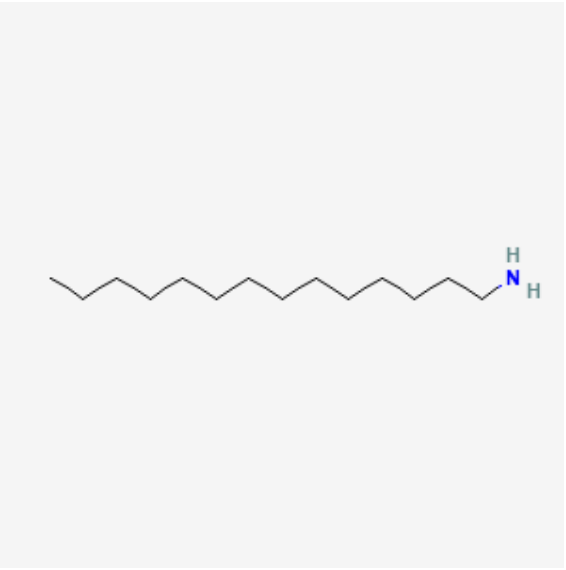


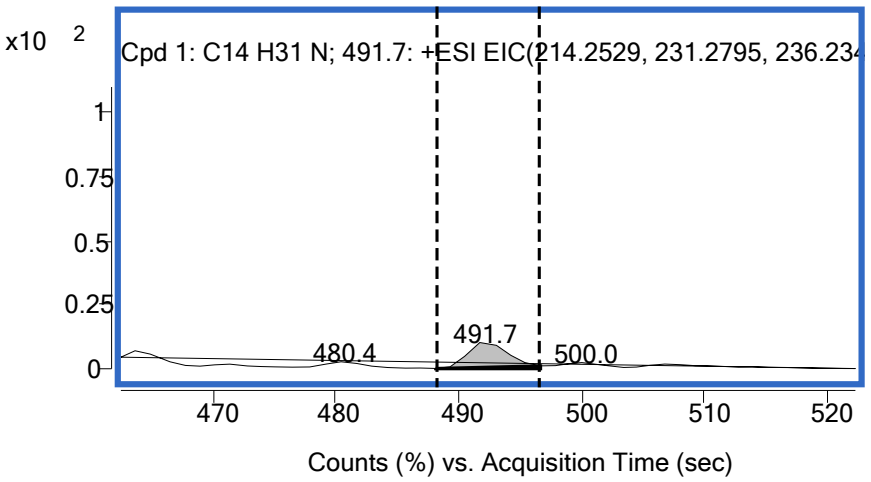
MONOELAIDIN

- surfact-active agent in foods, pharmaceuticals, and cosmetics



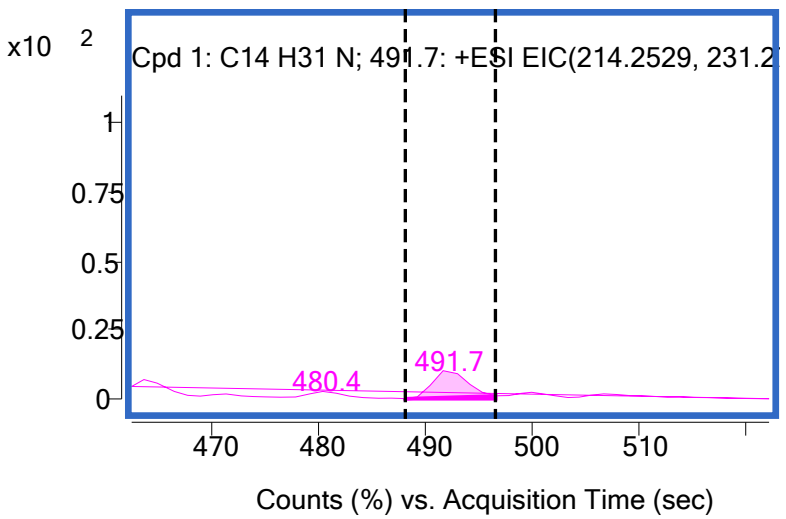


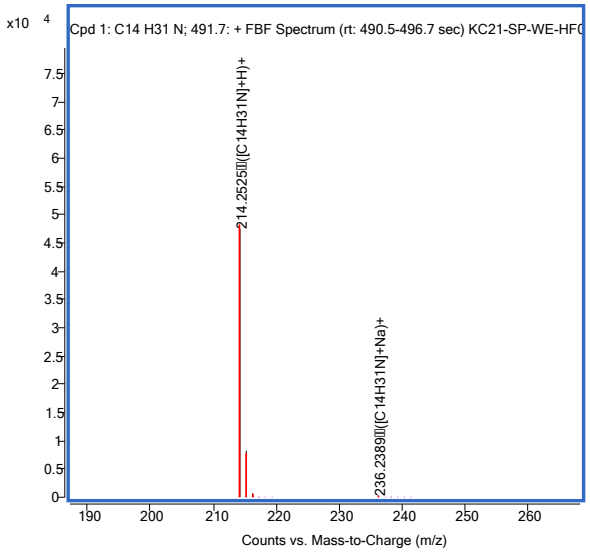


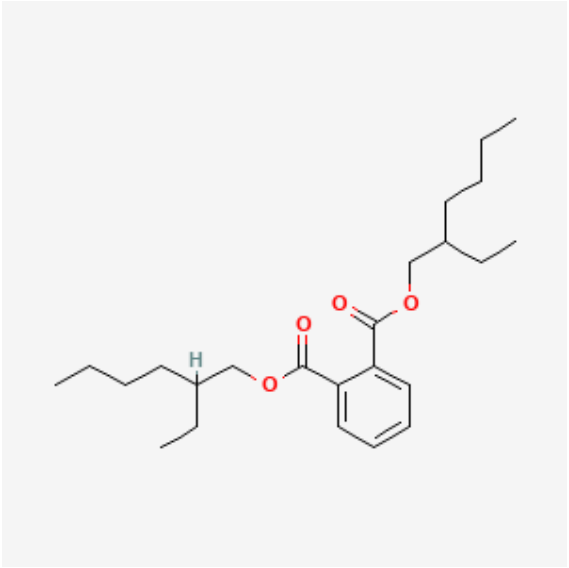


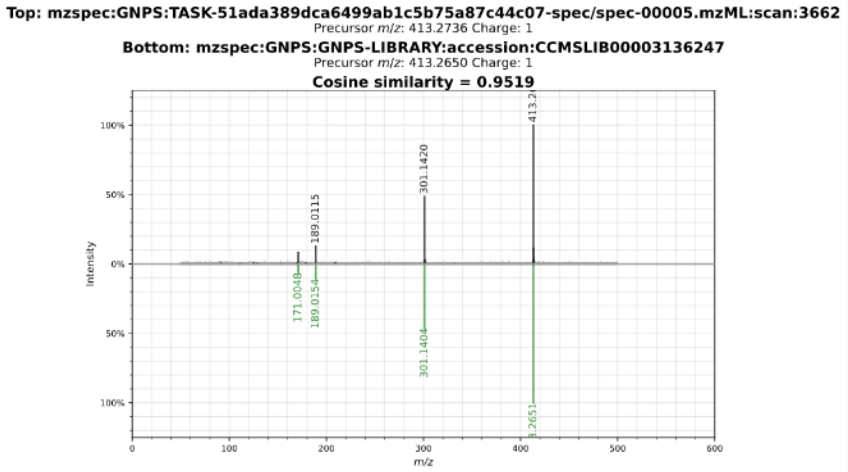
TETRADECYLAMINE

- surface active agent, asphalt paving, roofing, and coating



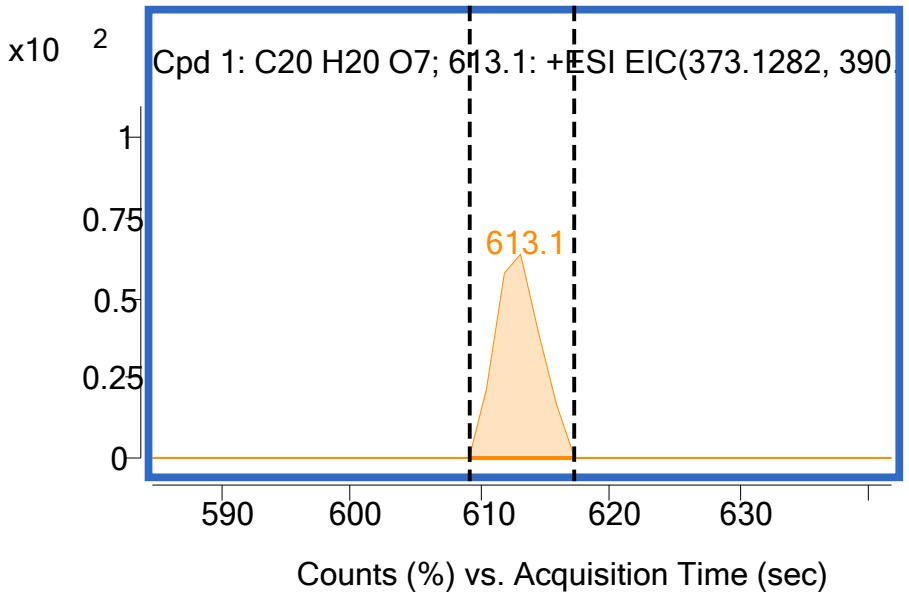


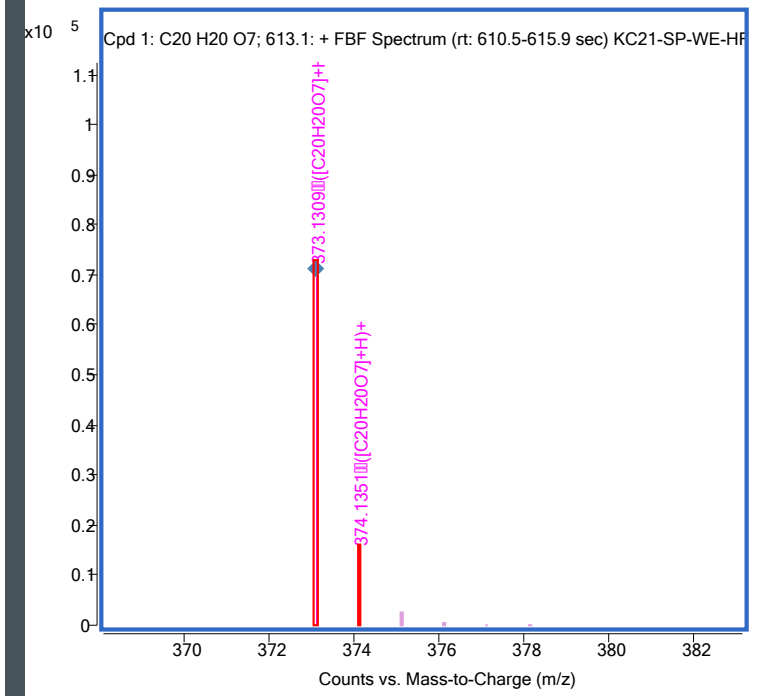


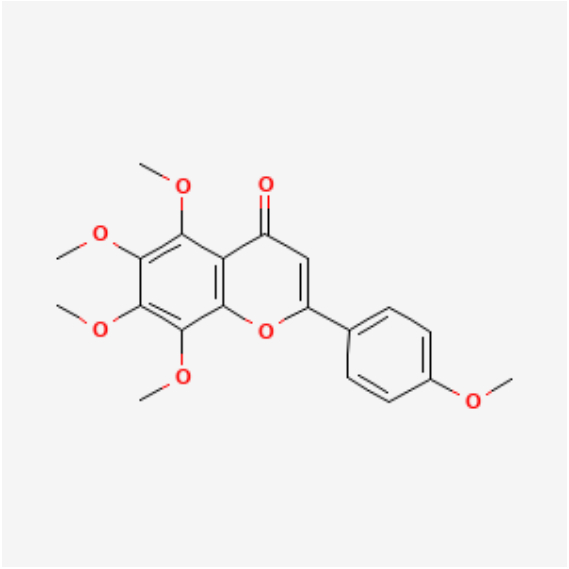


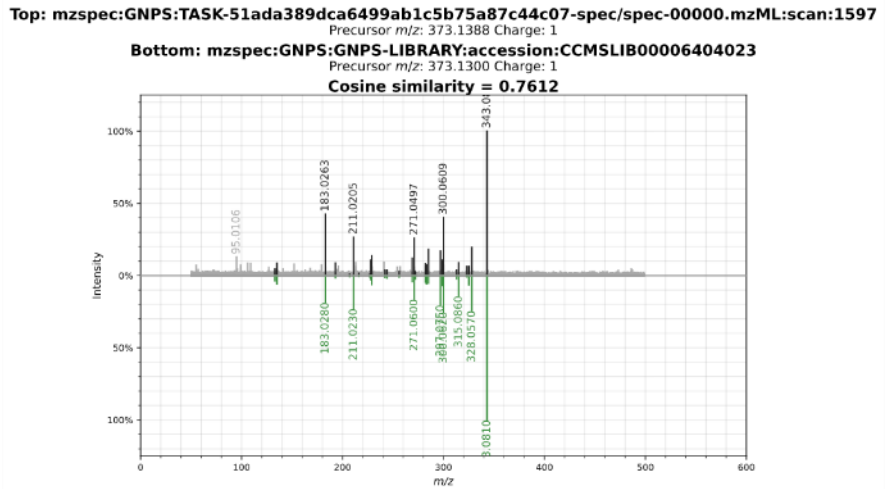
BIS(2-ETHYLHEXYL) PHTHALATE

- surface active agent, asphalt paving, roofing, and coating



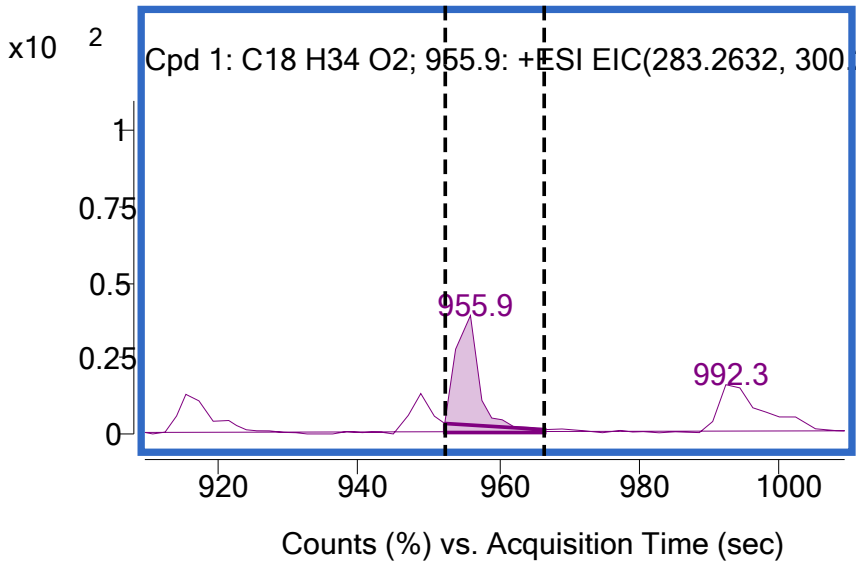


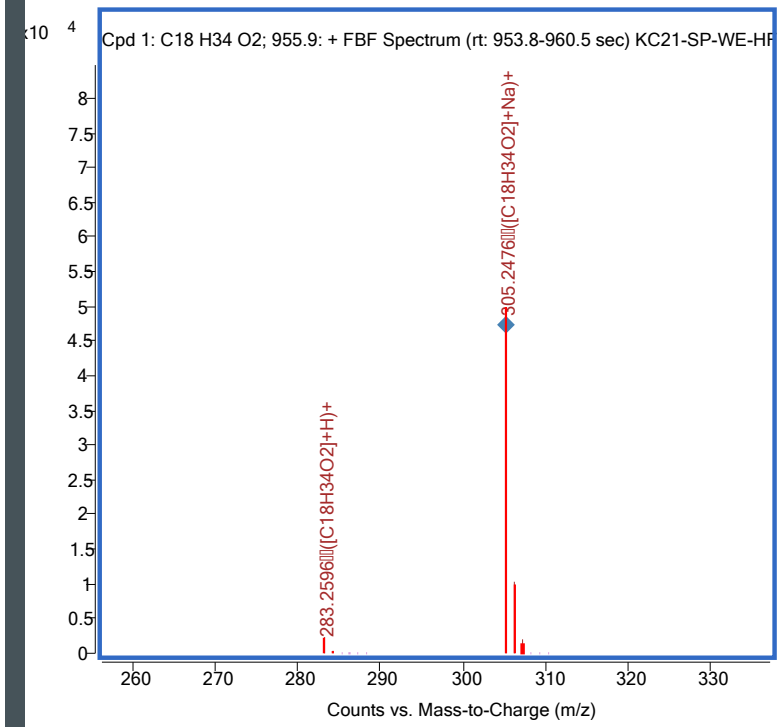


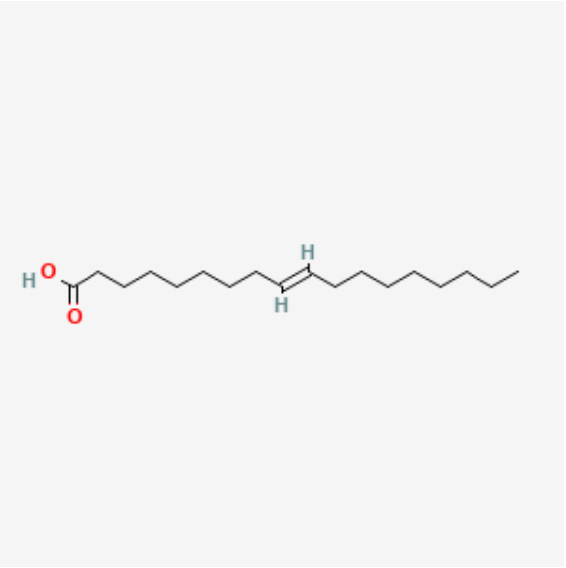


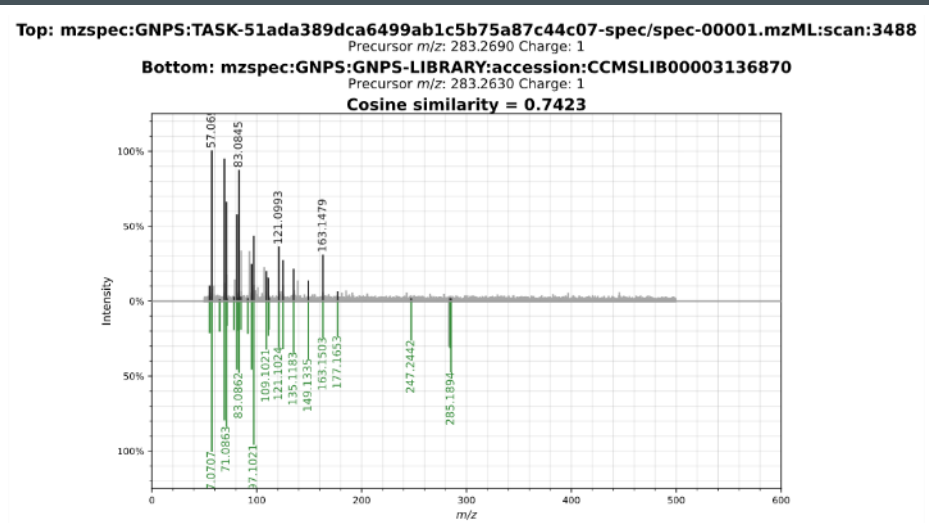
TANGERITIN

- antineoplastic agent and plant metabolite



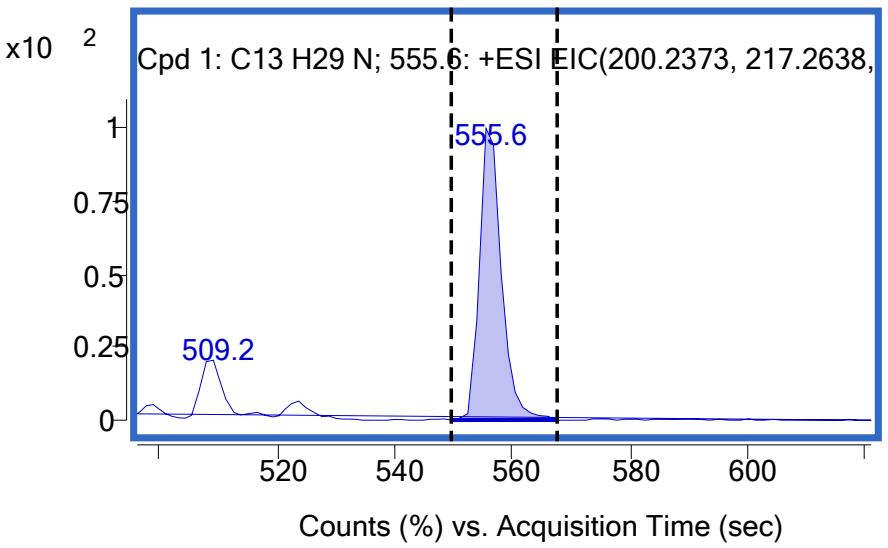


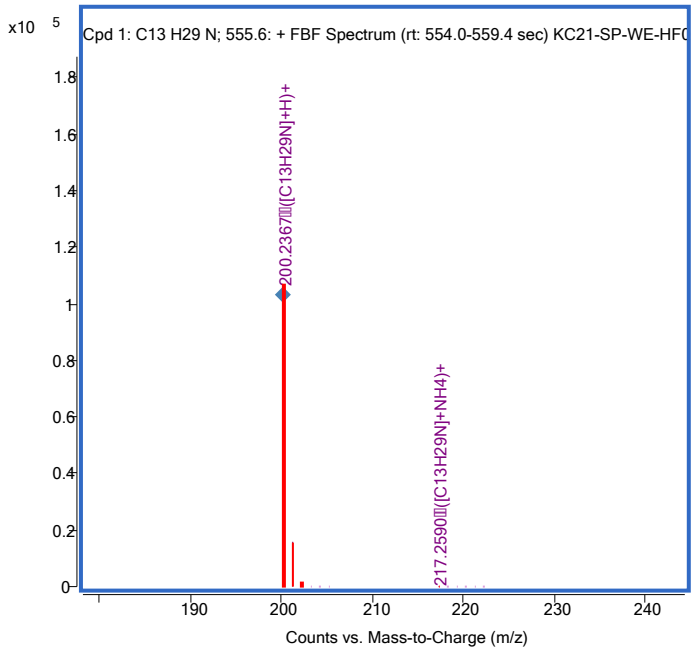


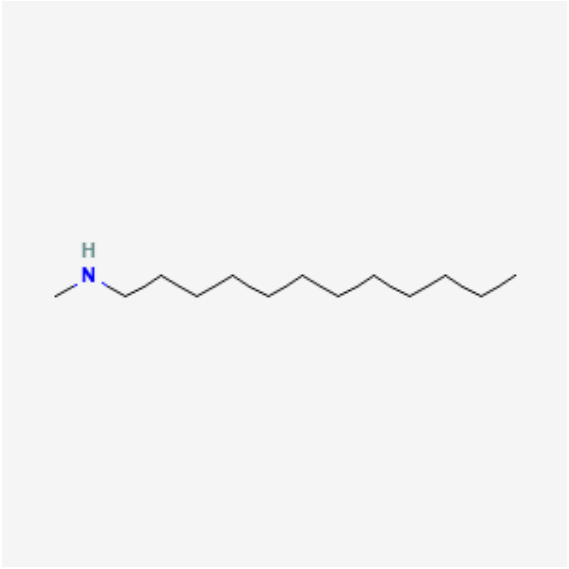


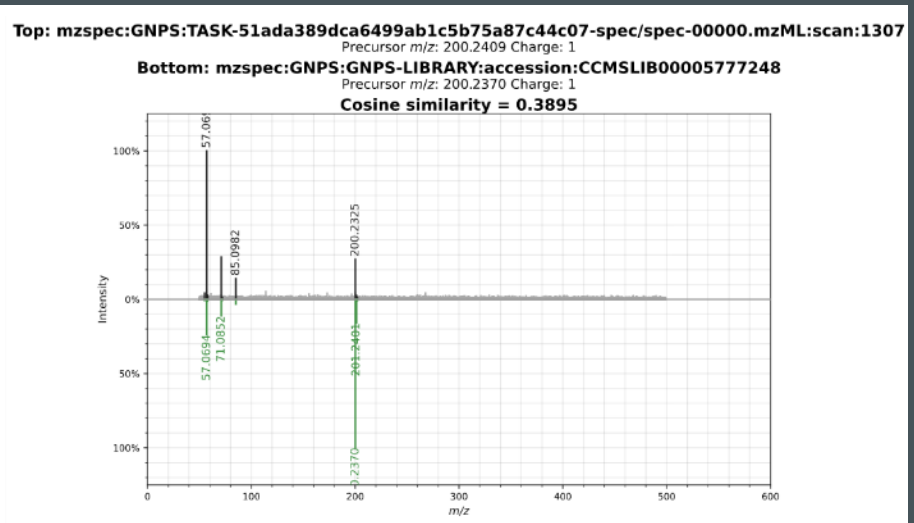
ELAIDIC ACID

- arts and crafts supplies- devices containing liquid or gel ink

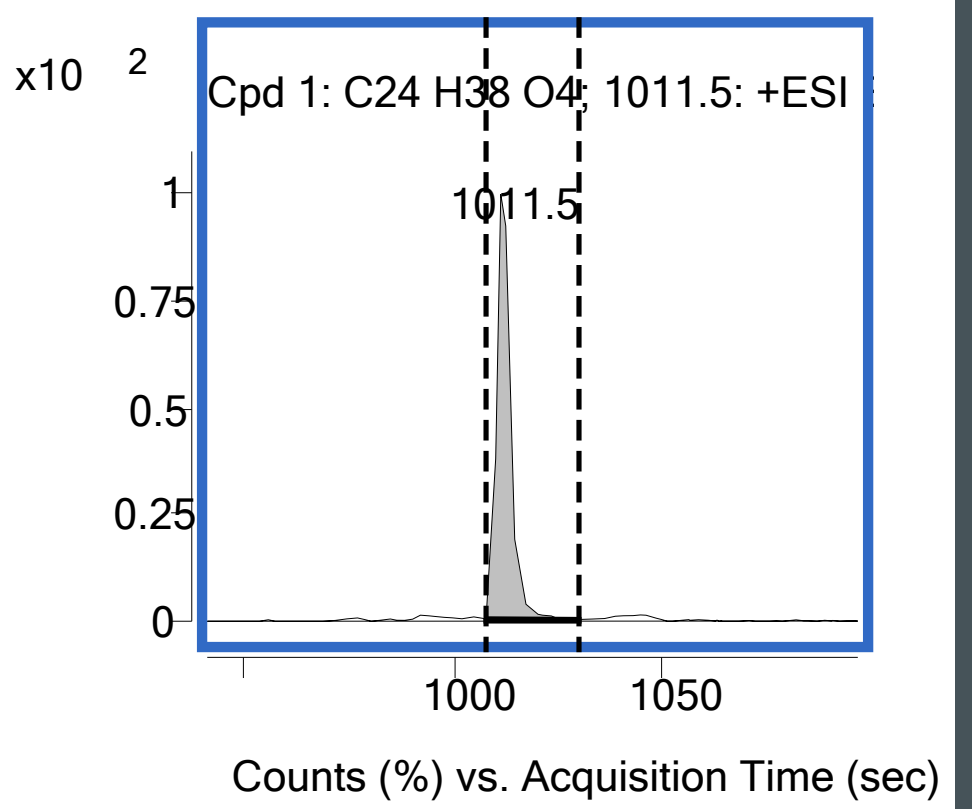


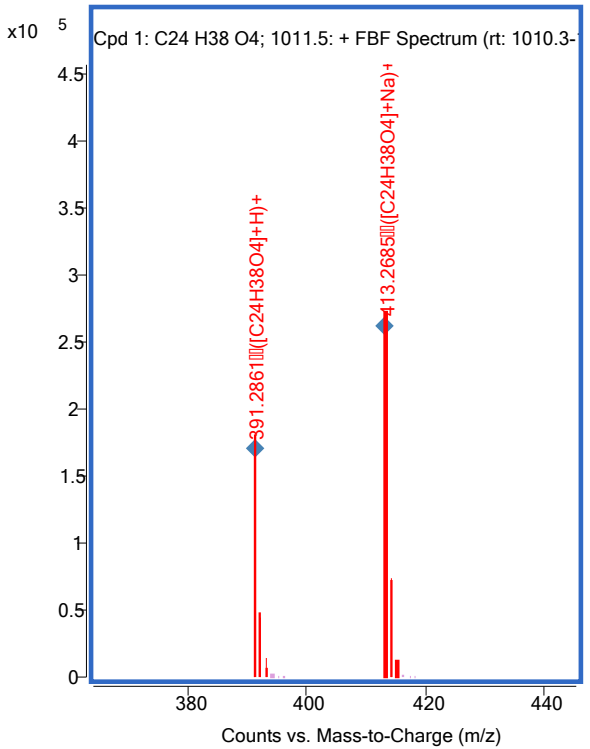


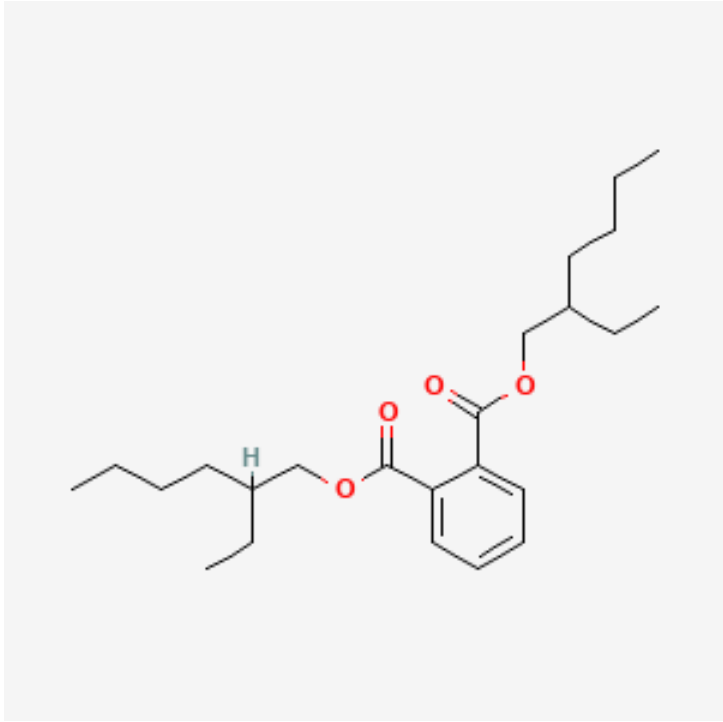


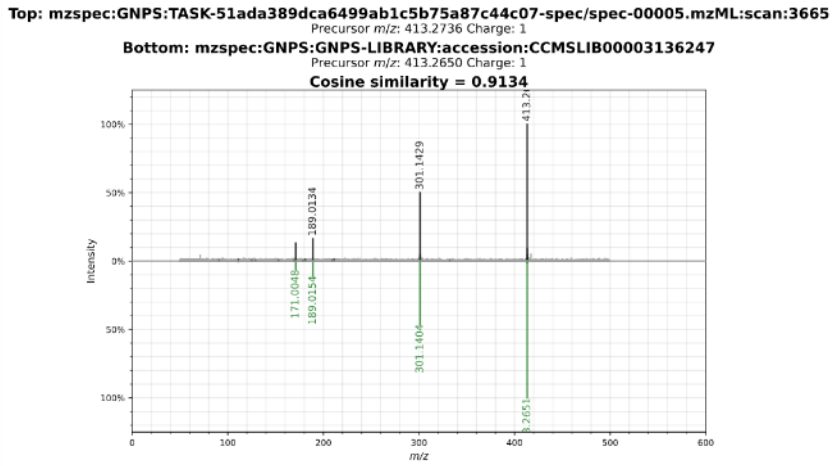


N-METHYLDODECYLAMINE



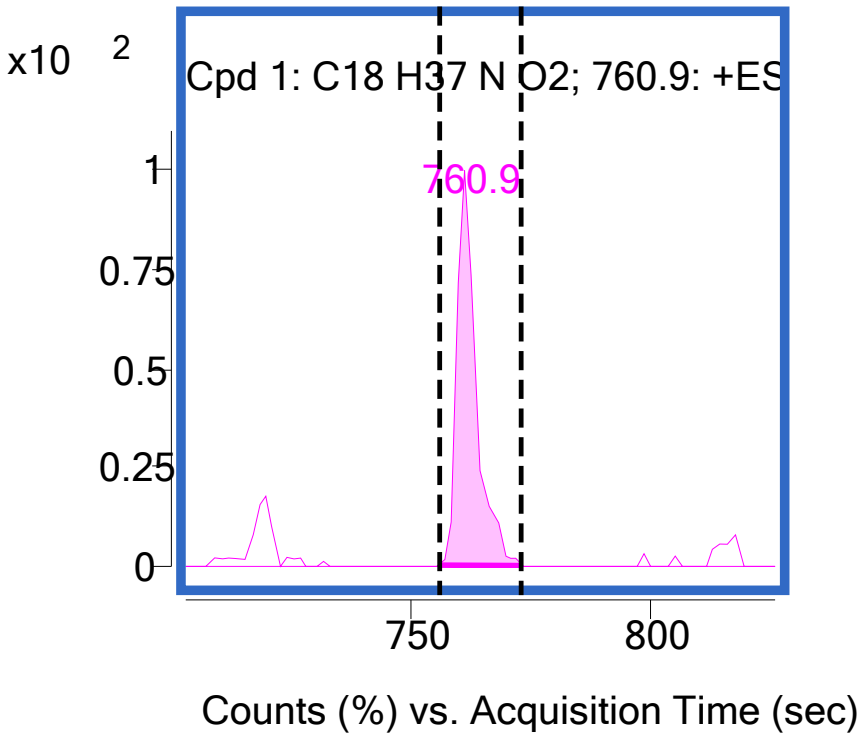


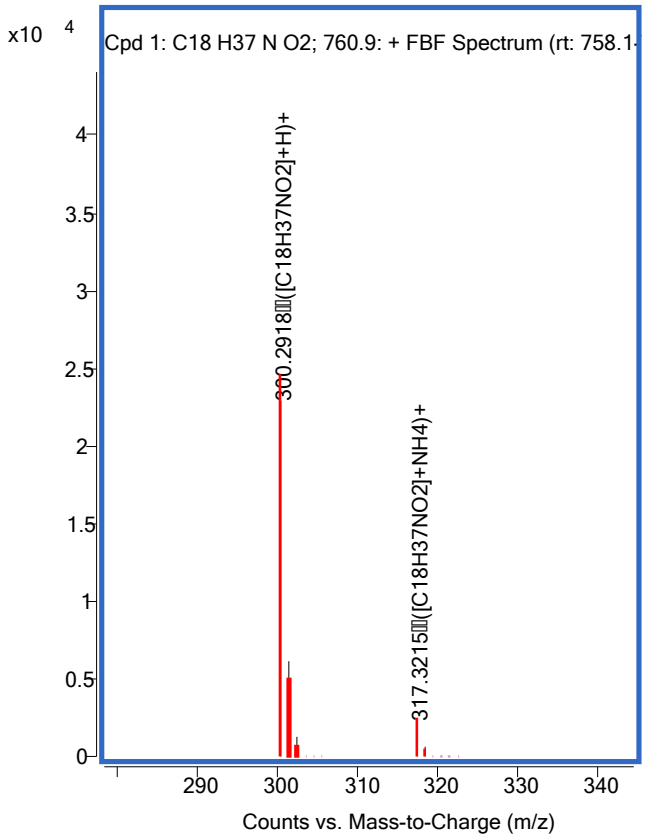


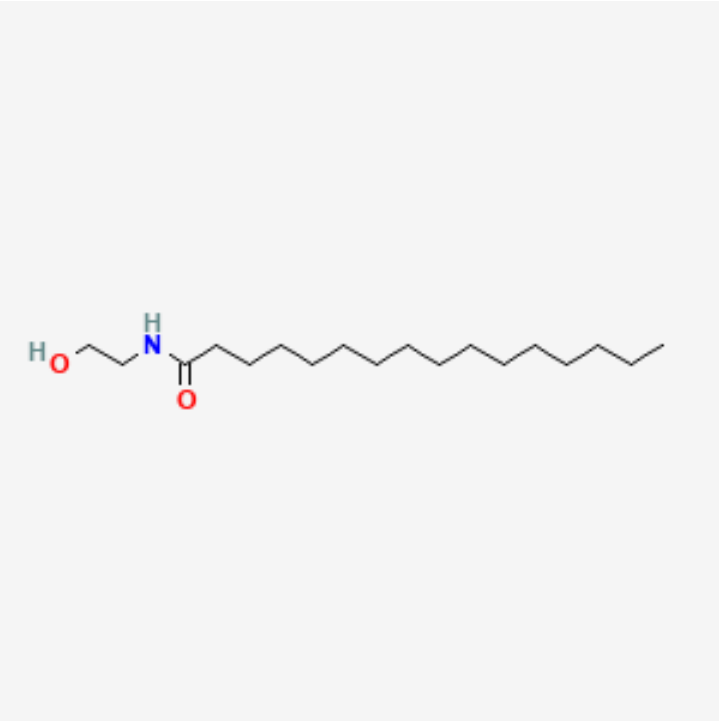


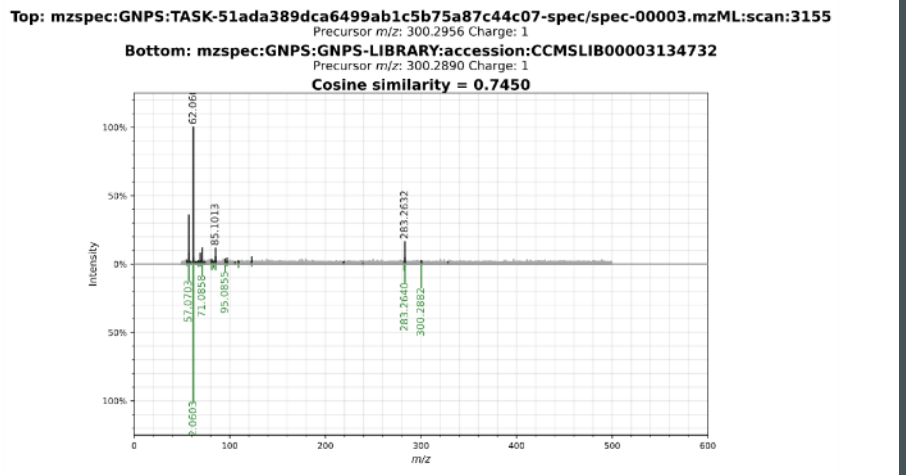
BIS(2-ETHYLHEXYL) PHTHALATE

ADDED TO PLASTICS TO MAKE THEM FLEXIBLE



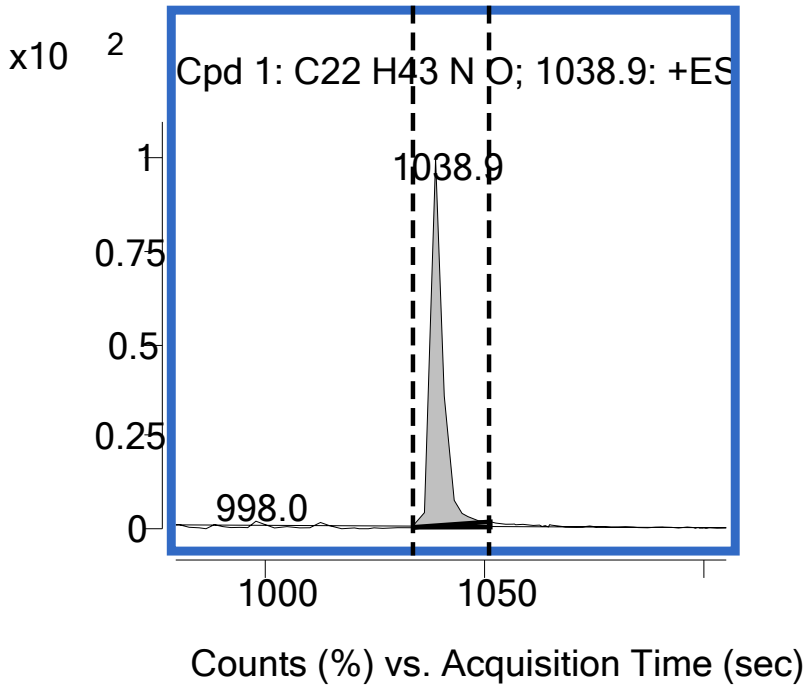


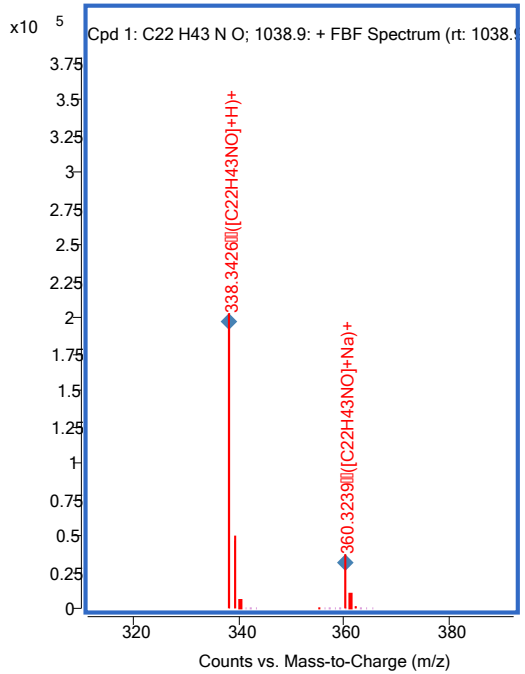


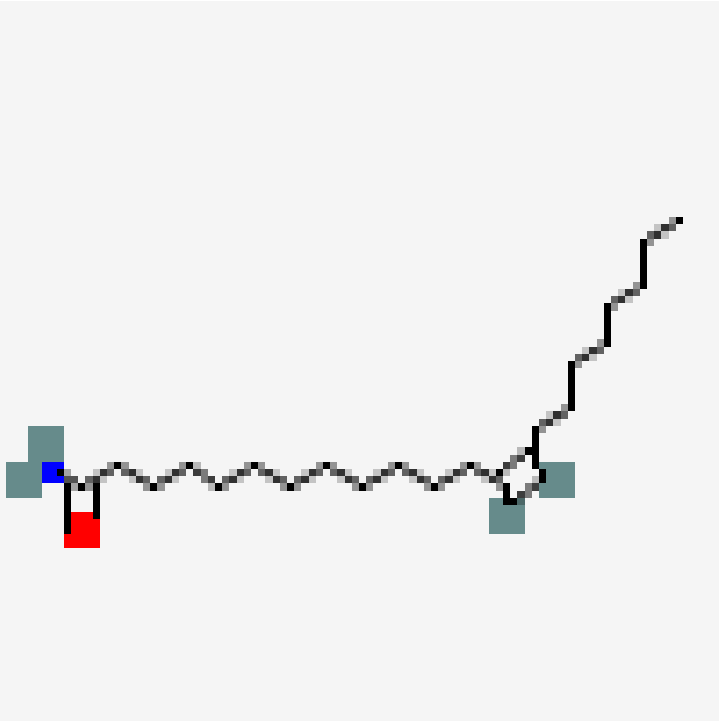


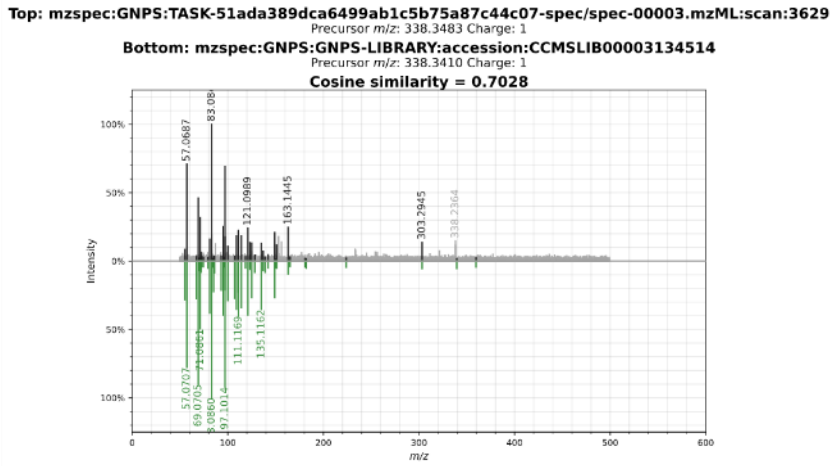
PALMITOYLETHANOLAMIDE

ANTISTATIC, ALSO HAS POTENTIAL ANALGESTIC AND ANTI-INFLAMMATORY ACTIVITIES



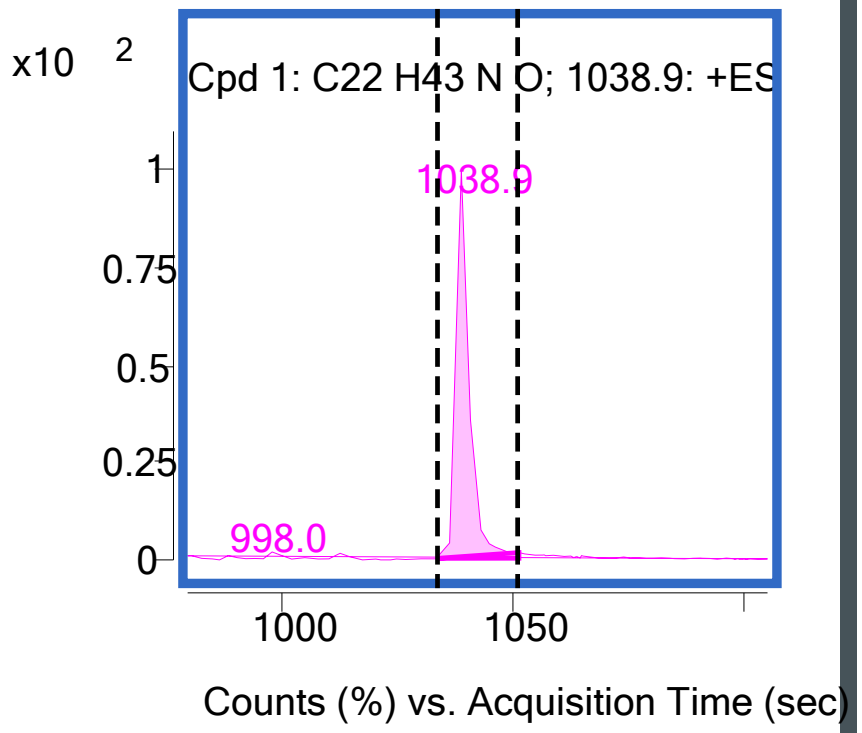


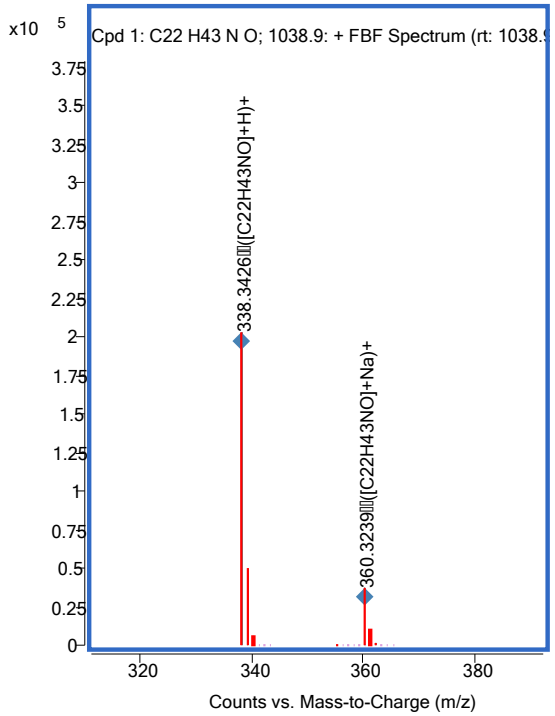


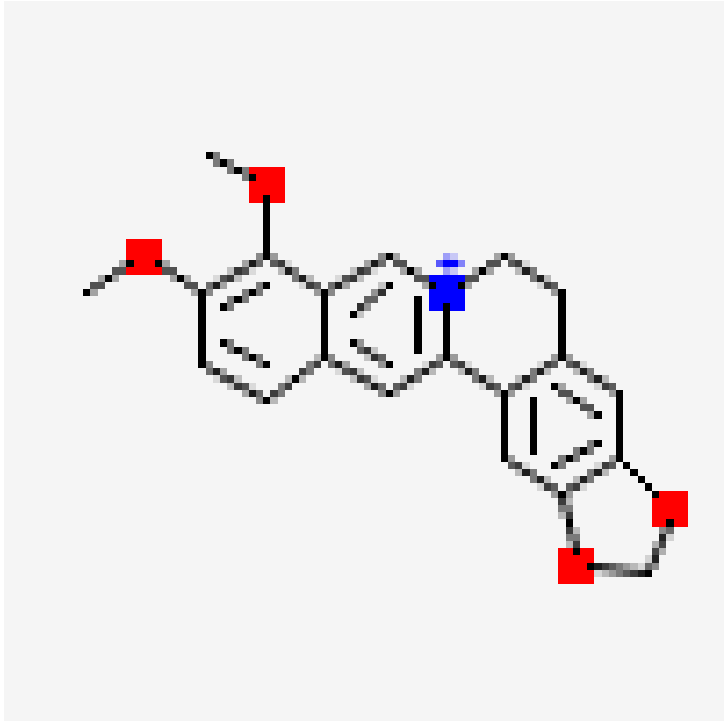


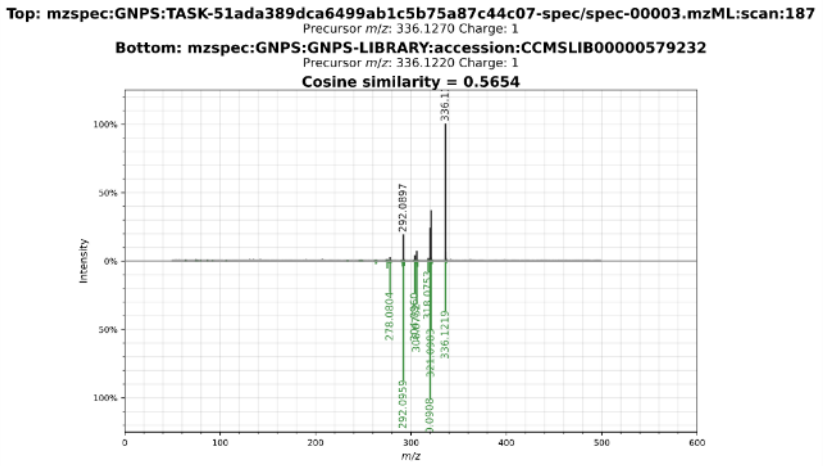
ERUCAMIDE

SLIP ADDITIVE IN PLASTIC MANUFACTURING, ALSO AN ANIMAL METABOLITE



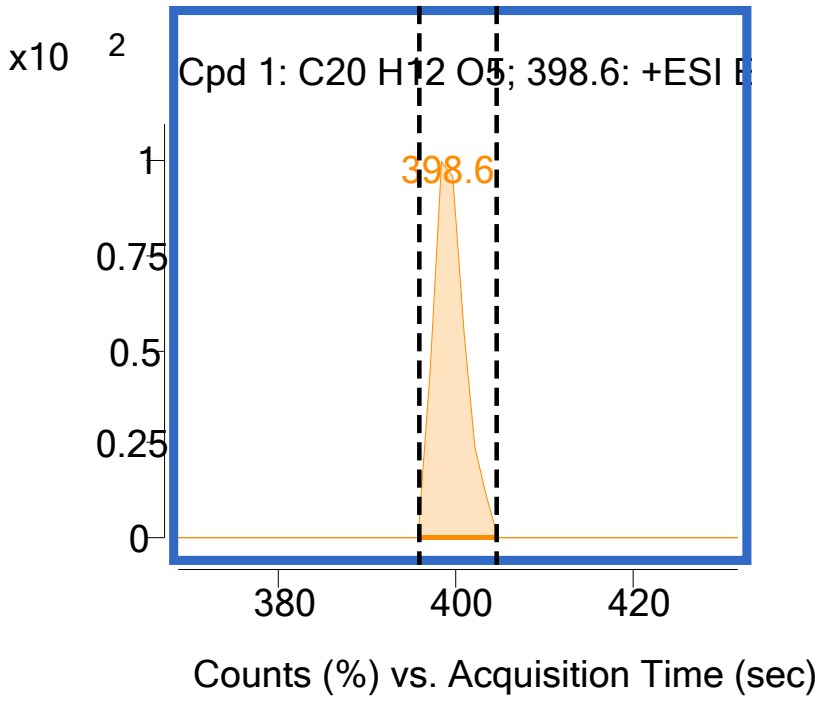


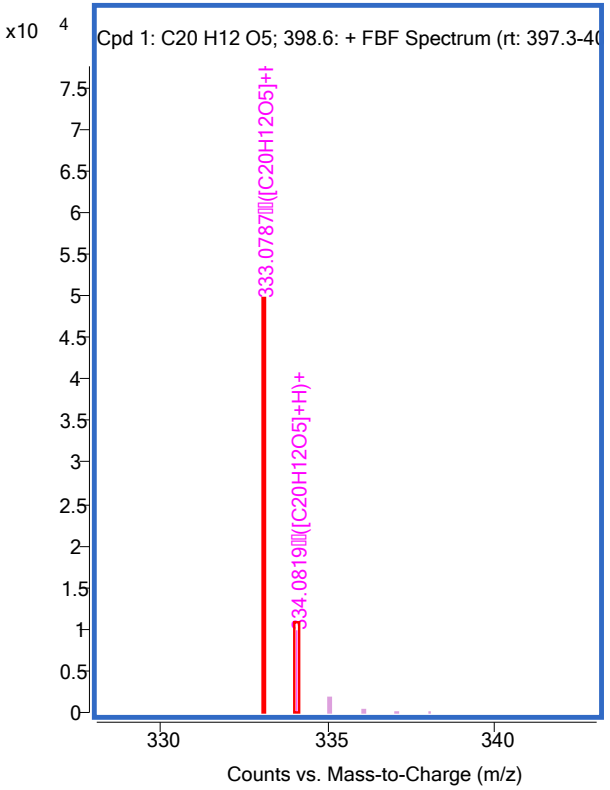


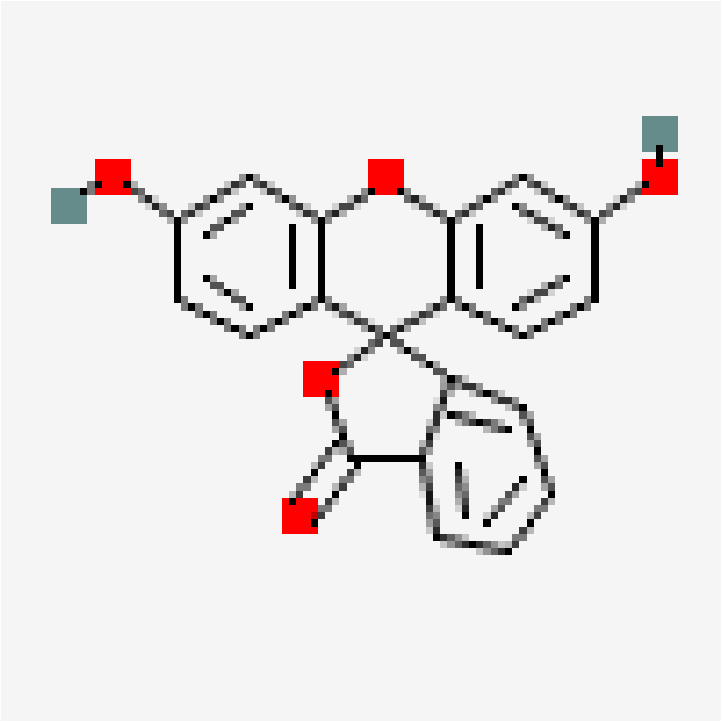


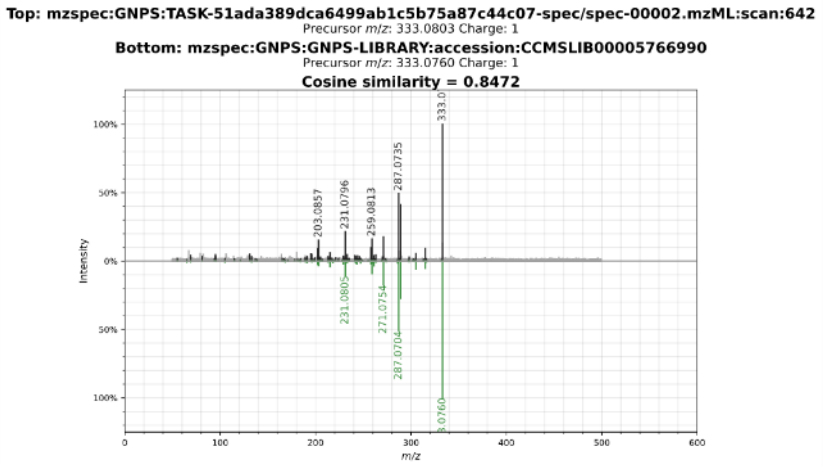
BERBERINE

HERBAL & DIETARY SUPPLEMENT: ANTIOXIDANT + ANTIMICROBIAL



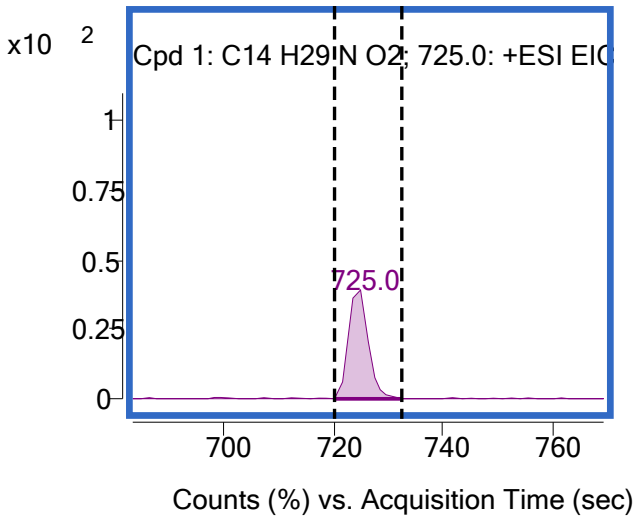


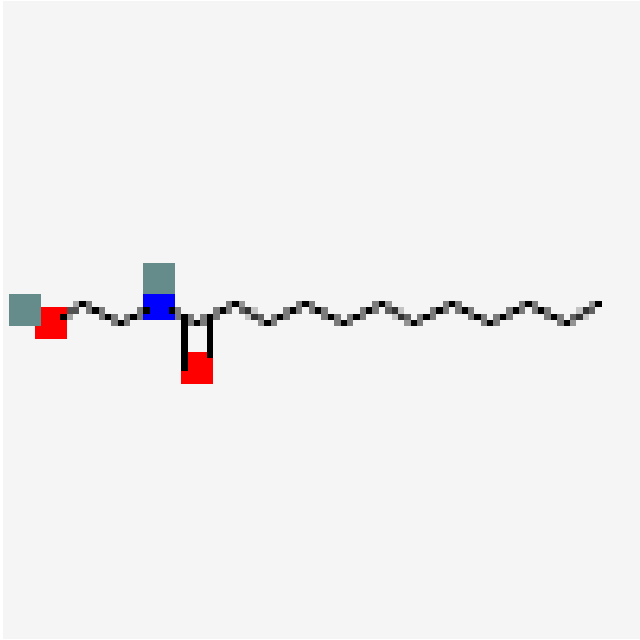


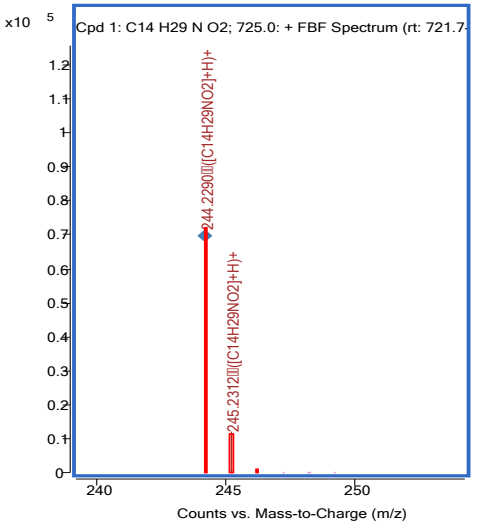


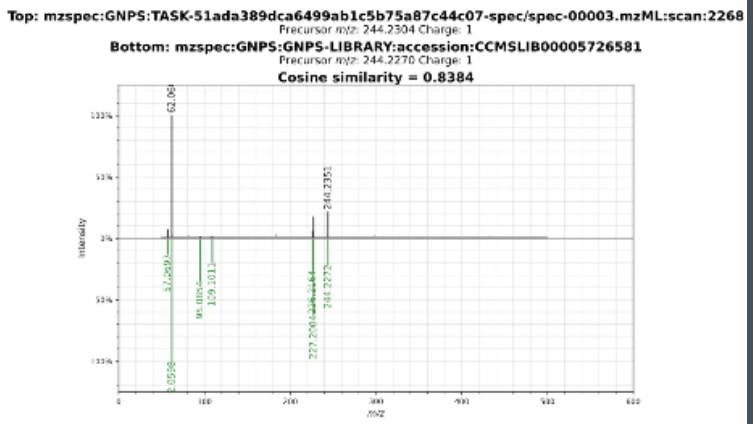
FLUORESCEIN

YELLOW DYE USED IN COSMETICS, HOUSEHOLD PRODUCTS, AND MORE



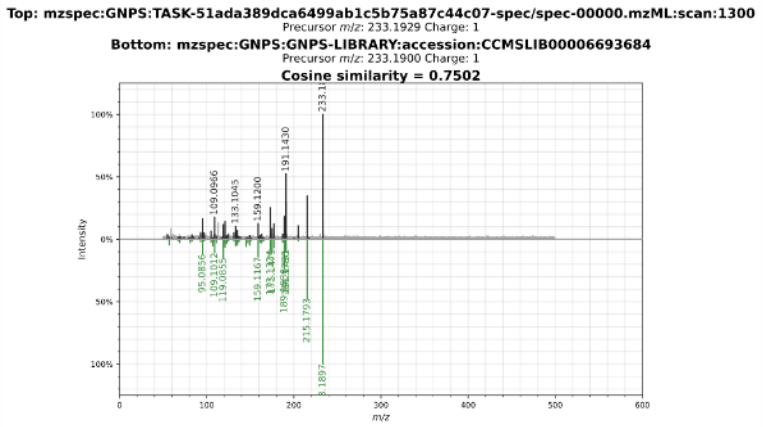


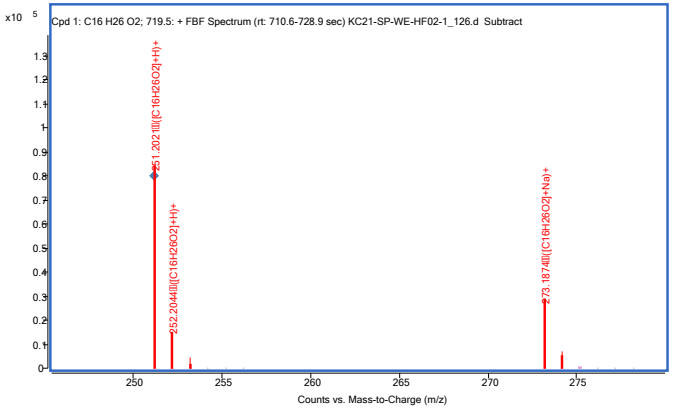


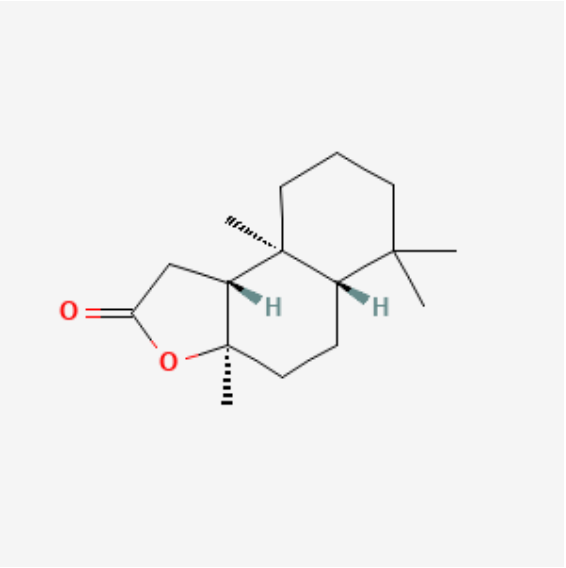


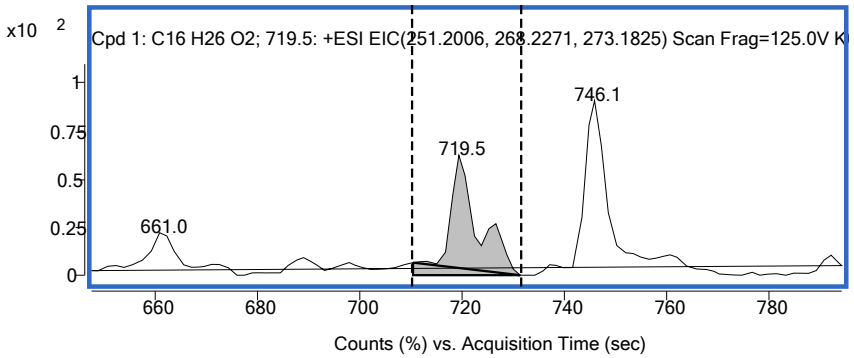
N-(2-HYDROXYETHYL)DODECANAMIDE

- in urinal cakes, toilet deodorizers, & hand/body lotion



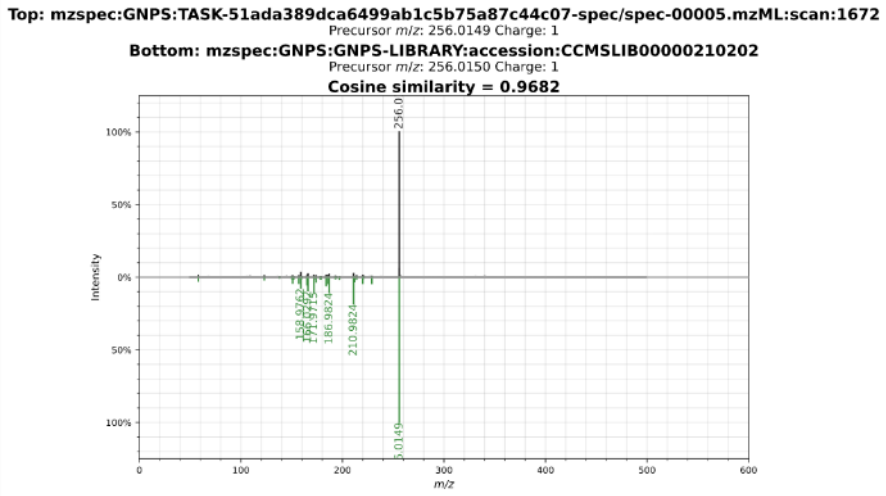


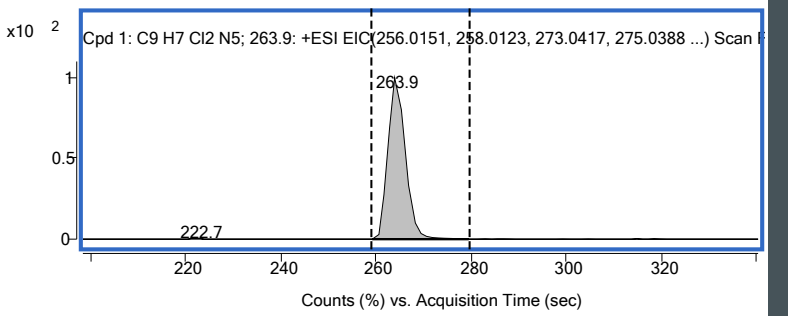


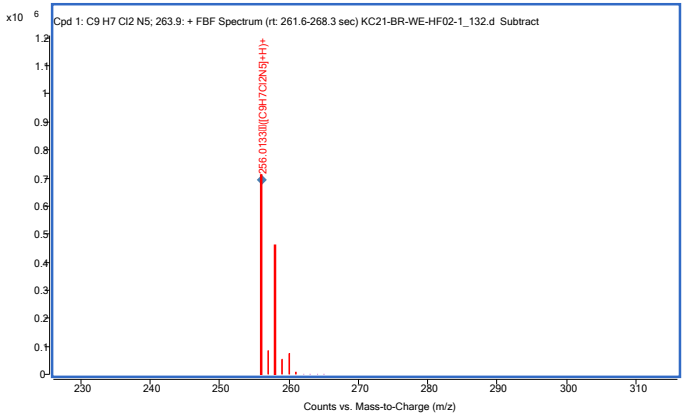


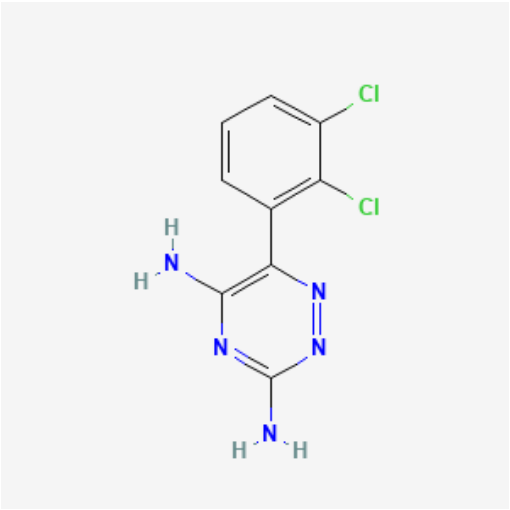
SCLAREOLIDE

- Flavoring agent



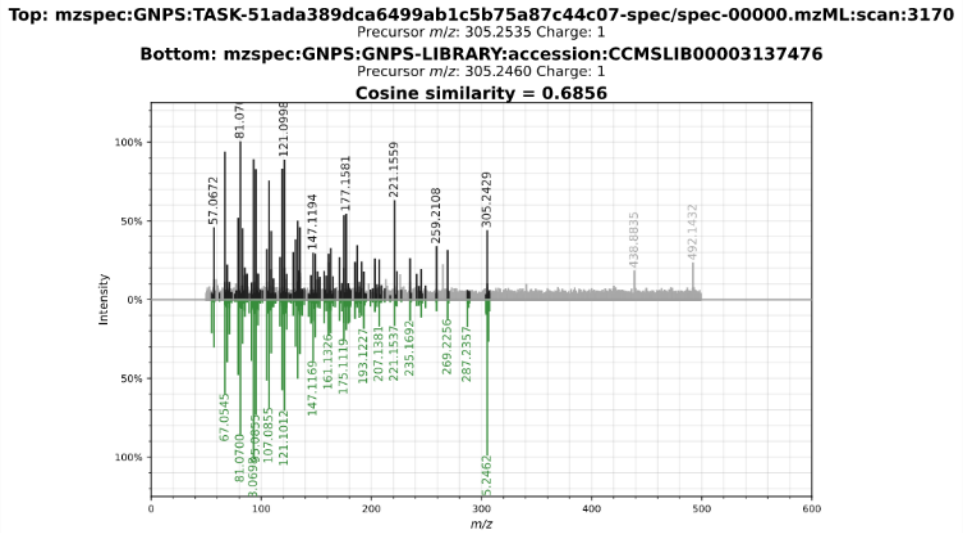


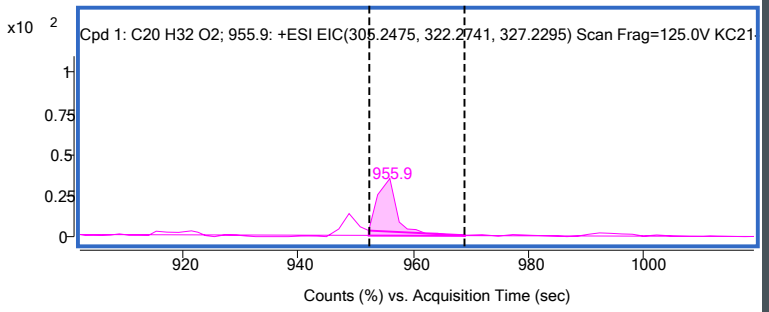


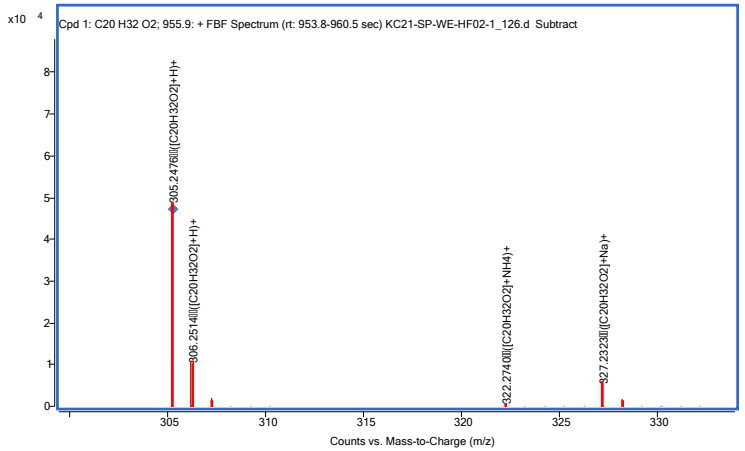


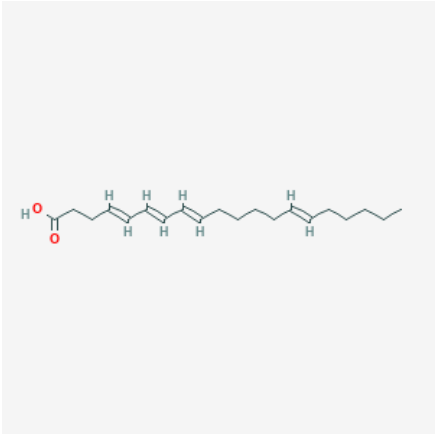
LAMOTRIGINE

ANTICONVULSANT



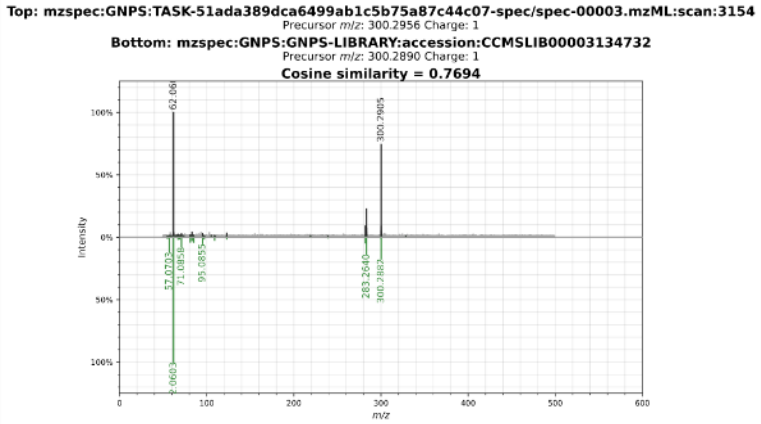


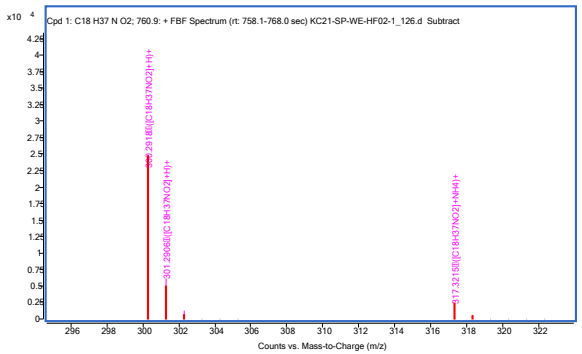


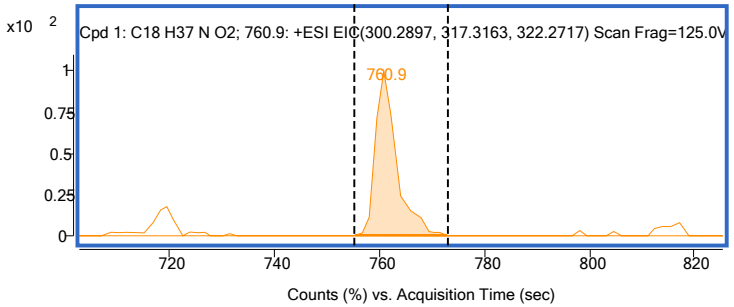


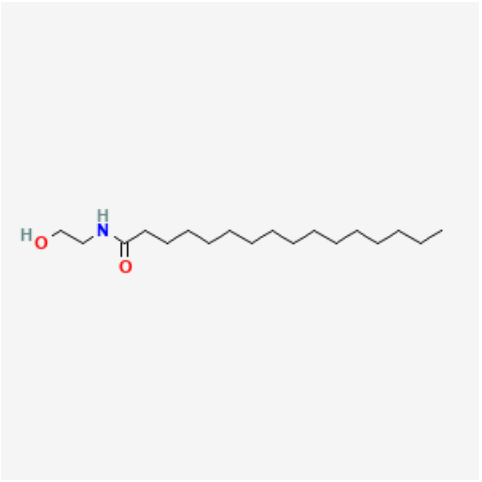
14-EICOSATETRAENOIC ACID

- Seems like a fatty acid based off of structure?



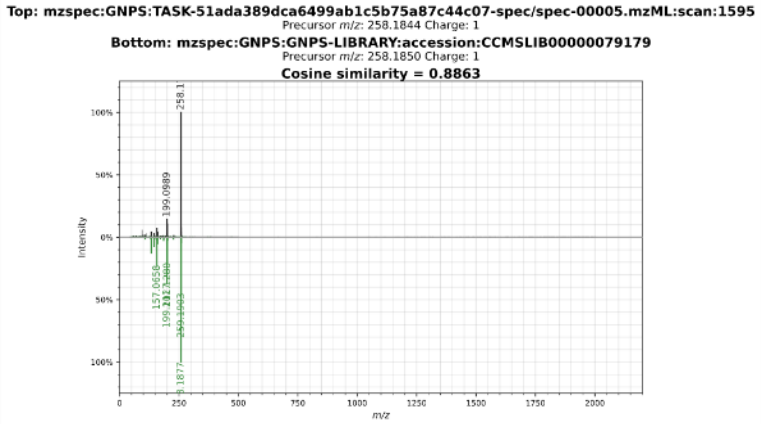


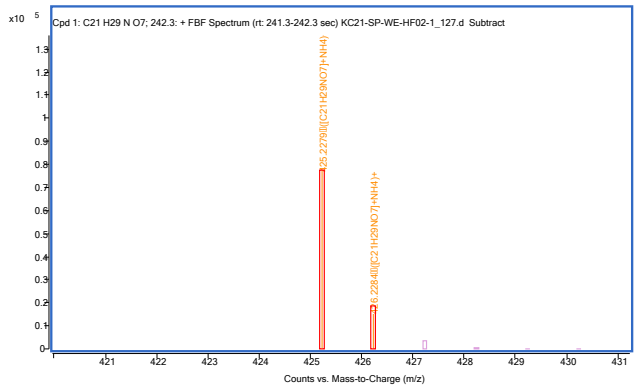


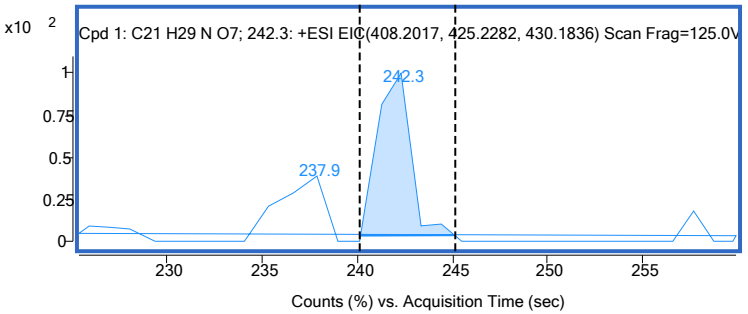


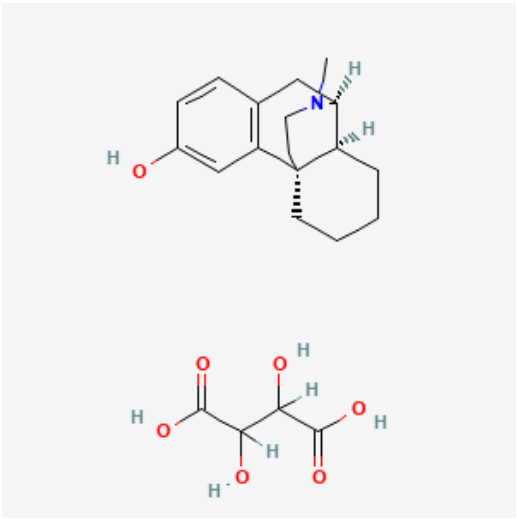
PALMITOYLETHANOLAMIDE

- antistatic; foam boosting; viscosity controlling, used in cosmetics, detergent, and agricultral chemicals (non-pesticidal)

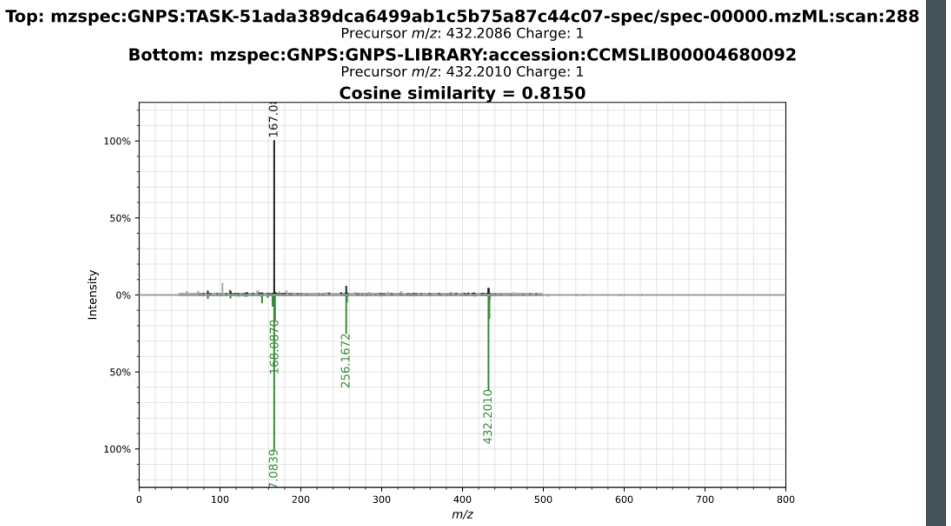


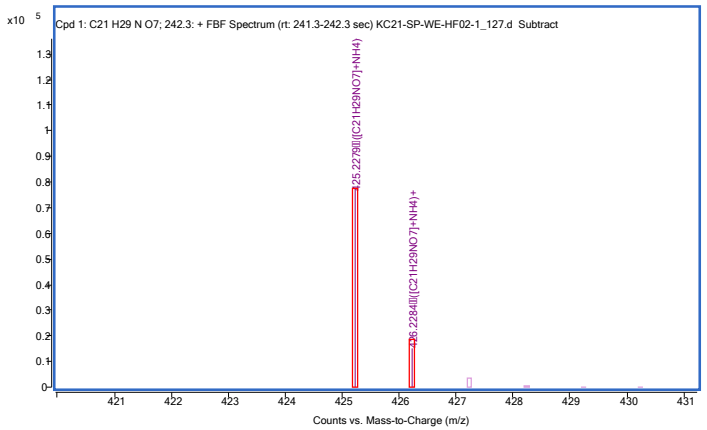


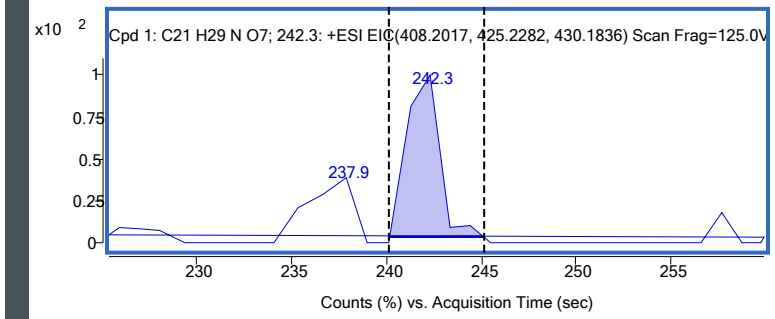


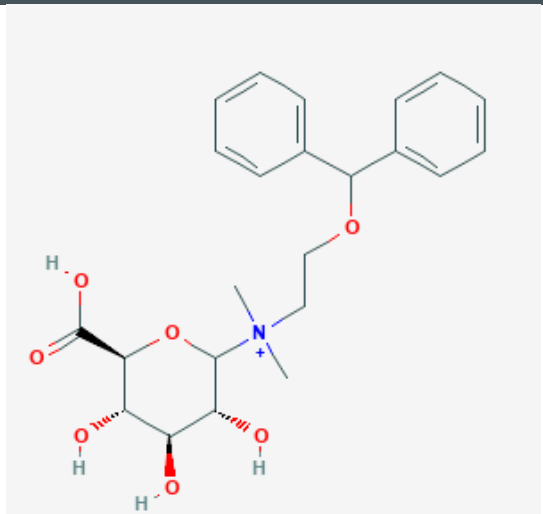


(+)-3-HYDROXY-N-METHYLMORPHINAN D-TARTRATE

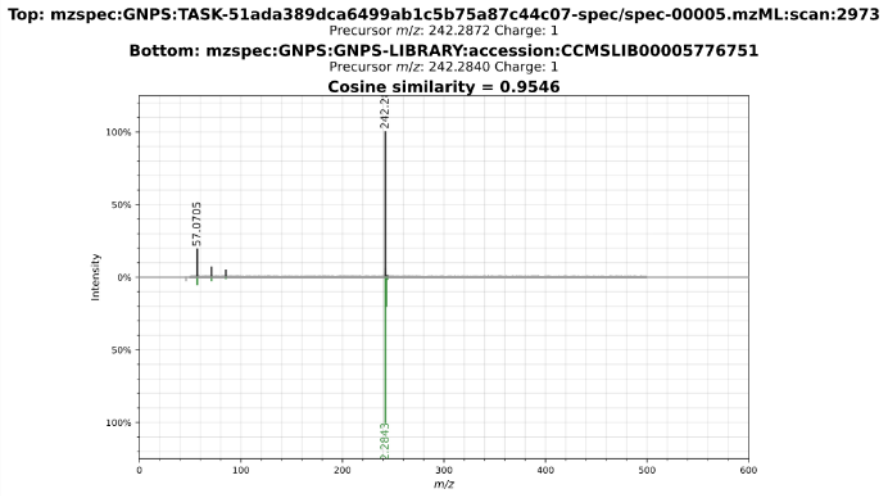


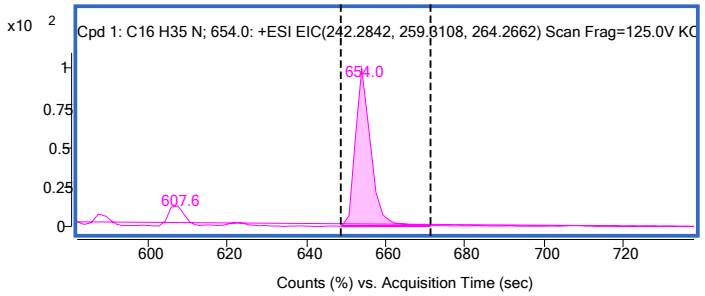


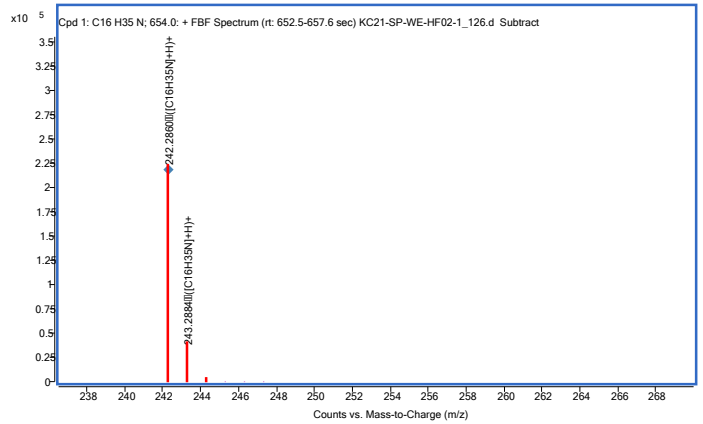


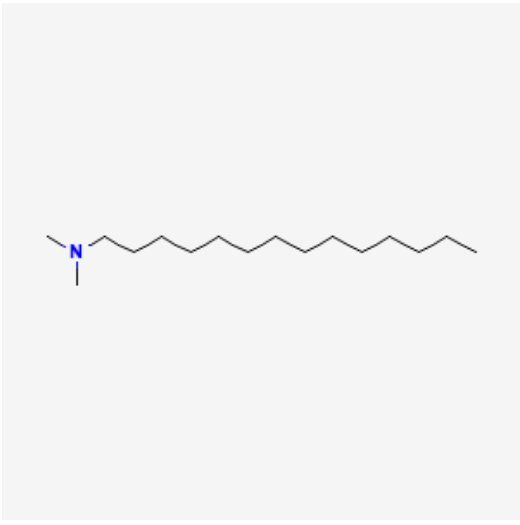


DIPHENHYDRAMINE-N-GLUCURONIDE



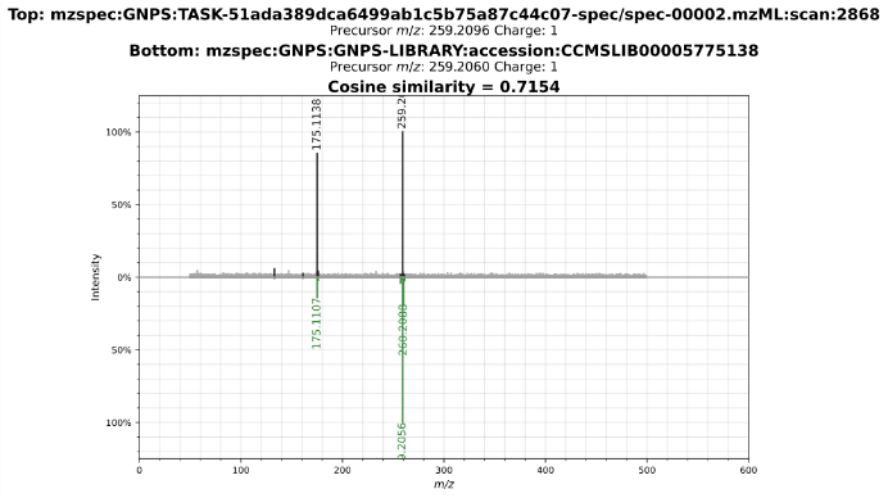


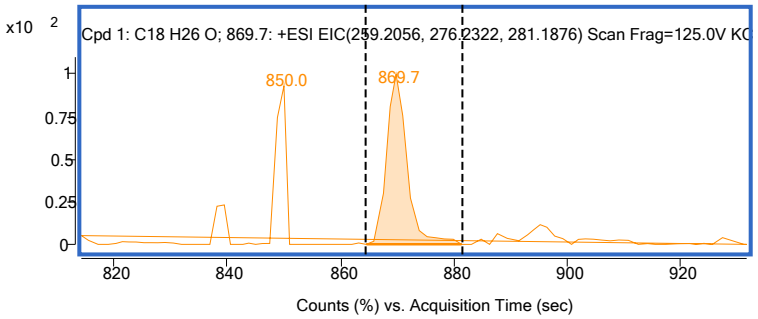


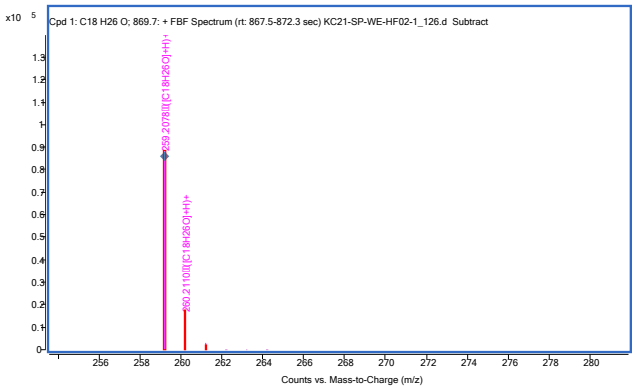


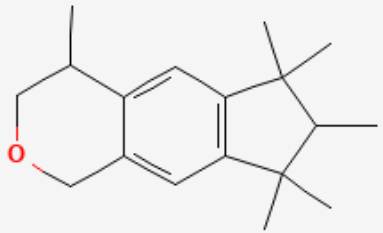
N,N-DIMETHYLTETRADECYLAMINE

CATIONIC DETERGENT AND CORROSION INHOBITOR, USED TO MAKE OTHER CHEMICALS



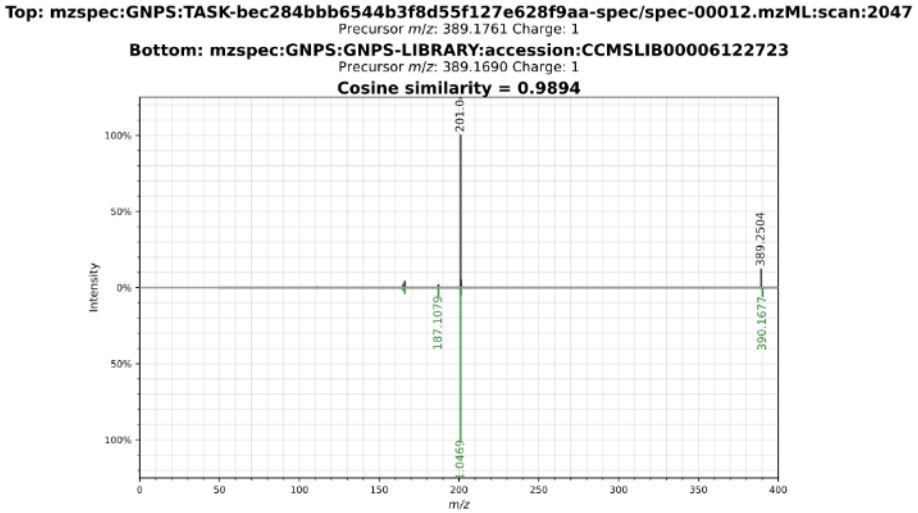


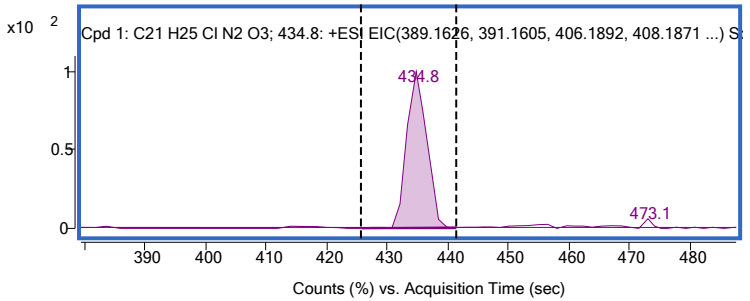


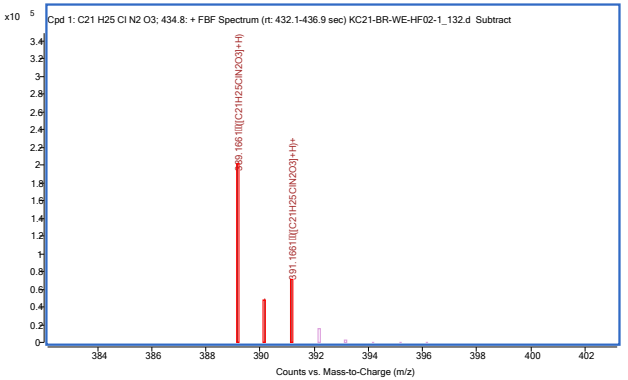


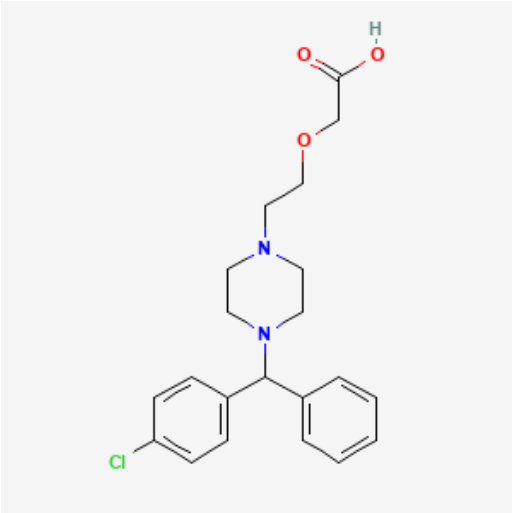
GALAXOLIDE

FRAGRANCE AGENT



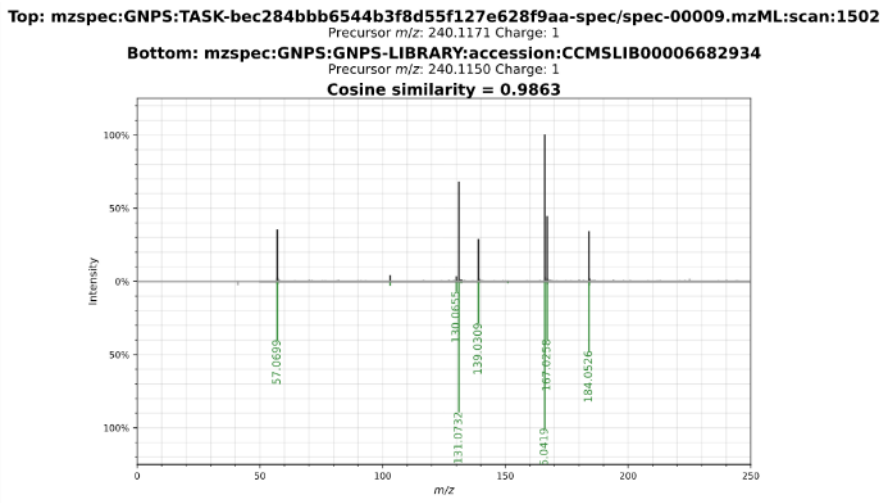


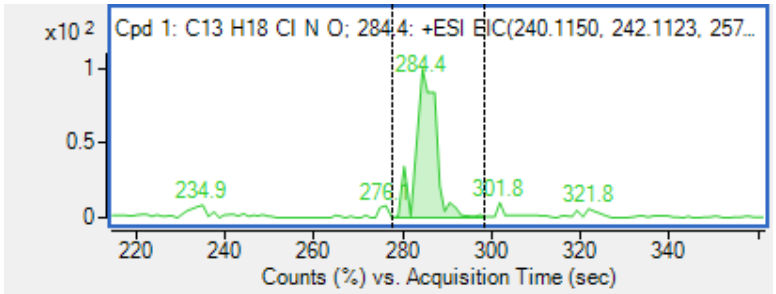


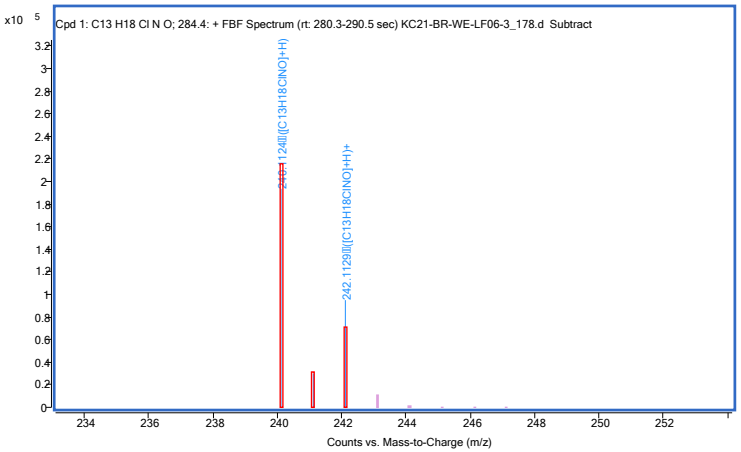


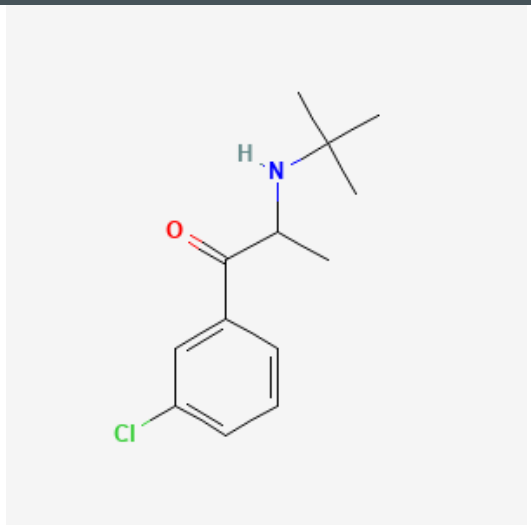
CETIRIZINE

RELIEVES ALLERGY SYMPTOMS; ANTIHISTAMINES



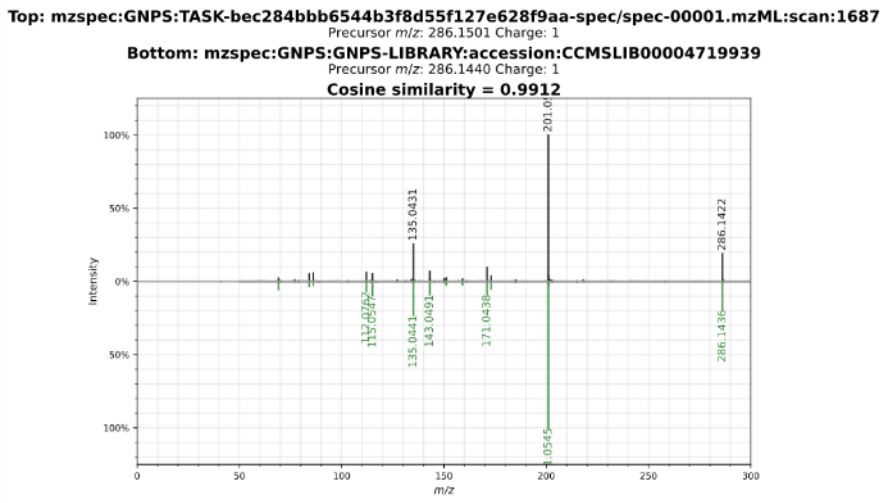


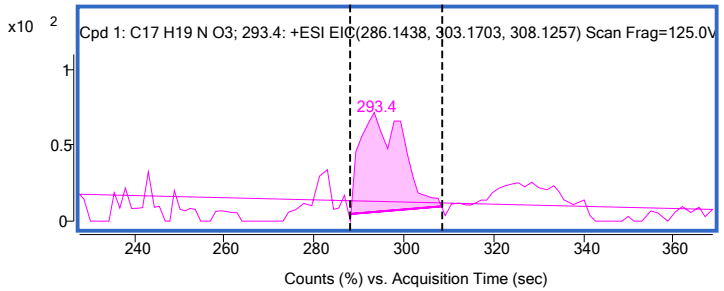


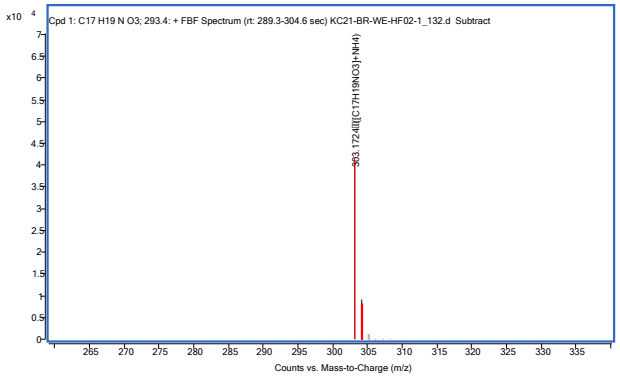


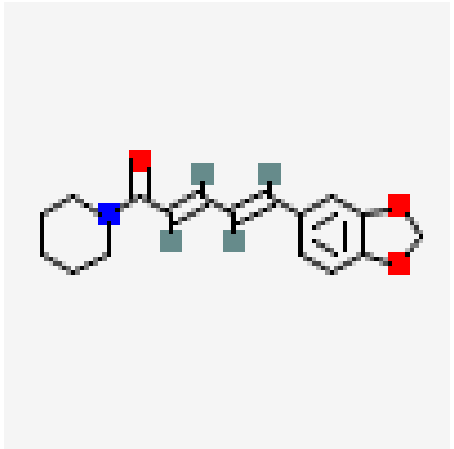
BUPROPION

ANTIDEPRESSANT (WELLBUTRIN)



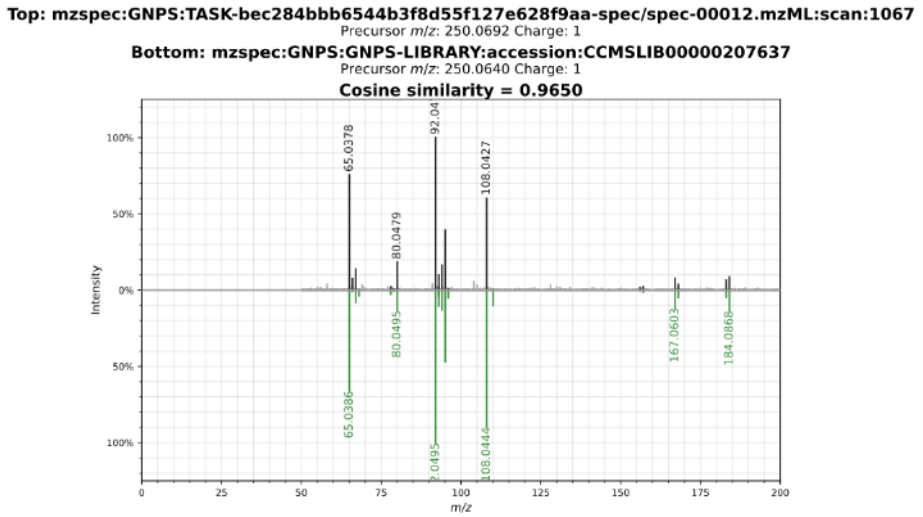


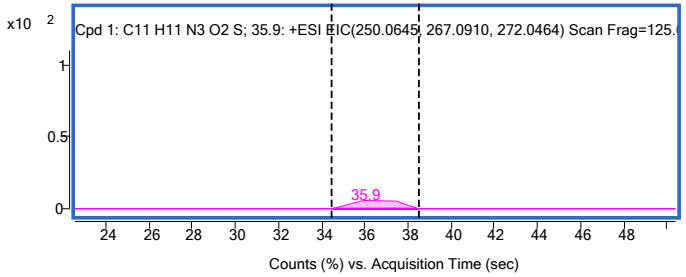


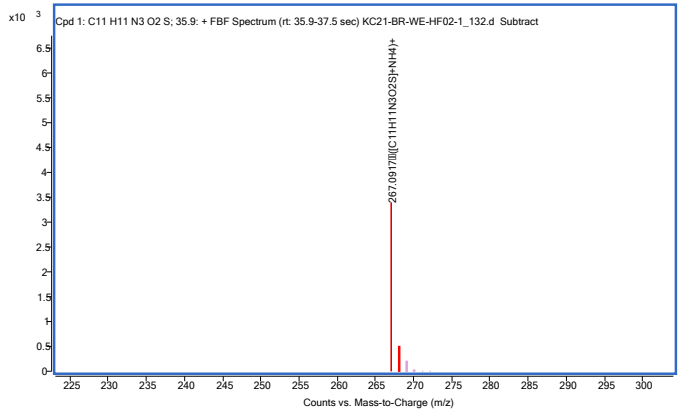


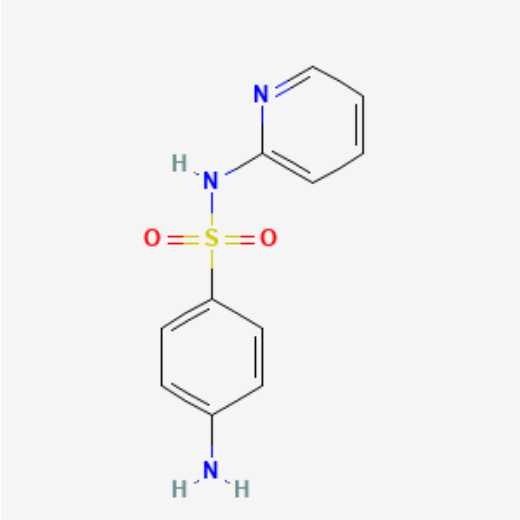
PIPERINE- 94-62-2

in brandy, also an insecticide & pest repellant



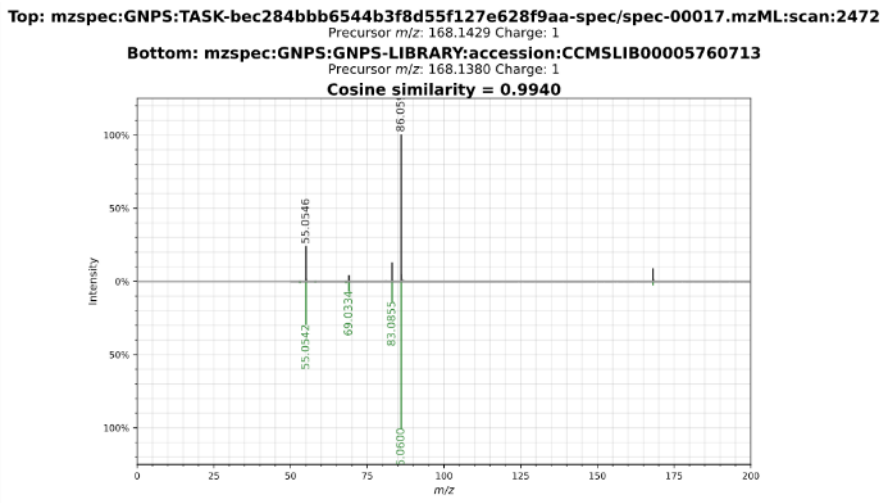


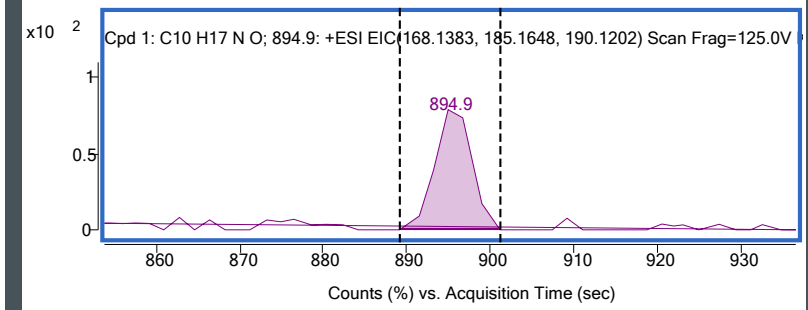


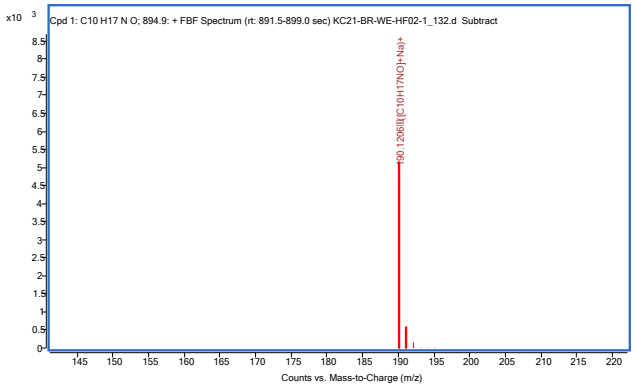


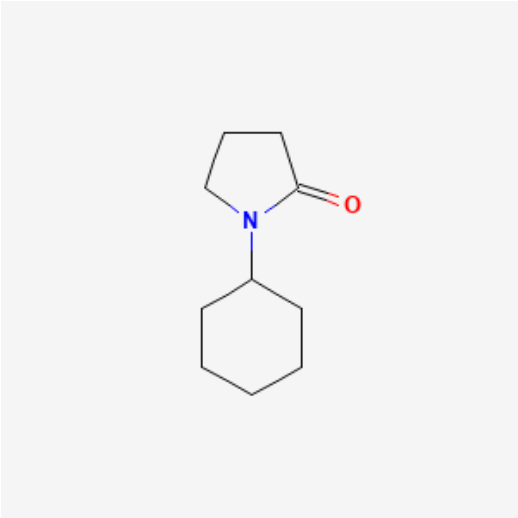
SULFAPYRIDINE- 144 83-2

- Antibiotic



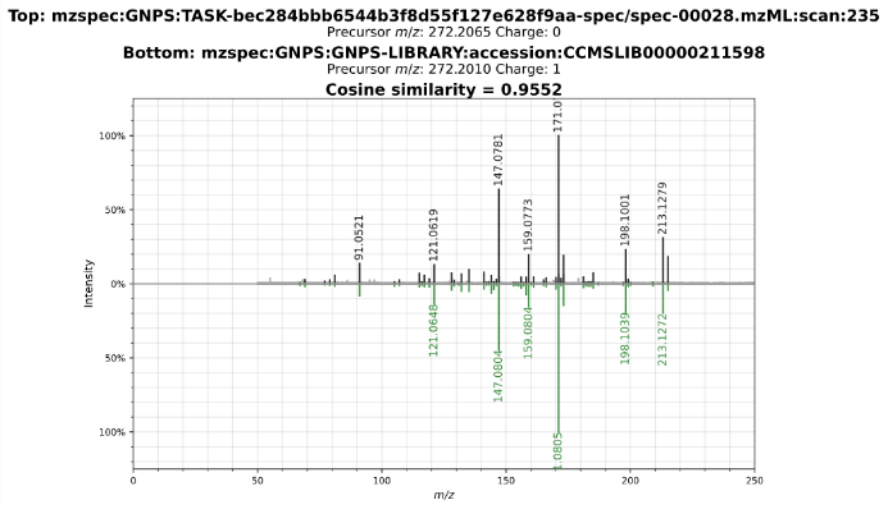


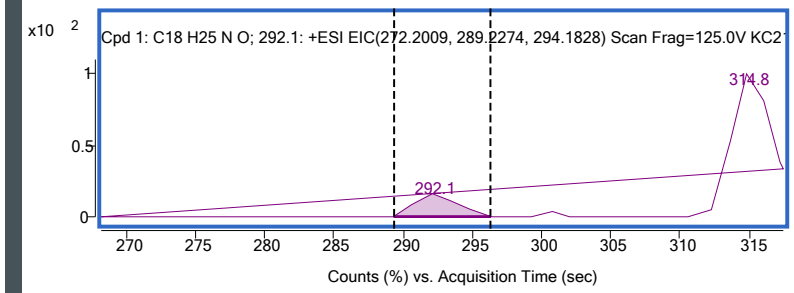


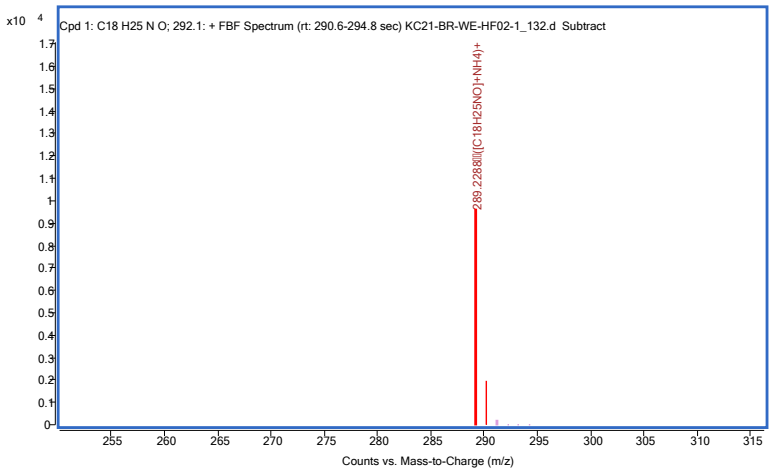


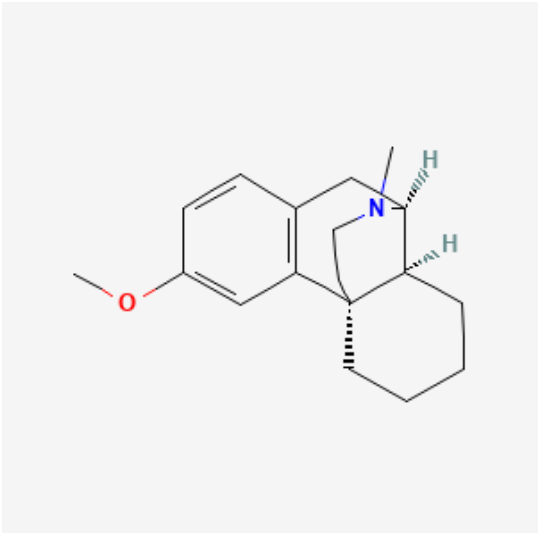
N-CYCLOHEXYL-2-PYRROLIDONE 6837-24-7

SYNTHETIC DYE AND PIGMENT MANUFACTURING, CHEMICAL PRODUCT MANUFACTURING



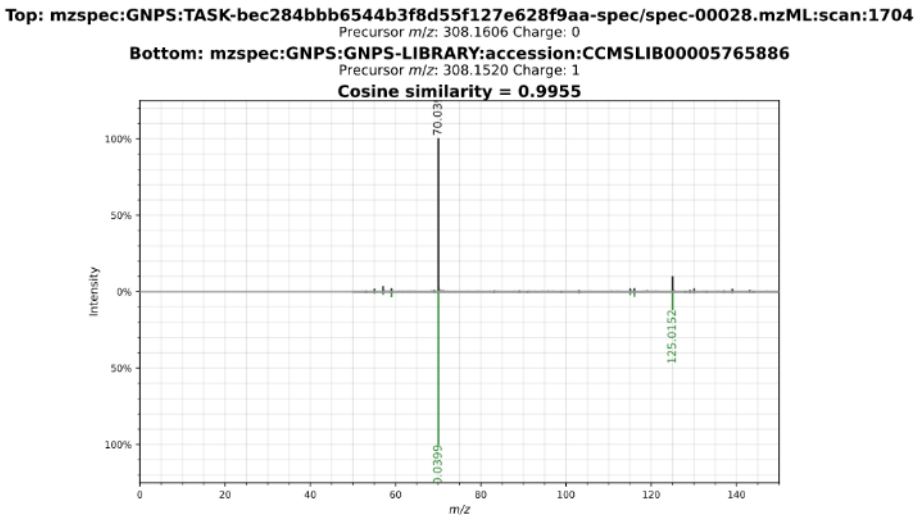


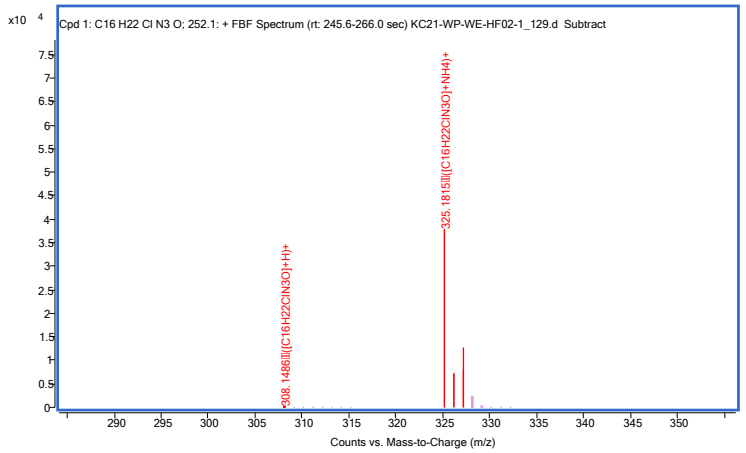


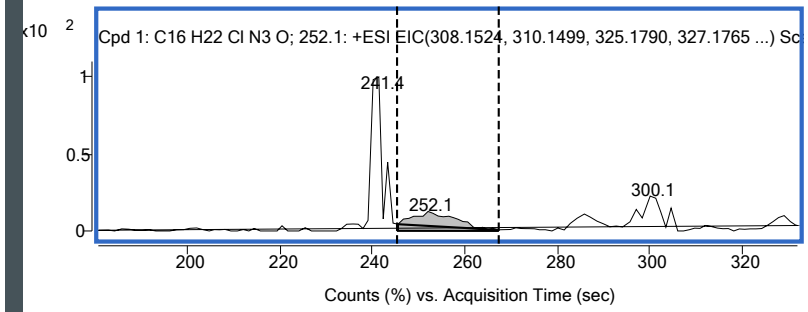


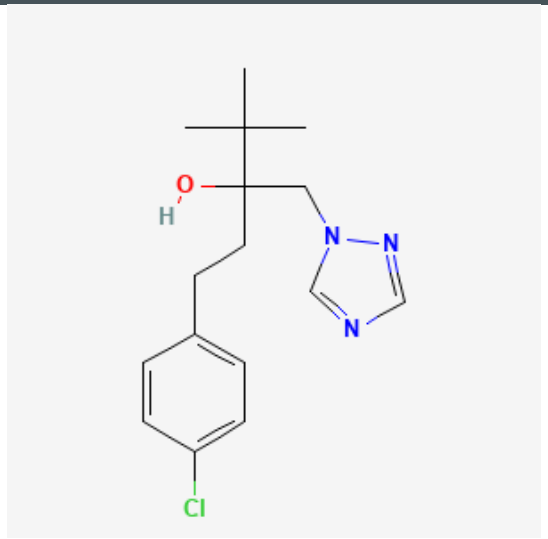
DEXTROMETHORPHAN 125-71-3

OTC COUGH SUPPRESSANT & COLD MEDICATION



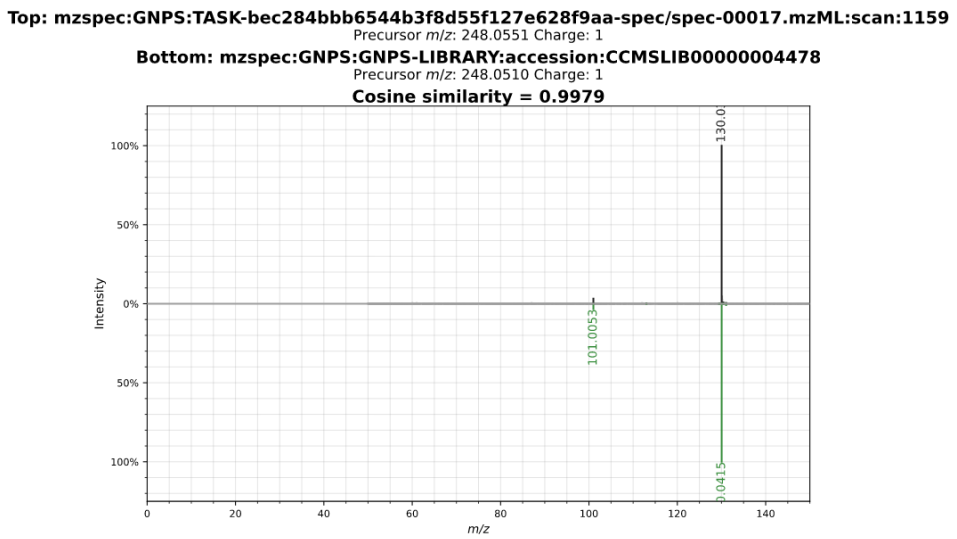


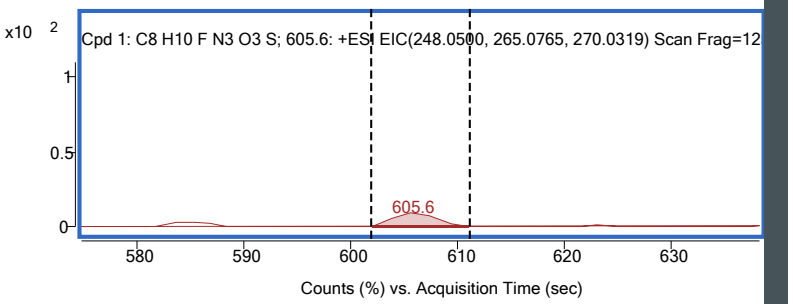


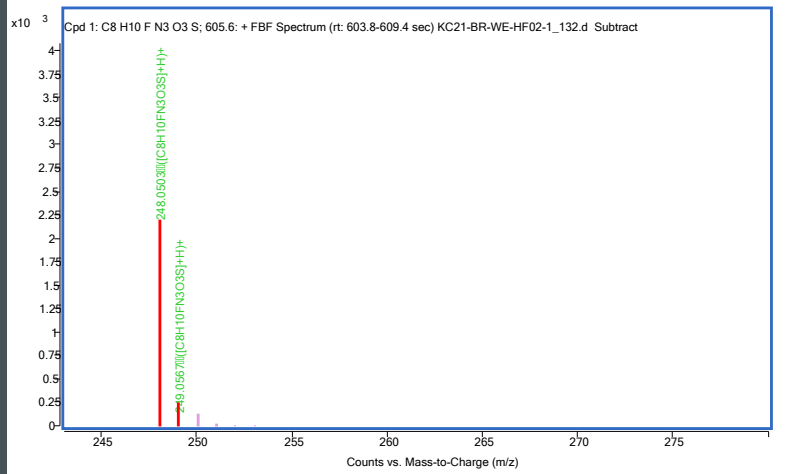


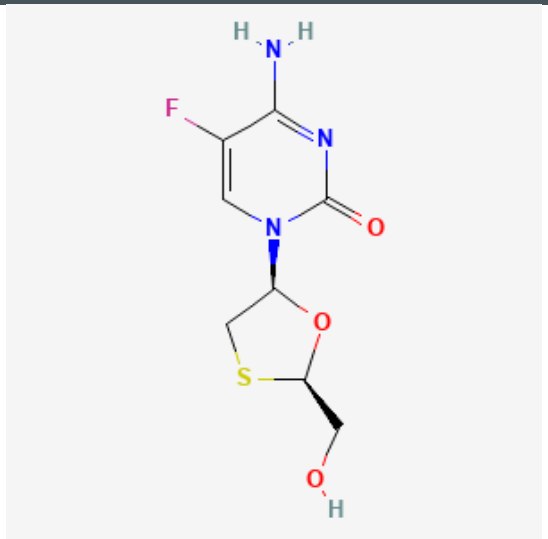
TEBUCONAZOLE 1107534-96-3

- Pesticide, fungicide specifically



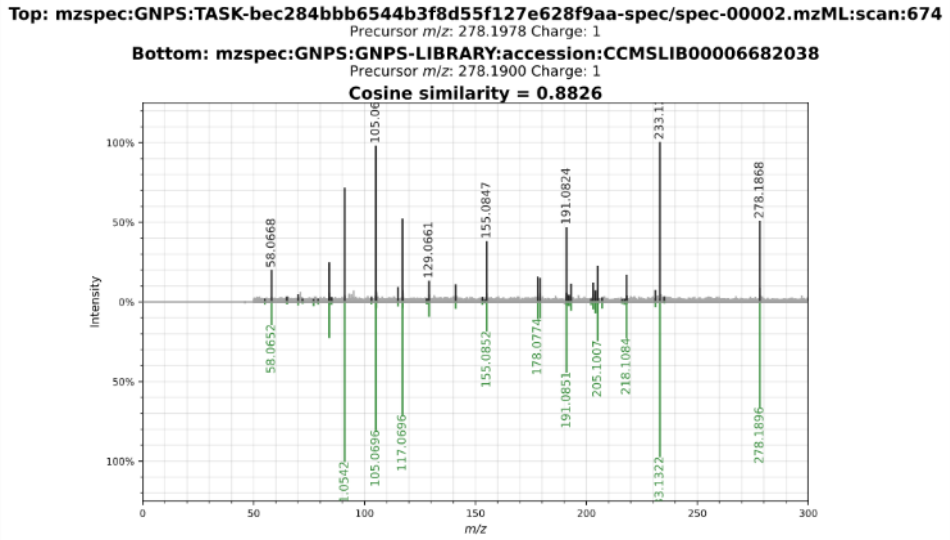


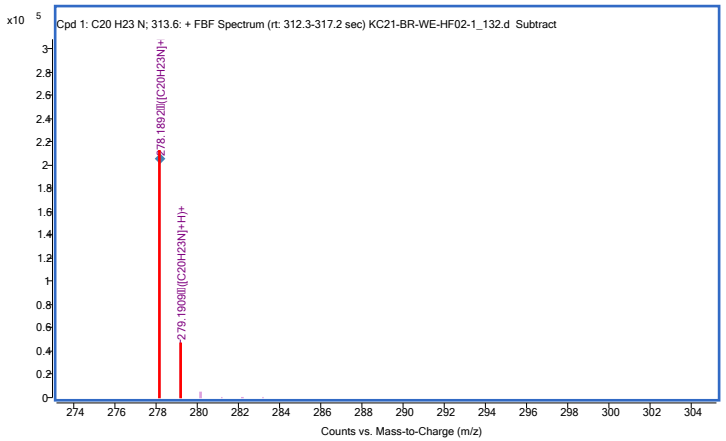


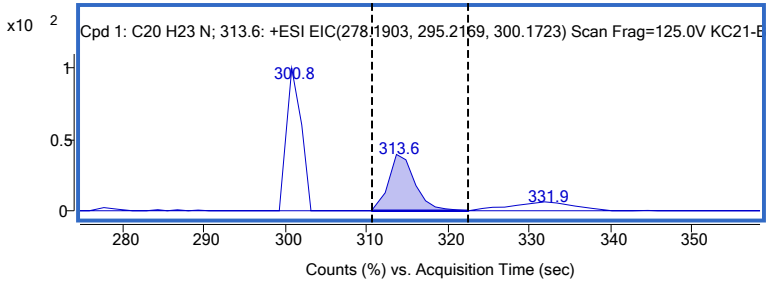


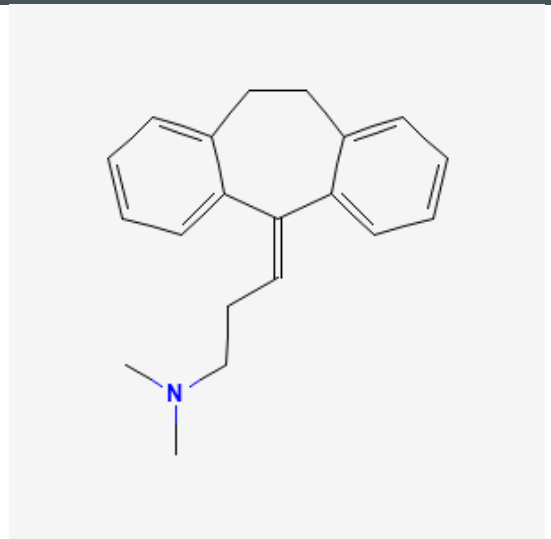
EMTRICITABINE 143491-57-0

- HIV medication



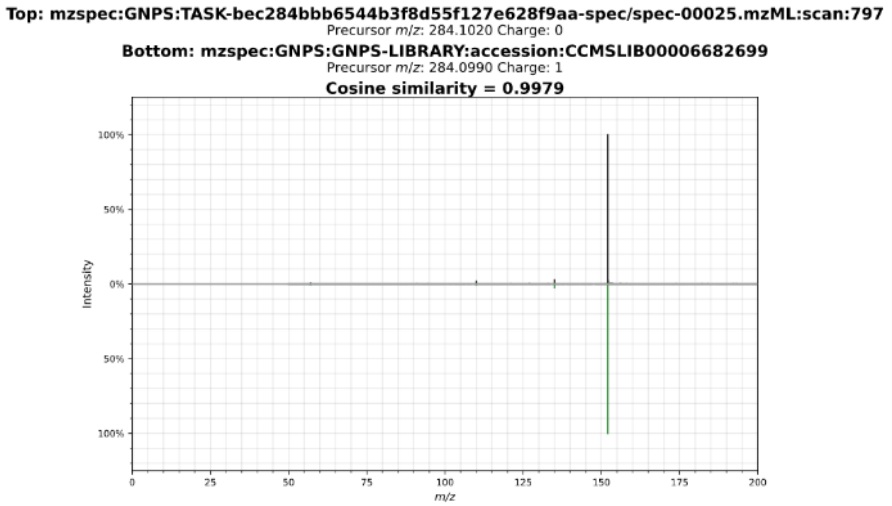


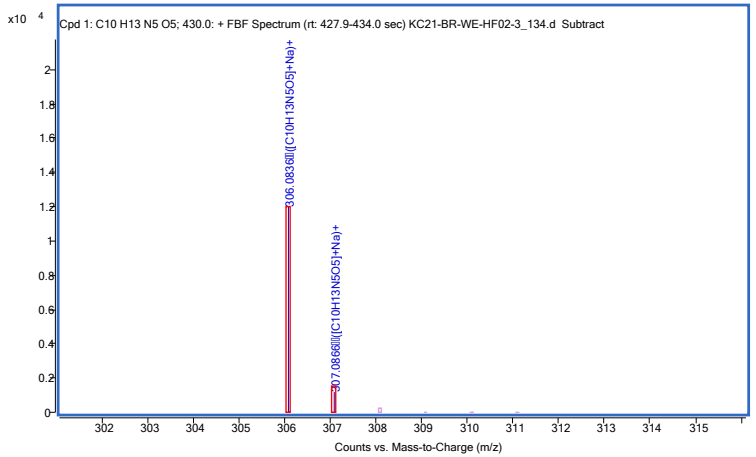


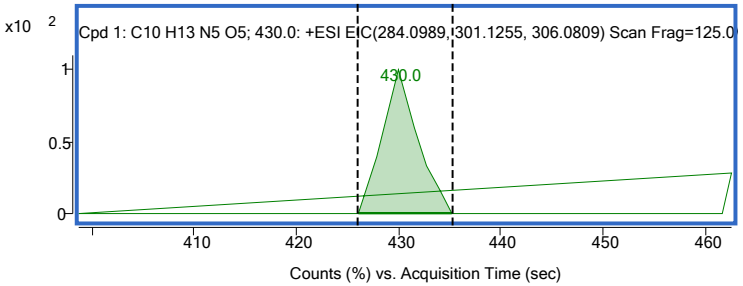


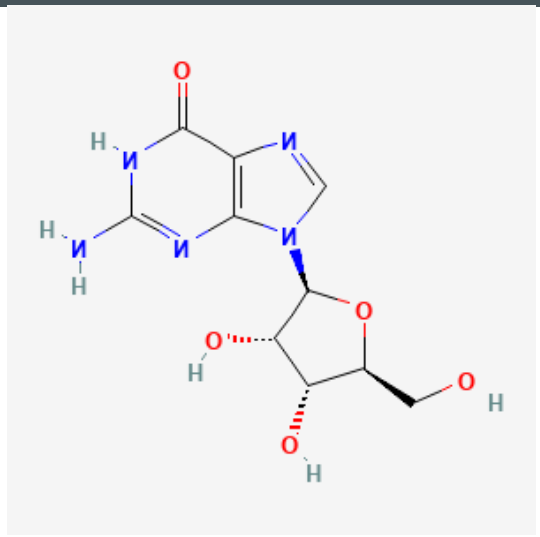
AMITRIPTYLINE - 50-48-6

- Antidepressant



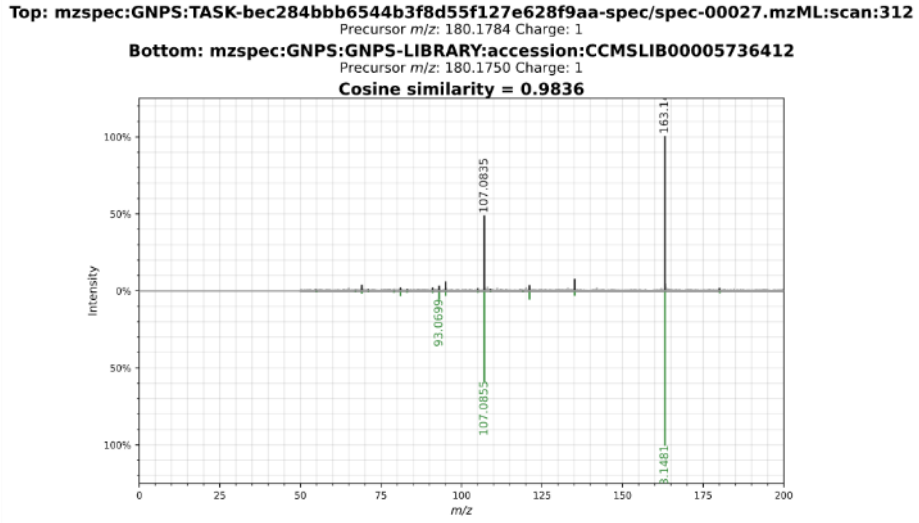


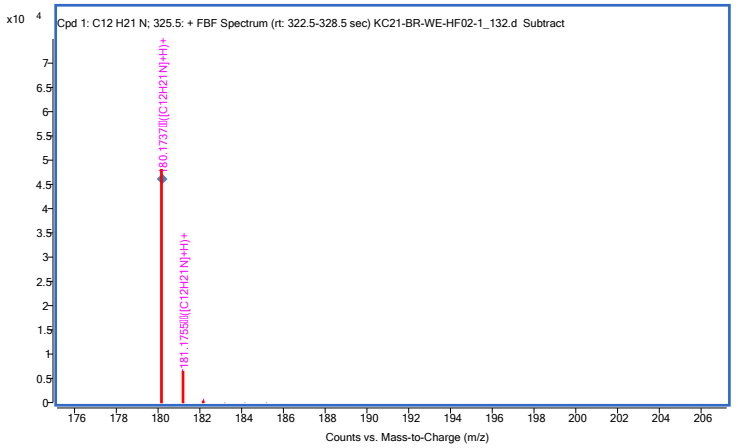


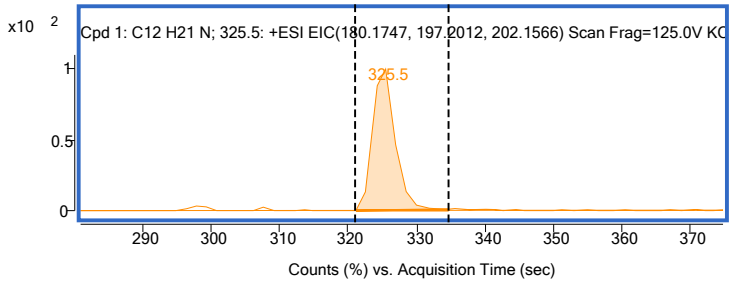


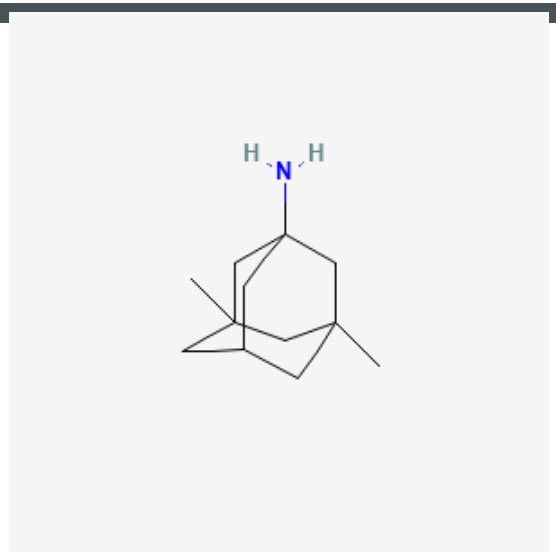
GUANOSINE

- Cosmetic



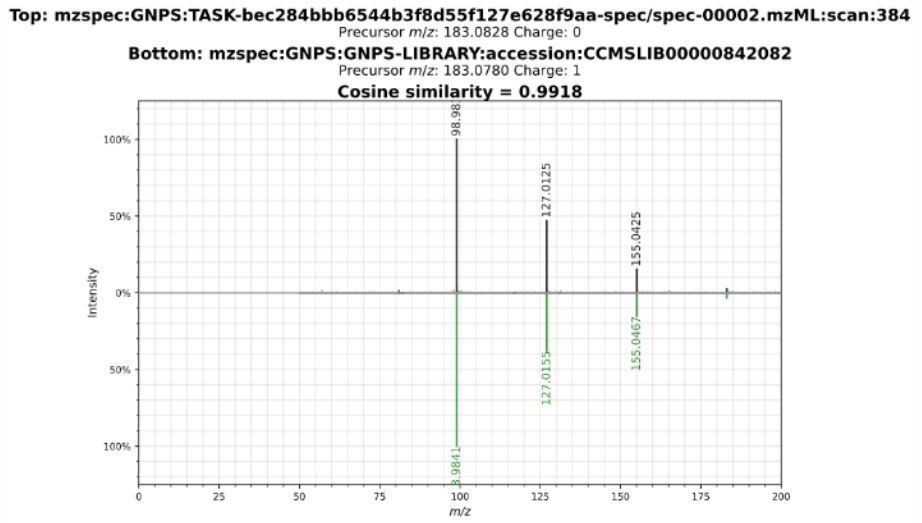


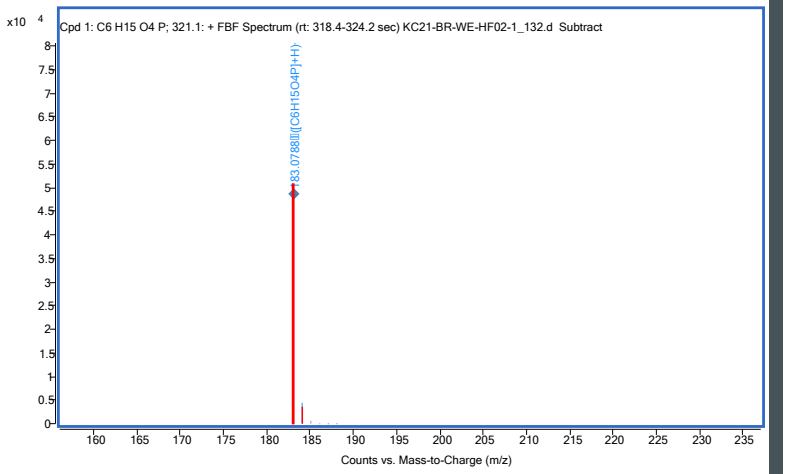


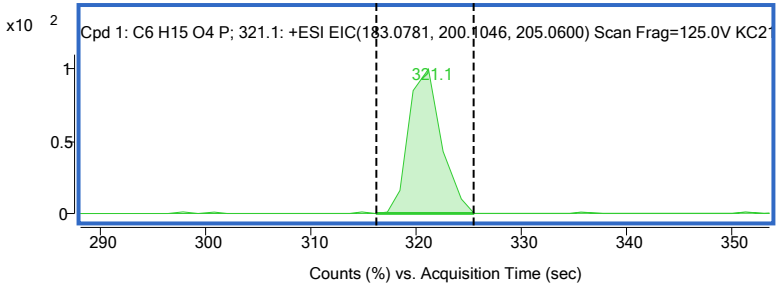


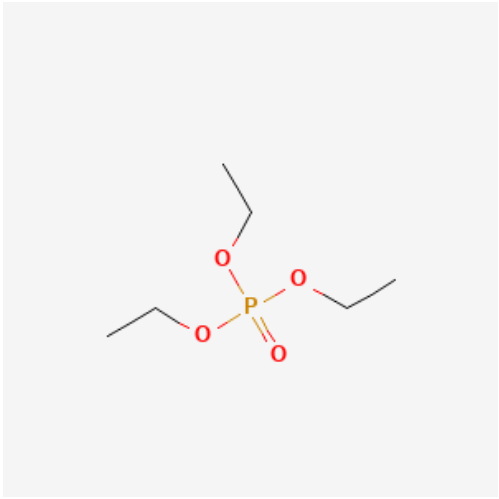
MEMANTINE 19982-08-2

- Used in Alzheimer's treatment



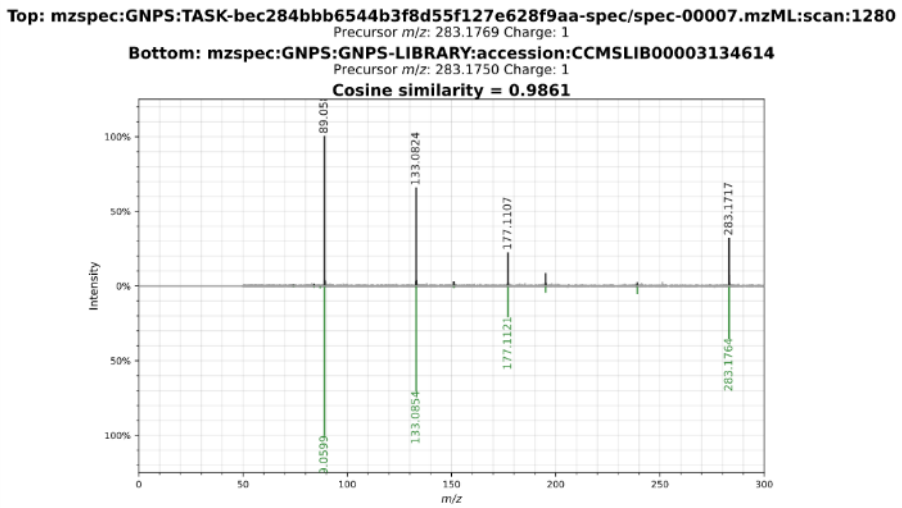


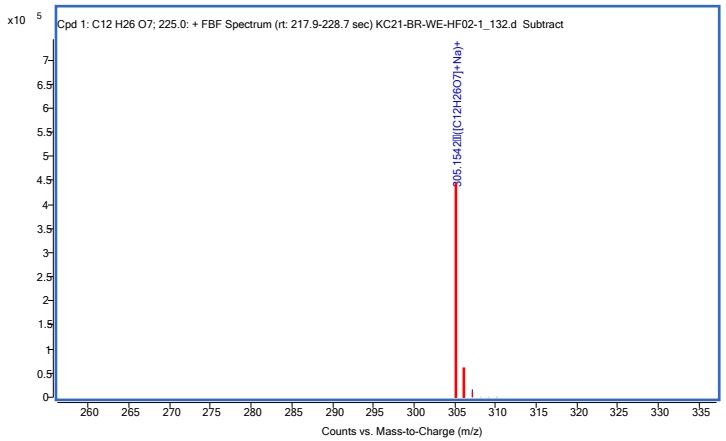


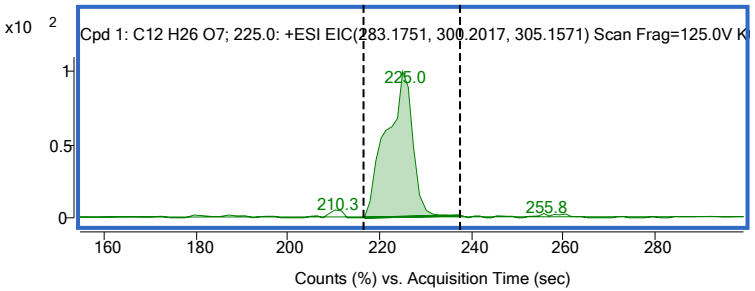


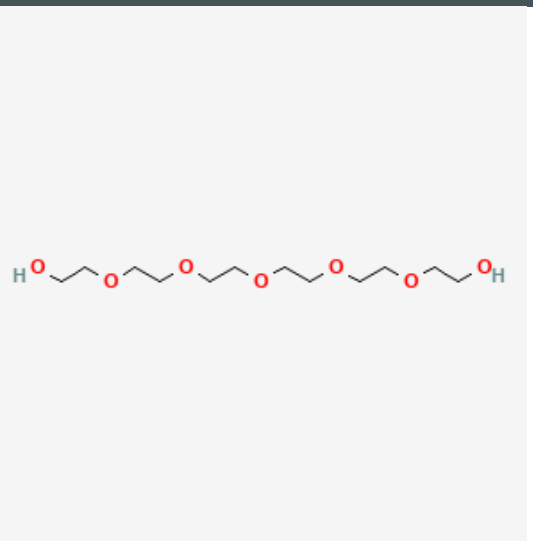
TRIETHYL PHOSPHATE 78-40-0

- Flame retardant



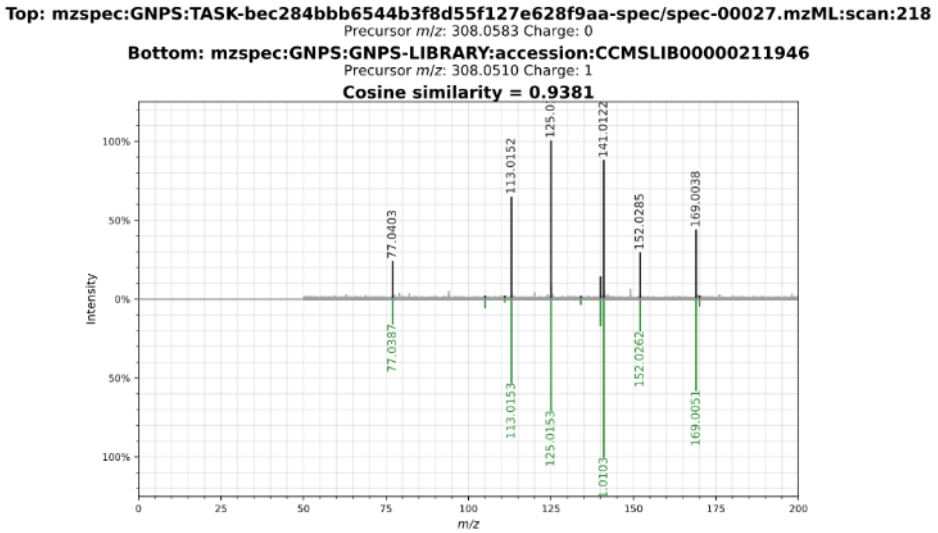


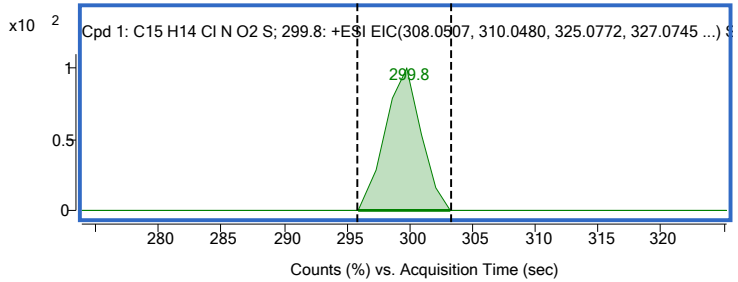


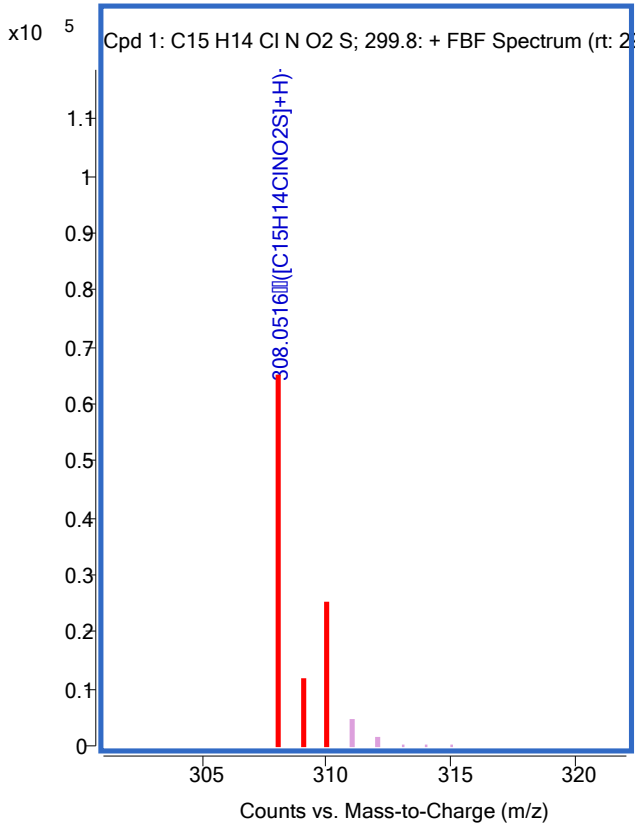


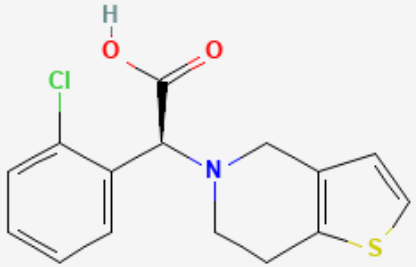
HEXAETHYLENE GLYCOL 2615-15-8

- makeup, engine maintenance, processing aid and additive



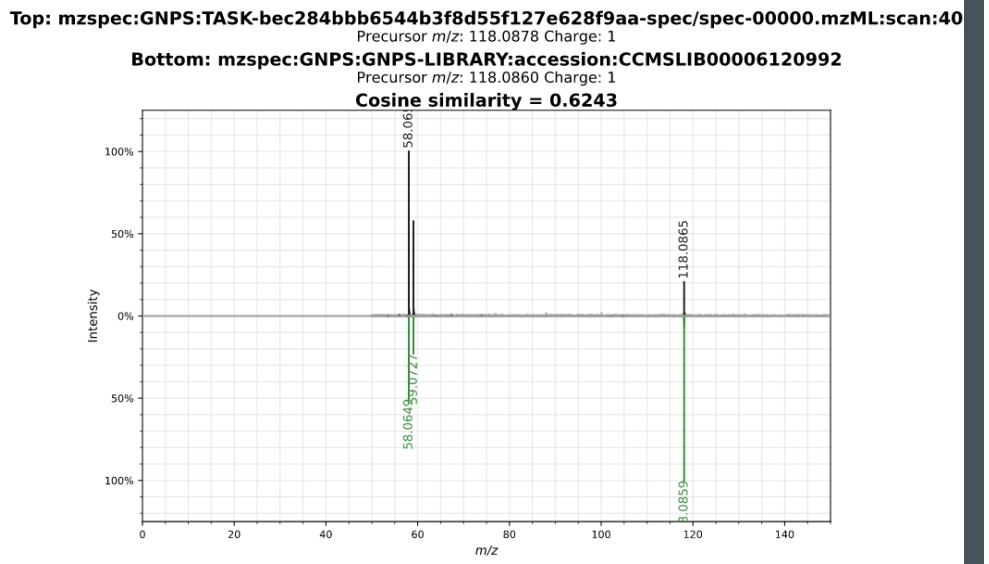


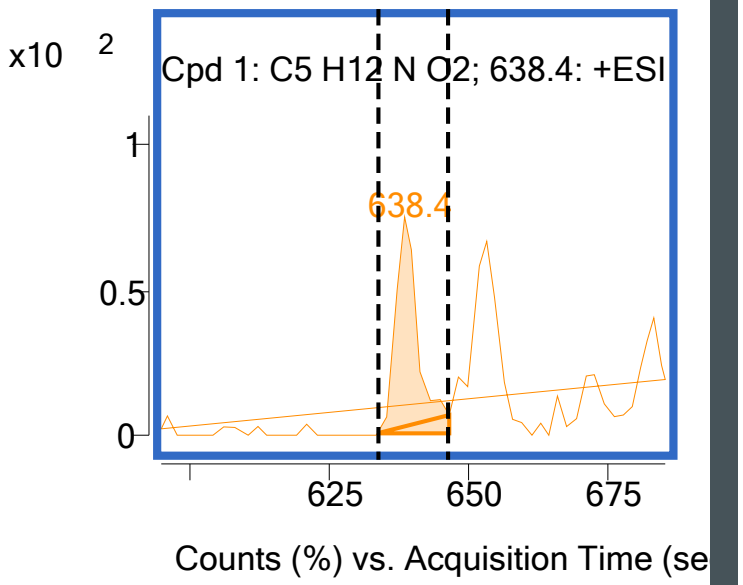


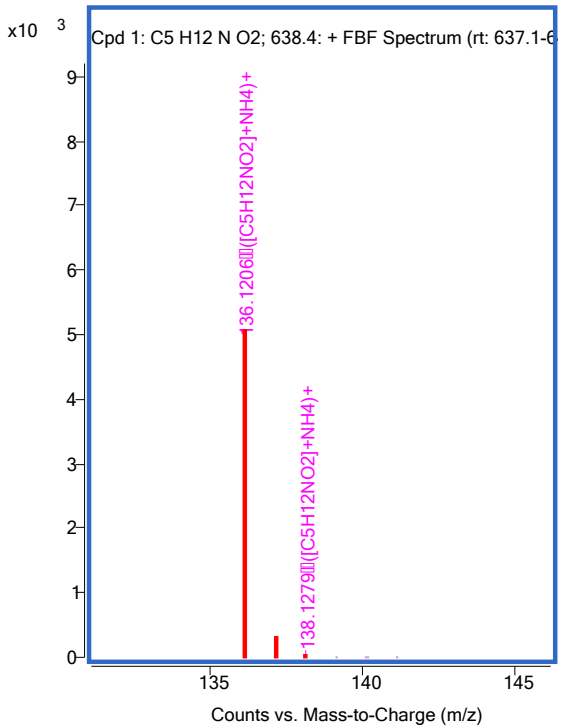


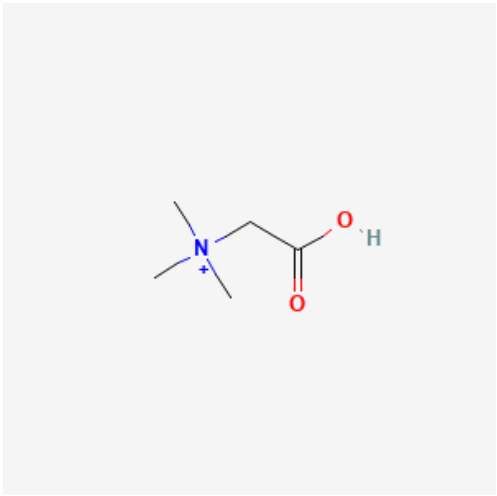
CLOPIDOGREL CARBOXYLIC ACID 144457-28-3

- ?



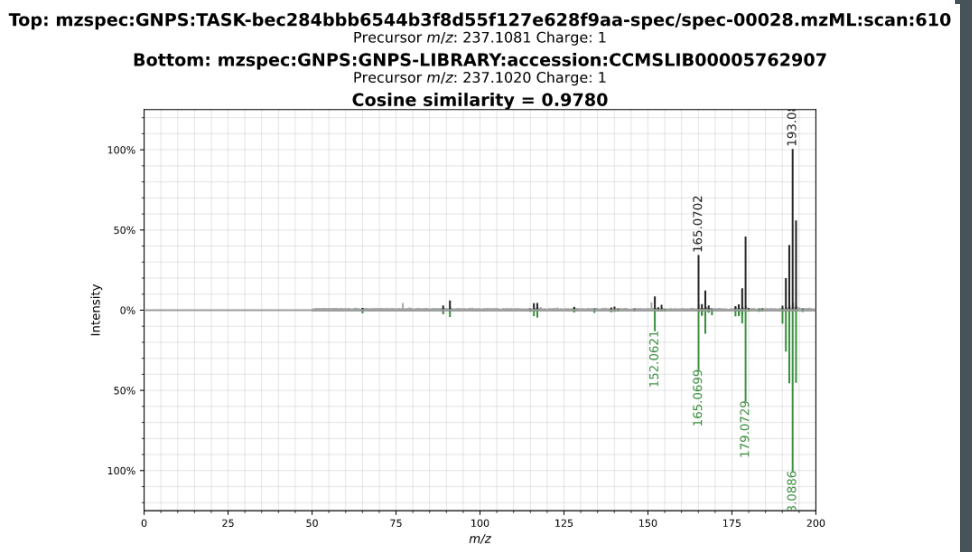


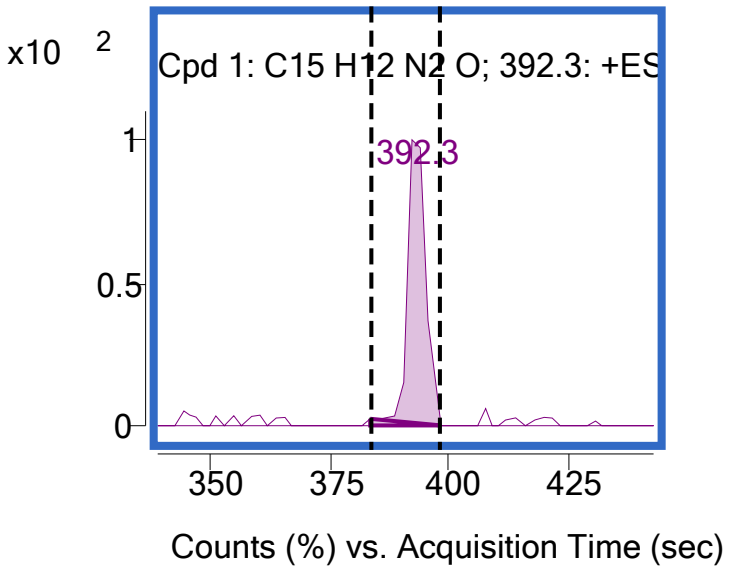


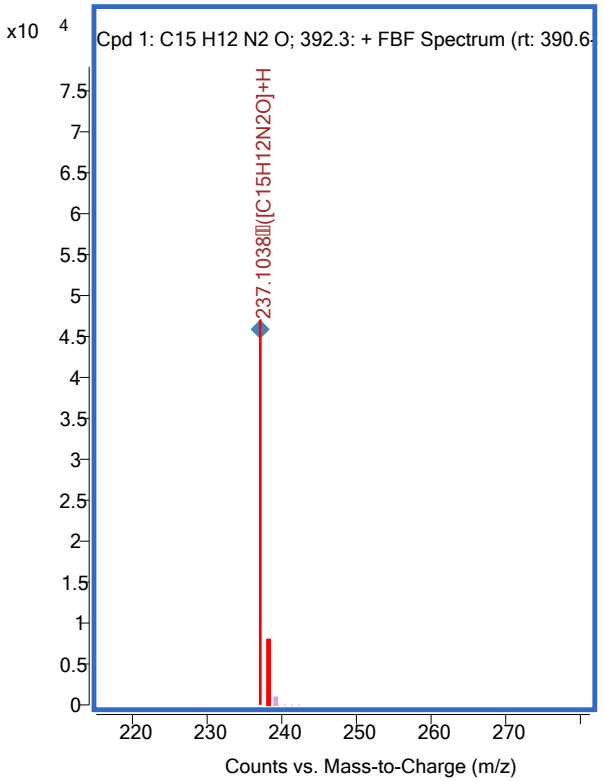


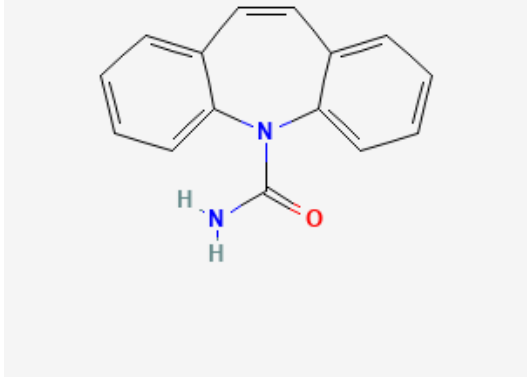
TRIMETHYL GLYCINE 6640-00-2

- gastrointestinal and lipotropic agent



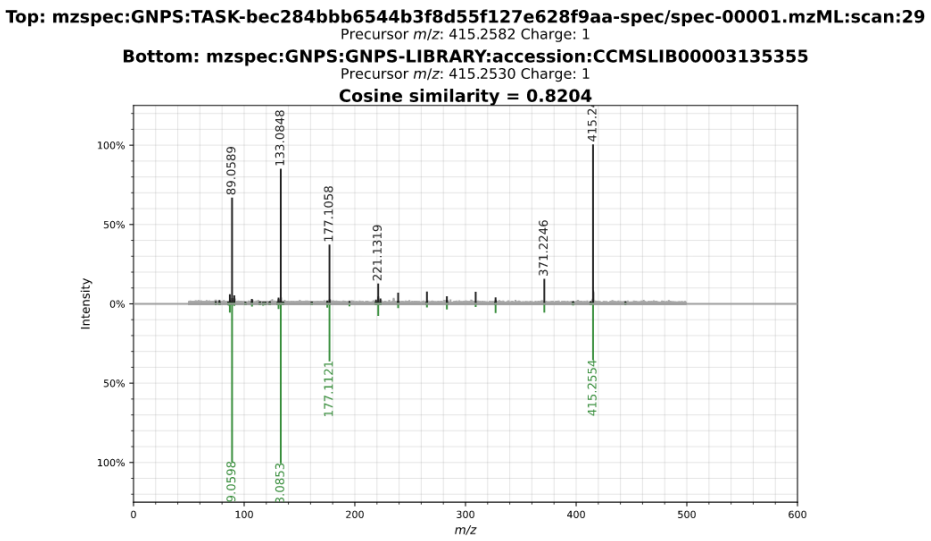


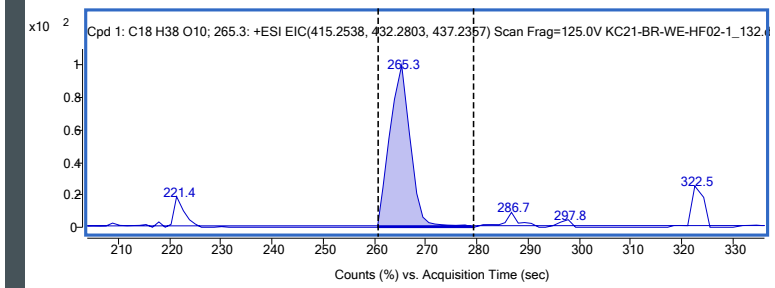


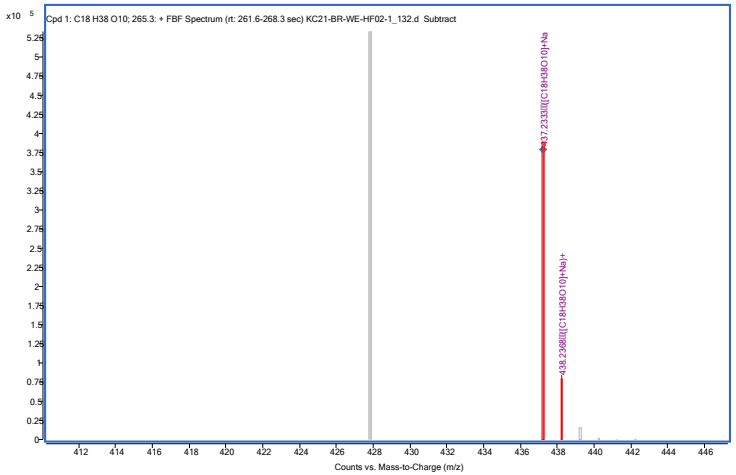


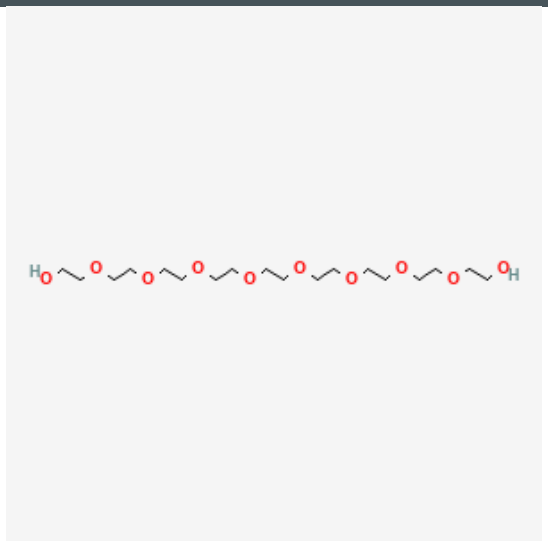
CARBAMAZEPINE 298-46-4

ANTIEPILEPTIC DRUG



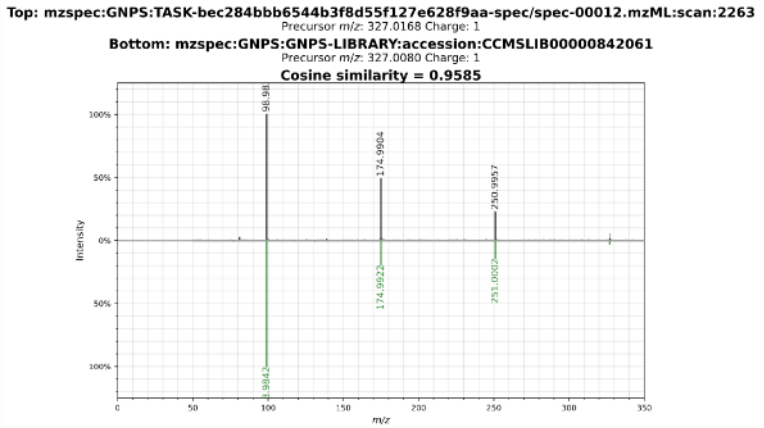


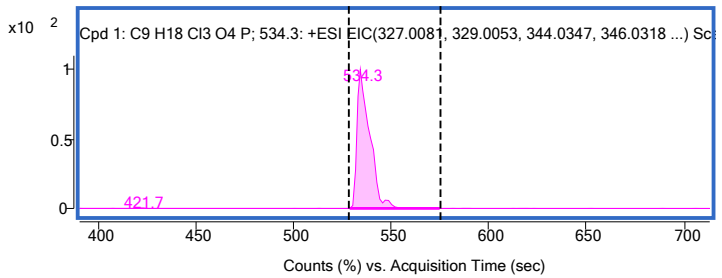


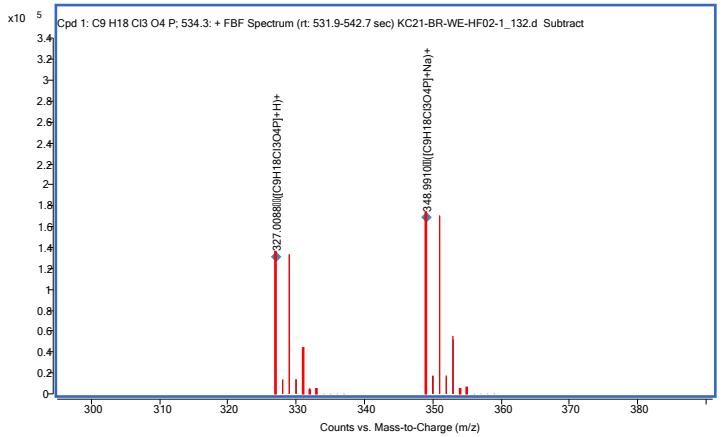


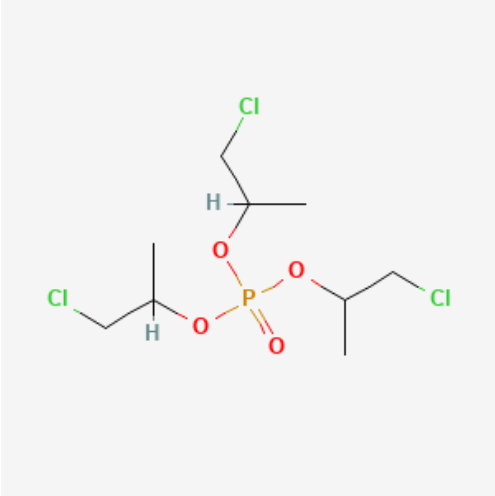
NONAETHYLENE GLYCOL

- processing aid and additive



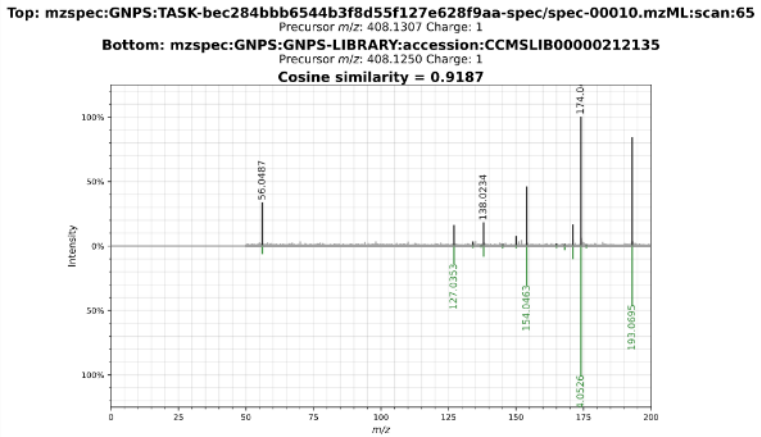


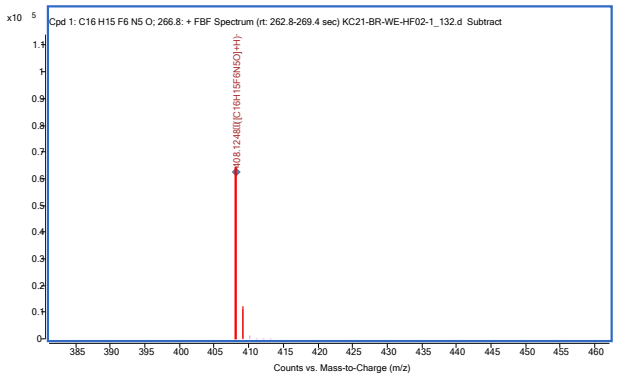


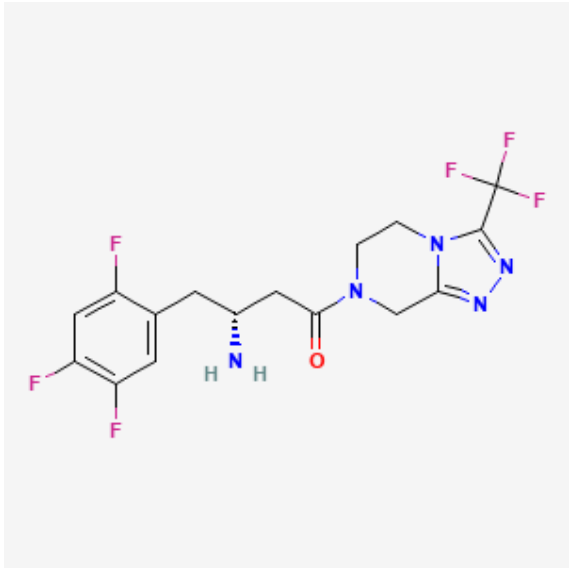


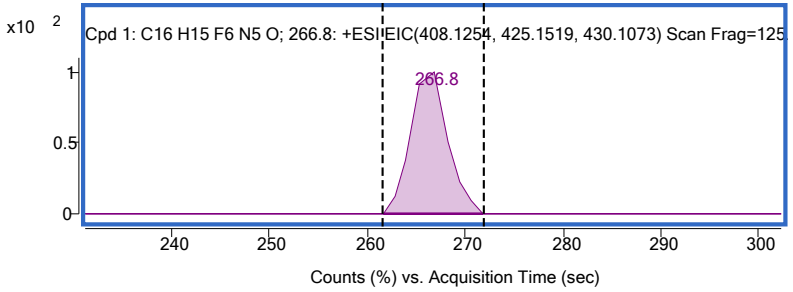
TRIS(1-CHLORO-2-PROPYL) PHOSPHATE 13674-84-5

- related/used for insulation



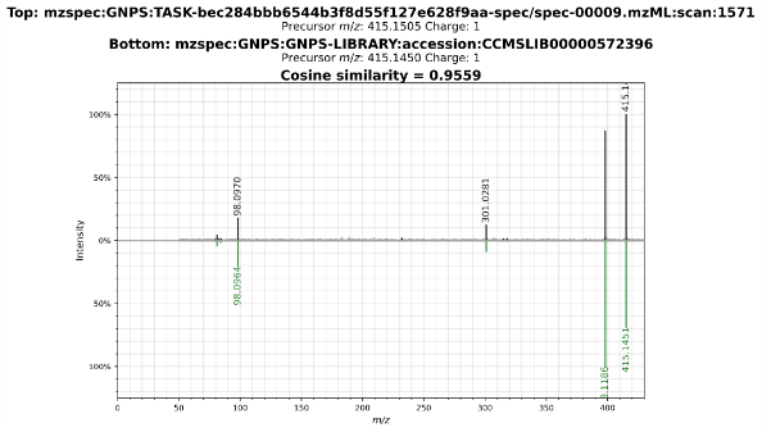


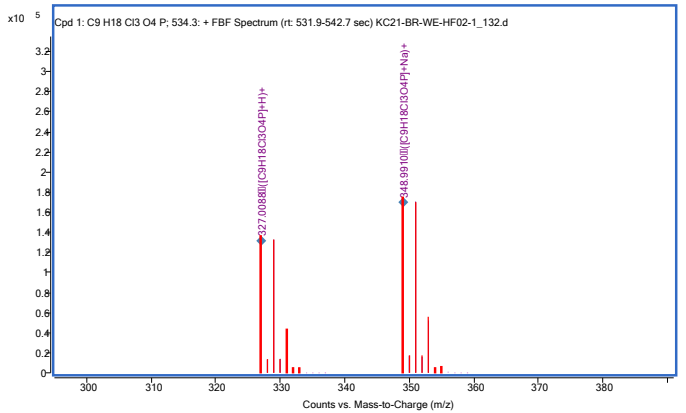


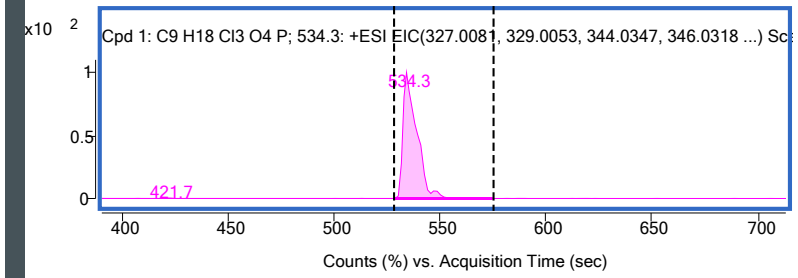


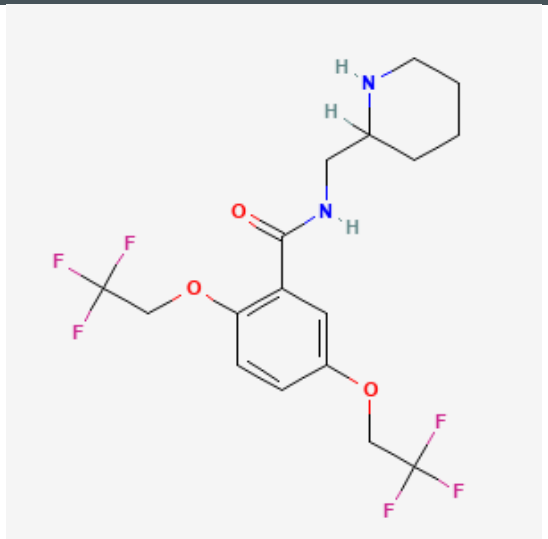
SITAGLIPTIN

- competitive, beta-amino acid-derived inhibitor of dipeptidyl peptidase 4 with hyperglycemic activity



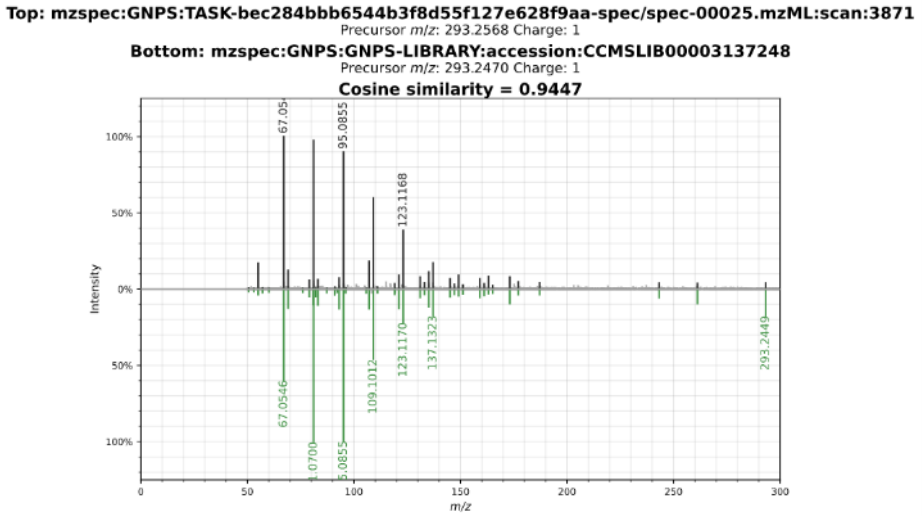


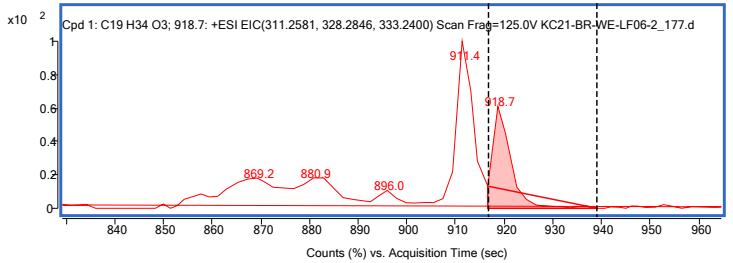


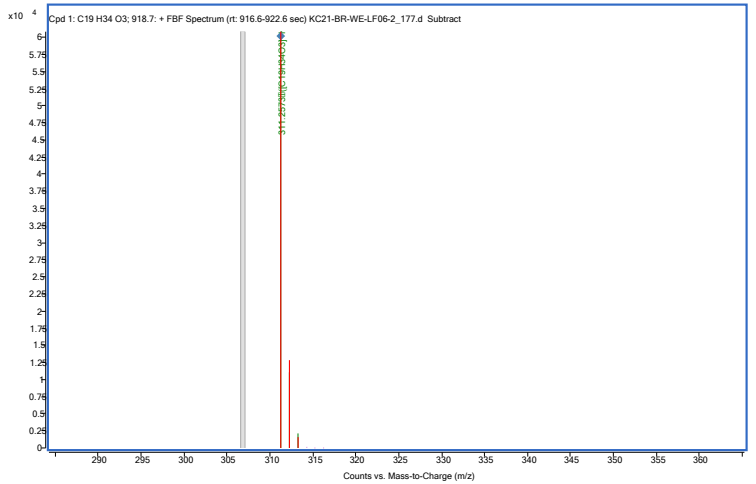


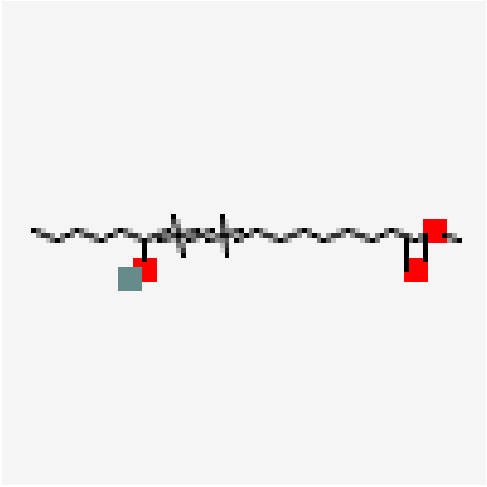
FLECAINIDE 54143-55-4

- Antiarrhythmic agent

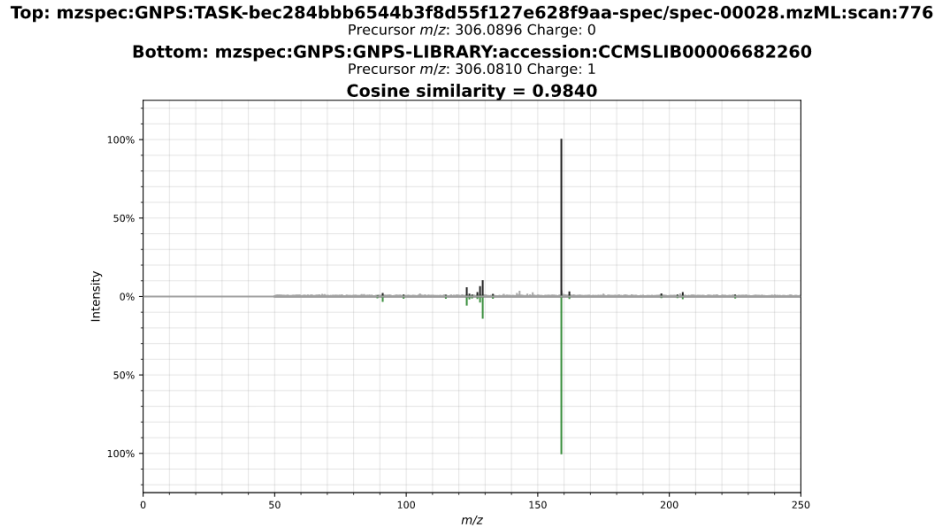


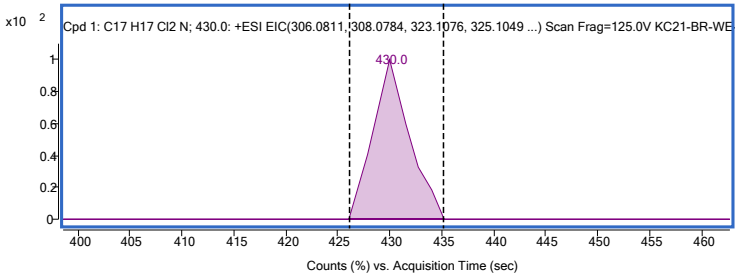


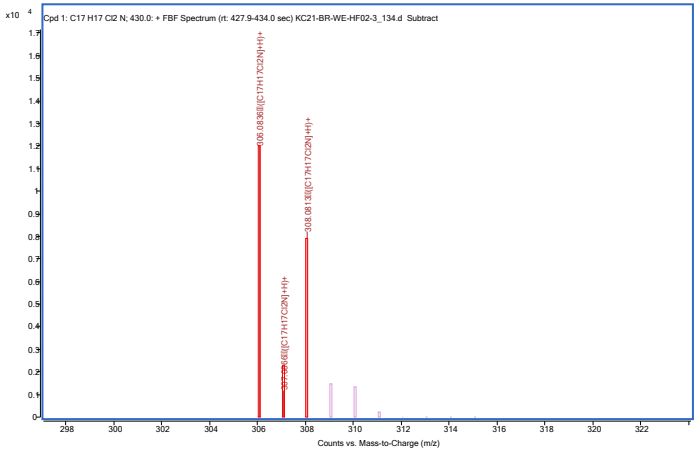


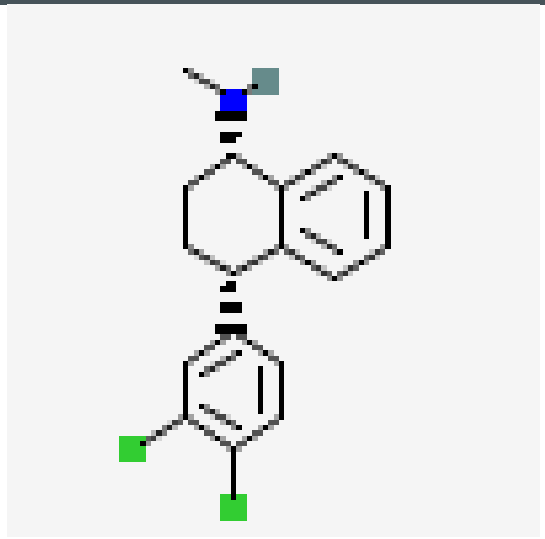


13(S)-HODE METHYL ESTER



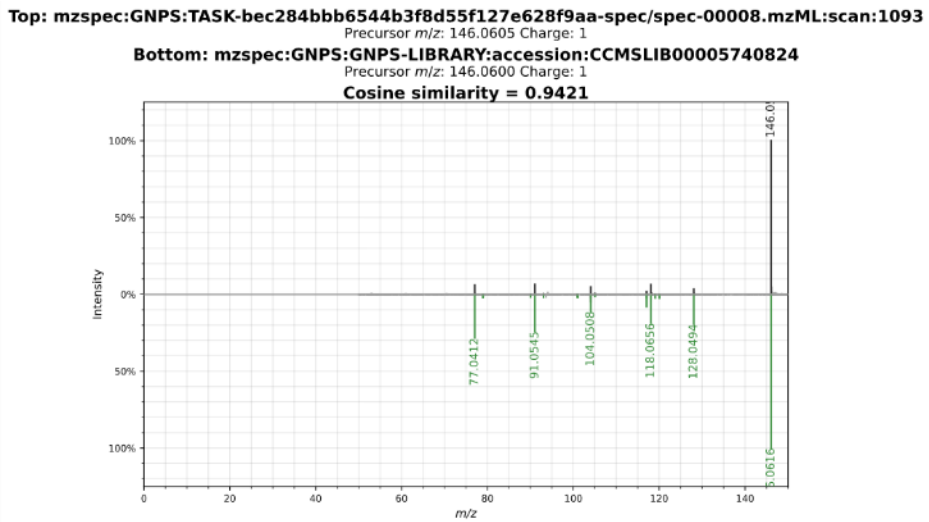


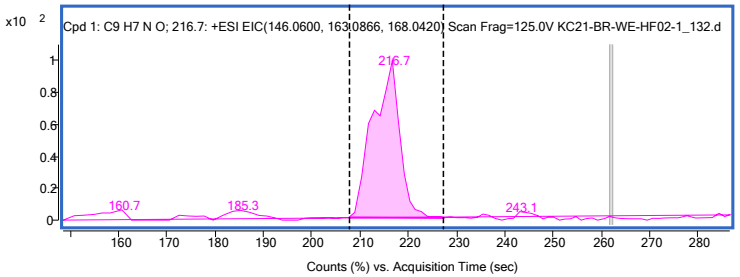


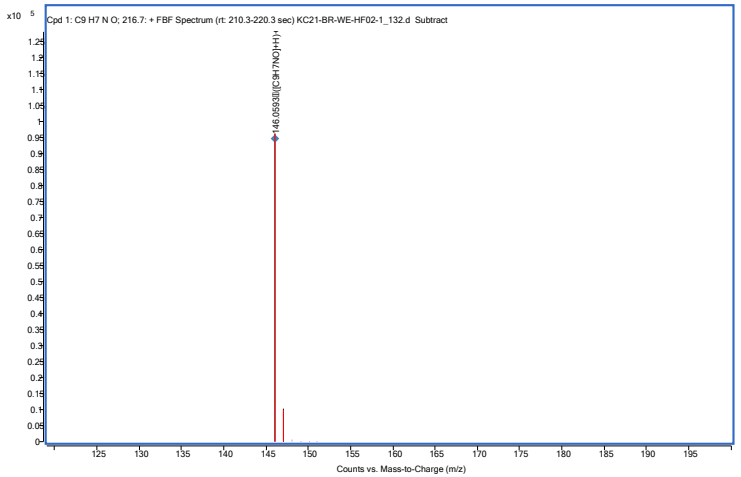


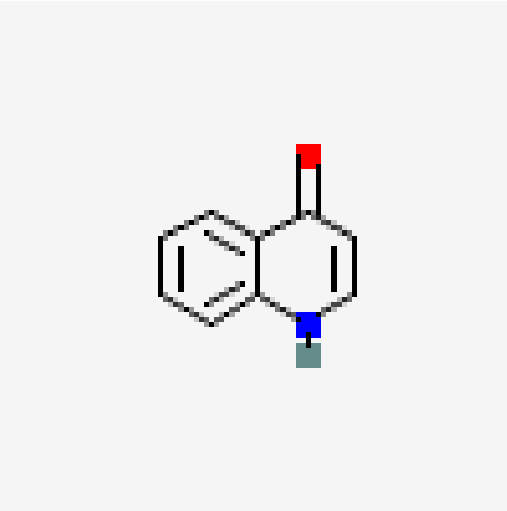
SERTRALINE

- SSRI used to treat depression, anxiety or OCD



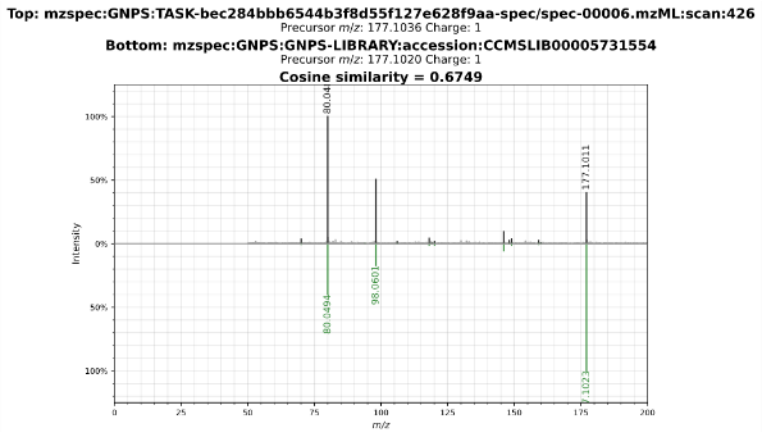


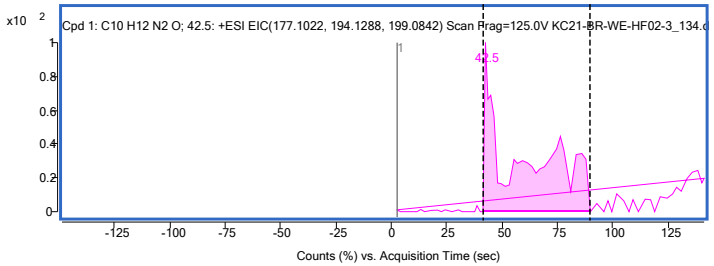


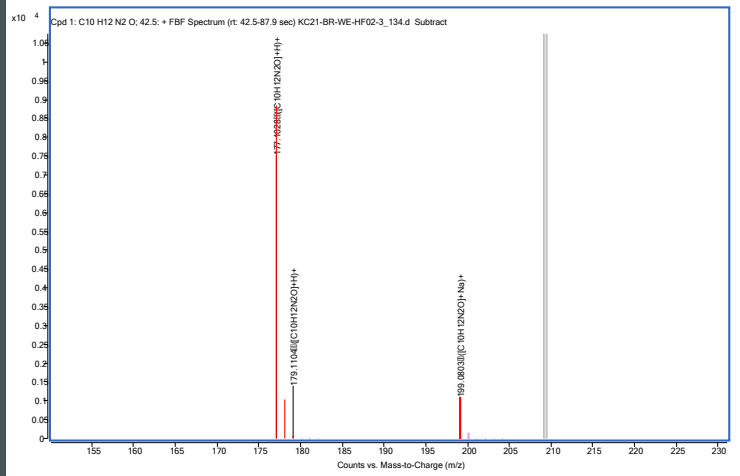


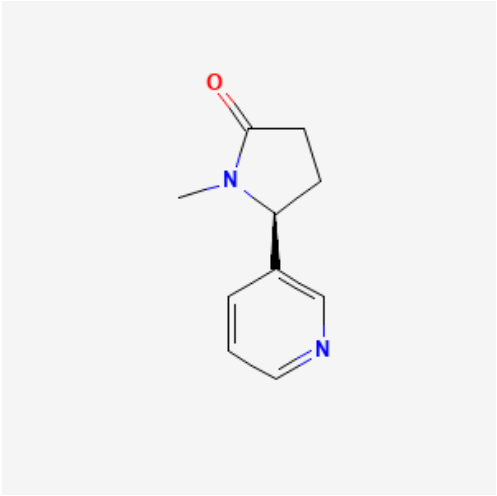
4-HYDROXYQUINOLINE

- forms the core moiety of antibacterials such as norfloxacin, nalidixic acid, ciprofloxacin and cinoxacin



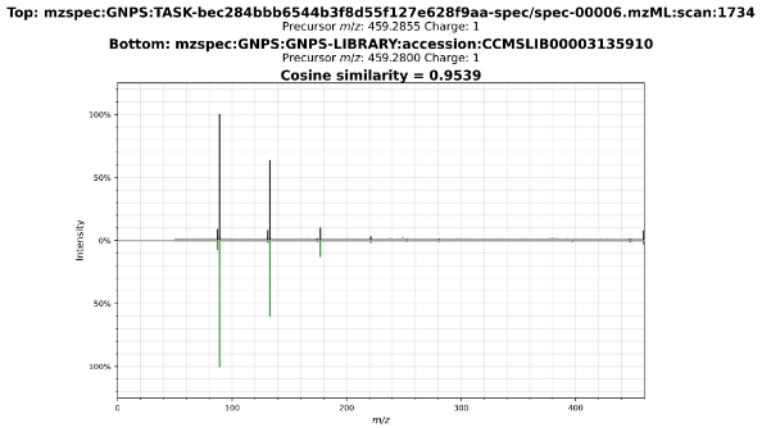


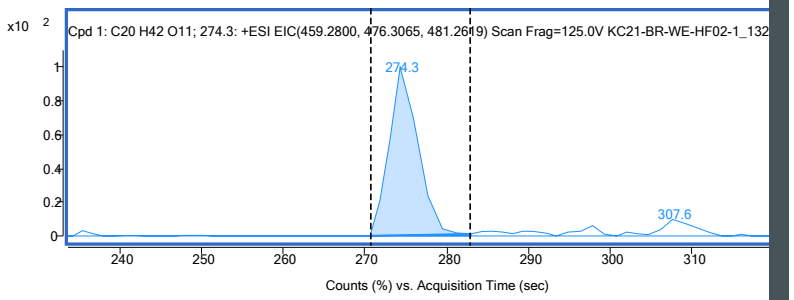


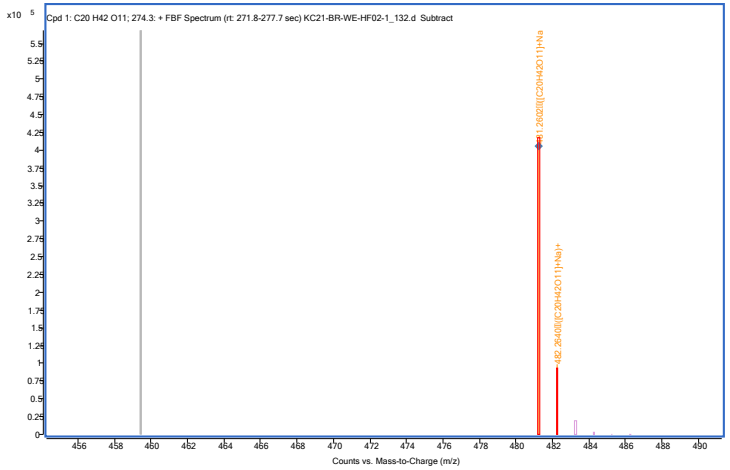


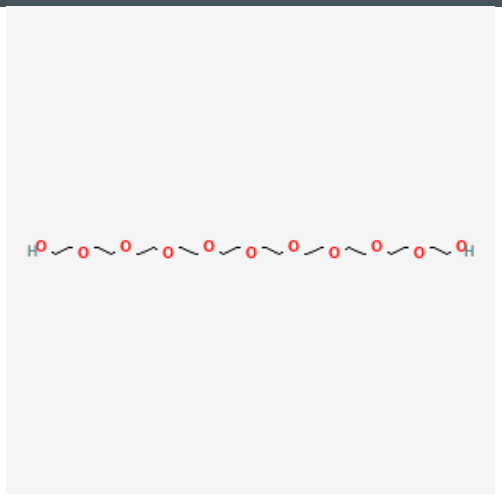
COTININE 486-56-5

- major urinary metabolite of nicotine, role as biomarker, antidepressant, and also plant metabolite



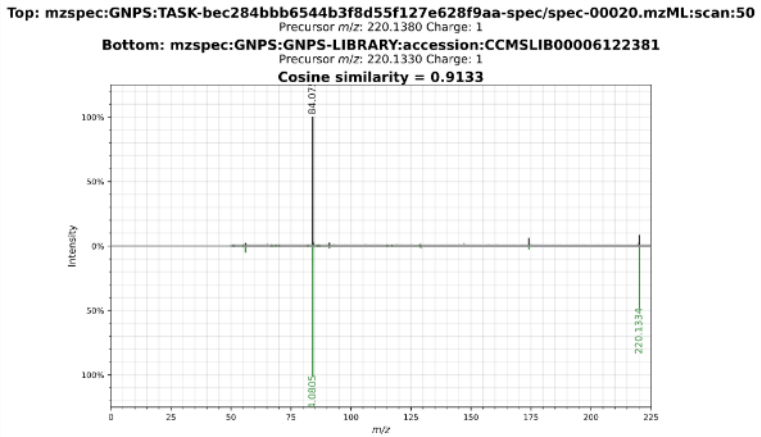


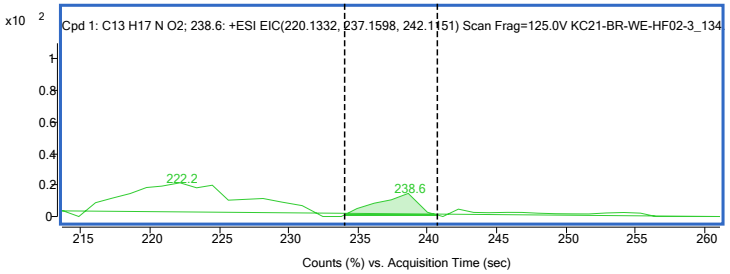


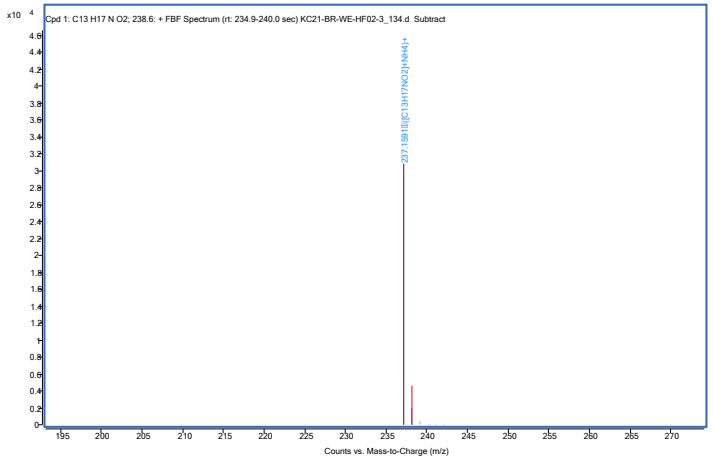


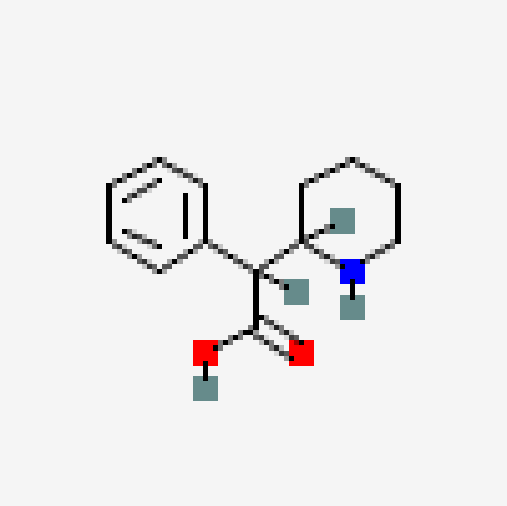
3,6,9,12,15,18,21,24,27-NONAOXANONACOSANE-1,29-DIOL

- Household & Commercial/Institutional Products- personal care; Processing Aids and Additives



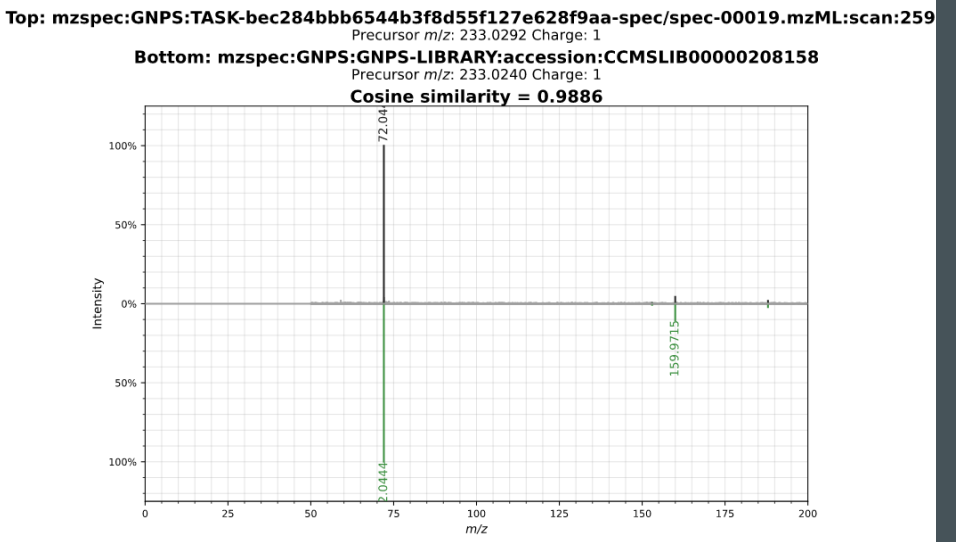


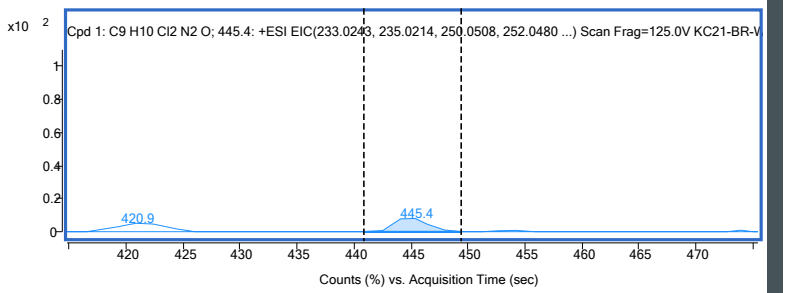


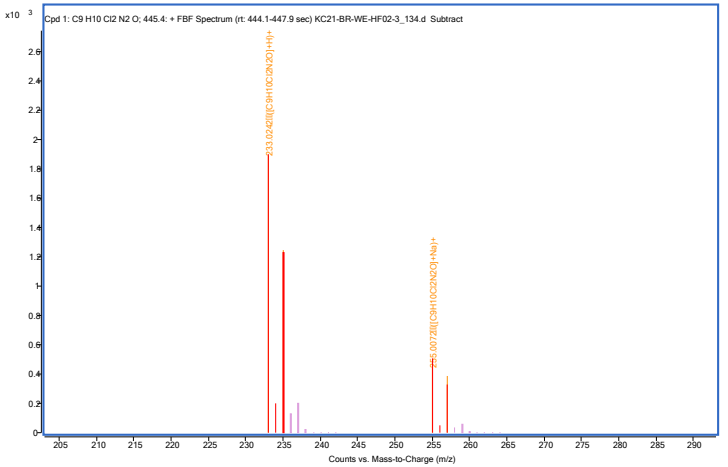


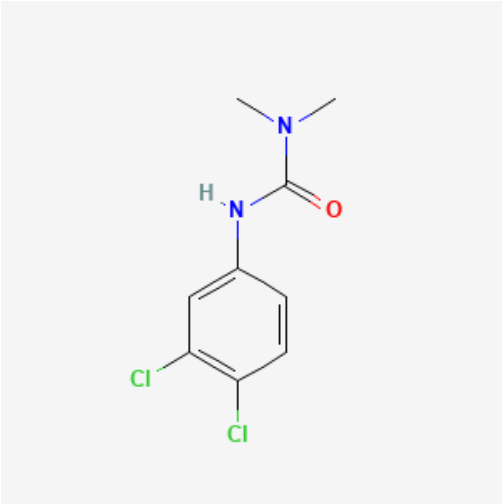
RITALINIC ACID 19395-41-6

- metabolite of methylphenidate



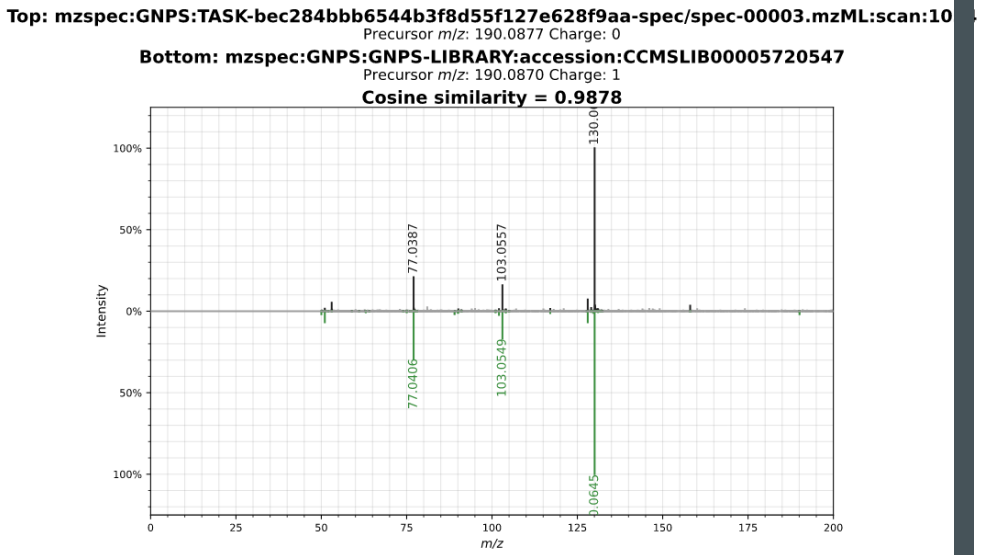


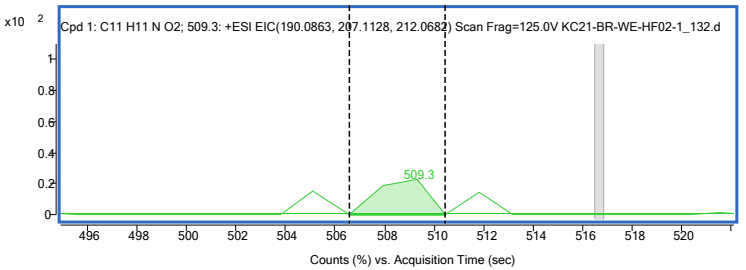


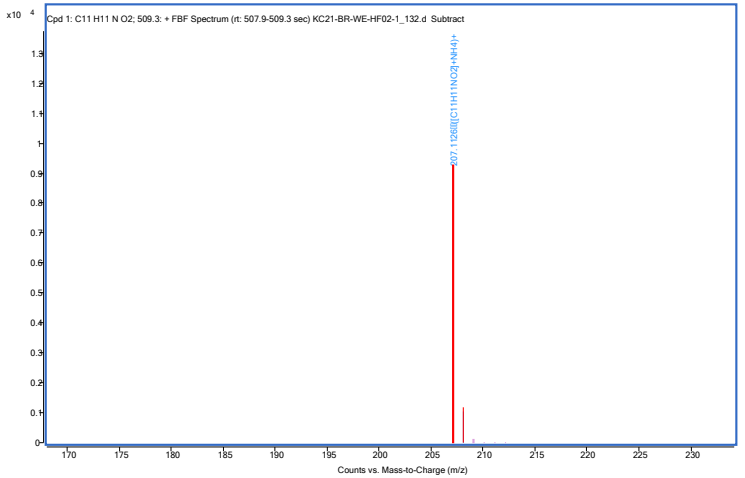


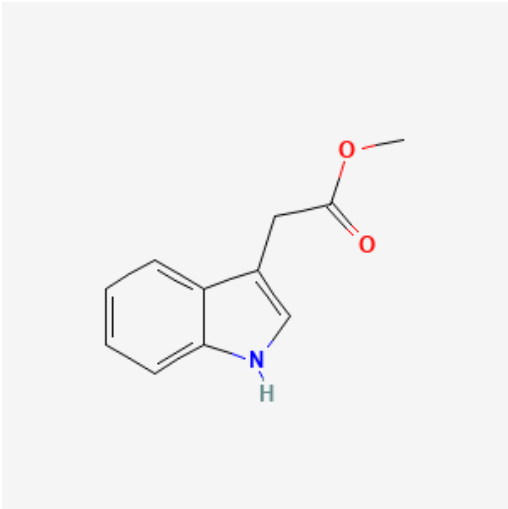
DIURON

- herbicide (main), adhesive and selant chemical, and paint additive



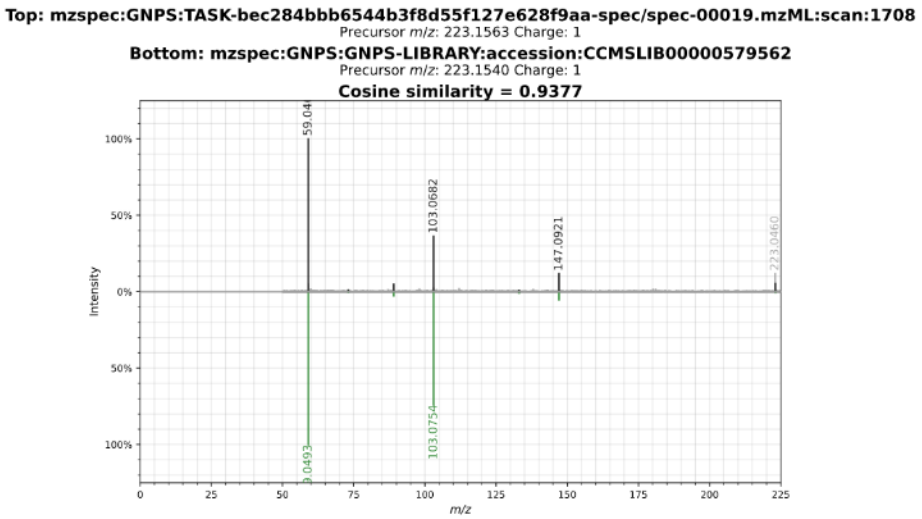


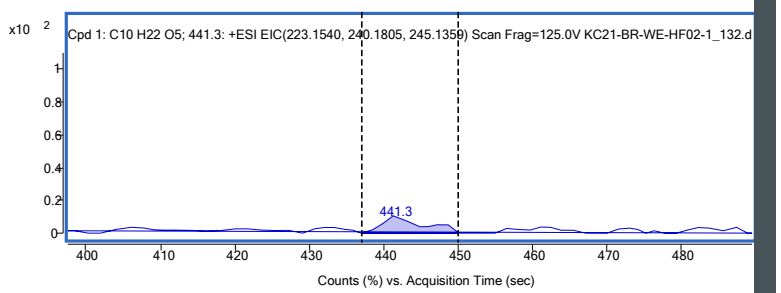


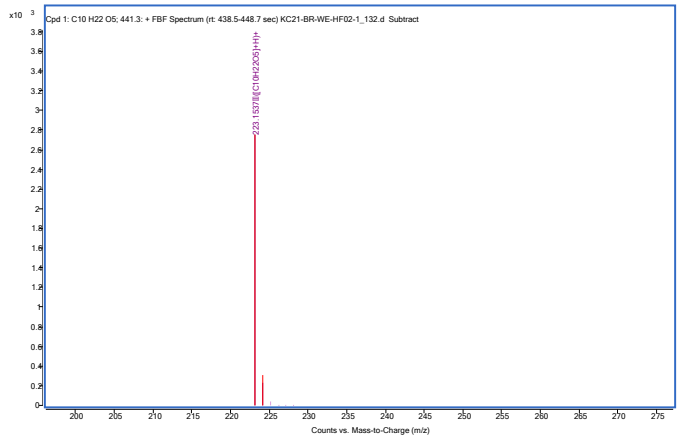


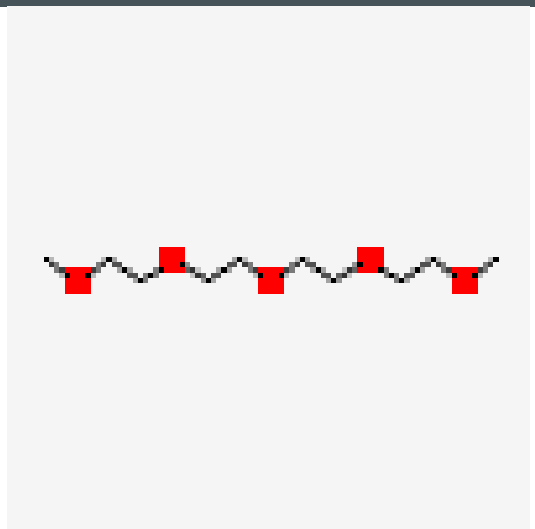
METHYL 2-(1H-INDOL-3-YL)ACETATE

- antineoplastic agent (cancer treatment), and metabolite



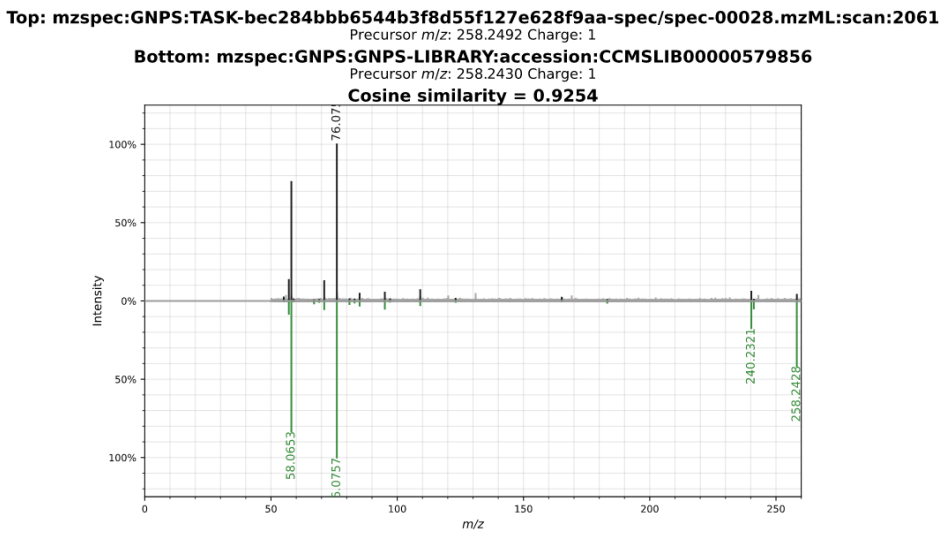


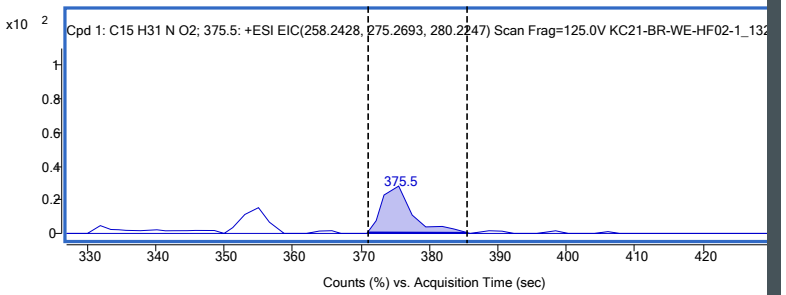


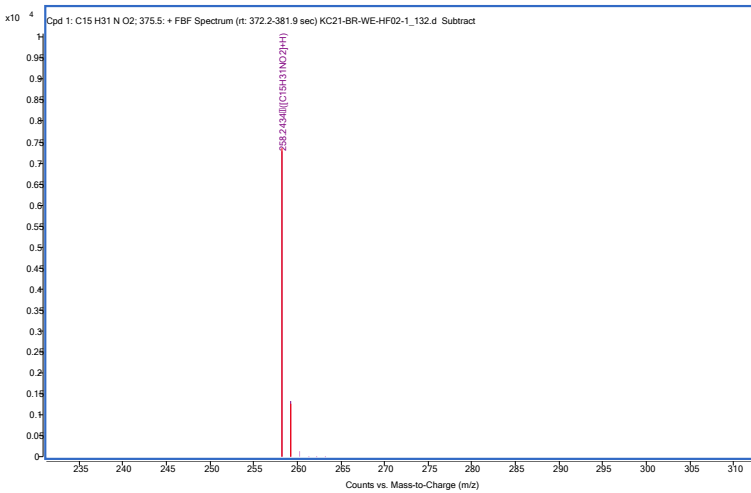


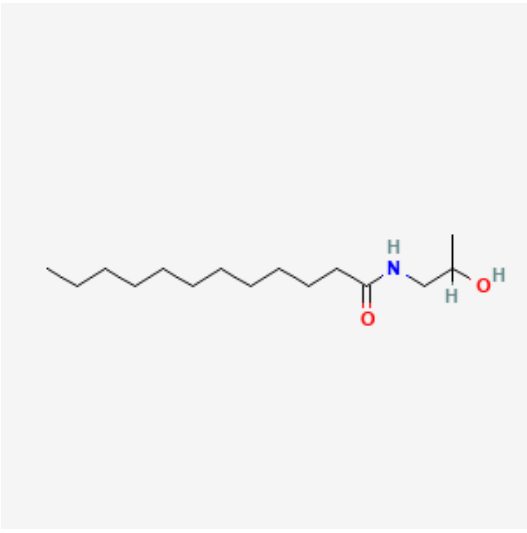
TETRAGLYME 143-24-8

- solvent, lubricant, colorant & fuel additive



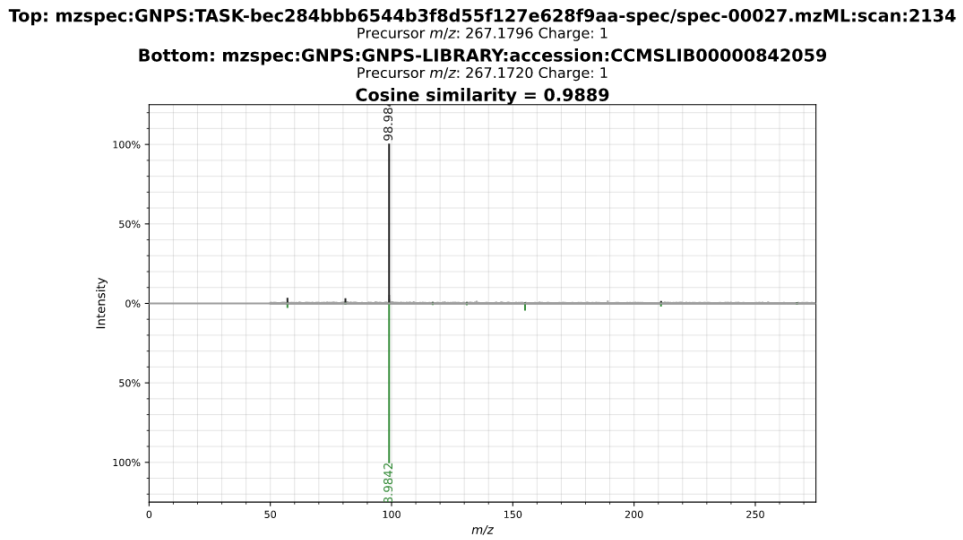


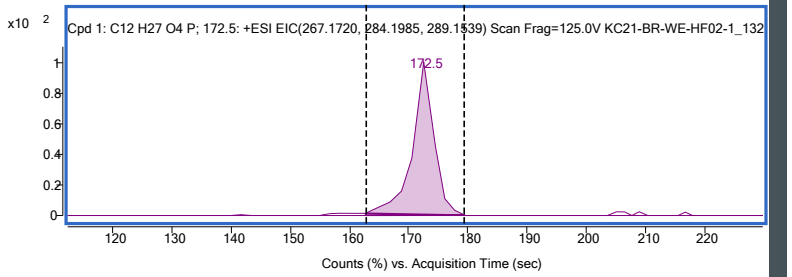


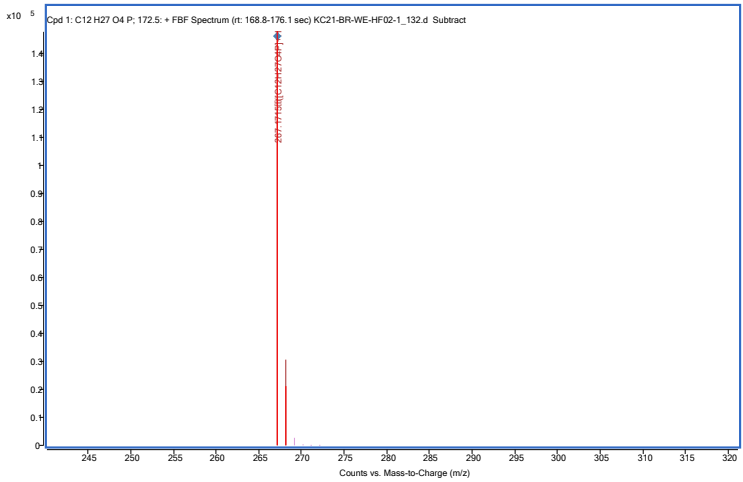


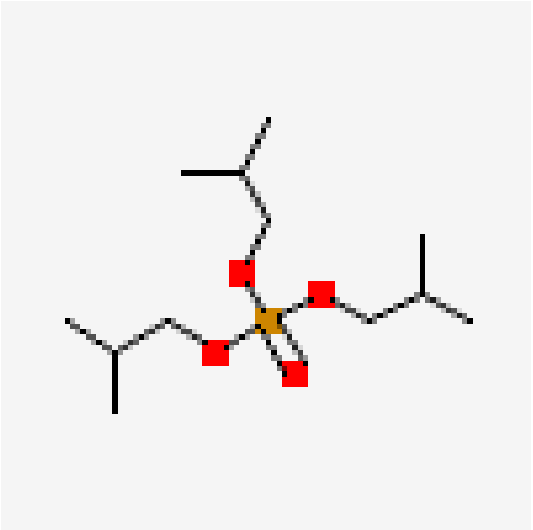
N-(2-HYDROXYPROPYL)DODECANAMIDE 142-54-1

- cosmetics, surfactant, and foam boosting



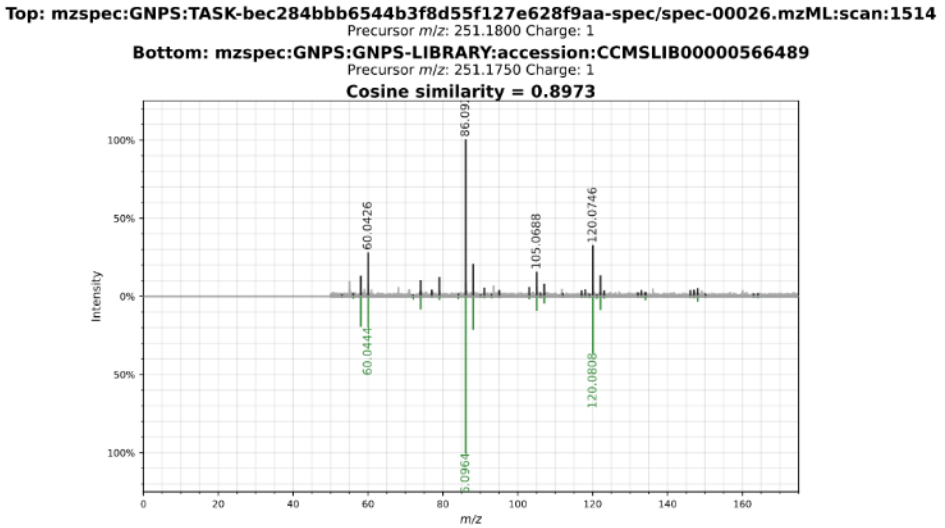


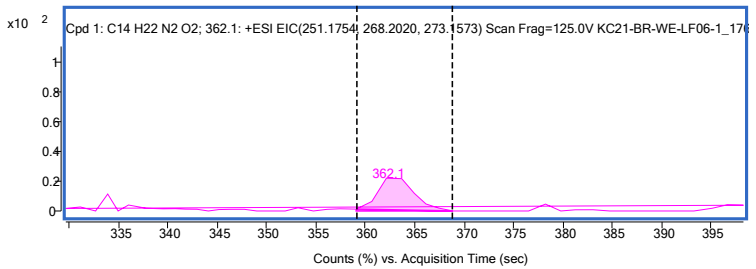


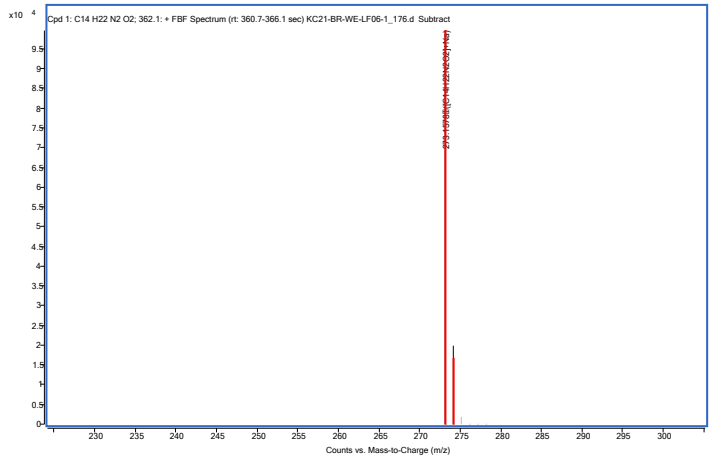


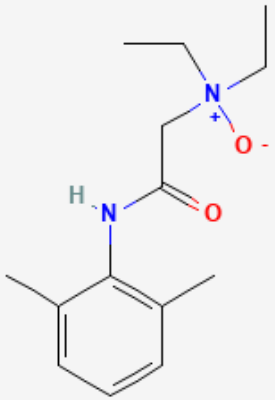
TRIISOBUTYL PHOSPHATE 126-71-6

- anti-foaming agent



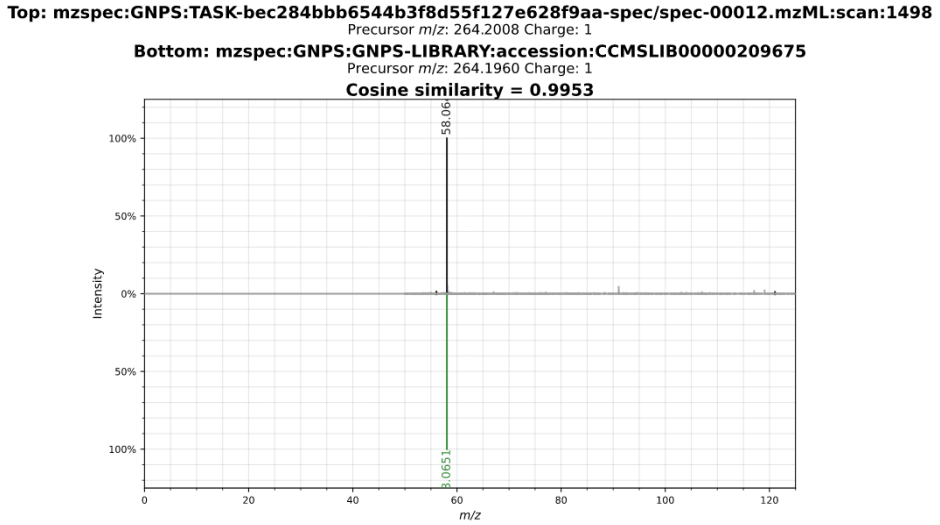


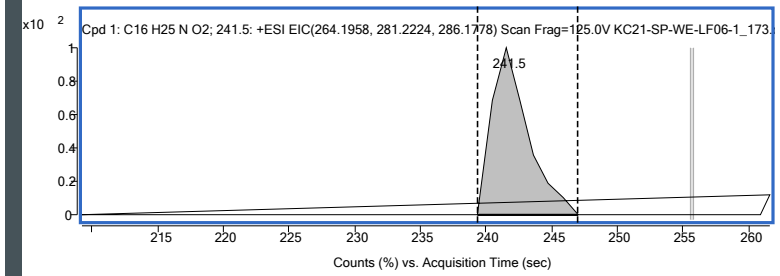


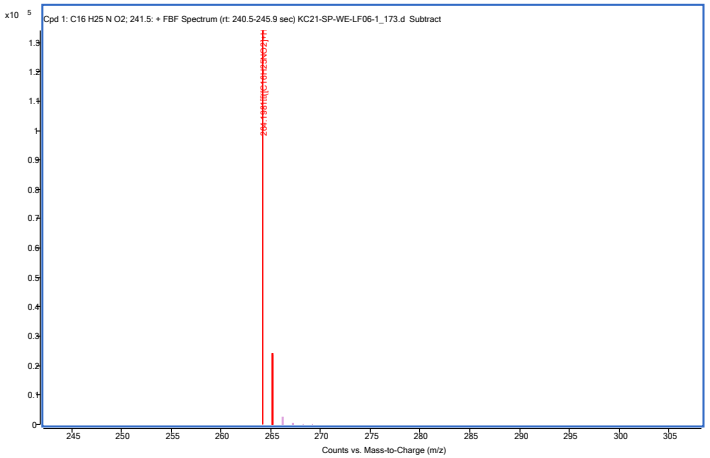


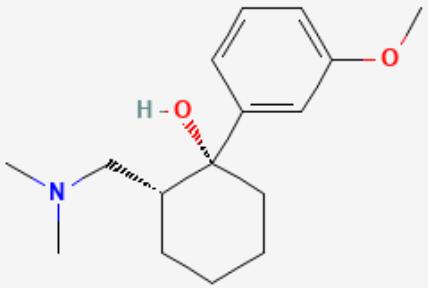
LIDOCAINE N-OXIDE 2903-45-9

TRANSFORMATION PRODUCT OF LIDOCAINE



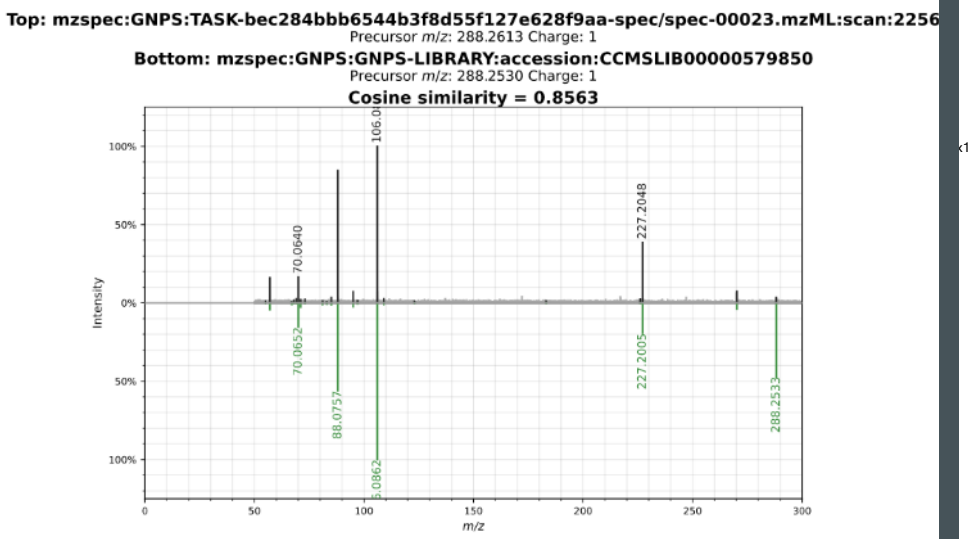


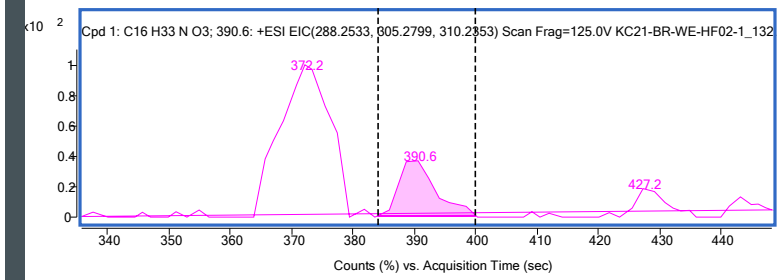


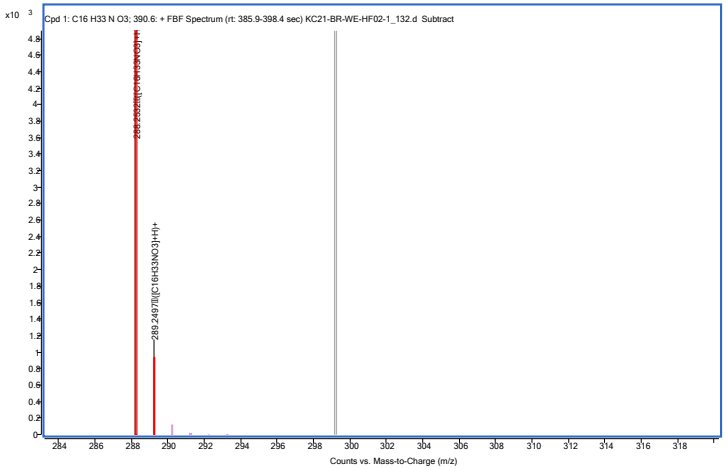


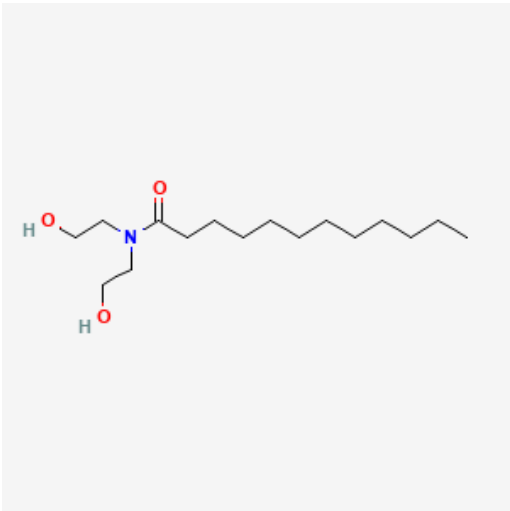
TRAMADOL

USED FOR MANAGEMENT OF MODERATE TO SEVERE PAIN IN ADULTS



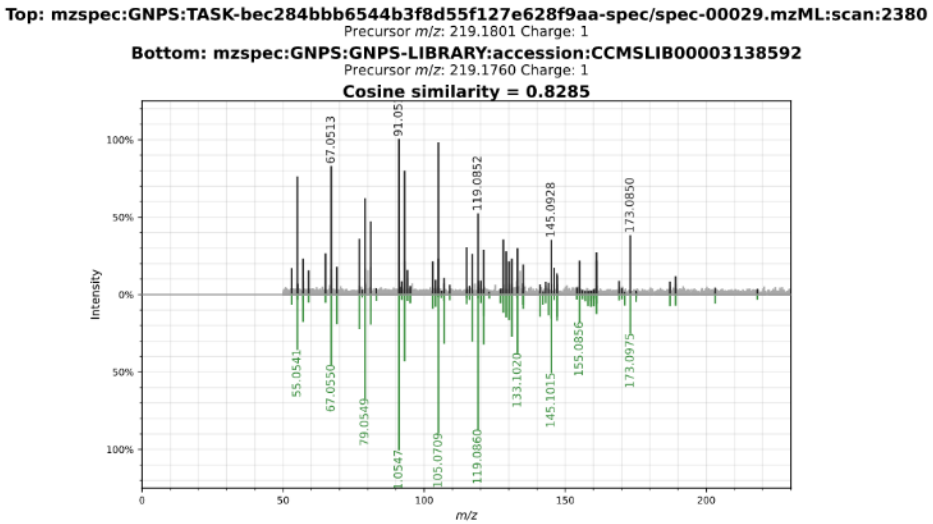


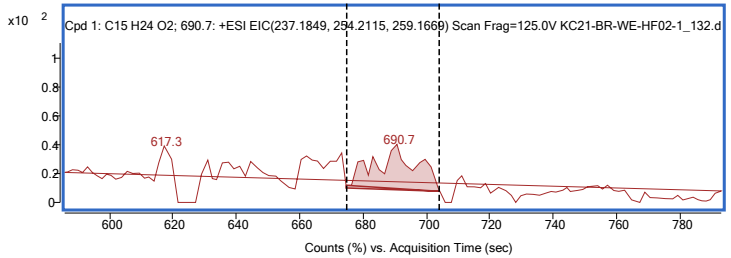


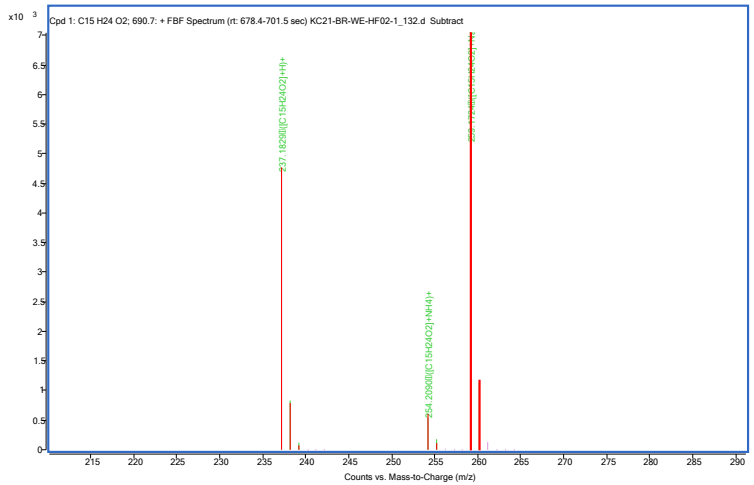


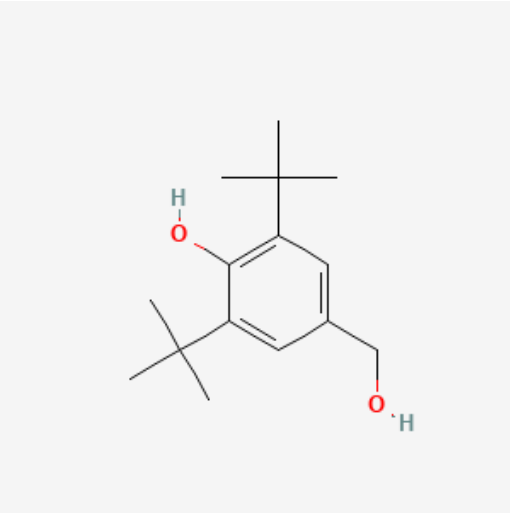
N,N-BIS(2-HYDROXYETHYL DODECANAMIDE

- foam stabilizer



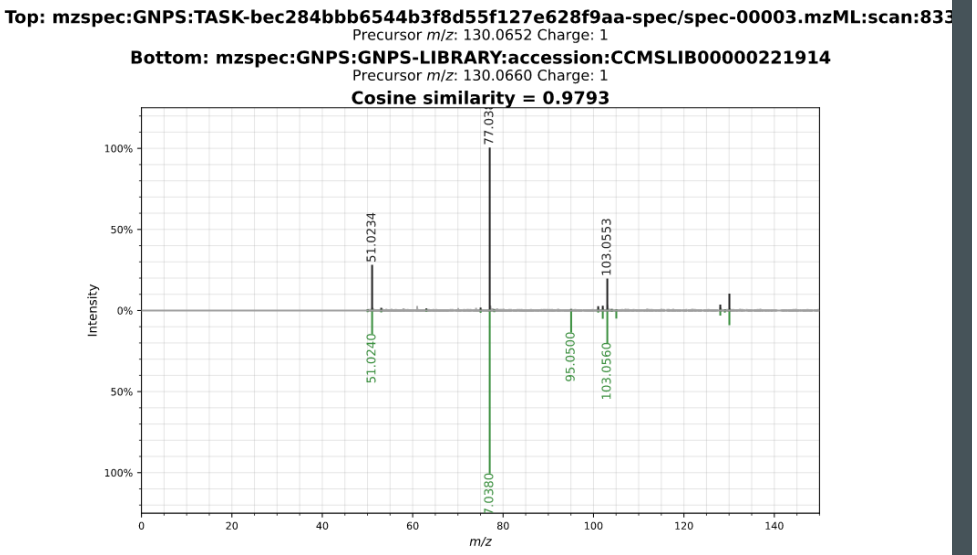


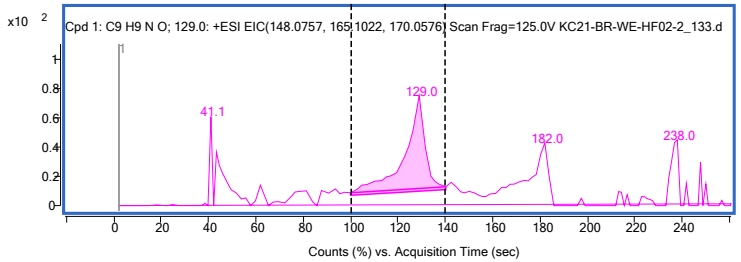


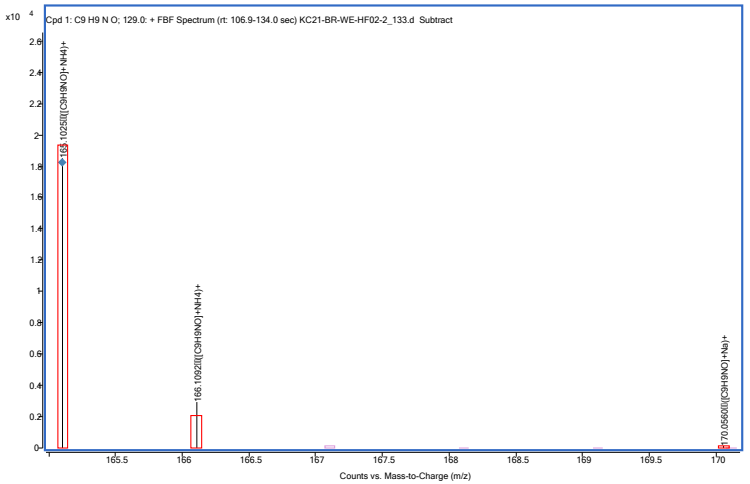


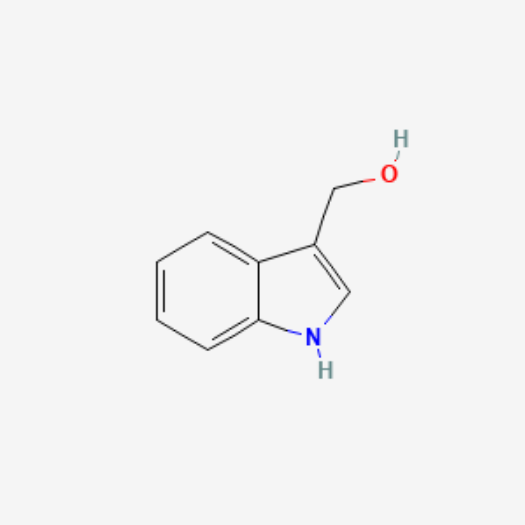
3,5-DI-TERT-BUTYL-4-HYDROXYBENZYL ALCOHOL 88-26-6

- antioxidant



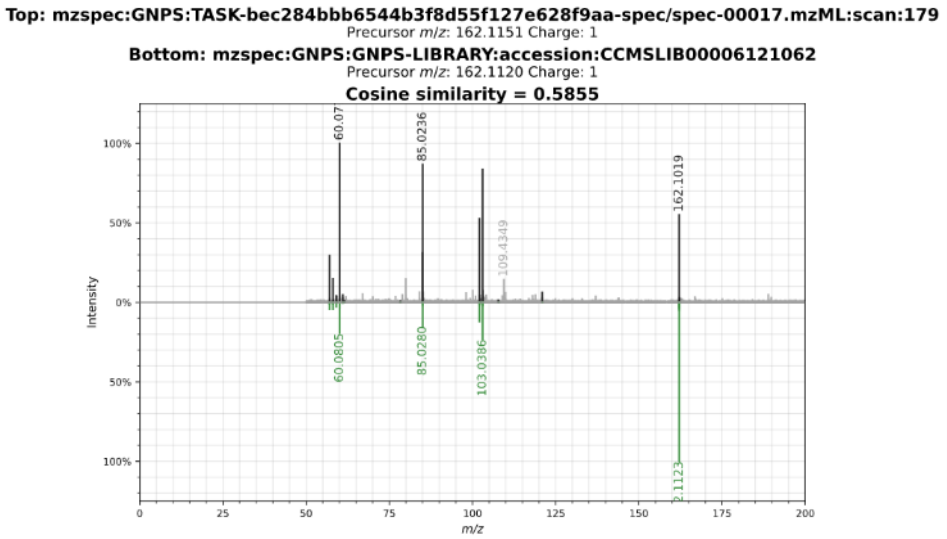


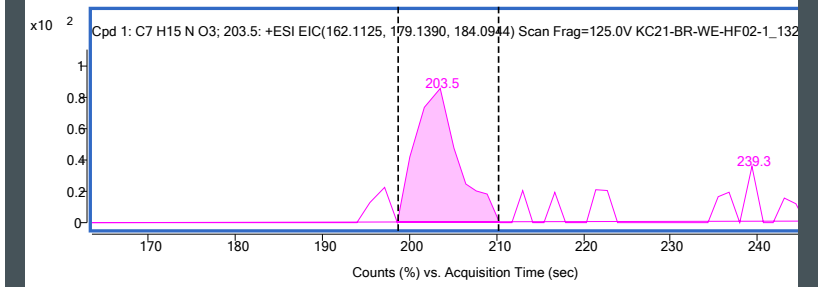


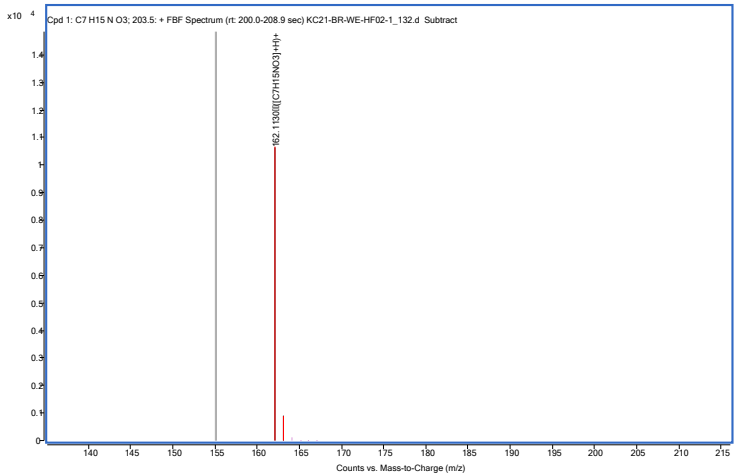


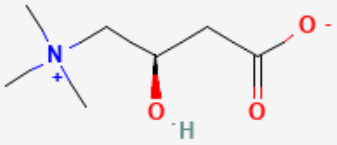
INDOLE-3-CARBINOL 700-06-1

Anticarcinogenic Agents



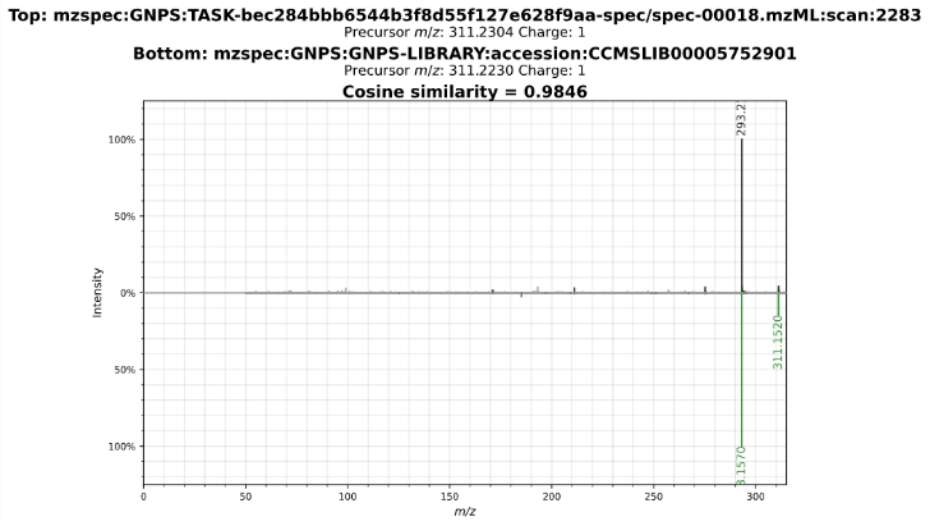


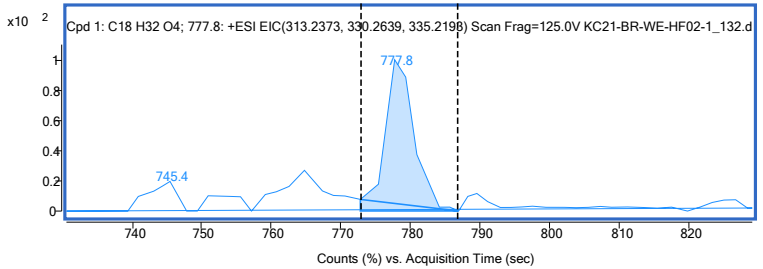


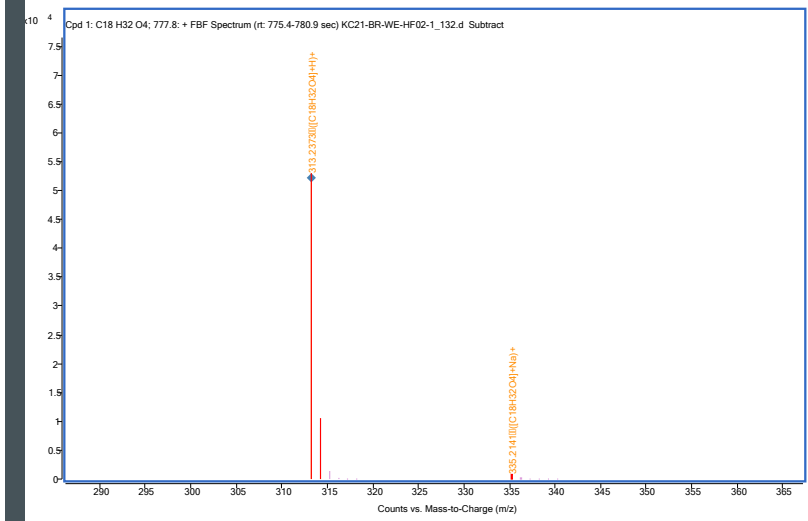


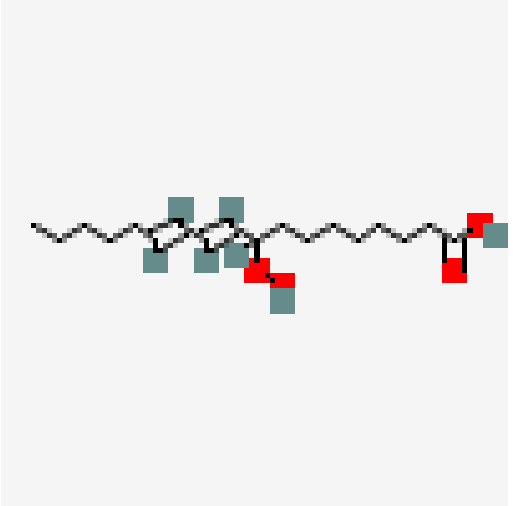
LEVOCARNITINE 541-15-1

- printer ink and toner, antistatic agent and cleaning. Used to stimulate gastric and pancreatic secretions

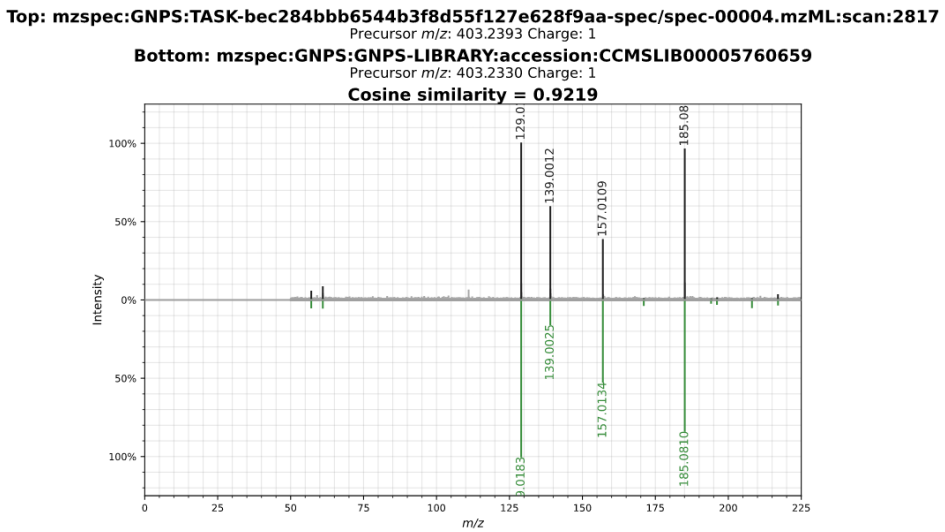


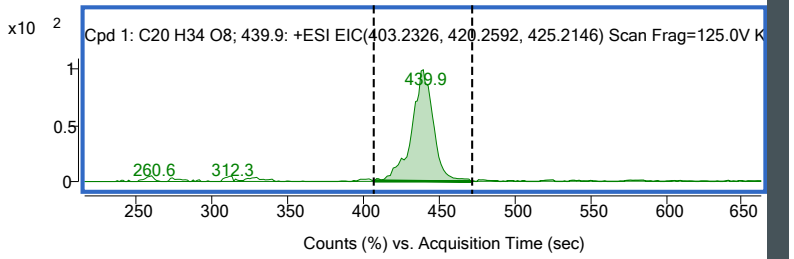


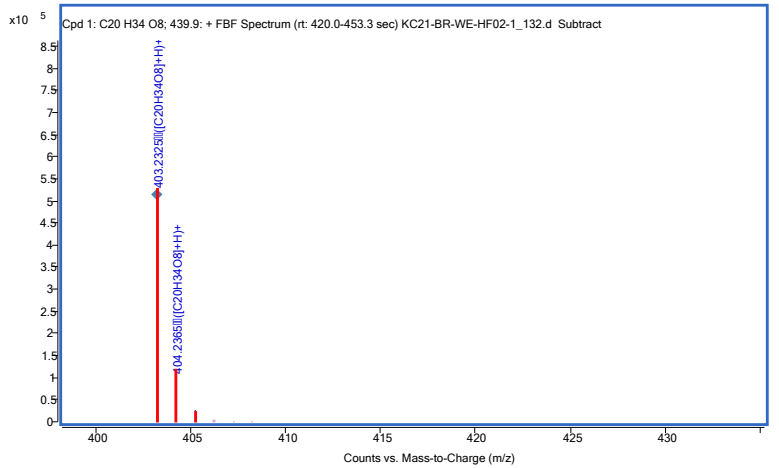


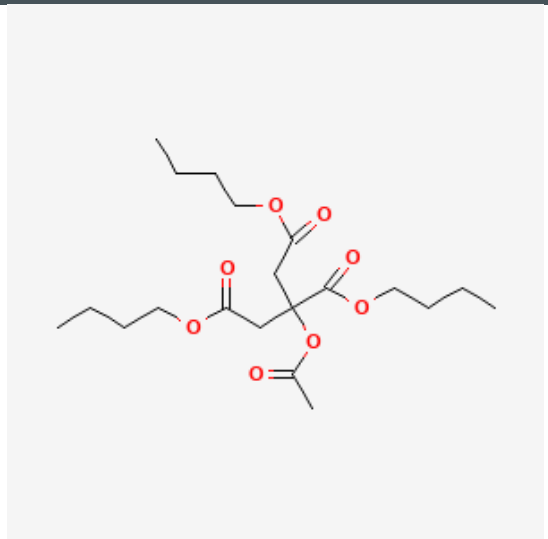


9-HPODE 63121-49-3



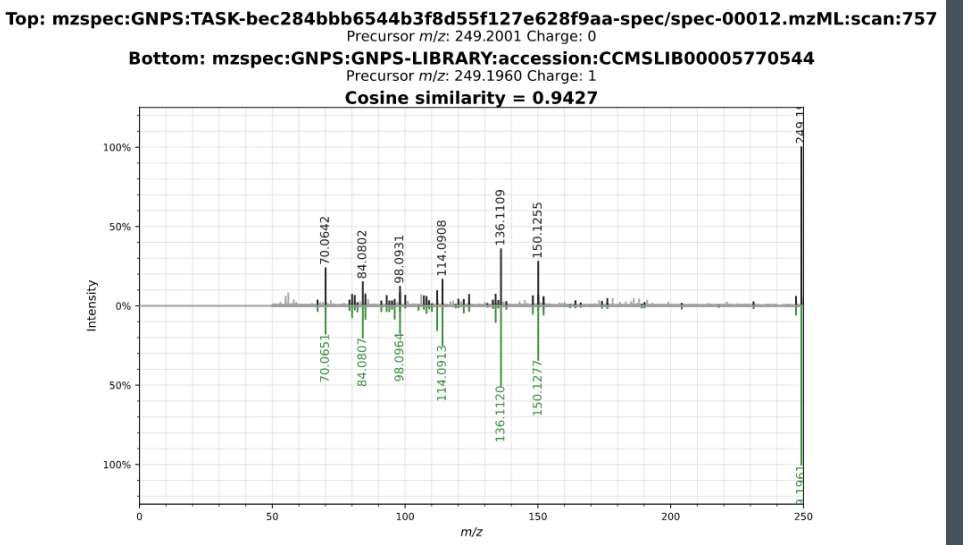


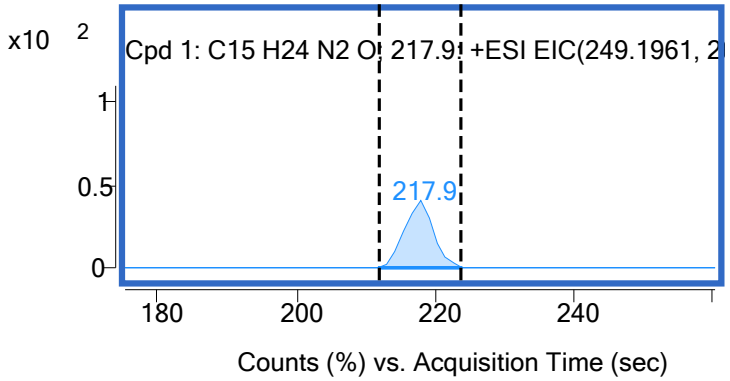


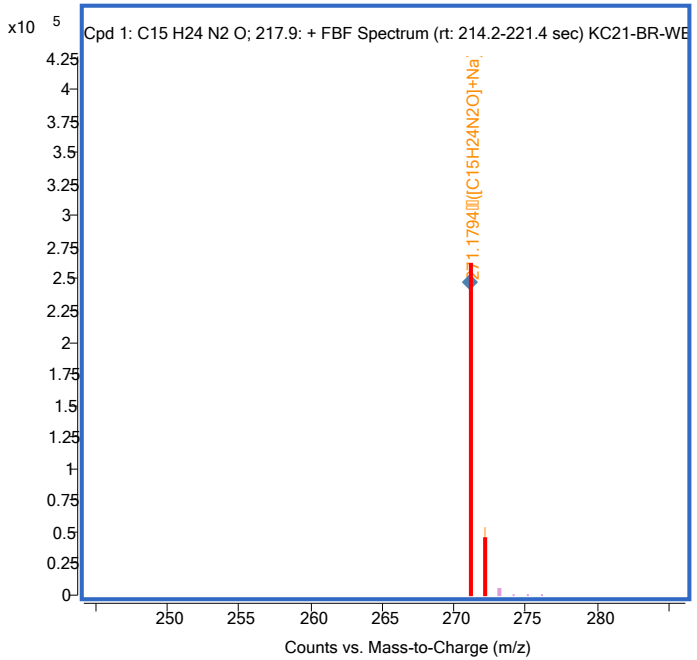


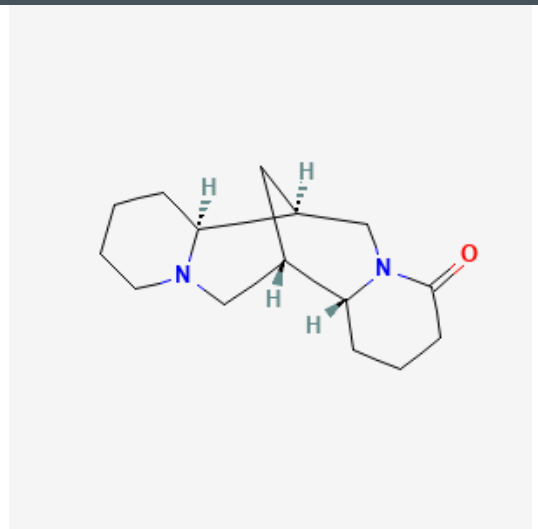
ACETYL TRIBUTYL CITRATE 77-90-7

- Flavoring agent, plasticizer, and to make food safe coatings



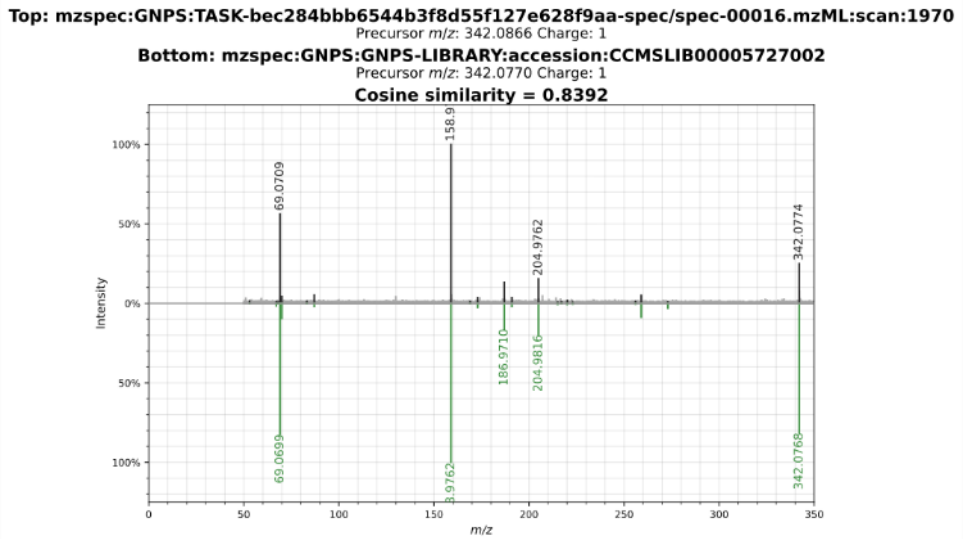


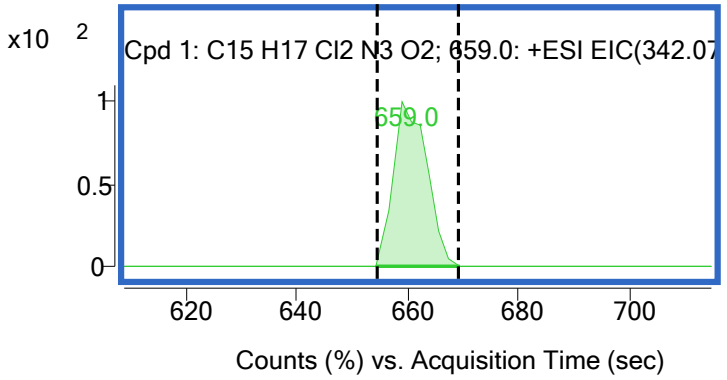


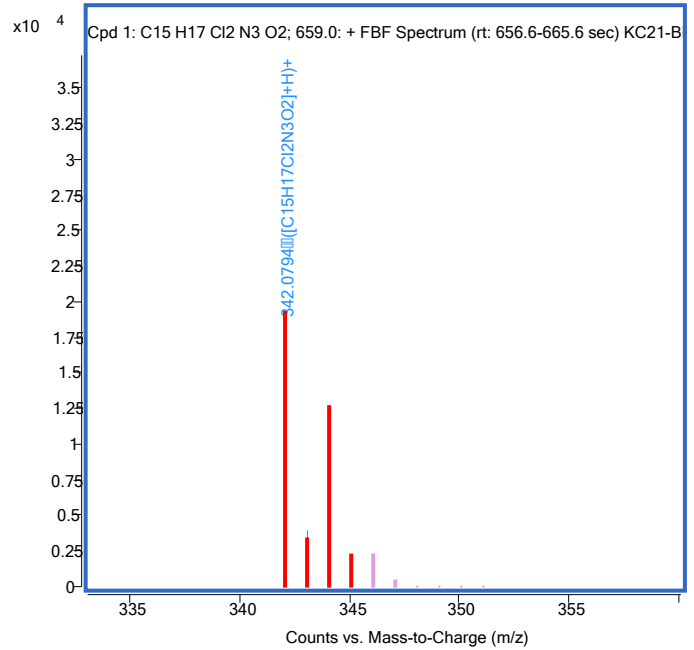


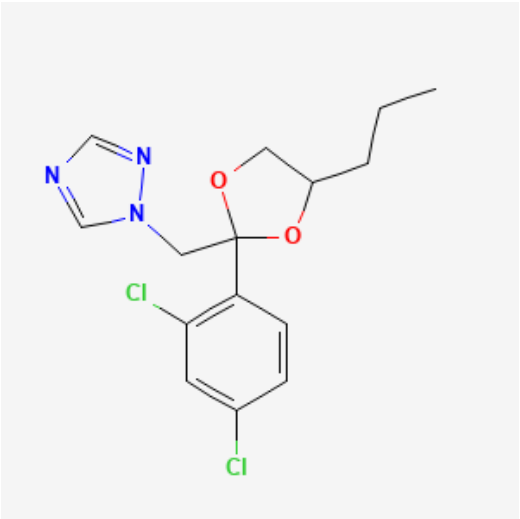
LUPANINE 550-90-3

NATURAL PRODUCT; MAJOR ALKALOID FROM LUPINUS EXALTATUS SEEDS



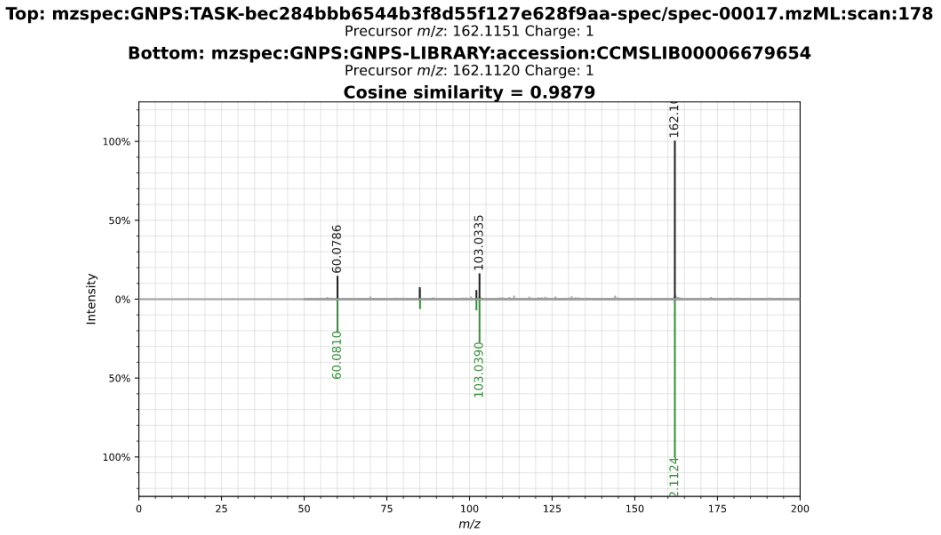


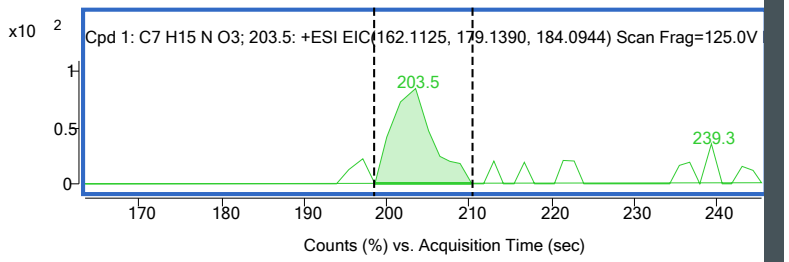


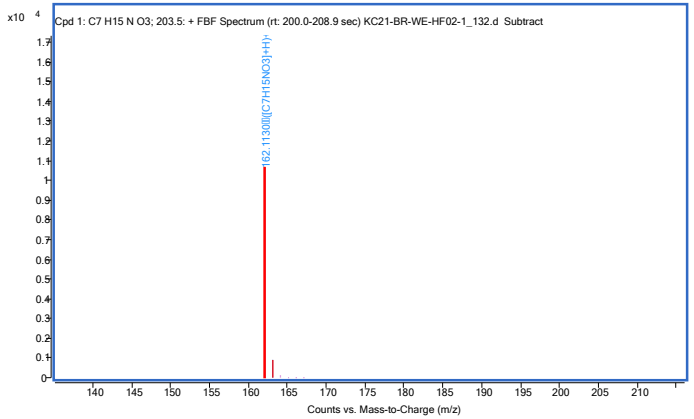


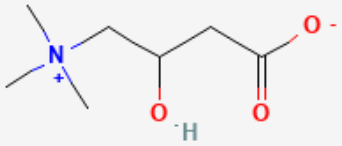
PROPICONAZOLE 60207-90-1

- fungicide



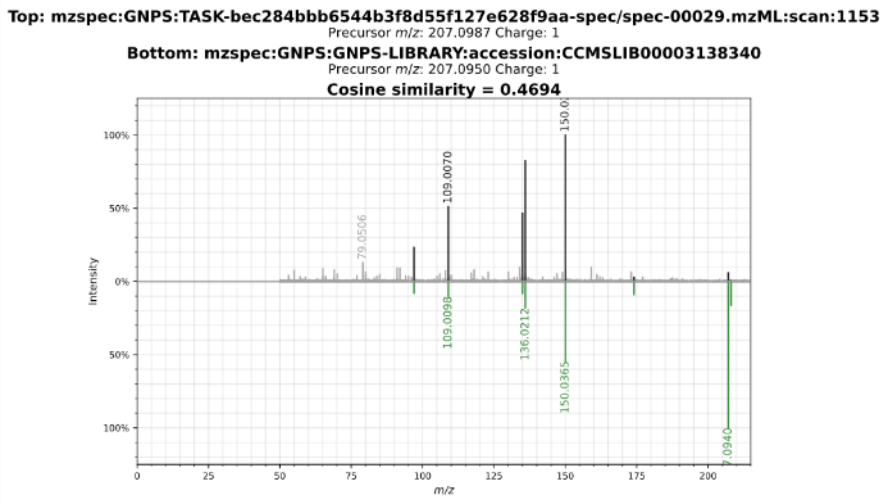


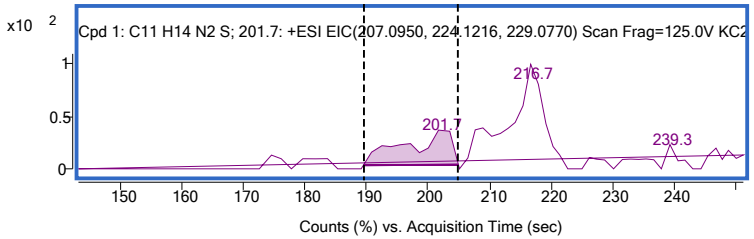


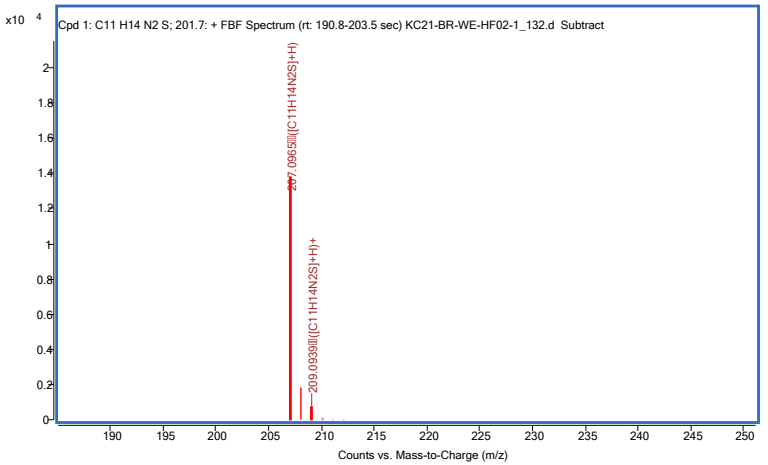


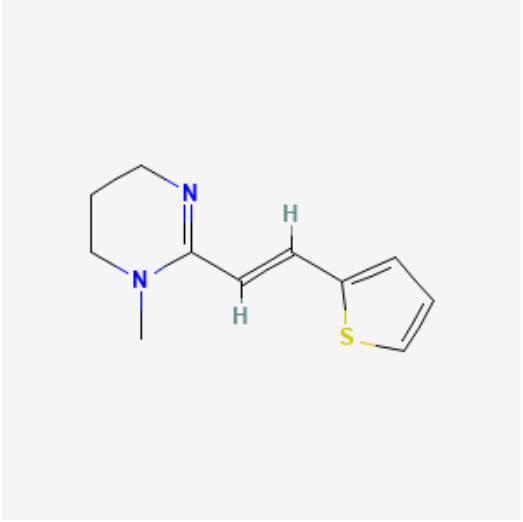
DL-CARNITIN E 406-76-8

- antistatic



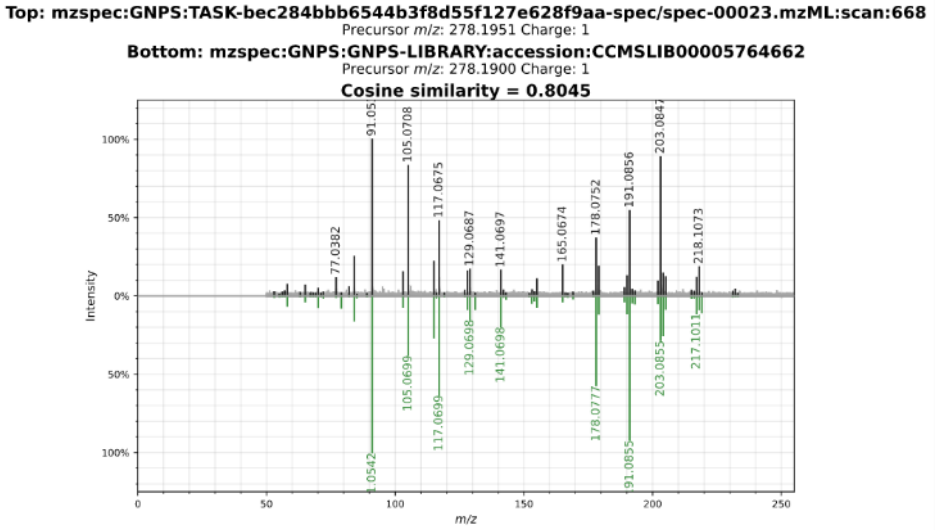


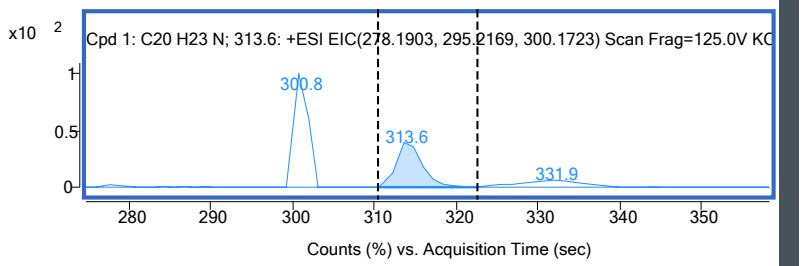


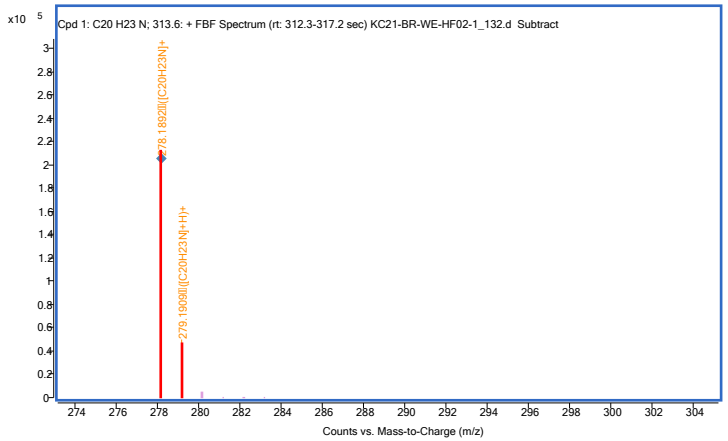


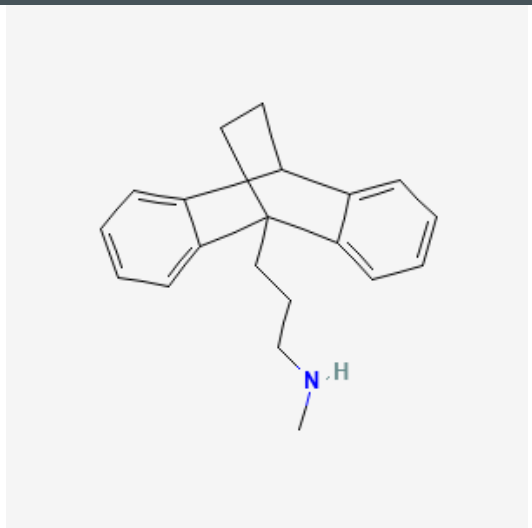
PYRANTEL

ANTINEMATODAL AGENTS, NEUROMUSCULAR DEPOLARIZING AGENT



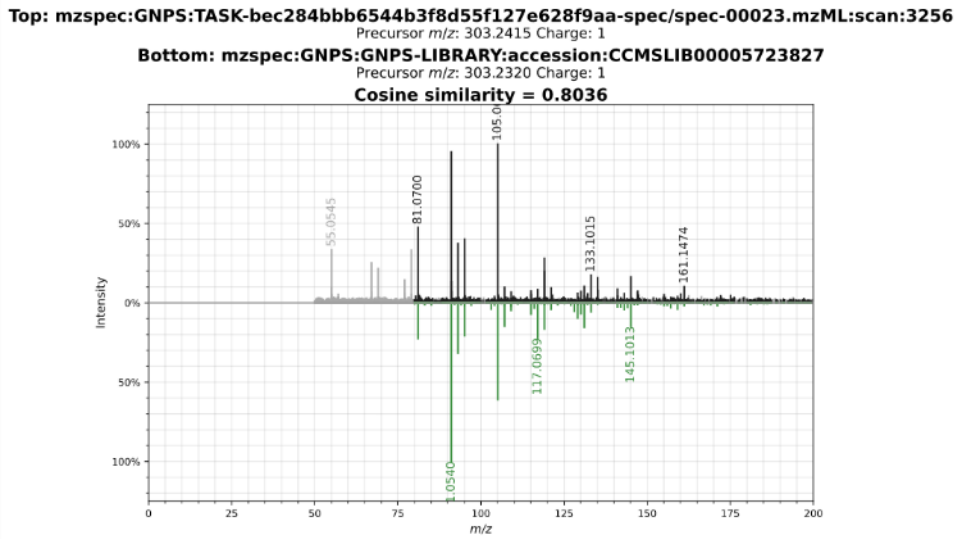


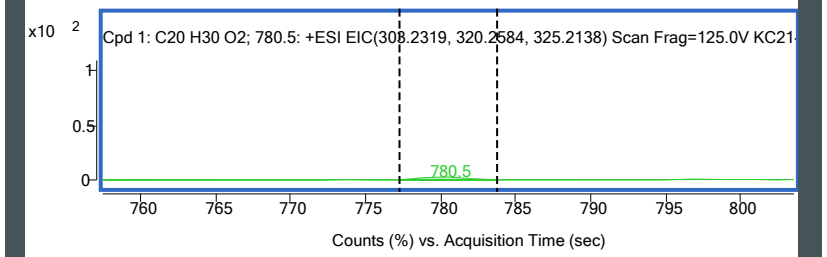


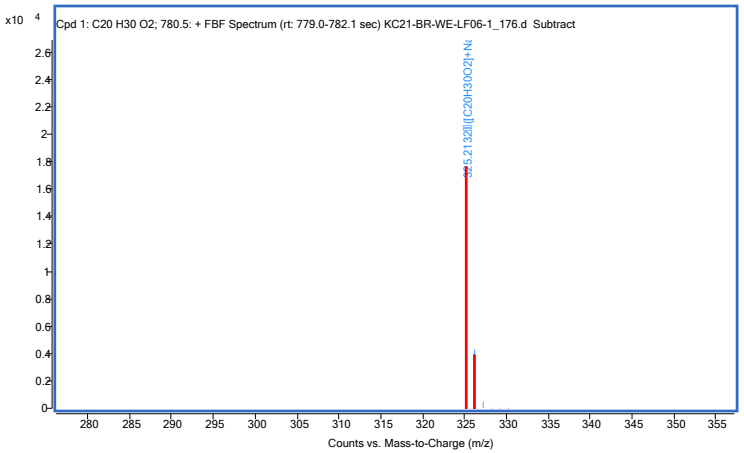


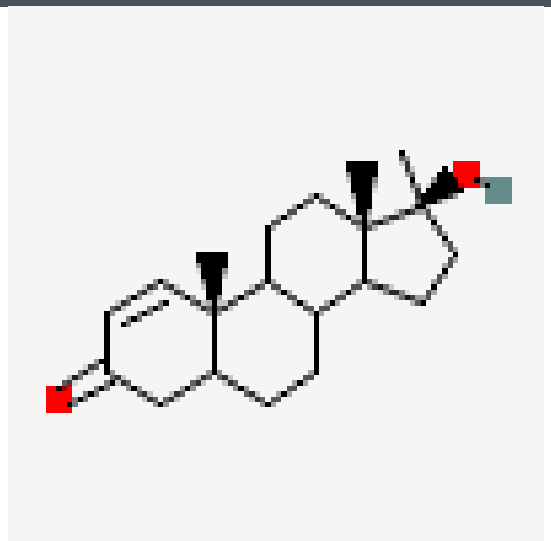
MAPROTILINE 10262-69-8

ANTIDEPRESSANT; ADRENERGIC UPTAKE INHIBITORS

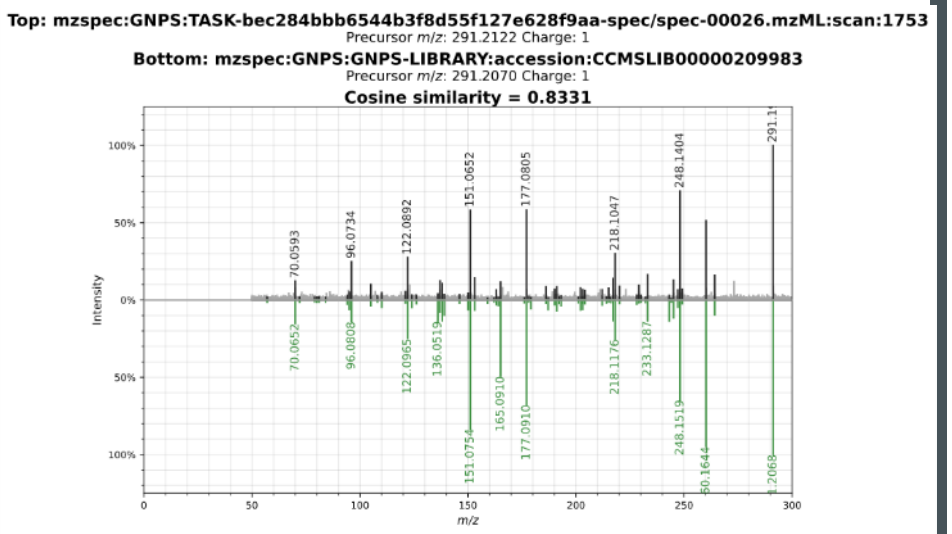


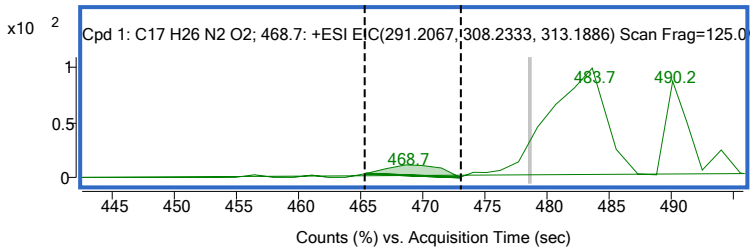


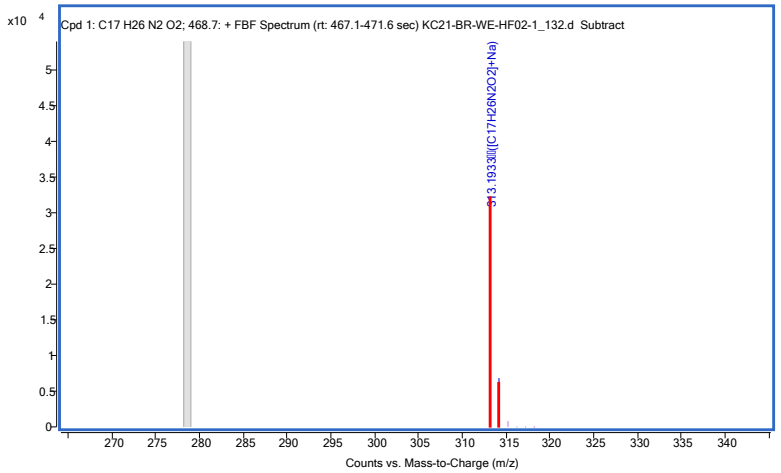


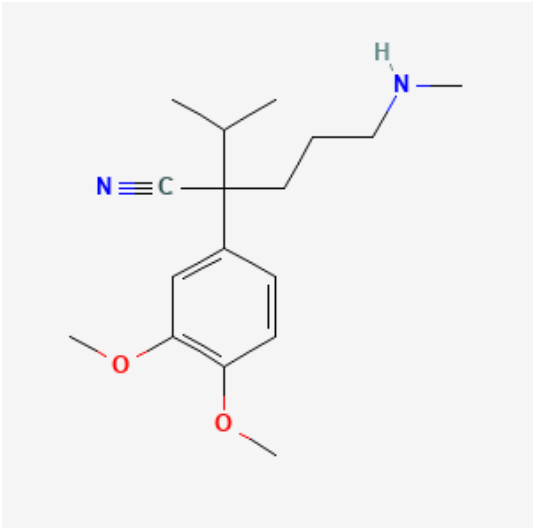


H_128_METHYL_1_TESTOSTERONE N/A



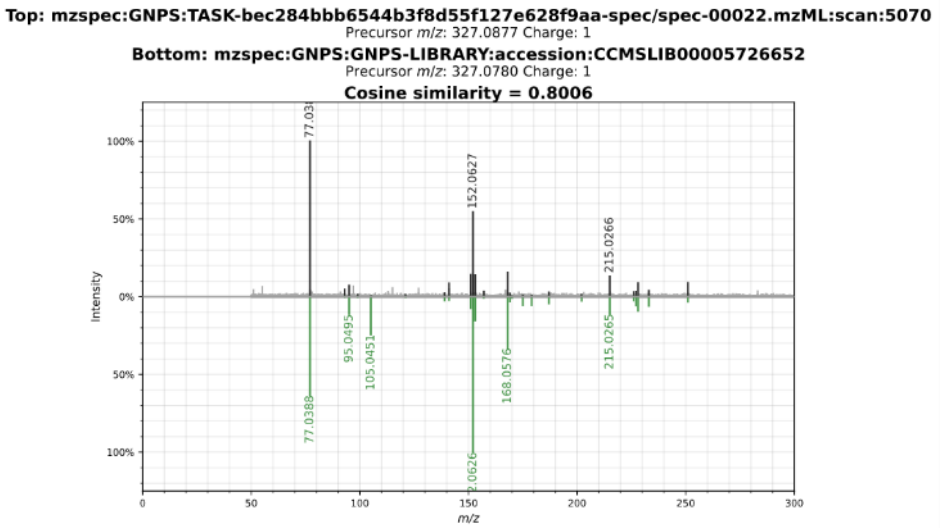


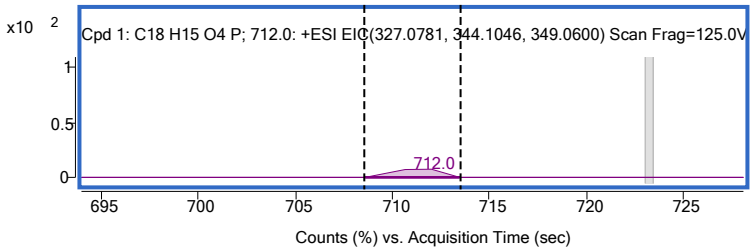


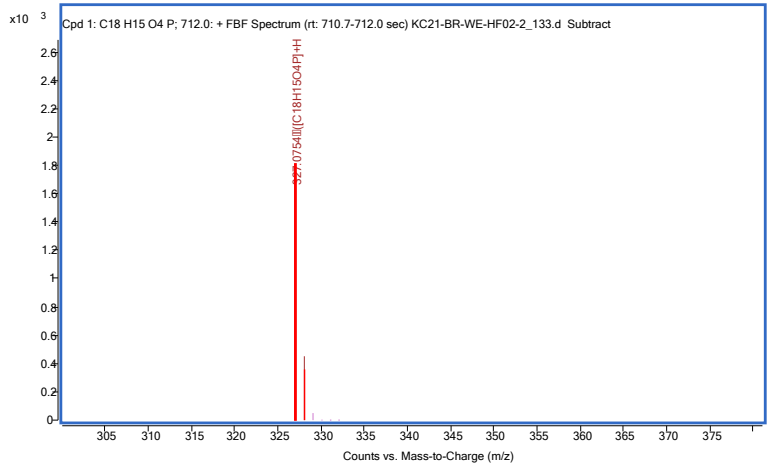


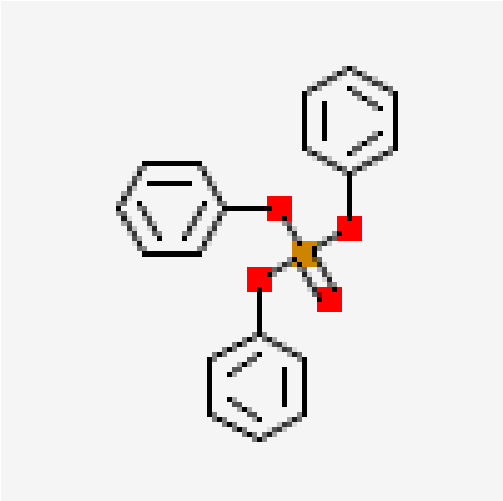
3-(3,4-DIMETHOXYPHENYL)-2-METHYL-6-METHYLAMINOHEXANE-3-CARBONITRILE

METABOLITE OF VERAPAMIL DRUG; CA CHANNEL BLOCKERS



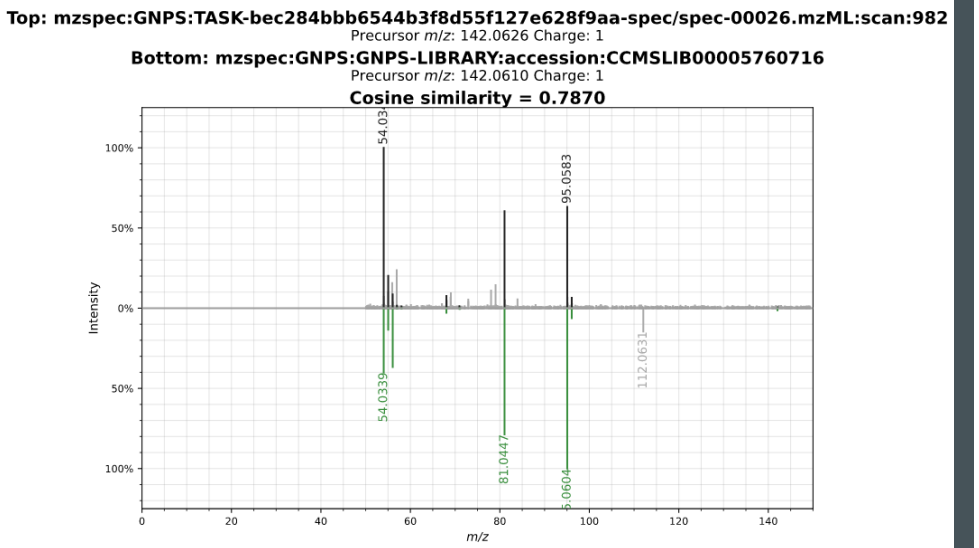


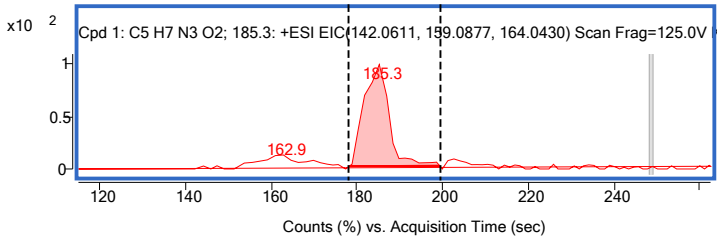


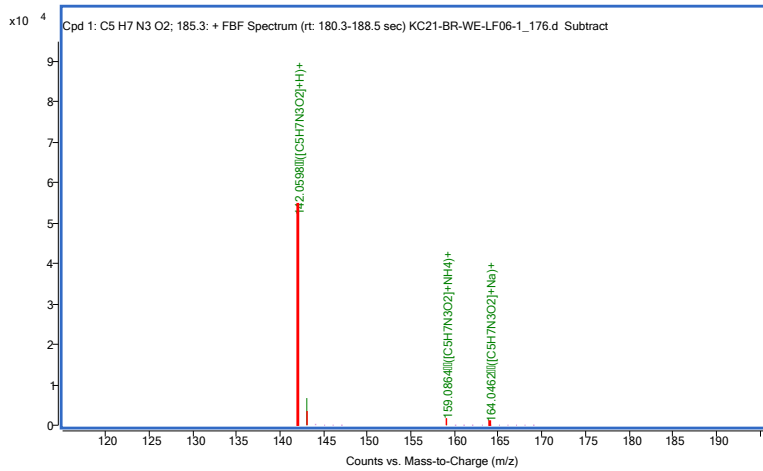


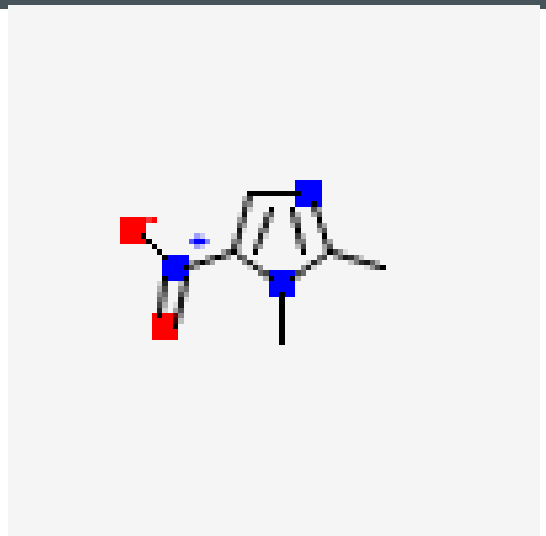
TRIPHENYL PHOSPHATE

FLAME RETARDANT, PLASTICIZER, CONSTRUCTION AND BUILDING MATERIAL



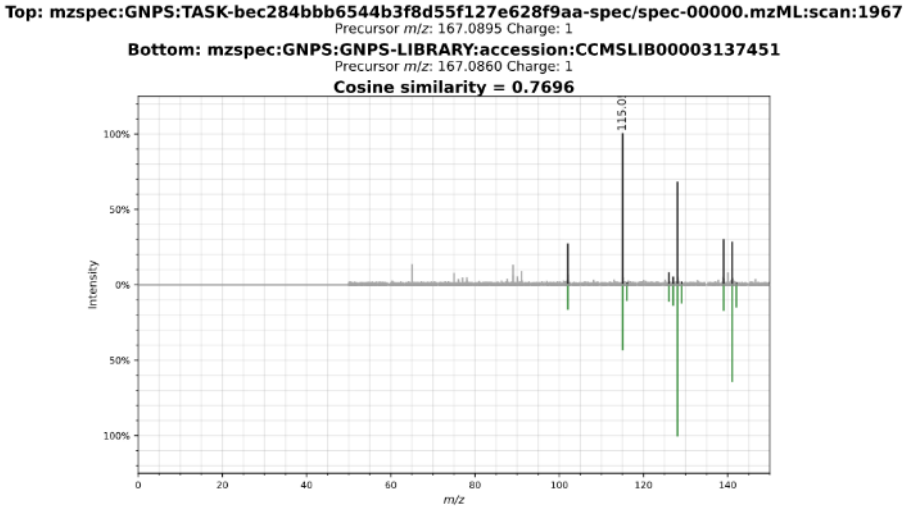


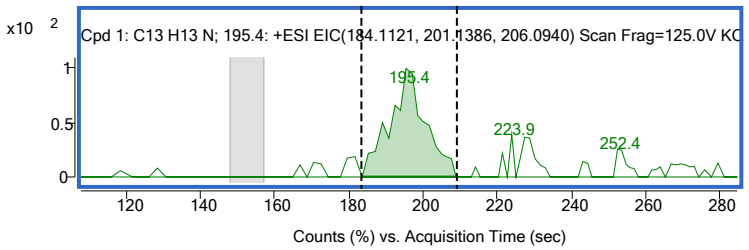


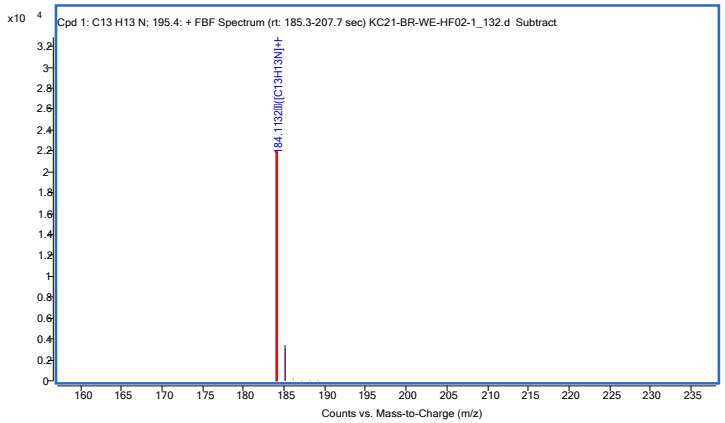


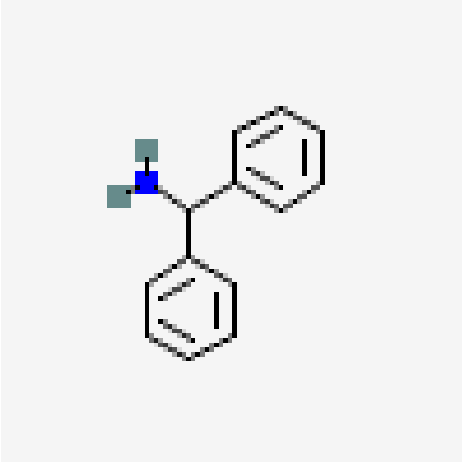
DIMETRIDAZOLE

- Veterinary drug

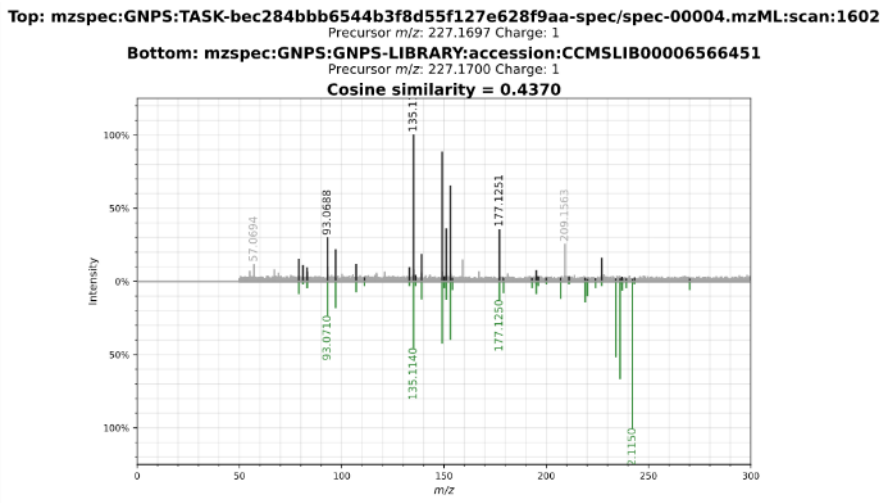


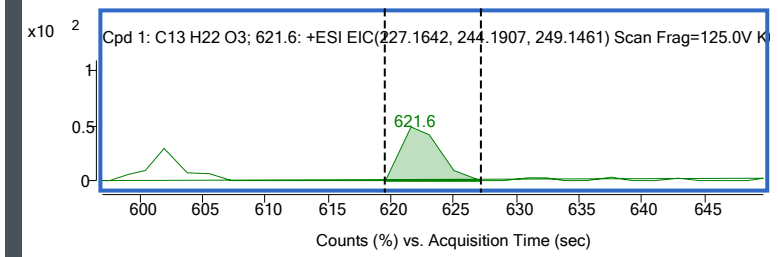


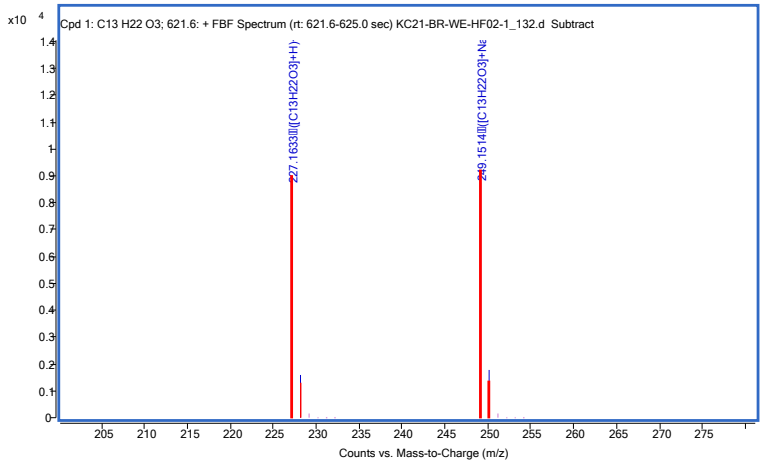


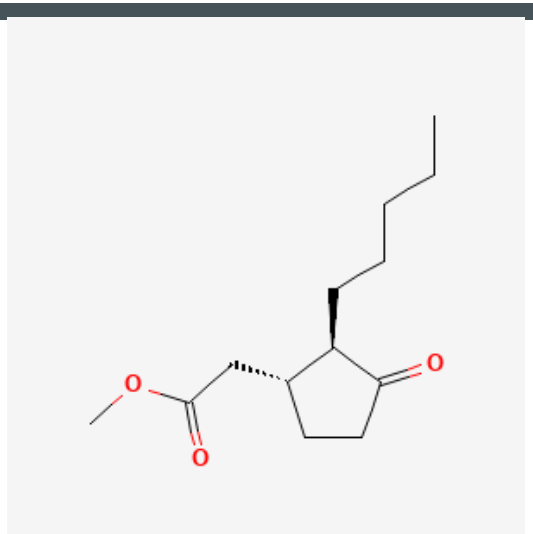


BENZHYDRYLAMINE 91-00-9



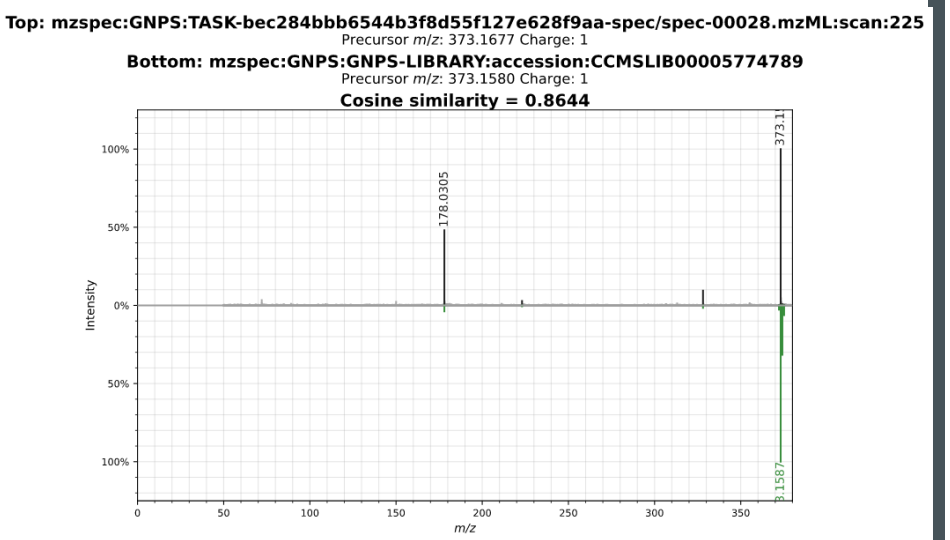


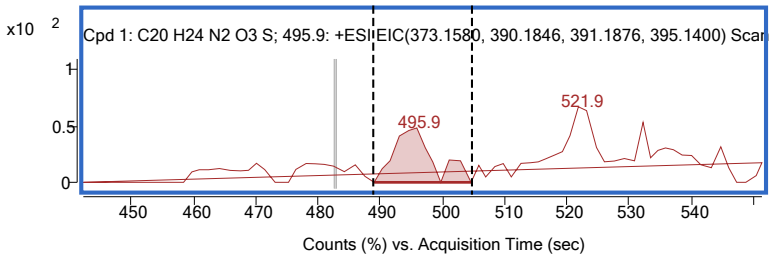


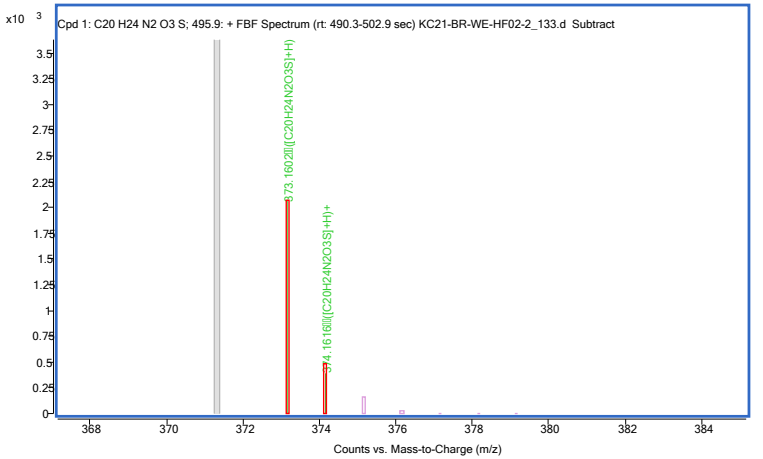


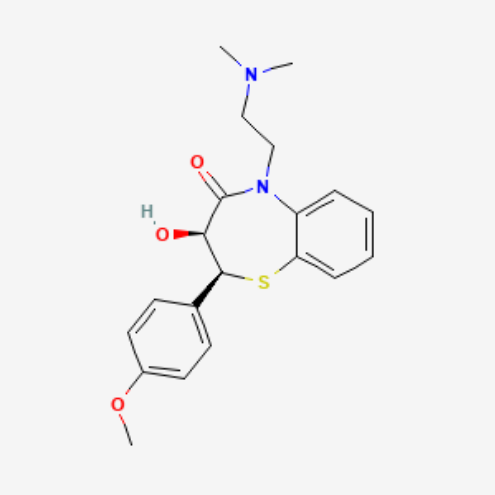
METHYL (1R-TRANS)-3-OXO-2-PENTYLCYCLOPENTANEACETATE 2630-39-9

USED AS FRAGRANCE

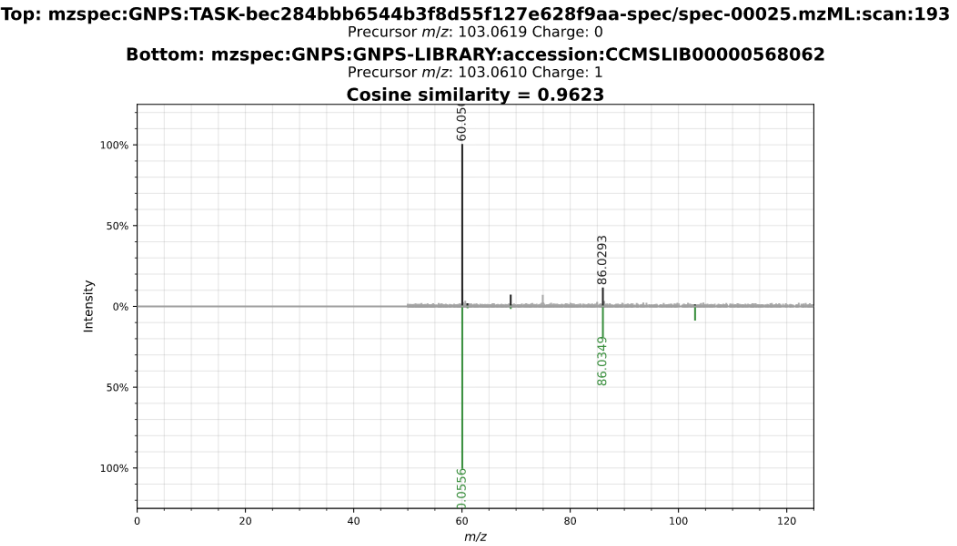


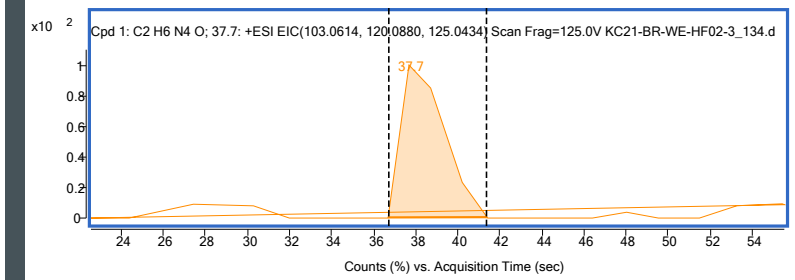


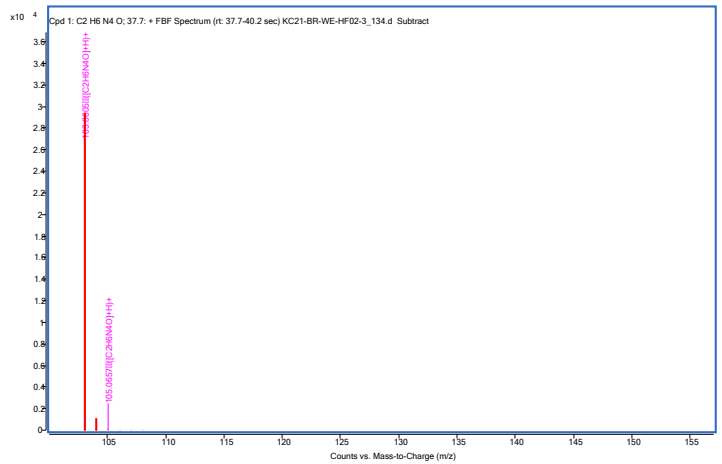


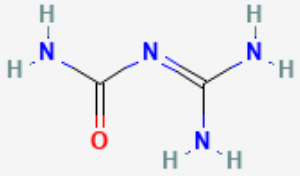


DEACETYLDILTIAZEM 42399-40-6

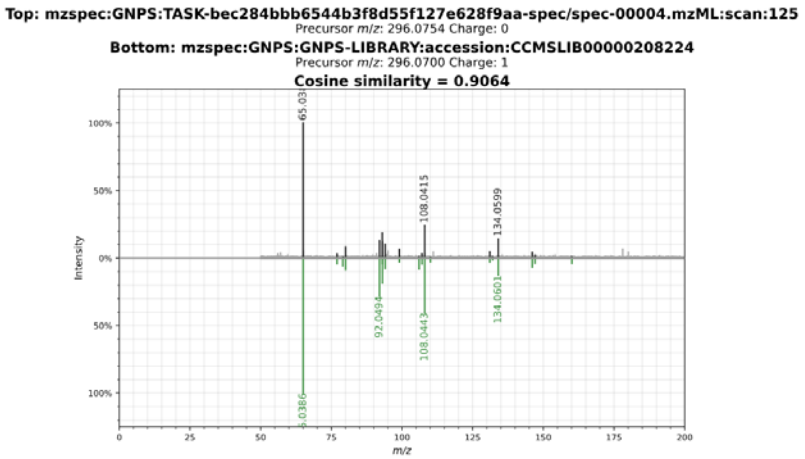


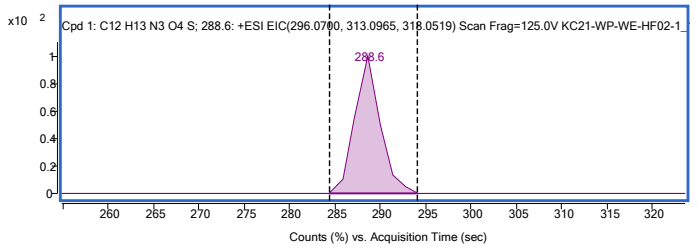


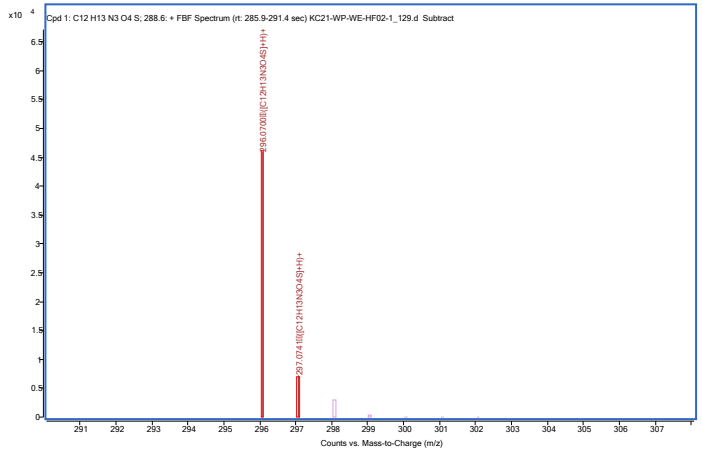


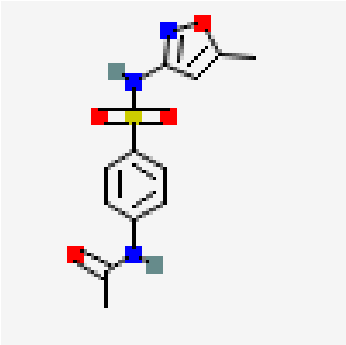


GUANYLUREA 141-83-3



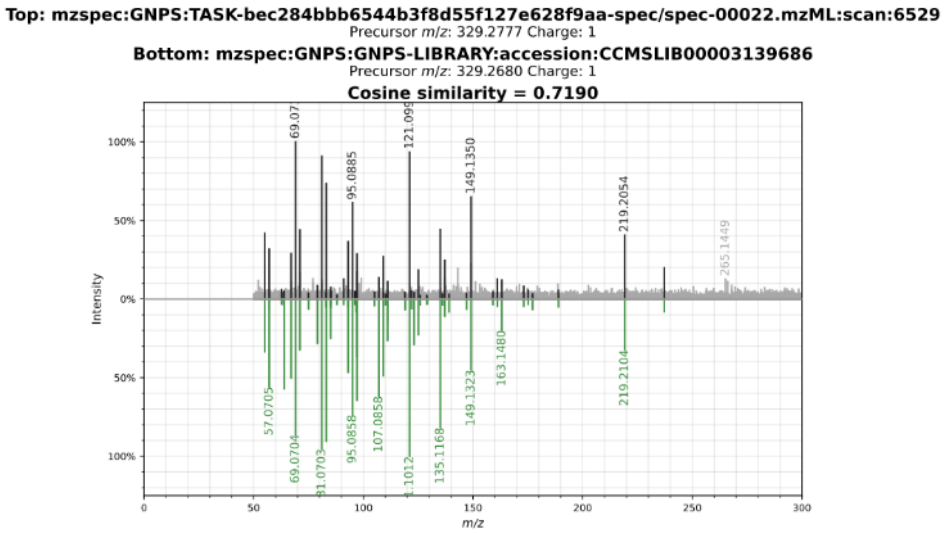


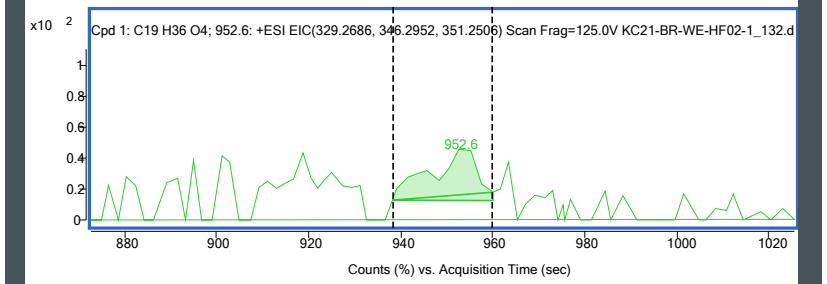


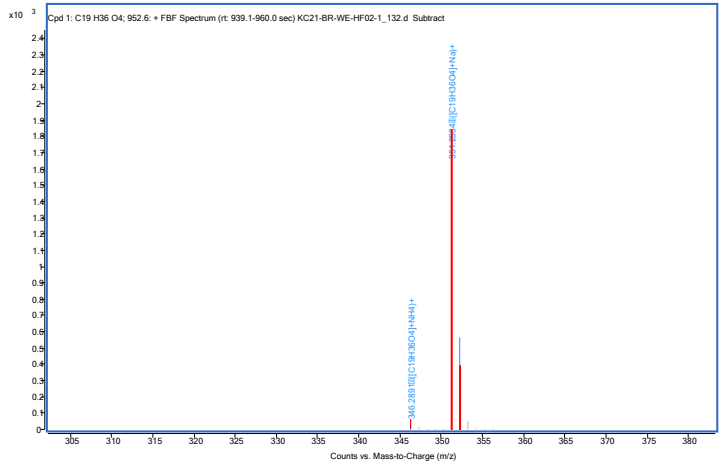


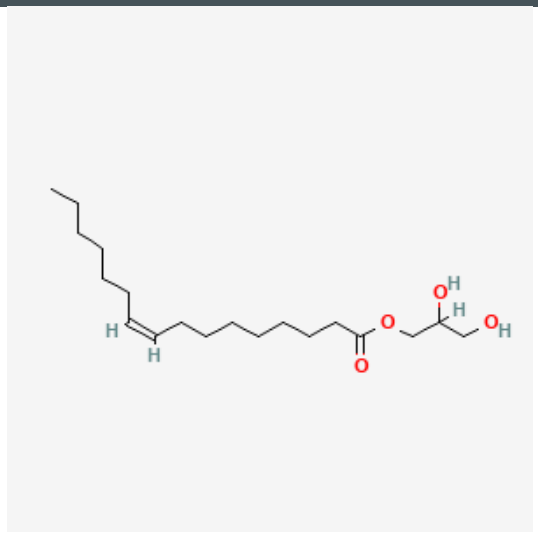
ACETYLSULFAMETHOXAZOLE 21312-10-7

- transformation product of sulfamethoxazole



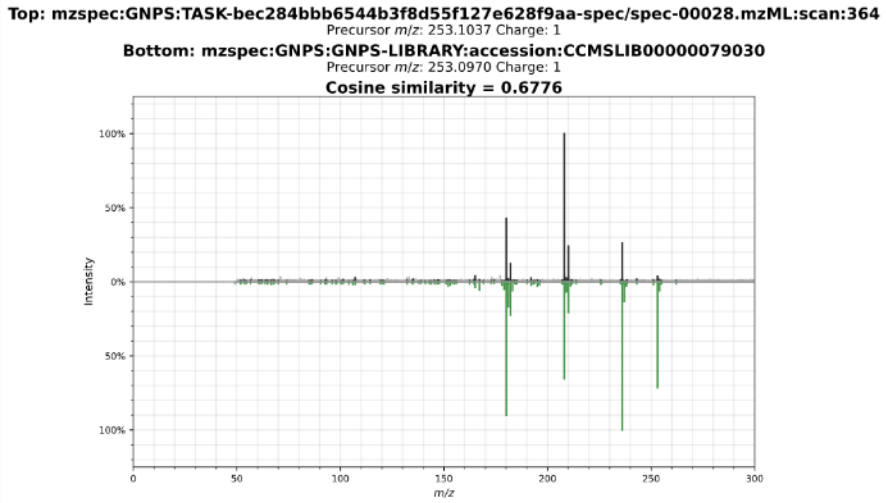


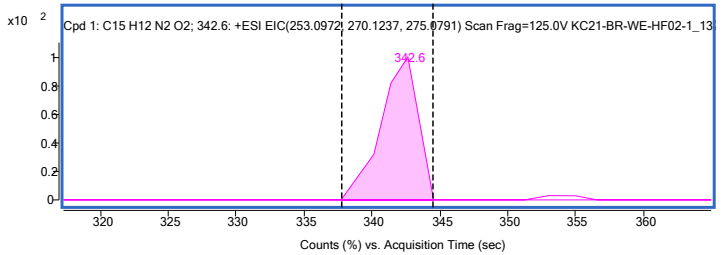


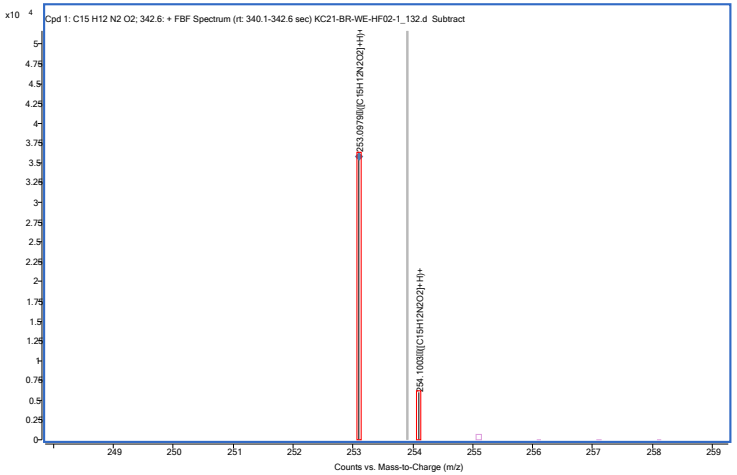


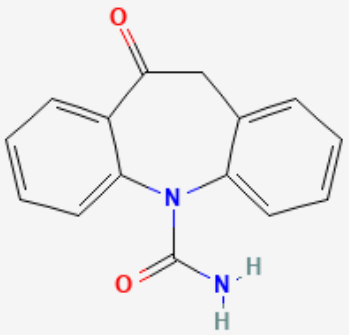
GLYCERYL PALMITOLEATE 37515-61-0

- cosmetics, emulsifying



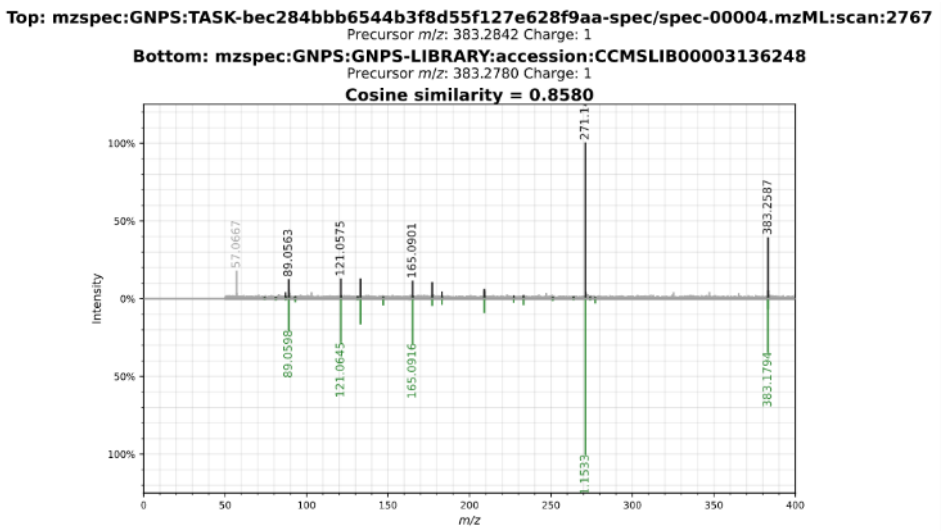


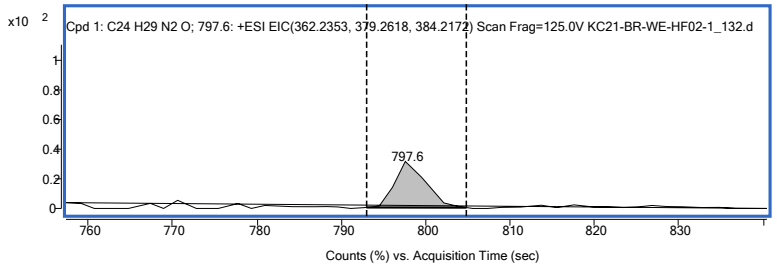


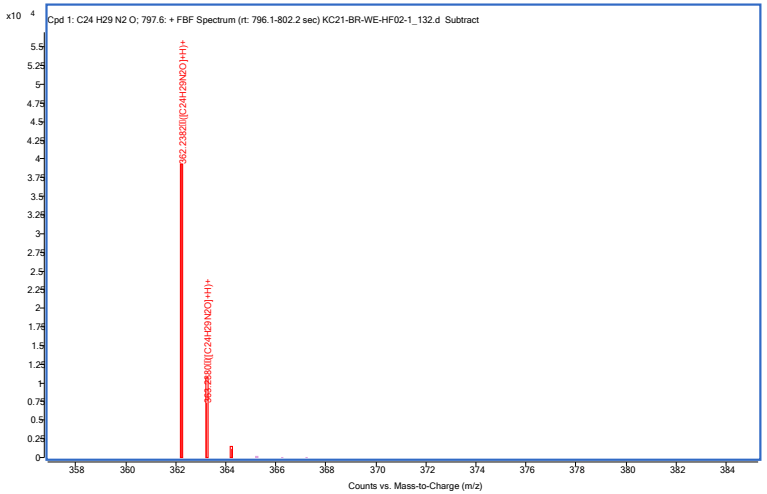


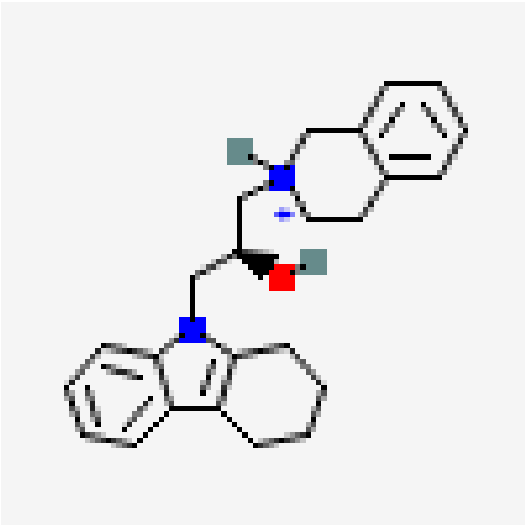
OXCARBAZEPINE 28721-07-5

ANTICONVULSANT, ANTIEPILEPTIC

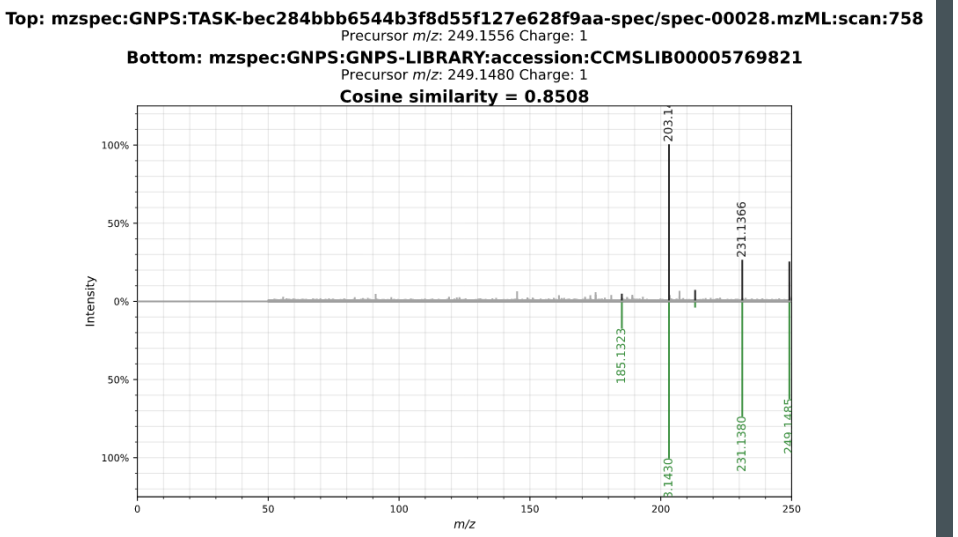


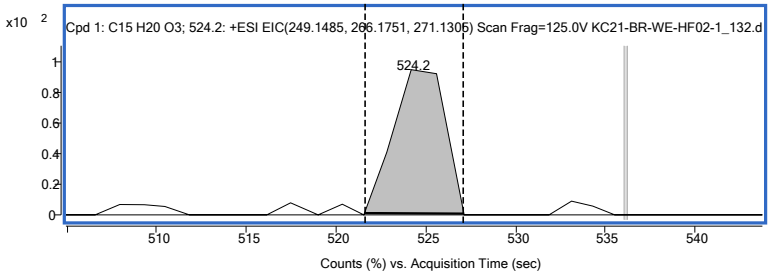


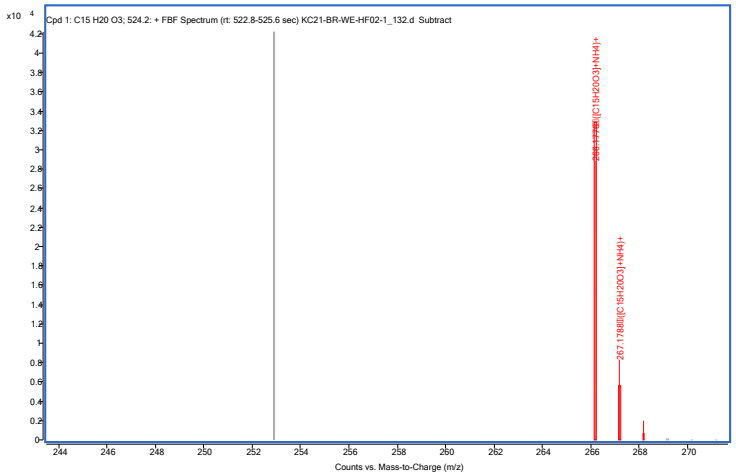


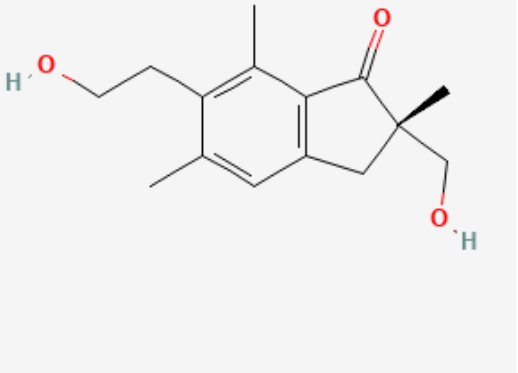


(2S)-1-(1,2,3,4-TETRAHYDROCARBAZOL-9-YL)-3-(1,2,3,4-TETRAHYDROISOQUINOLIN-2-IUM-2-YL)PROPAN-2-OL NO CAS



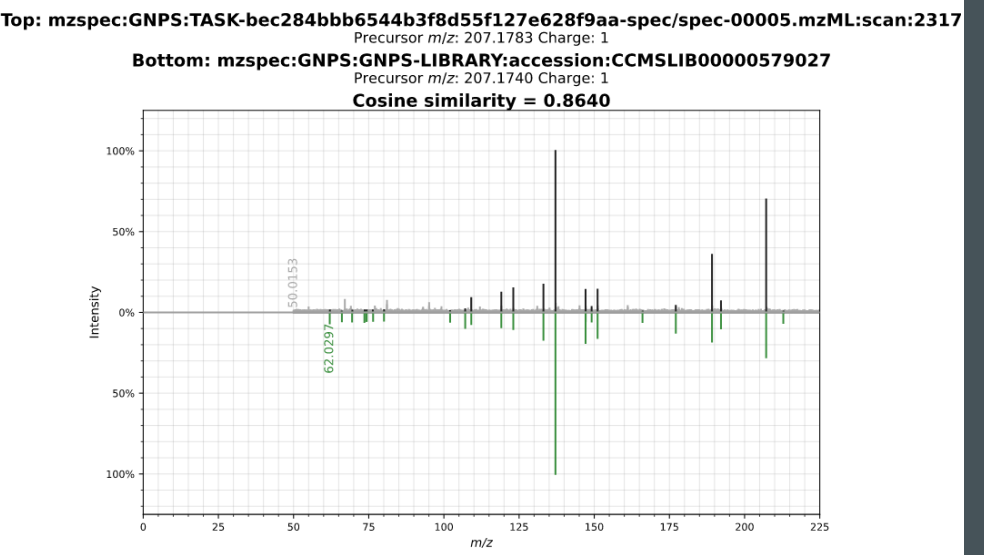


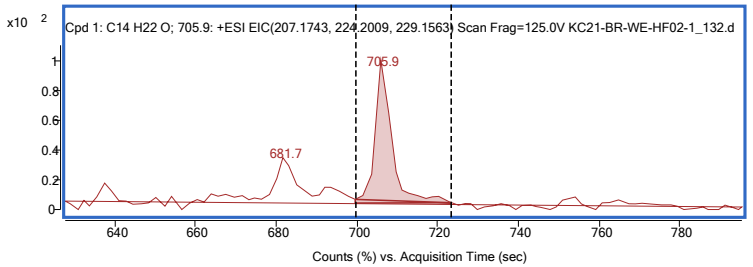


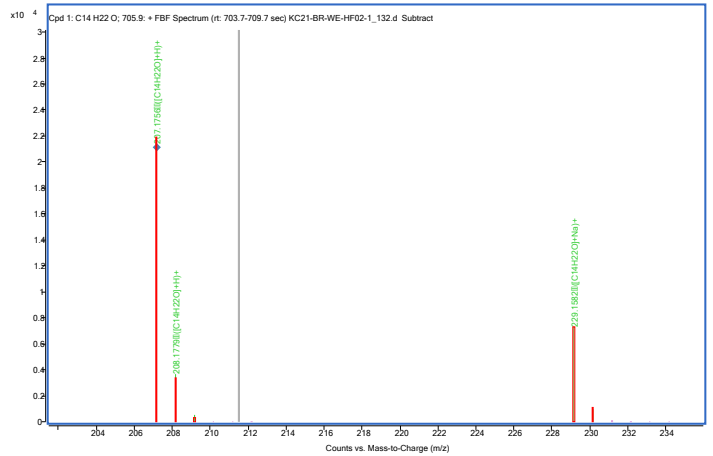


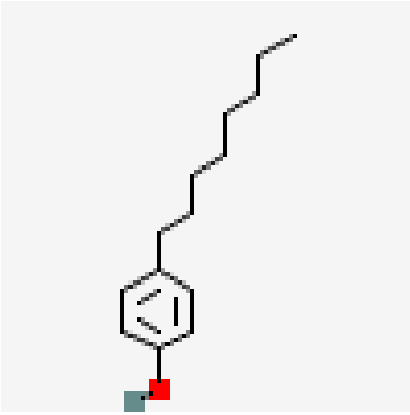
PTEROSIN A

ONGOING MEDICAL TRIALS AS WELL AS DRUG PATENTS



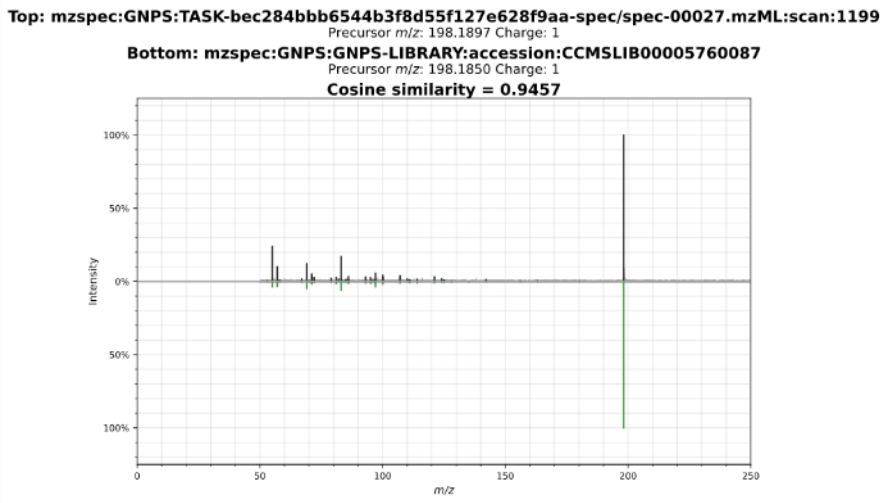


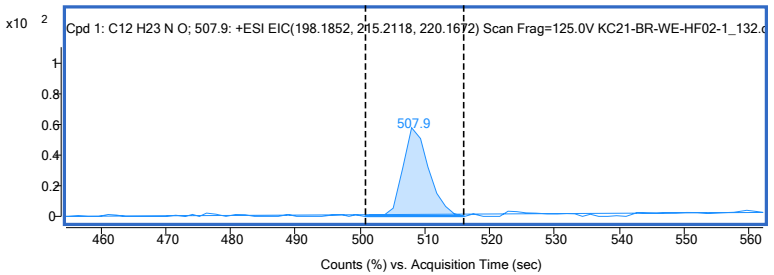


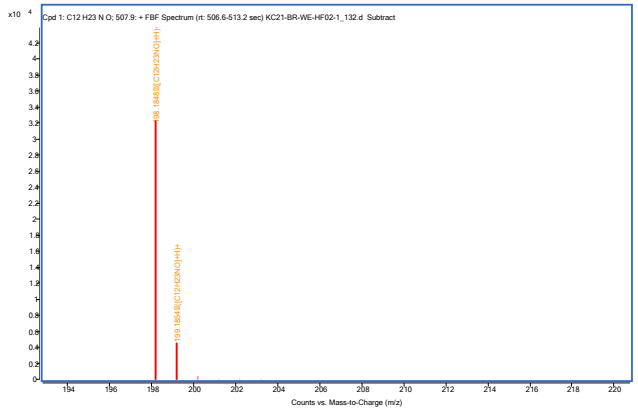


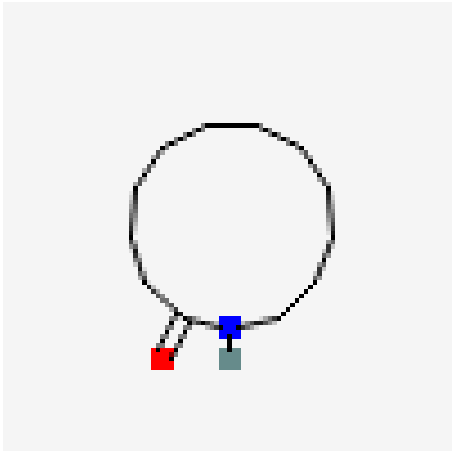
4-OCTYLPHENOL 1806-26-4

- Estrogens, Non-Steroidal; nonionic surfactants, resins, fungicides, bactericides, dyestuffs, adhesives, and rubber chemicals; Also used in plasticizers and antioxidants and as a fuel oil stabilize



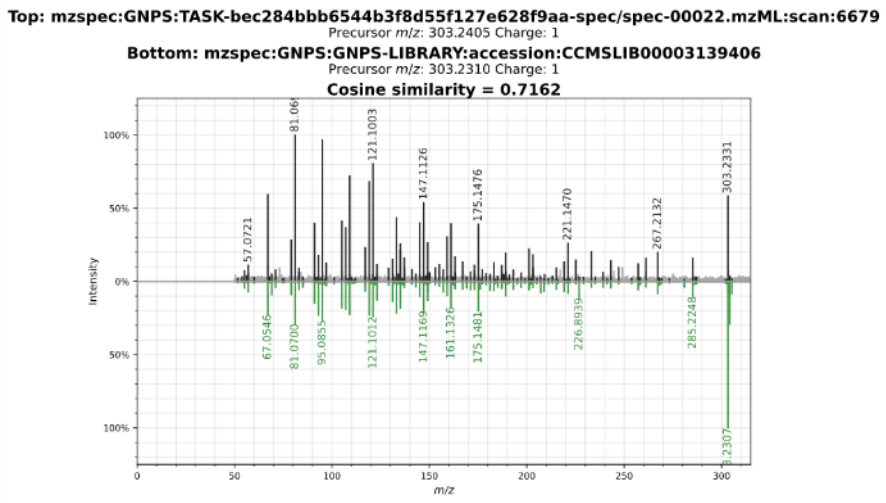


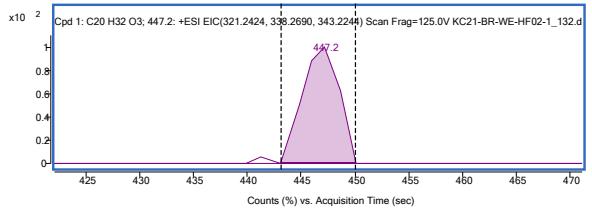


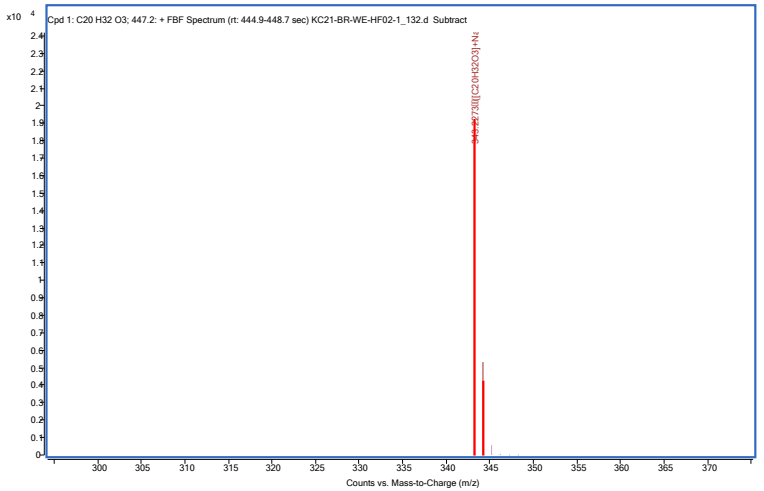


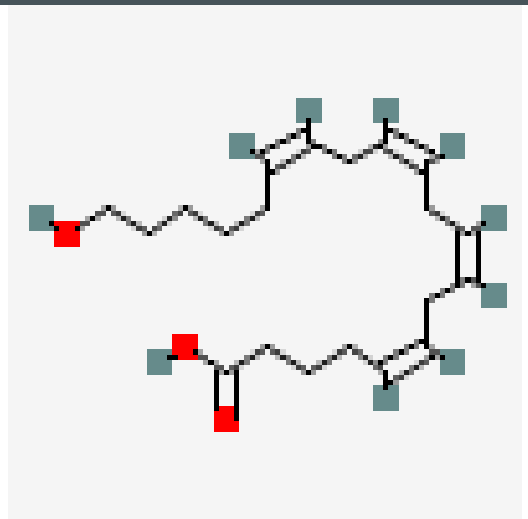
AZACYCLOTRIDECAN-2-ONE 947-04-6

COSMETICS, INTERMEDIATES, PERSONAL CARE PRODUCTS



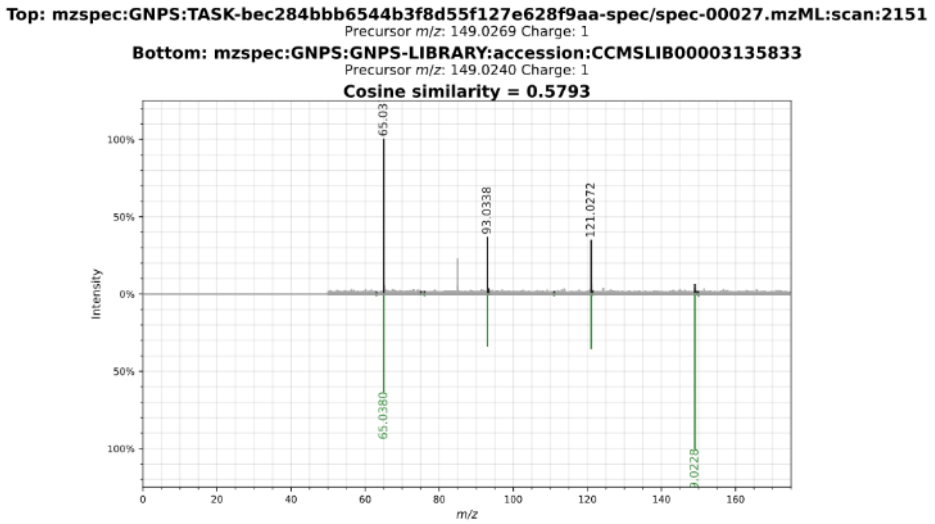


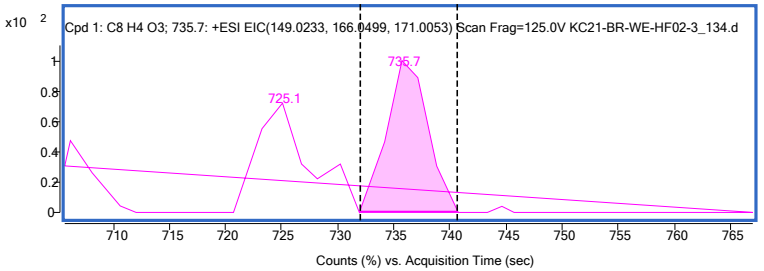


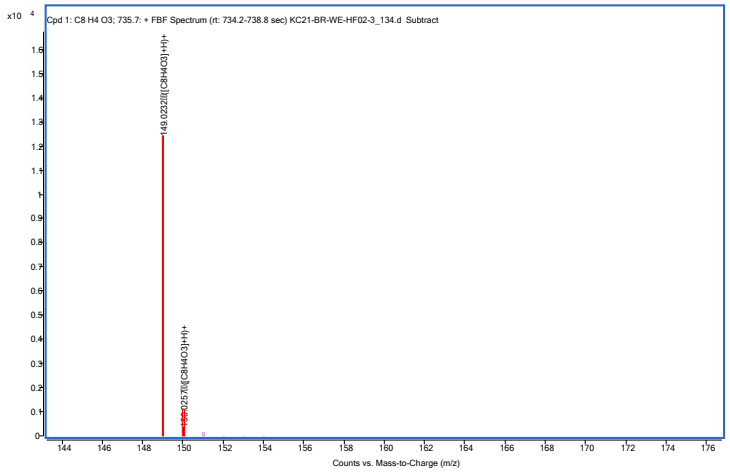


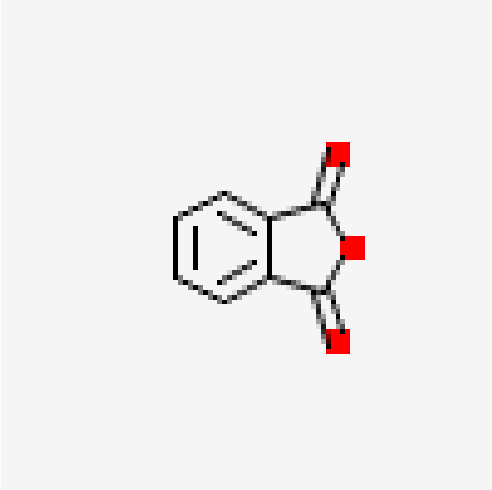
20-HYDROXYEICOSATETRAENOIC ACID 79551-86-3

HUMAN METABOLITE OF ARACHIDONIC ACID.



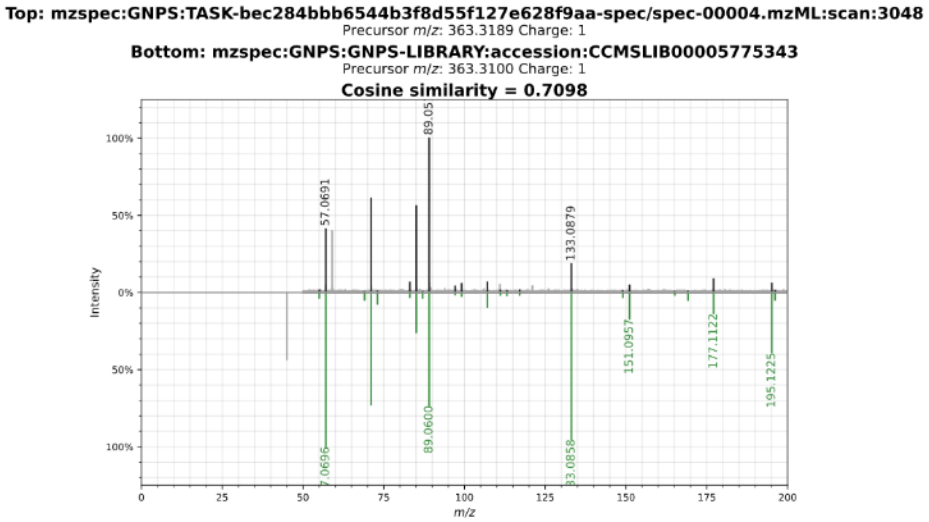


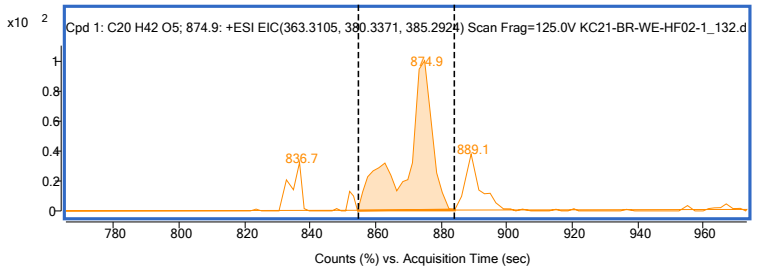


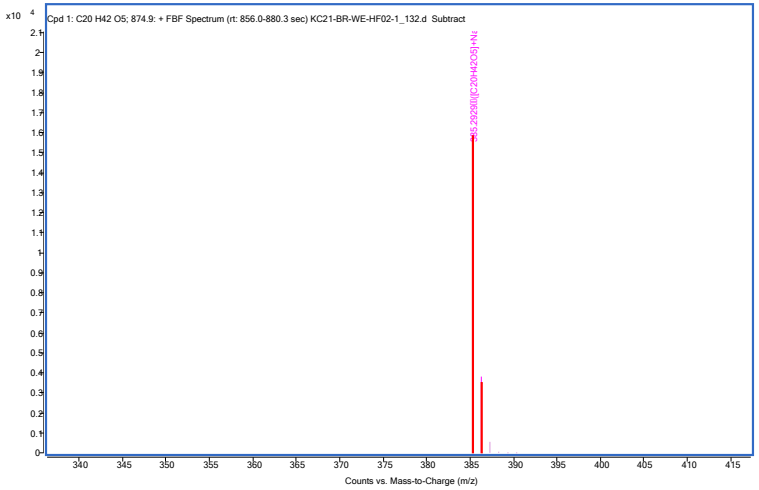


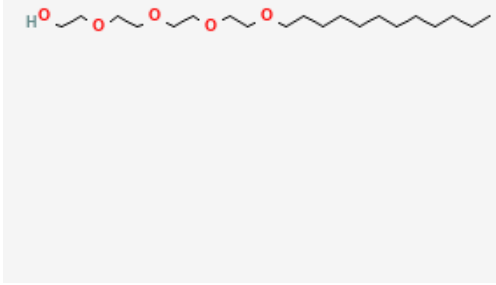
PHTHALIC ANHYDRIDE 85-44-9

CHEMICAL INTERMEDIATE IN THE PLASTICS INDUSTRY, IN NAIL POLISH, AND A FDA INDIRECT ADDITIVE



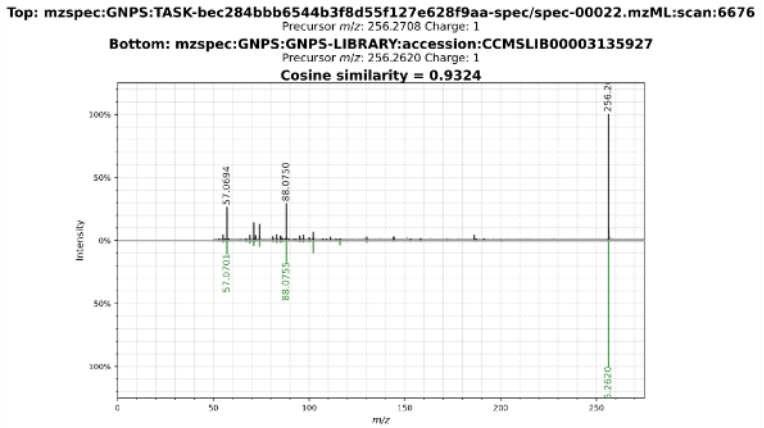


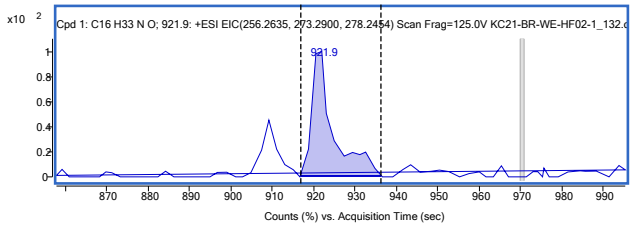


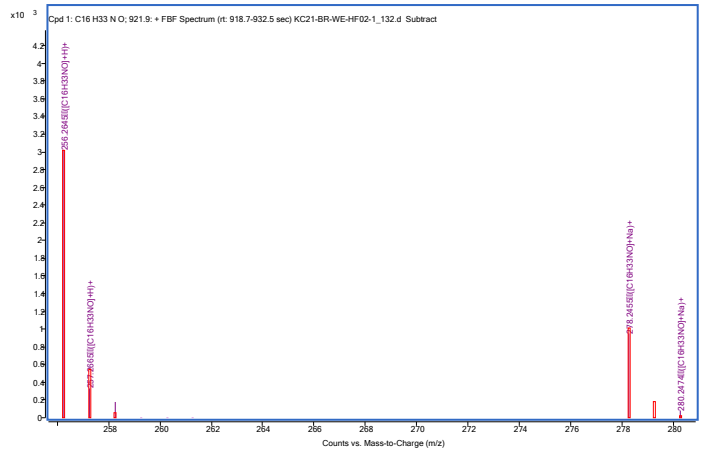


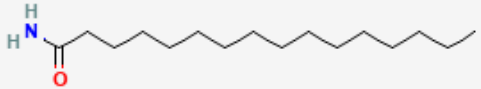
TETRAETHYLENE GLYCOL MONODODECYL ETHER 5274-68-0

- cleaning products, cosmetics, non-ionic surfactant commonly used in industrial formulations, FDA indirect food additive



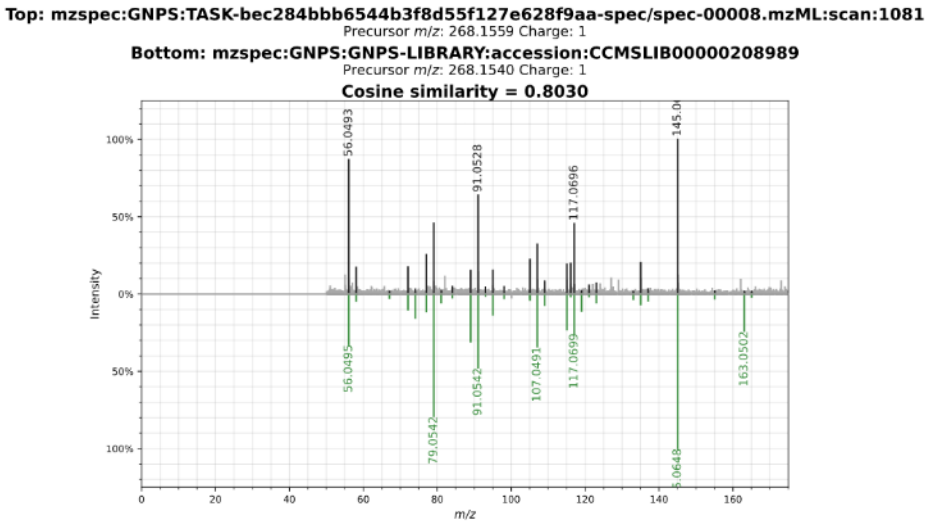


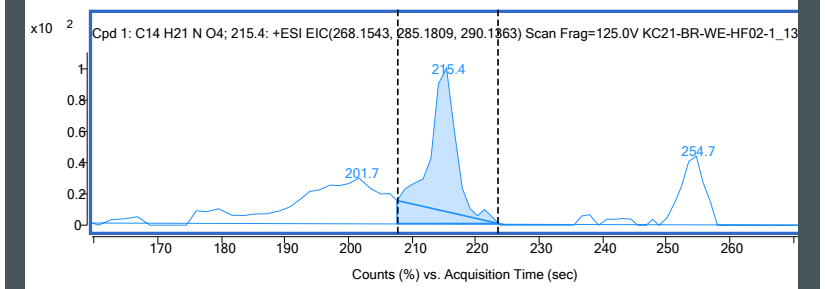


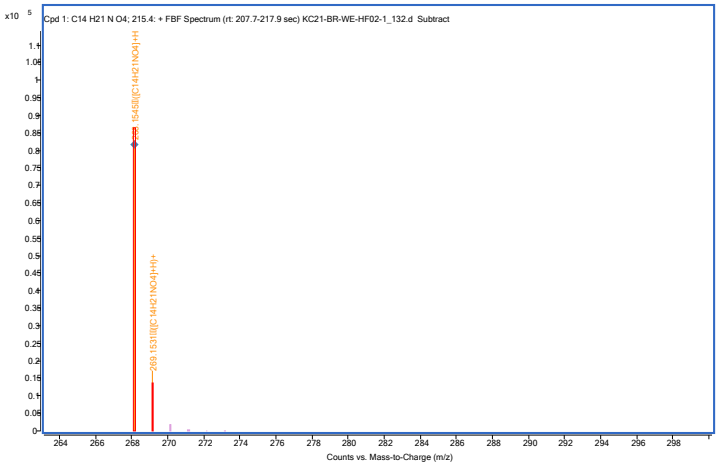


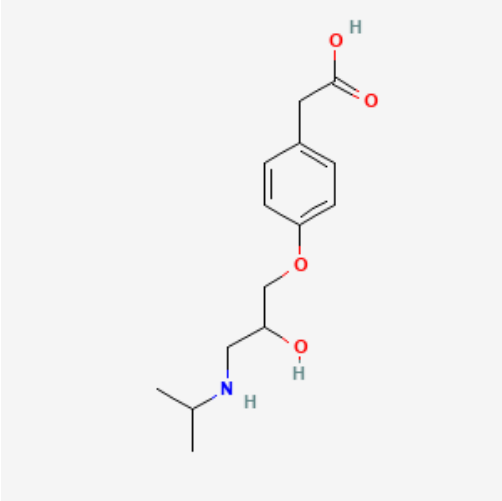
HEXADECANAMIDE 629-54-9

- Skin conditioning, FDA indirect food additive



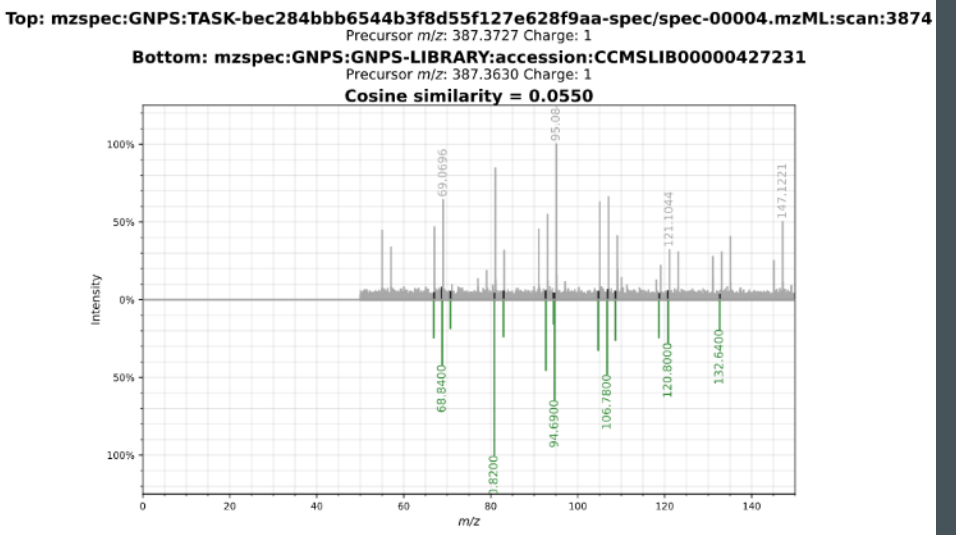


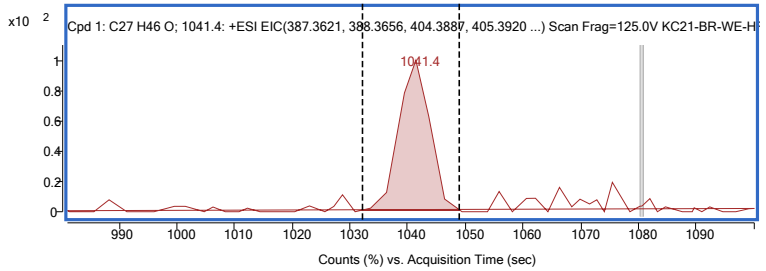


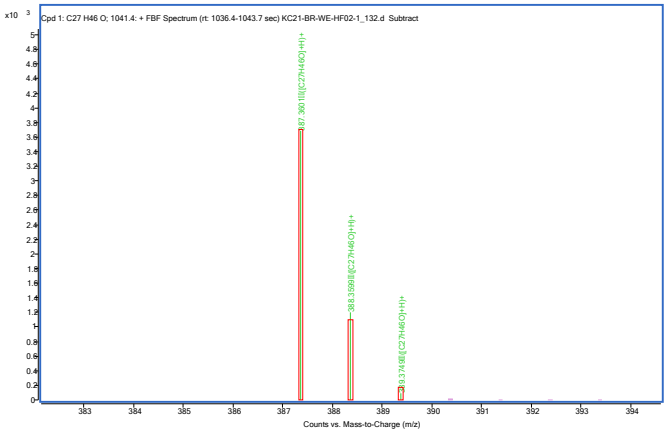


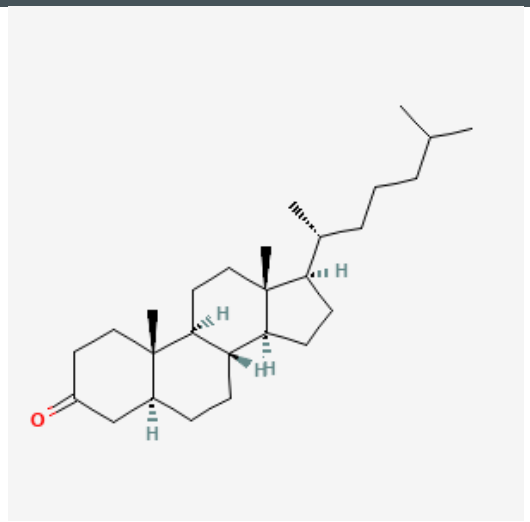
METOPROLOL ACID 56392-14-4

- beta blocking agent



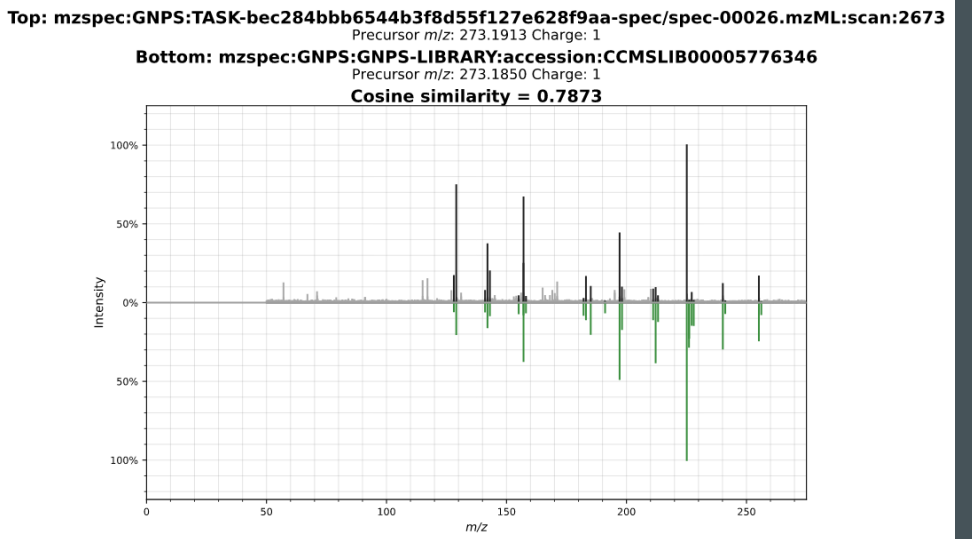


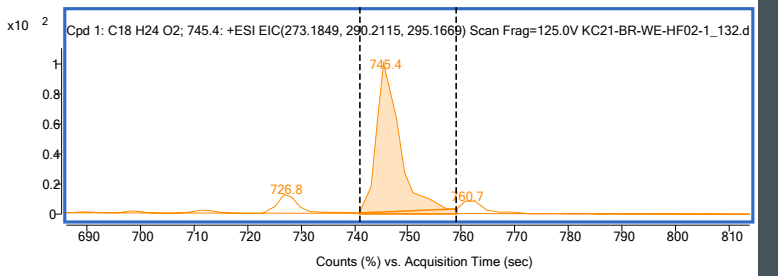


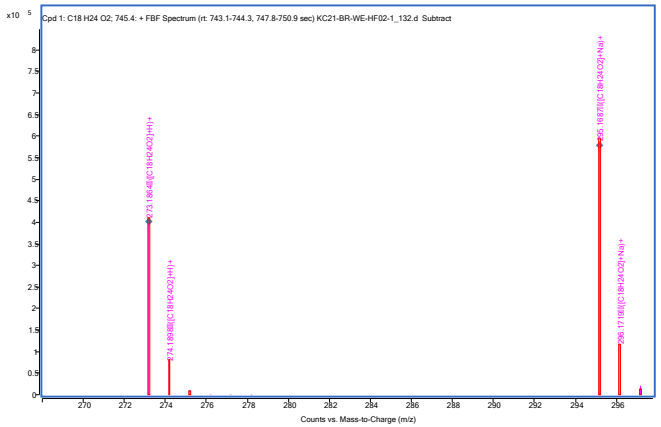


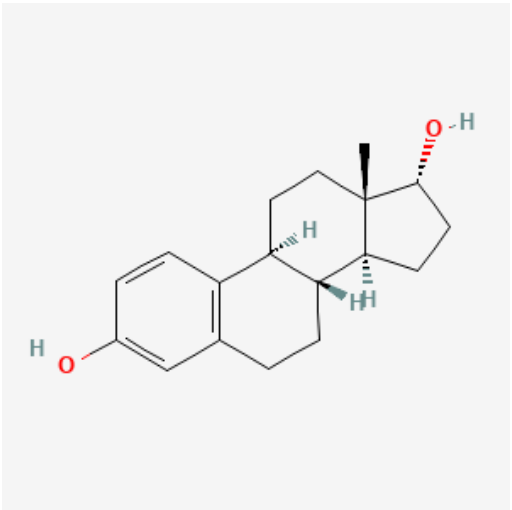
5ALPHA-CHOLESTAN-3-ONE 566-88-1

- cholesterol and derivatives



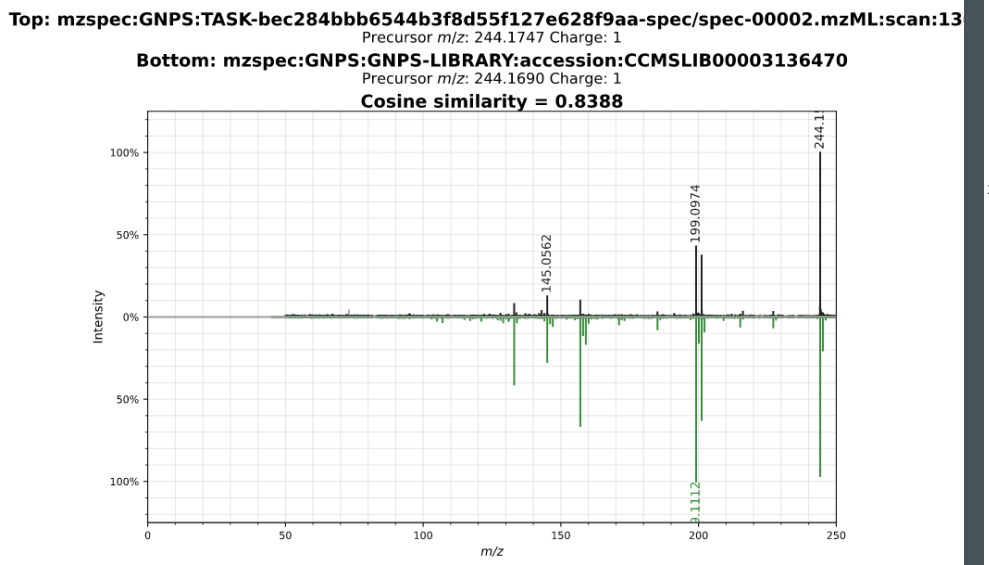


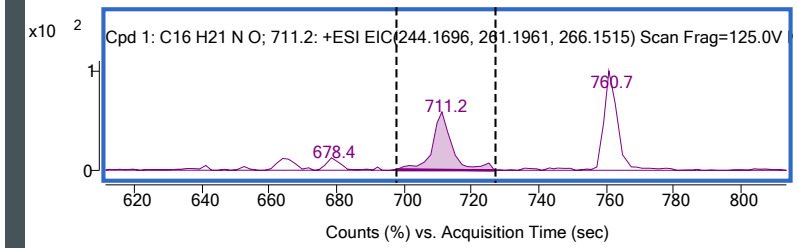


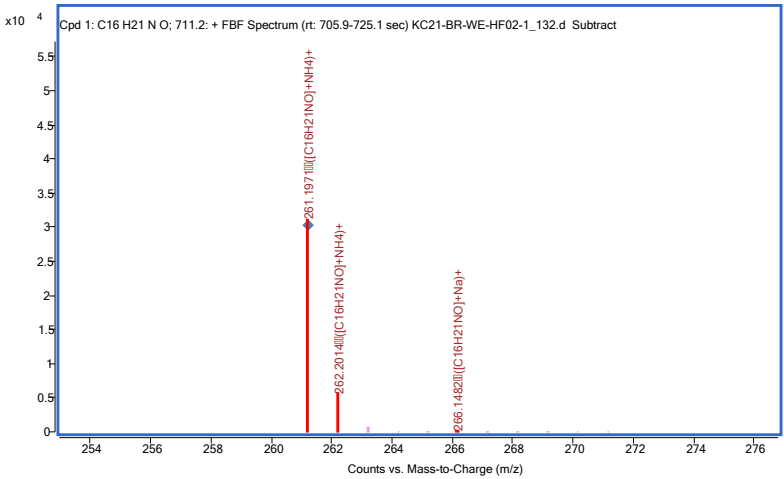


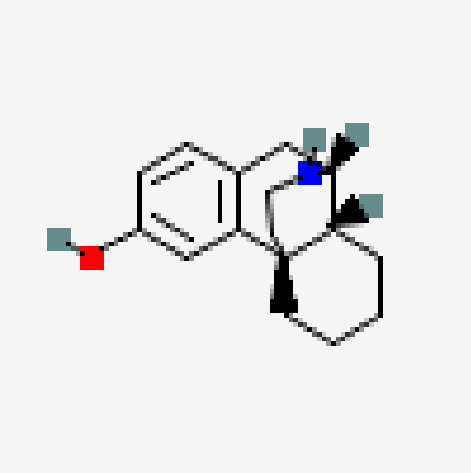
17ALPHA-ESTRADIOL 57-91-0

STEROL, DERMATOLOGIC AGENTS



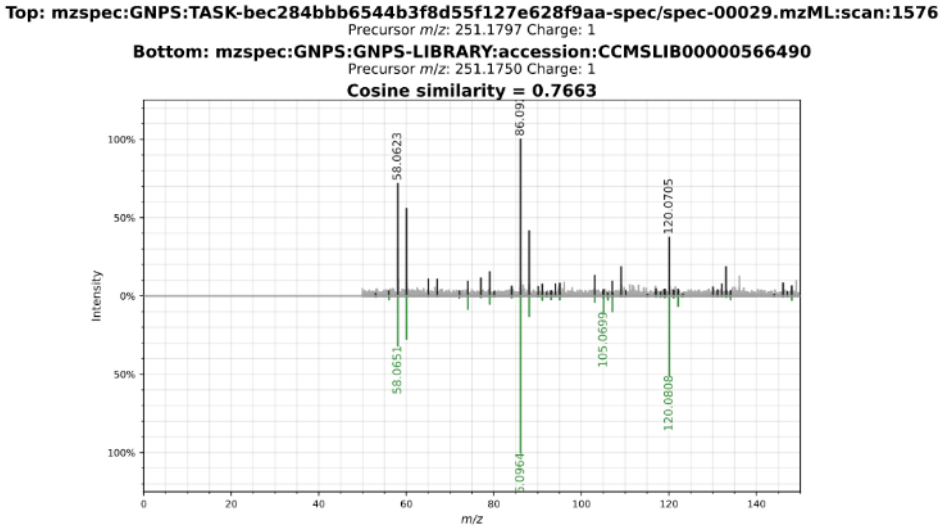


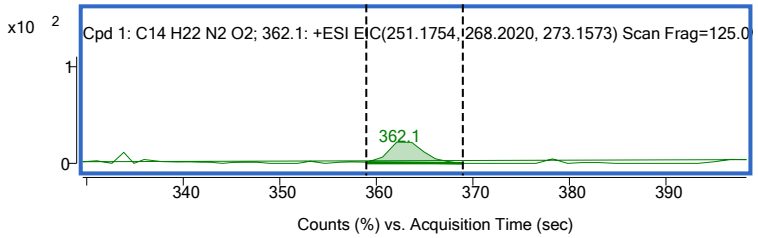


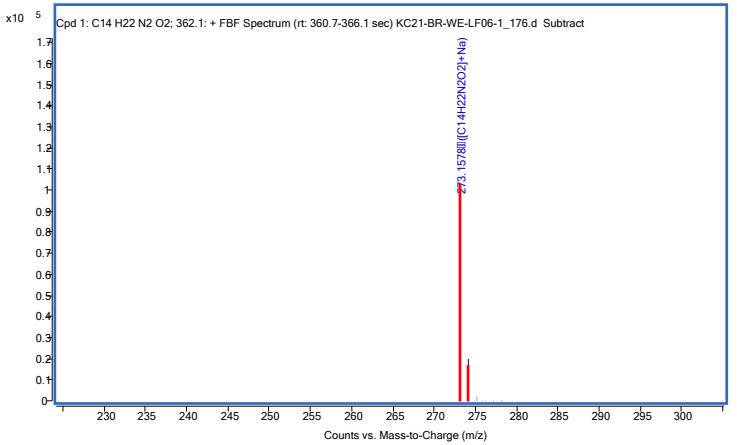


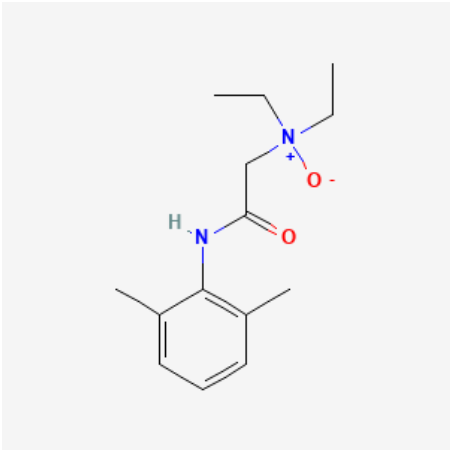
NORLEVORPHANOL

- schedule 1, no accepted medical use in US; Opiate



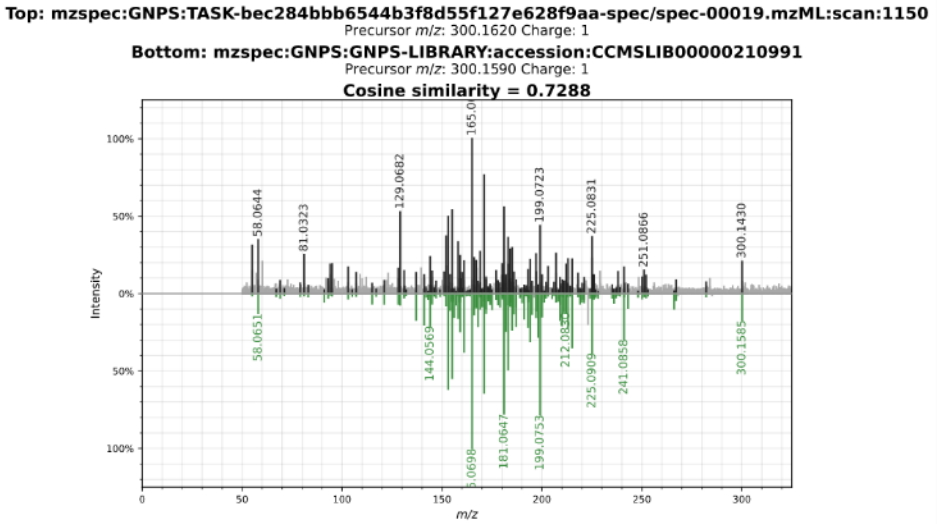


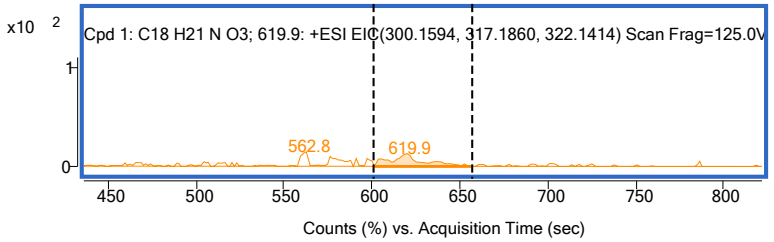


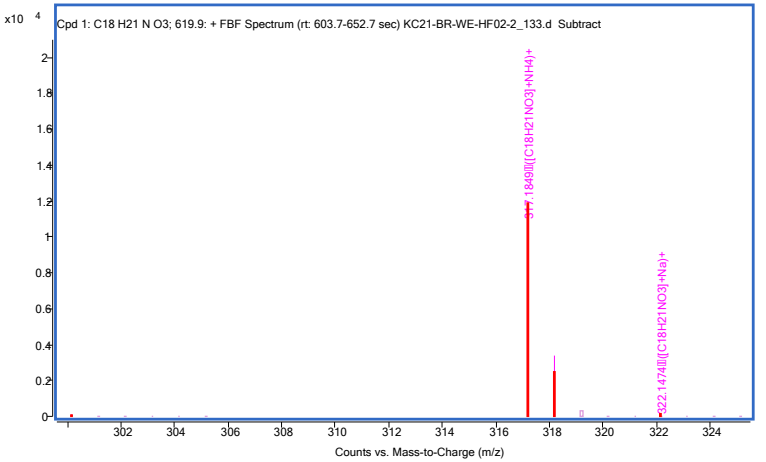


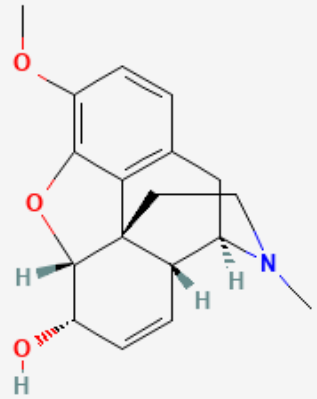
LIDOCAINE-N-OXIDE 2903-45-9

- transformation product includes lidocaine



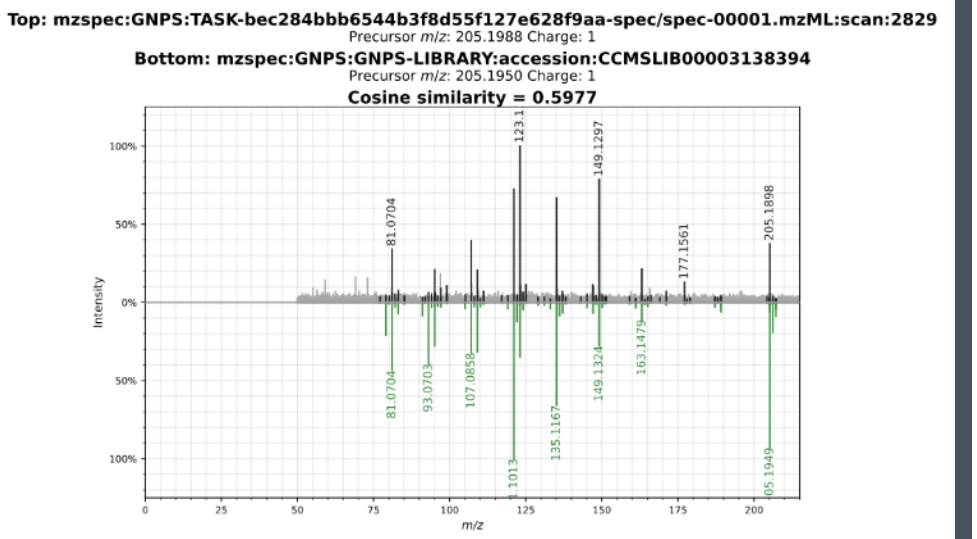


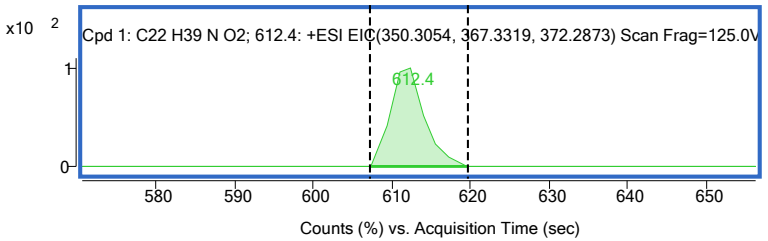


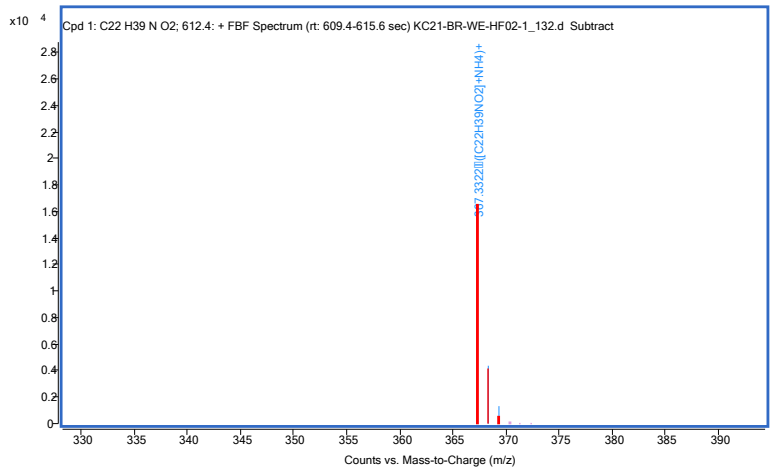


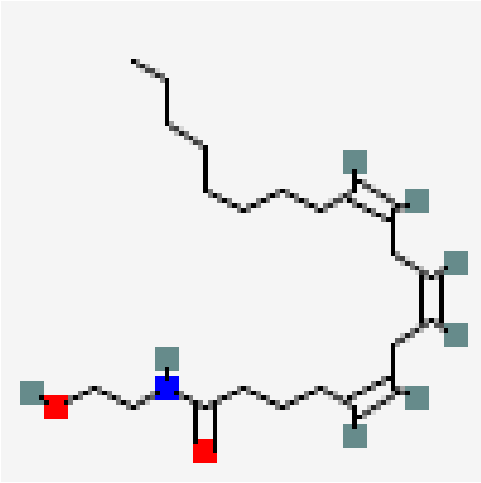
CODEINE 76-57-3

OPIOID



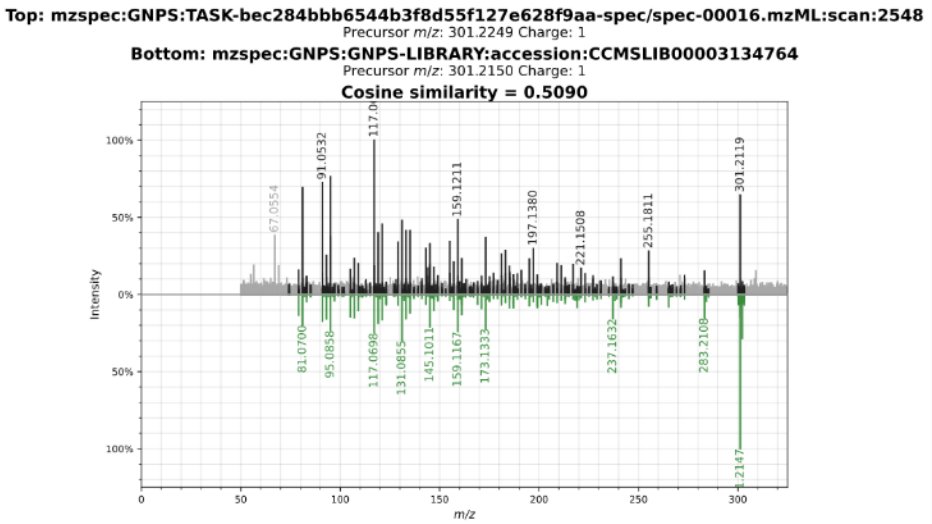


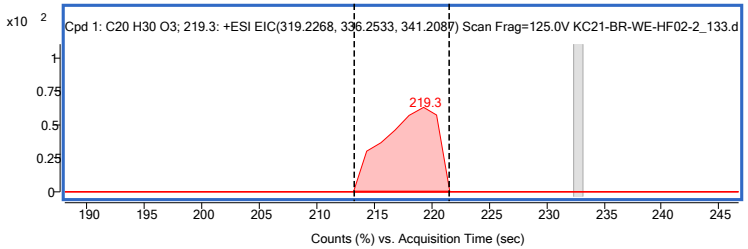


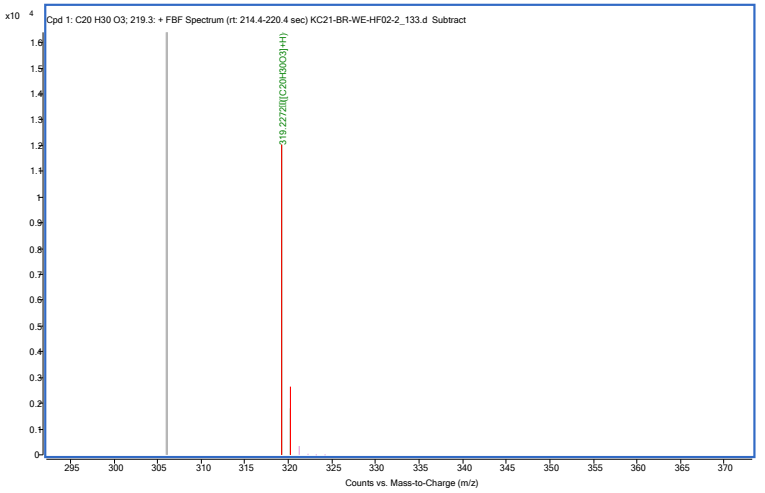


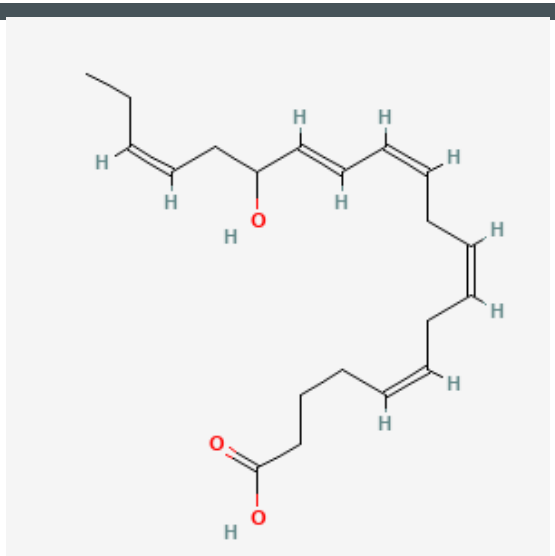
N-(5Z,8Z,11Z-EICOSATRIENOYL)-ETHANOLAMINE

FATTY AMIDE



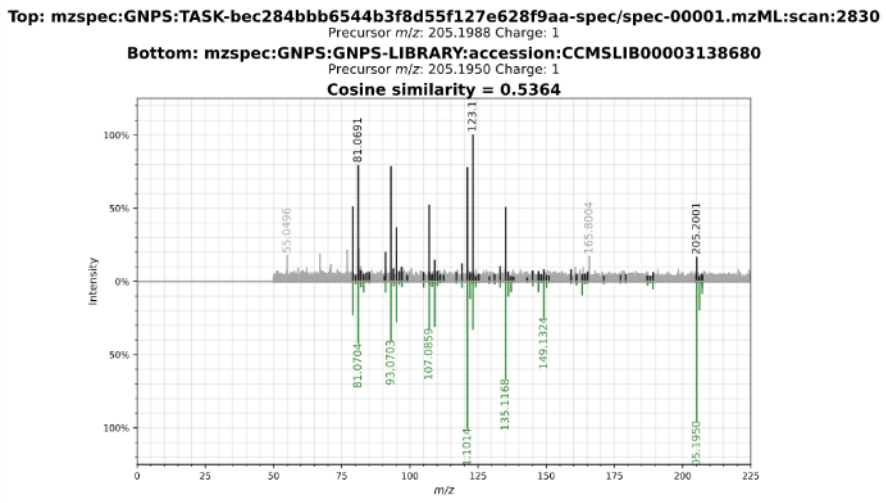


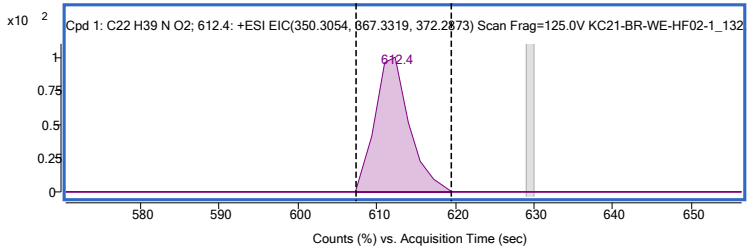


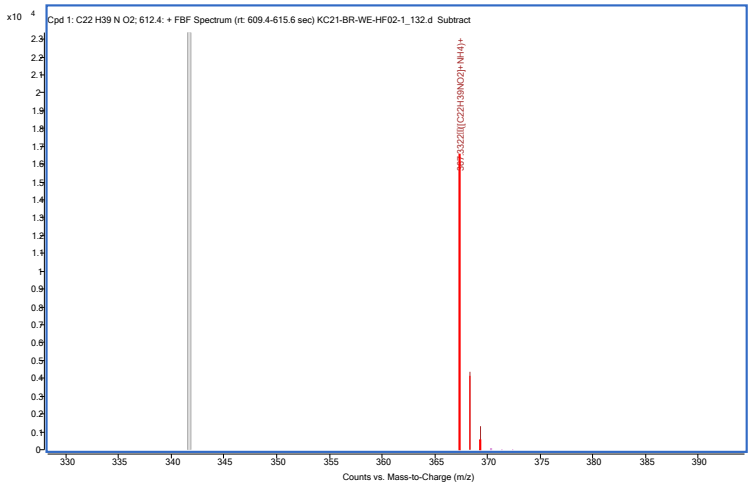


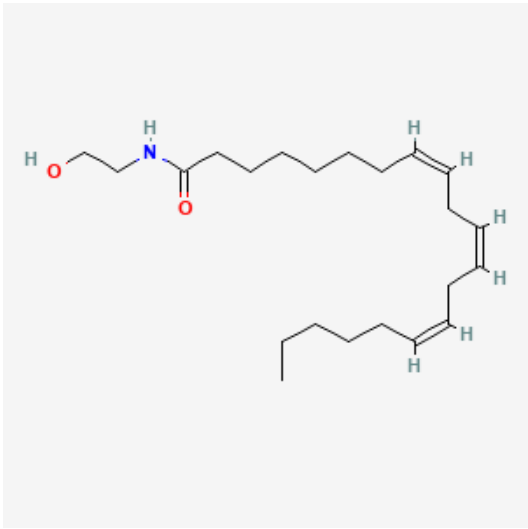
(+/-)-15-HYDROXY-5Z,8Z,11Z,13E,17Z-EICOSAPENTAENOIC ACID 88852-33-9

HYDROXY/HYDROPEROXYEICOSAPENTAENOIC ACIDS



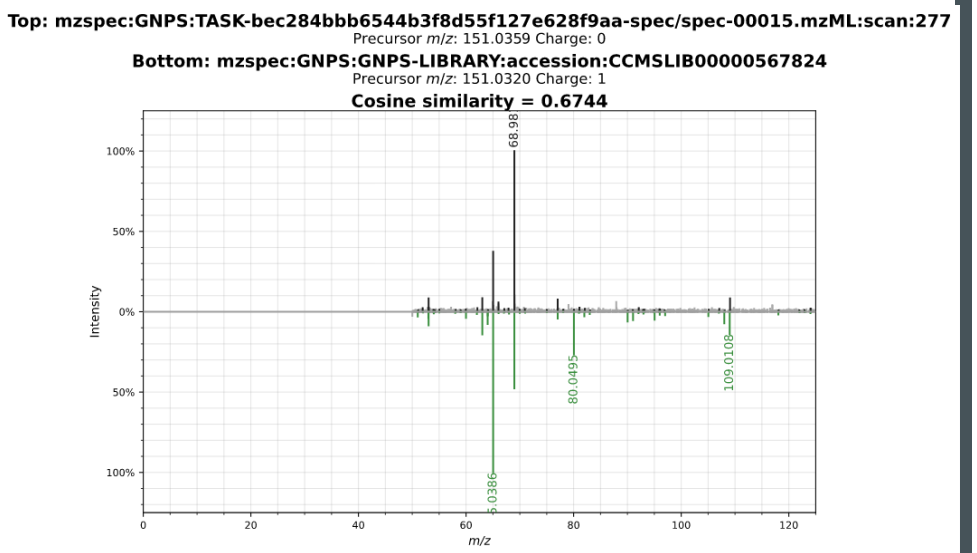


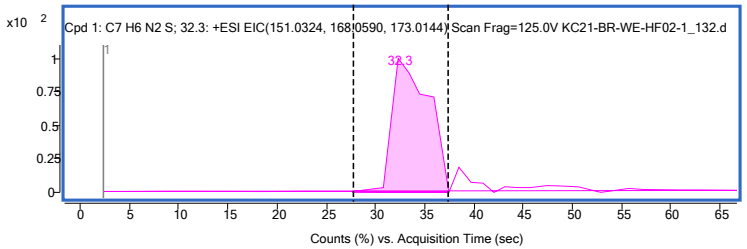


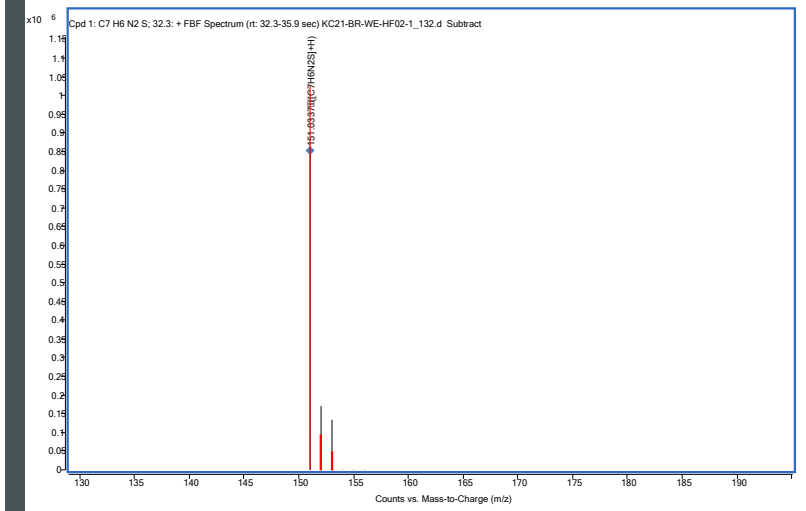


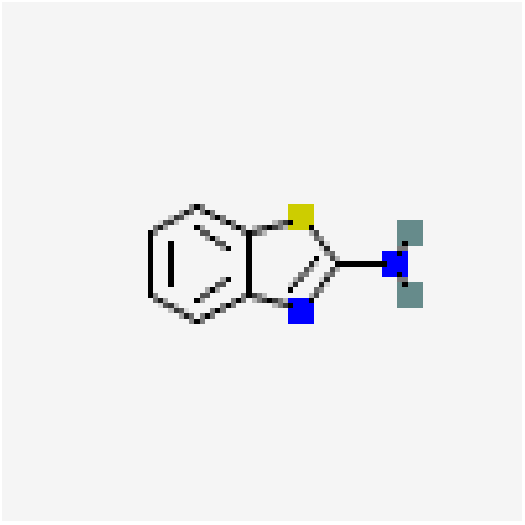
(8Z,11Z,14Z)-N-(2-HYDROXYETHYL)ICOSA-8,11,14-TRIENAMIDE 150314-34-4

FATTY AMIDE



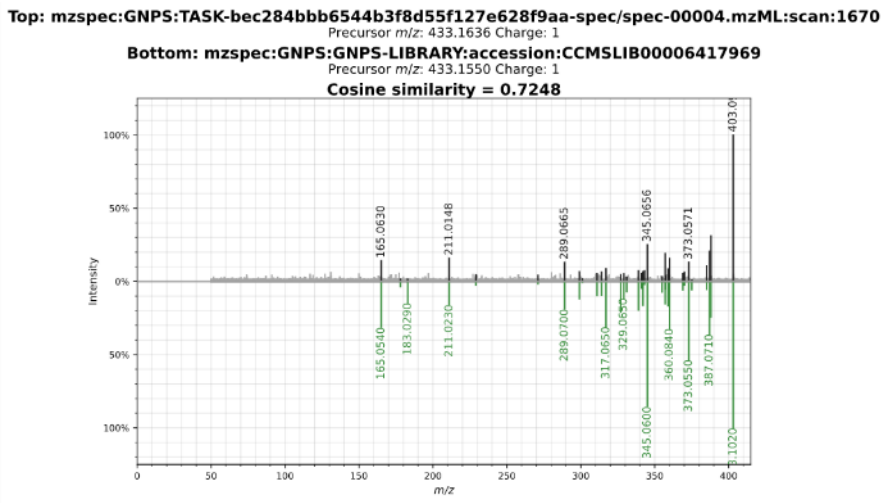


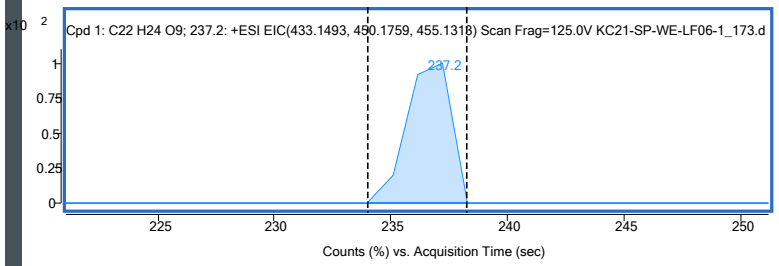


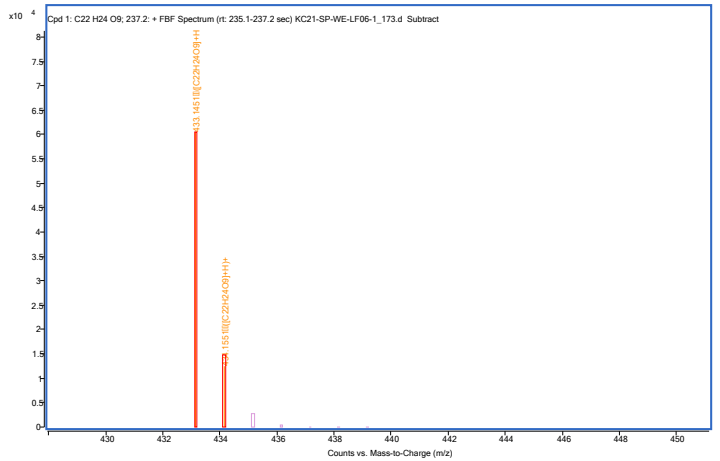


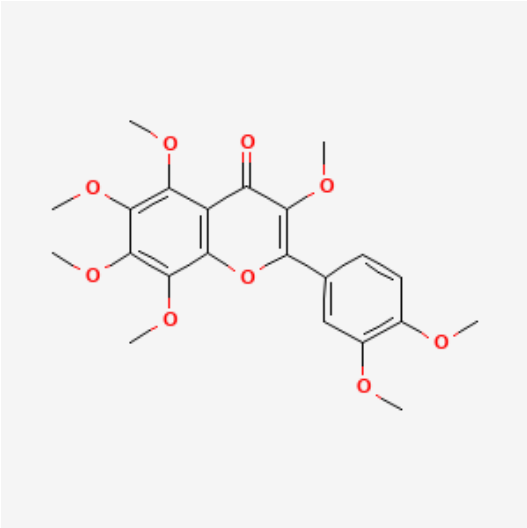
2-AMINOBENZOTHIAZOLE 136-95-8

AZO DYE INTERMEDIATE; PHOTOGRAPHIC CHEMICAL

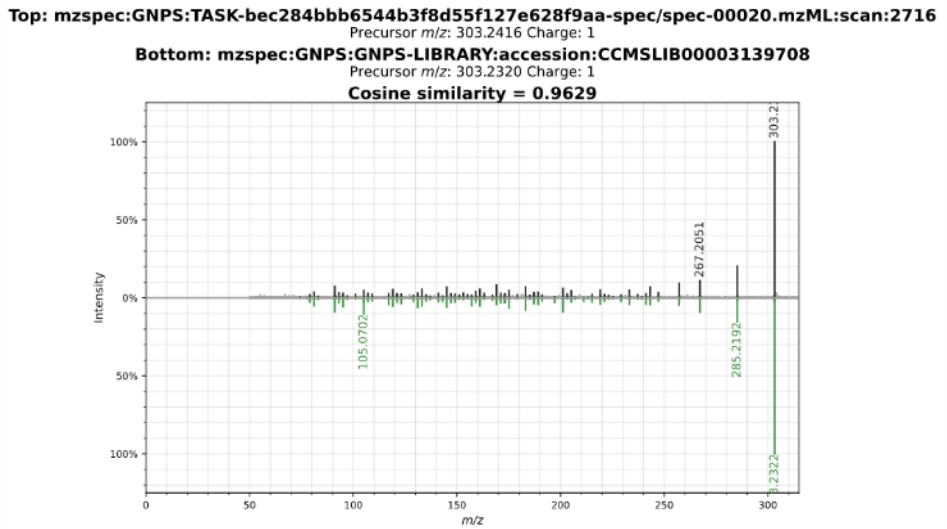


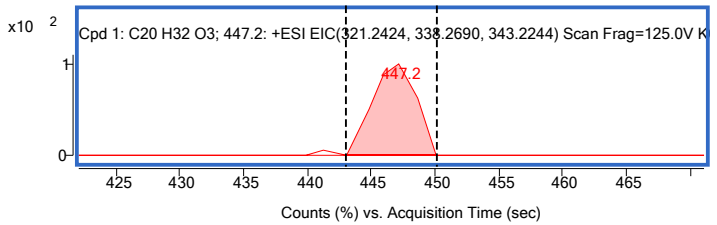


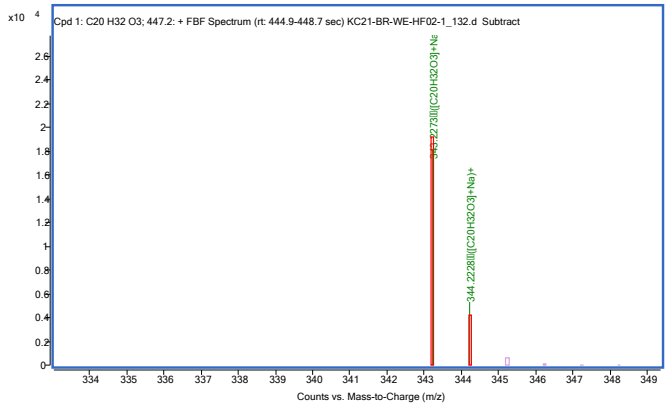


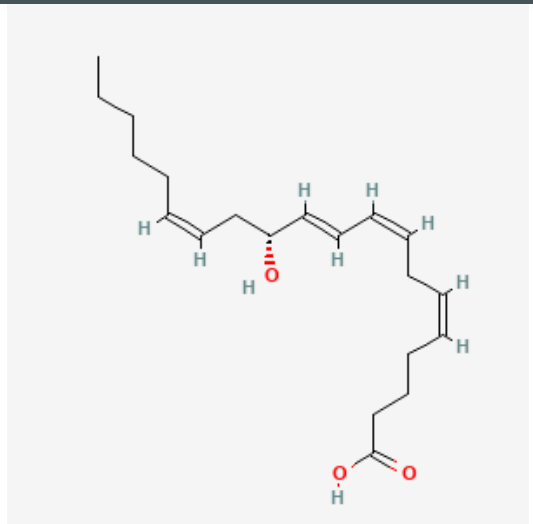


3,3',4',5,6,7,8-HEPTAMETHOXYFLAVONE 1178-24-1









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