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Levels of Selected Pharmaceuticals and Personal Care Products in the Aquatic Environment in Tromsø, Norway

Nivåer av utvalgte legemidler og personlig pleieprodukter i det akvatiske miljø i Tromsø, Norge

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Preface

This master thesis was written at the Faculty of Chemistry, Biotechnology and Food Science (KBM) at the Norwegian University of Life Sciences (NMBU) in Ås, Norway. The field work and some of the sample preparation was carried out at the Northern Research Institute (Norut) in Tromsø during October 2016, and at the Norwegian Institute for Air Research (NILU) at Kjeller during March 2017. The majority of the laboratory work was partly done at KBM and at the faculty of veterinary medicine (MatInf) NMBU. All of the instrumental analysis was performed at MatInf NMBU, during the period from August 2016 to May 2017.

Prof. Roland Kallenborn at the faculty of KBM at NMBU has been the chief supervisor during this period. Dr. Helene Thorsen Rønning and Associate Professor Terje Vasskog have been co-supervisors, at NMBU (MatInf) and Norut respectively.

Keywords: Pharmaceuticals and personal care products (PPCPs), Arctic, Tromsø, Aquatic Environment.

Julie Strømberg

Ås, 11.04.2017

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With the permission from Aasmund Fahre Vik, Research Director at NILU (Kjeller), Laboratory Technician Berit Helen Frogner, was very kind to arrange a visit their lab and do the sample preparation for the sediment samples collected in Tromsø. Berit helped me with every step of the preparation and made it a very nice experience.

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Abstract

Pharmaceuticals and personal care products (PPCPs) are acknowledged as environmental pollutants, and for the last decade have gotten a lot of attention. Studies conducted on contamination of PPCPs in the aquatic environment have identified sewage treatment plants (STP) as the predominant source. The Arctic environment is especially vulnerable to environmental pollutants. This is due to the low temperatures and lack of sunlight during the winter season leading to significantly lower degradation rates. There are several STPs in Tromsø located around Tromsøya. One of them, Breivika RA, receives sewage from private houses in Breivika area, UiT The Arctic University of Norway, and the University hospital of Northern Norway (UNN). This STP has a primary purification process where the solid phase is removed from the aqueous phase by a filter and a scrape. The aqueous phase is released by a pipeline about 100 m along the sea floor before emission 30 m under sea level into Tromsøysundet. It is difficult to make accurate measurements around Tromsøya because of strong currents and large differences in the tide.

In this study, sampling of seawater during a 7-day period, collection of liver from fish and sediments were carried out in Tromsø in October 2016 close to the emission point of Breivika STP. In addition, seawater was collected at two other locations in Tromsø. One location far away from the city collected from a boat and one location north of Breivika STP collected from the shore. The sample preparations were carried out by a solid phase extraction (SPE) method with a mixed-mode cation-exchange (MCX) sorbent for the seawater samples, a quick, easy, cheap, effective, rugged and safe (QuEChERS) method, specifically for lipid removal, were used for the preparation of fish liver, and an accelerated solvent extraction (ASE) were used for sediment samples. All of the samples were analysed for 30 compounds from different pharmaceutical groups by high performance liquid chromatography tandem mass spectrometry (HPLC -MS/MS).

Identification and quantification of the targeted compounds could only be carried out in seawater samples. The concentrations ranged from 90 to 300 ng/L for Acetaminophen and Caffeine, and 1 to 15 ng/L for Carbamazepine and Metoprolol during the 7-day period.

Norsk sammendrag

Legemidler og personlig pleieprodukter (PPCP) er en gruppe under miljøforurensinger som har fått mye oppmerksomhet verden rundt i det siste tiåret. PPCPer tilføres naturen via direkte utslipp eller som avrenning fra ulike kilder. En av hovedveiene og det som har vært mest forsket på er utslipp fra renseanlegg (RA) for avløp. Arktisk miljø er spesielt sårbart for miljøgifter på grunn av lave temperaturer og lite sollys om vinteren som gjør nedbrytningsprosessen tregere.

I Tromsø og rundt Tromsøya finnes det flere renseanlegg. Et av dem, Breivika RA, får kloakk fra husstander i Breivikaområdet, UiT Norges Arktiske Universitet og Universitetssykehuset Nord-Norge (UNN). Renseprosessen til Breivika RA går ut på å skille slam fra kloakken med hjelp av et filter og en skrape. Det er ingen videre renseprosess før vannet slippes ut i Tromsøysundet på 30 m dyp. Det er vanskelig å danne et godt bilde over eventuelle utslipp rundt Tromsøya på grunn av kraftige strømninger samt flo og fjære.

I denne studien ble det tatt sjøvanns-, fiskelever- og sedimentprøver nært utslippspunktet til Breivika RA i Oktober 2016. I tillegg ble det tatt sjøvannsprøver nord for Breivika RA og i et området langt unna mulige forurensningskilder. For sjøvannsprøvene ble det benyttet fastfaseekstaksjon (SPE) med en "mixed-mode cation-exchange" som sorbent (MCX), for fiskeleverprøver ble en "quick, easy, cheap, effective, rugged and safe" (QuEChERS) metode brukt som var spesifikk for fjerning av lipider, og for sedimentprøvene ble det bruk en "accelerated solvent extraction" (ASE) hvor selve ekstraksjonen var automatisert. For alle prøvene ble det undersøkt for 30 legemidler av ulike kategorier ved hjelp av væskekromatografi tandem massespektrometri (HPLC-MS/MS).

Identifisering og kvantifisering av analyttene var bare mulig i sjøvannsprøvene. Konsentrasjonene gjennom ukedagene varierte fra 90 til 300 ng/L for Koffein og Acetaminophen, og 1 til 15 ng/L for Carbamazepin og Metoprolol.

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Abbreviations

ASE	Accelerated Solvent Extraction
ATC	Anatomical Therapeutic Chemical
DDD	Defined Daily Dose
HPLC	High Performance Liquid Chromatography
ISTD	Internal Standard
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
MAP	Moisture Absorbing Polymer
MCX	Mixed-mode Cation-eXchange
MMCC	Matrix Matched Calibration Curve
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MP	Mobile Phase
m/z	Mass to charge ratio
NA	Not available/analysed
NMBU	Norwegian University of Life Science
Norut	Northern Research Institute
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
OTC	Over the Counter
PPCP	Pharmaceuticals and Personal Care Products
QqQ	Triple quadrupole
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
R^T	Total recovery of the method
R^{MS}	Recovery of the LC-MS/MS method
R^{PO}	Recovery of the sample preparation
RT	Retention Time
S/N	Signal-to-Noise ratio
SOP	Standard Operation Procedure
SPE	Solid Phase Extraction
SP	Stationary Phase
SSRIs	Selective Serotonin Reuptake Inhibitors
STP	Sewage Treatment Plant
UiT	University in Tromsø
VEAS	Vestfjorden Avløpsselskap
WHO	World Health Organization

1 Introduction

Pollution in the Arctic has been studied since the 1970s[1, 2] and continuous monitoring has been conducted since the establishment of the Arctic Monitoring and Assessment Programme (AMAP) in 1991. The monitoring has proven that the Arctic environment acts as a "sink" for certain pollutants because of its geographical location and climate. The climate is unique with low year-around temperatures in the water, the Gulf Stream, and seasonal variations in sunlight. The low temperatures and sunlight during the winter season are some of the factors that gives pollutants longer half-life, lower degradation rates and accumulation in higher trophic levels from long-range transport of local pollution sources[3, 4]. Pharmaceuticals and personal care products (PPCPs) are acknowledged as potential contaminants and e.g. Caffeine (CAF), Ibuprofen (IBU) and Diclofenac (DCF) has been identified in the Arctic aquatic environment[5]. In sensitive environments they are associated with adverse effects including endocrine disruption, teratogenic effects and resistance to antibiotics[6-8].

Since the beginning of the 19th century, PPCPs have improved the health of human society, the world's agriculture and animal husbandry. Diseases causing death, or long term damage, have been eliminated and living conditions have improved. Over the years, human society has grown dependent on medicinal science. The pharmaceuticals consumed are often metabolised in the human body into more polar and water soluble compounds. The mechanism is either hydrolysis, oxidation, reduction or conjugation reactions[9]. Hence, the vast majority of the PPCPs consumed by humans are excreted and will ultimately end up in the aquatic environment as a metabolite or a parent compound. The most likely pathway for PPCPs to enter the environment is via fish farms, private sewage systems or from discharged sewage effluent from STPs[10, 11]. A primary STP is designed to remove the solid material from the aqueous phase by a filtration system. The aqueous effluent is released into the water which means that the only removal step for PPCPs are adsorption to solid material. Whereas a larger and more complex STP has several cleaning steps (e.g. heating and/or biodegradation) before releasing the effluent. There are no specific procedures made for the removal of PPCPs and it has been observed in studies that the removal of these compounds in the STPs are poor[5, 12]. In Norway, the cleaning steps at a STP varies. According to Norwegian Environment Agency there are 1844 listed STPs where 30 % are undefined, 30 % are using degradation of either chemical, biological, or both, 28.5 % are equipped with mechanical separator, 1.5 % are nature based and 10 % do not have any form of cleaning steps[13].

There are many methods available for extracting PPCPs from water or other sample matrices. Solid-phase extraction (SPE) is one of the most common methods for extracting analytes in different matrices and it has a variety of applications. The aim of a sample preparation is to remove matrix components which can interfere with the analysis without losing the targeted analytes. An advantage of the SPE is that it gives the opportunity to go from large sample volumes to small and thus detection of trace levels are possible. It is important to take into account when choosing a method, which matrix and the physical-chemical properties of the analytes that are going to be analysed. Therefore it is difficult to find one method suitable for a group of different compounds, and also optimal for every compound. It is especially important to have a sensitive method and instrumentation in order to detect the compounds in low concentrations. In seawater where the dilution factor is extremely high, or biota samples where matrix effects can have a big impact on the analysis.

The concentration of organic environmental pollutant are often found in trace levels which needs very sensitive instruments to be detected. Preferred instruments are gas chromatography (GC) for volatile compounds, or liquid chromatography (LC) for the more polar compounds, combined with a mass spectrometer (MS). The GC-MS combination has been used since 1950s[14, 15] and separates the sample in a gaseous mobile phase (MP). It is limited to thermally stable and volatile samples and thus many compounds need derivatisation before analysis. The benefit of using GC-MS is general lower detection limits and matrix effects. Measurements using the LC-MS system was started in the 1970s[16, 17] and provides an advantage when measuring polar and non-volatile compounds.

In order to assess the above reasons, investigation and monitoring of PPCPs in the environment is necessary to evaluate implications of long-term exposure. By constructing an effective method that can address multiple compound groups with enough precision and accuracy, more studies and monitoring can be performed and prevent possible adverse effects in humans and environment due to unintentional exposure.

1.1 Environmental relevance

Pharmaceuticals in the environment have been identified as an environmental issue since the early 1970s, where hormones were found in sewage[18]. The consumption of hormonal contraceptives has increased significantly since it came on the market in the 1960's[19]. Synthetic and natural hormones are exerted from the human body. There has been several reports on endocrine disruption in different fish living in sewage effluent dominated environments[6, 7, 20]. The findings of PPCPs in later years has increased and the focus of preventing emissions into the environment have gotten more attention worldwide. The different pathways for PPCPs to reach the aquatic environment are illustrated in Figure 1. Leaching from landfill and soils or direct emissions from STPs or fish farms are some of the possible routes.

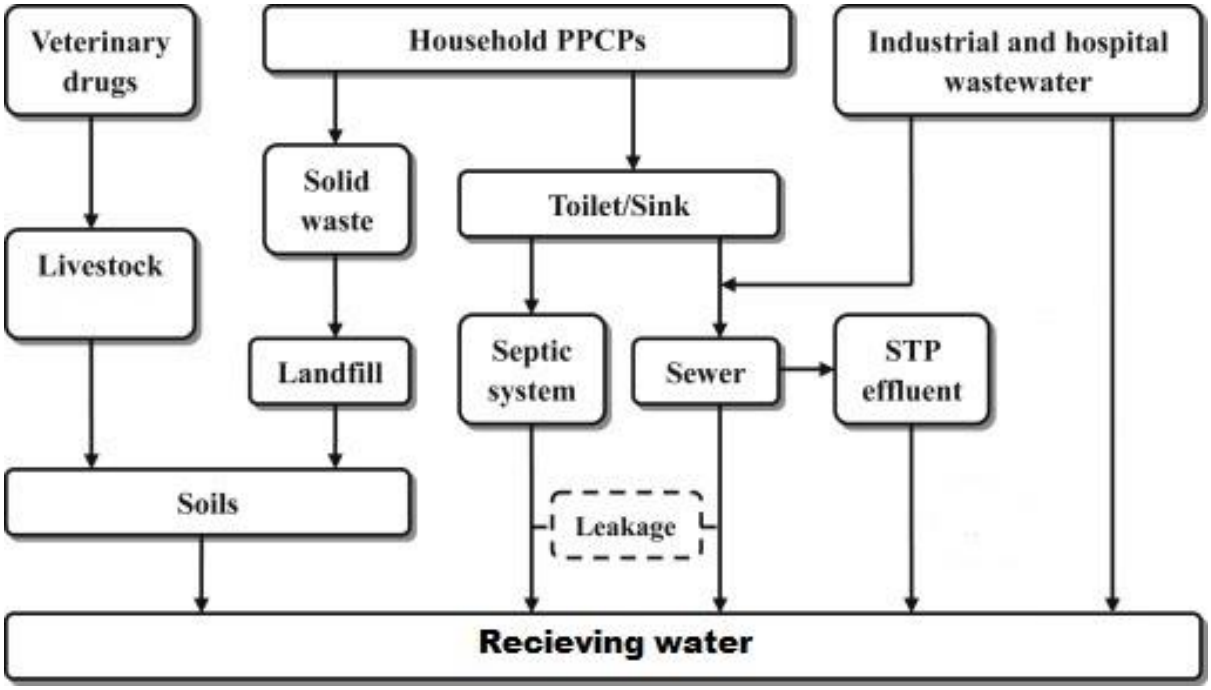


Figure 1: Environmental pathways of PPCPs adapted from [21]

The risk assessment of certain compounds and what risk it poses to the environment is often described as the ratio between predicted environmental concentration (PEC) and predicted no-effect concentration (PNEC), where a ratio lower than one is considered as low risk. However, the model does not take into account combined effects of multiple compounds present at the

same time, variability in concentrations or effects of long-term exposure of low concentrations. Risk assessment studies are also conducted with a predicted concentration and are often performed in a closed environment. This may not always be directly equivalent to exposure and effects in their natural environment. The general findings of PPCPs in the environment are in the parts per trillion (PPT) and parts per billion (PPB) area of concentrations[22] but is also proven to be found at higher concentrations. A study done on sewage effluents from STPs in Sweden, Italy, France and Greece revealed concentrations of Carbamazepine (CBZ) up to 1.2 µg/L[23]. CBZ is a drug used mainly as epilepsy medication, and is an example of a compound that has a narrow therapeutic index. Therapeutic index compares the ratio between toxic effects at lethal doses (LD_{50}) in 50 % of the subjects, with therapeutic effects or effective dose (ED_{50}) on 50 % of the subjects. This means that it has a low safety margin between safe doses and toxic doses, and small changes in concentration can lead to a fatal response[24].

In extreme cases there has been detected high concentrations of PPCPs in effluents from drug manufacturers. Concentrations of an antibiotic drug, Ciprofloxacin (CIP), up to 31 000 µg/L was discovered in effluent from a STP connected to about 90 drug manufactures in India[25]. Even though Norway is one of the countries in Europe with the lowest consumption of antibiotics both in agriculture and medicine[26], traces can be detected in the Norwegian effluents and elimination rates in STPs has been proven to be poor[27]. Large consumption of antibiotics can lead to growth of antibiotic resistant bacteria and the antibiotic agent will no longer have an effect. This will occur if the bacteria is partially resistant, or if they are exposed over time. Bacteria also have a rapid growing rate and are multiplying fast. Therefore, a chance of mutation resulting in resistance is elevated.

Over the counter (OTC) pharmaceuticals are sold without prescription and are frequently used in Norway for minor issues (e.g. headache and inflammation). Amongst the most popular OTC pharmaceuticals there are Acetaminophen (Paracetamol) (APAP), Acetylsalicylic acid (ASA), Diclofenac (DCF), and Ibuprofen (IBU), which are often used for pain relief. A commonly used example of adverse effects on nature as a cause of pharmaceutical is DCF and vultures (*Gyps bengalensis*) in Pakistan. There was a decline in > 95 % in the population because of renal failure[28], and the source was identified as dead domestic livestock which had been treated with Diclofenac, which the vultures had been feeding of.

Fertilizers of recycled manure are often used in agriculture and are designed to work as a promoter of the soils moisture, organic content and the plants health. STPs have been producing

fertilizers and by subjecting the solid phase to heat and drying it for a period of time, it can be used to promote growth in agriculture[29]. Potential exposure of pharmaceuticals in the food as a result using fertilizers from STPs is a growing concern. Pharmaceutical residues in fertilizer from different animals[30] shows that stronger legislations and broader studies needs to be conducted and the right precautions needs to be taken into account in order to prevent major adverse effects, similar to the incidence with the vultures.

1.2 Aim of this study

During two previous studies conducted in the Breivika area and around Tromsøya in 2004 and 2008[5, 31] PPCPs were confirmed both in sewage effluents and receiving waters near STPs around Tromsøya.

The aim of this study was to expand the investigation of PPCPs in the aquatic environment in Tromsø by monitoring additional groups of PPCPs within the same method, and try to investigate the weekly occurrence around Tromsø city. In addition, it is necessary to investigate the possible correlation between the concentration in the water and the ambient environment (e.g. fish and sediment samples).

1.3 Analytes

In this investigation, 30 PPCPs were chosen (Table 3 and Figure 2) as target analytes based on sale statistics in Norway and availability of analytical standards. All of the compounds are frequently used in Norway and eight of them (Table 1) are on the top transacted active ingredients in defined daily dose (DDD) in Norway 2015[32]. The five internal standards (ISTD) used for quantification were Caffeine $^{13}C_3$, Carbamazepine-d_{10}, Metoprolol-d_7, Sulfadoxine-d_3 and Trimethoprim-d_9

Table 1: Top 25 transacted active ingredients in Norway 2015, adapted from[32].

1 Atorvastatin	6 Cetirizine	11 Candesartan	16 Ascorbic acid	21 Calcium
2 Acetylsalicylic acid	7 Amlodipine	12 Levothyroxine	17 Esomeprazole	22 Ibuprofen
3 Simvastatin	8 Ramipril	13 Metoprolol	18 Pantoprazole	23 Cetirizine
4 Paracetamol	9 Zopiclone	14 Vitamin B Complex	19 Escitalopram	24 Metformin
5 Natrium fluoride	10 Xylomethazolin	15 Levonorgestrel and estrogen	20 Hydroksokobalamin	25 Losartan

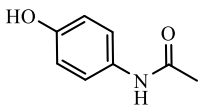
There are different ways to classify pharmaceuticals. The anatomical therapeutic chemical (ATC) classification system, from the Worlds Health Organization (WHO), gives an overview and access to a classification system that is easy to apply to the different pharmaceuticals. The classification system is grouped into five levels, where the first level is shown in the table below (Table 2). The second, third and fourth levels are divided after chemical pharmacological or therapeutic subgroups, and the fifth level is the chemical substance[33, 34].

Table 2: First level of the ATC classification system adapted from[34].

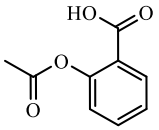
A	Alimentary tract and metabolism
B	Blood and blood forming organs
C	Cardiovascular system
D	Dermatologicals
G	Genito urinary system and sex hormones
H	Systemic hormonal preparations, excluding sex hormones and insulins
J	Anti-infective for systemic use
L	Antineoplastic and immunomodulating agents
M	Musculo-skeletal system
N	Nervous system
P	Antiparasitic products, insecticides and repellents
R	Respiratory system
S	Sensory organs
V	Various

Table 3: List of abbreviation, IUPAC-name, ATC category and mode of action of the targeted analytes.

Analyte	Abbreviation	IUPAC-name	ATC 1st level	Mode of action
Acetaminophen	APAP	N-(4-hydroxyphenyl)acetamide	N	Analgestics and antipyretics, Fever lowering agent
Acetylsalicylic acid	ASA	2-acetyloxybenzoic acid	A, B, C, N	NSAIDs
Amitriptyline	AMT	3-(5,6-dihydrodibenzo[2,1-b:2',1'-f][7]annulen-11-ylidene)-N,N-dimethylpropan-1-amine	N	Antidepressants, TCA, Non-selective monoamine reuptake inhibitors
Amlodipine	ADP	3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate	C	Antihypertensive drugs, Calcium channel blockers
Atenolol	ATN	2-[4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl]acetamide	C	Antihypertensive drugs , Adrenergic receptor antagonists
Atorvastatin	AVS	(3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid	C	Antihyperlipidemic agents, Cholesterol lowering agent
Caffeine	CAF	1,3,7-trimethylpurine-2,6-dione	N, V	Stimulates the CNS
Carbamazepine	CBZ	benzo[b][1]benzazepine-11-carboxamide	N	Antipiletic
Cephalexin	CEP	(6R,7R)-7-[[(2R)-2-amino-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid	J	Antibacterial, β-Lactams, Cephalosporins
Chlorphenamine	CPN	3-(4-chlorophenyl)-N,N-dimethyl-3-pyridin-2-ylpropan-1-amine	R	H_1-antihistamine
Ciprofloxacin	CIP	1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid	J, S	Antibacterial, Fluoroquinolones
Diclofenac	DCF	2-[2-(2,6-dichloroanilino)phenyl]acetic acid	D, M, S	NSAIDs
Diethyltoluamide	DEET	N,N-diethyl-3-methylbenzamide	P	Repellents, insecticides
Ethinylestradiol	EE2	(8R,9S,13S,14S,17R)-17-ethynyl-13-methyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthrene-3,17-diol	G, L	Contraception , Estrogen
Estrone	E1	(8R,9S,13S,14S)-3-hydroxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthren-17-one	G	Estrogen
Fluoxetine	FLX	N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine	N	Antidepressants, SSRI
Ibuprofen	IBP	2-[4-(2-methylpropyl)phenyl]propanoic acid	C, G, M, N, R	NSAIDs
Losartan	LAT	[2-butyl-5-chloro-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]imidazol-4-yl]methanol	C	Angiotensin II receptor antagonists
Metformin	MET	3-(diaminomethylidene)-1,1-dimethylguanidine	A	Biguanudes, blood glucose lowering drugs
Metoprolol	MPL	1-[4-(2-methoxyethyl)phenoxy]-3-(propan-2-ylamino)propan-2-ol	C	Beta blocking agents
Metronidazole	MNZ	2-(2-methyl-5-nitroimidazol-1-yl)ethanol	A, D, G, J, P	Antibiotic agents, Nitroimidazoles
Penicillin G	PEN G	(2S,5R,6R)-3,3-dimethyl-7-oxo-6-[(2-phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid	J, S	Antibiotic agents, β-Lactams
Prednisolone	PNS	(8S,9S,10R,11S,13S,14S,17R)-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthren-3-one	A, C, D, H, R, S, V	Corticosteroid
Ranitidine	RAN	(E)-1-N'-[2-[[5-[(dimethylamino)methyl]furan-2-yl]methylsulfanyl]ethyl]-1-N-methyl-2-nitroethene-1,1-diamine	A	H_2-receptor antagonist
Salicylic acid	SCA	2-hydroxybenzoic acid	A, B, C, D, J, M, N, P, S,	Anti- bacterial, inflammatory, fungial, NSAIDs
Simvastatin	SIV	[(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] 2,2-dimethylbutanoate	A, C	Antihyperlipidemic agents, Cholesterol lowering agent
Sulfadoxine	SFD	4-amino-N-(5,6-dimethoxypyrimidin-4-yl)benzenesulfonamide	J	Antibiotic agents, Sulphonamides
Sulfamethoxazole	SMX	4-amino-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide	J	Antibiotic agents, Sulphonamides
Trimethoprim	TMP	5-[(3,4,5-trimethoxyphenyl)methyl]pyrimidine-2,4-diamine	J	Antibiotic agents
Warfarin	WAR	4-hydroxy-3-(3-oxo-1-phenylbutyl)chromen-2-one	B	Antithrombotic agents, Vitamin K antagonists



Acetaminophen
Chemical Formula: $C_8H_9NO_2$
Exact Mass: 151,06
CAS #: 103-90-2



Acetylsalicylic acid
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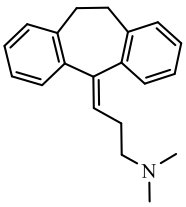
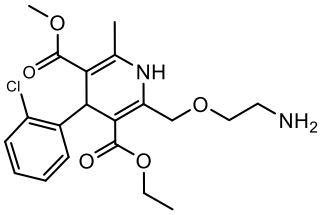
Exact Mass: 180,04
CAS #: 50-78-2



Amitriptyline
Chemical Formula: $C_{20}H_{23}N$
Exact Mass: 277,18
CAS #: 549-18-8



Amlodipine
Chemical Formula: $C_{20}H_{25}ClN_2O_5$
Exact Mass: 408,15
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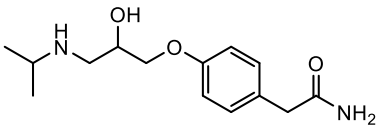

Atenolol
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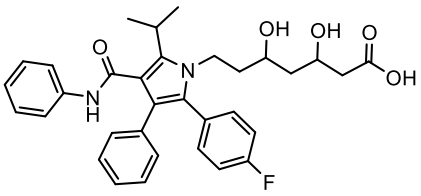
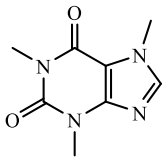
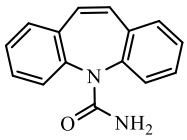
Exact Mass: 266,16
CAS #: 29122-68-7



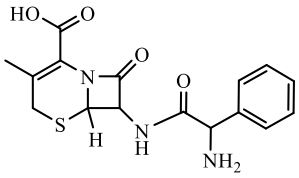
Atorvastatin
Chemical Formula: $C_{33}H_{35}FN_2O_5$
Exact Mass: 558,25
CAS #: 134523-00-5



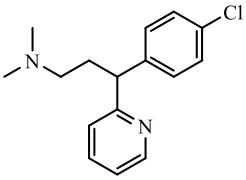
Caffeine
Chemical Formula: $C_8H_{10}N_4O_2$
Exact Mass: 194,08
CAS #: 58-08-2



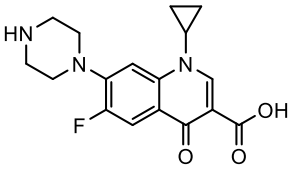
Carbamazepine
Chemical Formula: $C_{15}H_{12}N_2O$
Exact Mass: 236,09
CAS #: 298-46-4



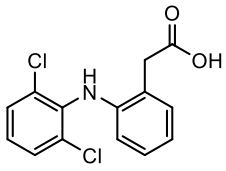
Cephalexin
Chemical Formula: $C_{16}H_{17}N_3O_4S$
Exact Mass: 347,09
CAS #: 15686-71-2



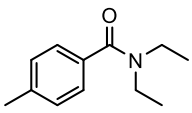
Chlorphenamine
Chemical Formula: $C_{16}H_{19}ClN_2$
Exact Mass: 274,12
CAS #: 132-22-9



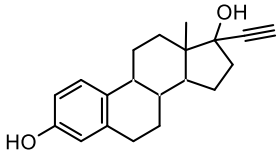
Ciprofloxacin
Chemical Formula: $C_{17}H_{18}FN_3O_3$
Exact Mass: 331,13
CAS #: 85721-33-1



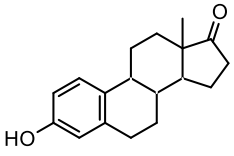
Diclofenac
Chemical Formula: $C_{14}H_{11}Cl_2NO_2$
Exact Mass: 295,02
CAS #: 15307-86-5



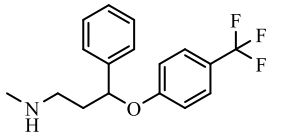
Diethyltoluamide
Chemical Formula: $C_{12}H_{17}NO$
Exact Mass: 191,13
CAS #: 134-62-3



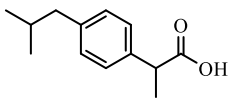
Ethinylestradiol
Chemical Formula: $C_{20}H_{24}O_2$
Exact Mass: 296,18
CAS #: 57-63-6



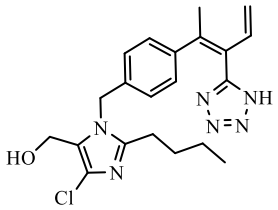
Estrone
Chemical Formula: $C_{18}H_{22}O_2$
Exact Mass: 270,16
CAS #: 53-16-7



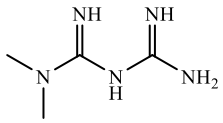
Fluoxetine
Chemical Formula: $C_{17}H_{18}F_3NO$
Exact Mass: 309,13
CAS #: 54910-89-3



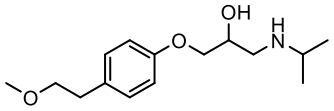
Ibuprofen
Chemical Formula: $C_{13}H_{18}O_2$
Exact Mass: 206,13
CAS #: 15687-27-1



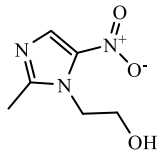
Losartan
Chemical Formula: $C_{22}H_{23}ClN_6O$
Exact Mass: 422,16
CAS #: 114798-26-4



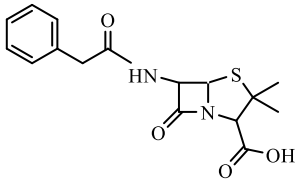
Metformin
Chemical Formula: $C_4H_{11}N_5$
Exact Mass: 129,10
CAS #: 657-24-9



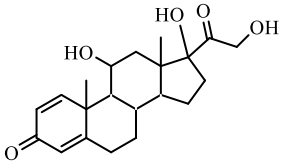
Metoprolol
Chemical Formula: $C_{15}H_{25}NO_3$
Exact Mass: 267,18
CAS #: 37350-58-6



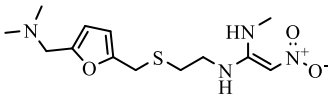
Metronidazole
Chemical Formula: $C_6H_9N_3O_3$
Exact Mass: 171,06
CAS #: 443-48-1



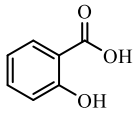
Penicillin G
Chemical Formula: $C_{16}H_{18}N_2O_4S$
Exact Mass: 334,10
CAS #: 61-33-6



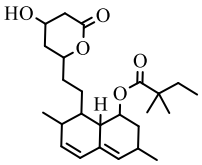
Prednisolone
Chemical Formula: $C_{21}H_{28}O_5$
Exact Mass: 360,19
CAS #: 50-24-8



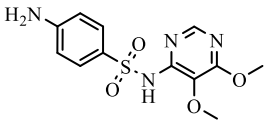
Ranitidine
Chemical Formula: $C_{13}H_{22}N_4O_3S$
Exact Mass: 314,14
CAS #: 66357-35-5



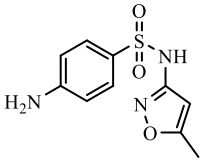
Salicylic acid
Chemical Formula: $C_7H_6O_3$
Exact Mass: 138,03
CAS #: 69-72-7



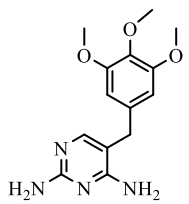
Simvastatin
Chemical Formula: $C_{25}H_{38}O_5$
Exact Mass: 418,27
CAS #: 79902-63-9



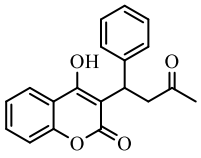
Sulfadoxine
Chemical Formula: $C_{12}H_{14}N_4O_4S$
Exact Mass: 310,07
CAS #: 2447-57-6



Sulfamethoxazole
Chemical Formula: $C_{10}H_{11}N_3O_3S$
Exact Mass: 253,05
CAS #: 723-46-6



Trimethoprim
Chemical Formula: $C_{14}H_{18}N_4O_3$
Exact Mass: 290,14
738-70-5



Warfarin
Chemical Formula: $C_{19}H_{16}O_4$
Exact Mass: 308,10
CAS #: 81-81-2

Figure 2: Page 9 and 10 displays chemical structure and formula, monoisotopic mass and CAS number (from ChemDraw®) of the target analytes.

1.3.1 Pharmaceuticals and metabolites

In this study, metabolites are considered as pharmaceuticals that have been through some form of metabolic reaction in the human body. Foreign compounds are in the human body, targeted by metabolic enzymes which has the ability to degrade or modify the foreign compounds. The aim of the modification in general is to make the foreign compound more easily excreted. This modification often makes the metabolite loose the activity of the parent compound, but in some cases, some activity is still retained. In extreme cases, the metabolite are even more active or has a different activity than the parent compound, which can result in serious side effects of toxicity [35].

1.3.1.1 Example of metabolites of a target analyte

The terms analgesics and antipyretic derives from Greek, and can roughly be translated to "without pain" and "against the fire of fever" respectively. A more common word is painkiller which covers a large group of pharmaceuticals that have a diverse mode of action. The terms include Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and opioids. Mild analgesics and antipyretics e.g. Ibuprofen, are typically OTC drugs which means that they can easily be bought in the local shop and are readily available. The common general structure of NSAIDs consist of an acidic centre, (hetero)-aromatic ring, and a lipophilic centre (alkyl chain or additional aromatic ring) [36]. IBU is metabolised into Hydroxy-ibuprofen (OH-IBU) and Carboxy-ibuprofen (CX-IBU) by hydroxylation and carboxylation respectively (Figure 3). Studies conducted on the occurrence of Ibuprofen and its metabolites both in the STP and receiving environments, shows that it is considerable higher concentrations of the metabolites than the parent compound [5, 37].

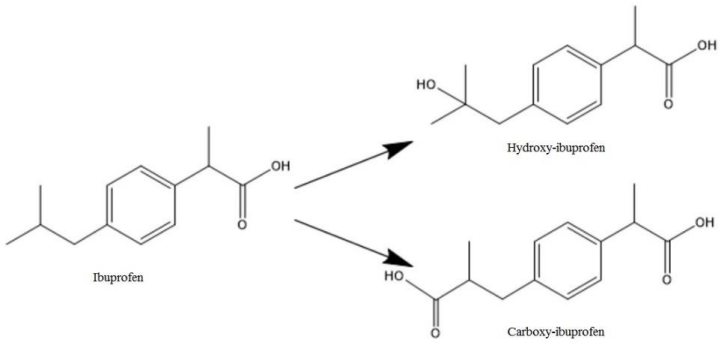


Figure 3: Parent compound of IBU and the metabolites OH-IBU and CX-IBU.

1.4 Sample preparations

1.4.1 Solid phase extraction

The general SPE setup is a tube or a cartridge packed with a sorbent, chosen based on its affinity to the analytes. The extraction is done by using a sorbent that the target compounds adsorbs to while impurities are either eluted or retained on the sorbent. Further clean-up is done by a washing step, before eluting the compounds with a suitable solvent that has a higher affinity to the target compound than the sorbent[38]. The clean-up will reduce matrix effects, and can up-concentrate large volume samples if eluted in smaller volumes than the sample added. The Mixed-mode Cation-eXchange (MCX) from Oasis®, is a sorbent consisting of sulfonic acid and a reversed-phase retention mode. This gives a selectivity for retaining positively charged compounds at the sulfonic functional group and hydrophobic retention on the reversed phase of the sorbent retain (Figure 4). It has a wide stability range in pH (pH 0-14), and can maintain its stability even if the cartridge goes dry. By applying the cartridge on a vacuum manifold, the process can be automated to simplify the extraction of large sample volumes.

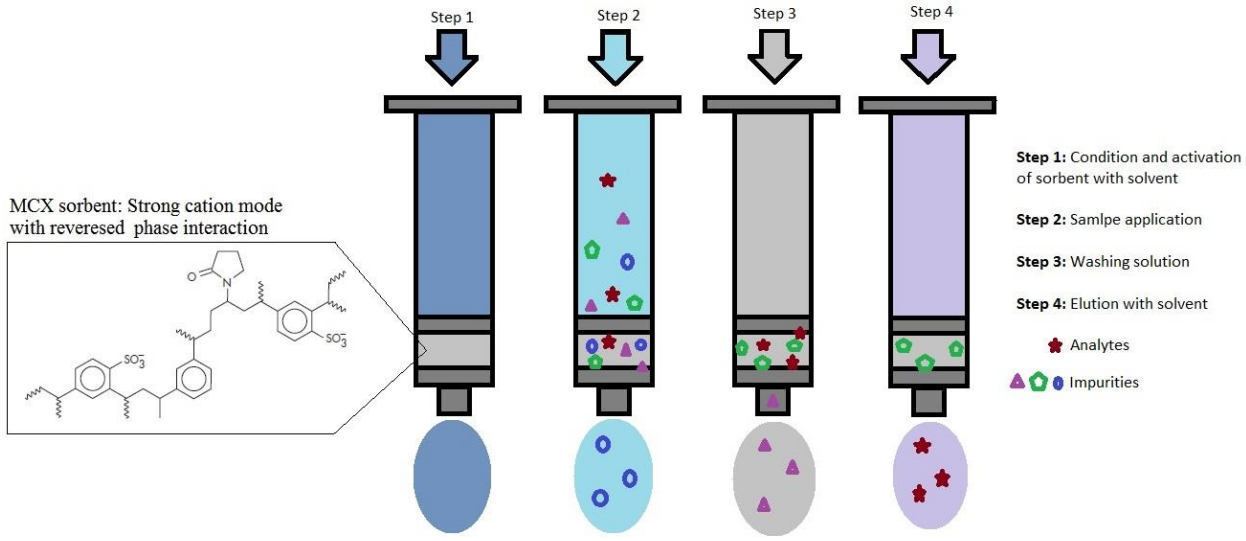


Figure 4: The standard extraction procedure steps in SPE.

1.4.2 Accelerated Solvent Extraction

Accelerated solvent extraction (ASE) is an automated extraction method which combines high temperature, pressure and liquid solvent. It is an extraction method for (semi) solid samples, and is typically used for targeting organic compounds. The sample is homogenized in a mixture of diatomaceous earth, moisture absorbing polymer and Florisil®. The mixture is packed in an extraction cell, and applied in an ASE device (Figure 5). A high pressure keeps the solvent in a liquid state under high temperatures, and this will accelerate the extraction. When 1 mL is collected in the collection vial and the extraction cell is filled with solvent, a static valve closes. The extraction cell is heated to the desired temperature and the static valve opens periodically to maintain pressure in the cell. After one cycle with high temperature and pressure, the solvent in the extraction cell is flushed into the collection vial and is ready for analysis or further clean up.

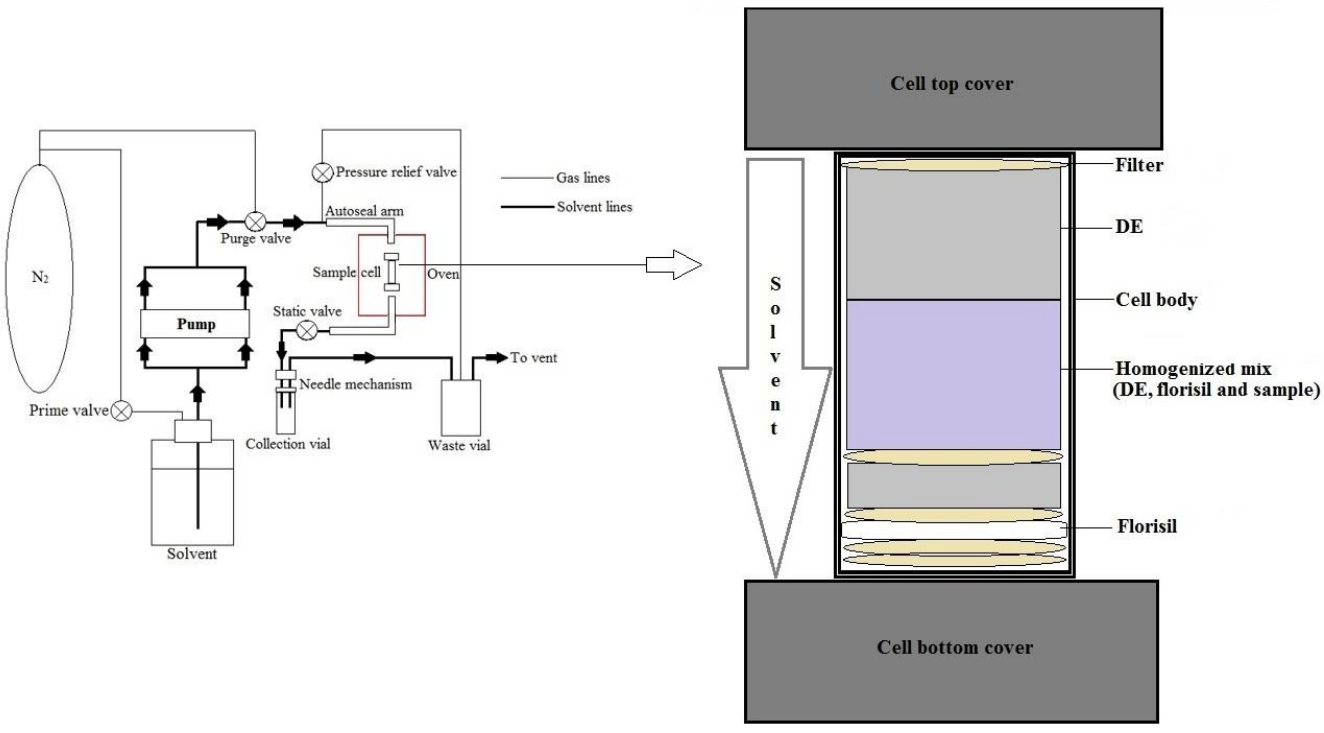


Figure 5: Schematic representation of ASE instrument and a packed extraction cell.

1.4.3 QuEChERS

QuEChERS, an abbreviation for "Quick, Easy, Cheap, Effective, Rugged and Safe", is a sample preparation technique originally developed for analysis of veterinary drugs in animal tissue, but has later been widely used for pesticides in vegetables, fruit and other matrixes [39, 40]. The method is designed, unlike SPE, where the sorbent is packed in a column, to remove matrix interferences by adding and mixing a sorbent material into a sample, and thereafter separate the extract by centrifugation. The QuEChERS method is used in different ways, depending on the working matrix and the chosen analytes. The general idea is to have a homogenised sample in an aqueous phase, and mix it with a suitable organic solvent. Sorbent material is added and mixed into the sample, and induces a dispersive SPE where matrix interferences binds to the sorbent. The mixture is centrifuged and the two phases with extract and sorbent material can be separated. By adding a drying agent (e.g. a hygroscopic salt) to the extract, the agent will bind to water molecules, and distribution of the analytes into the organic phase is enhanced. By shaking the mixture of sample and drying agent, the distribution into the organic phase is promoted. By centrifugation, the dried extract in the organic phase can be separated from the drying agent.

1.5 Liquid chromatography - tandem mass spectrometry

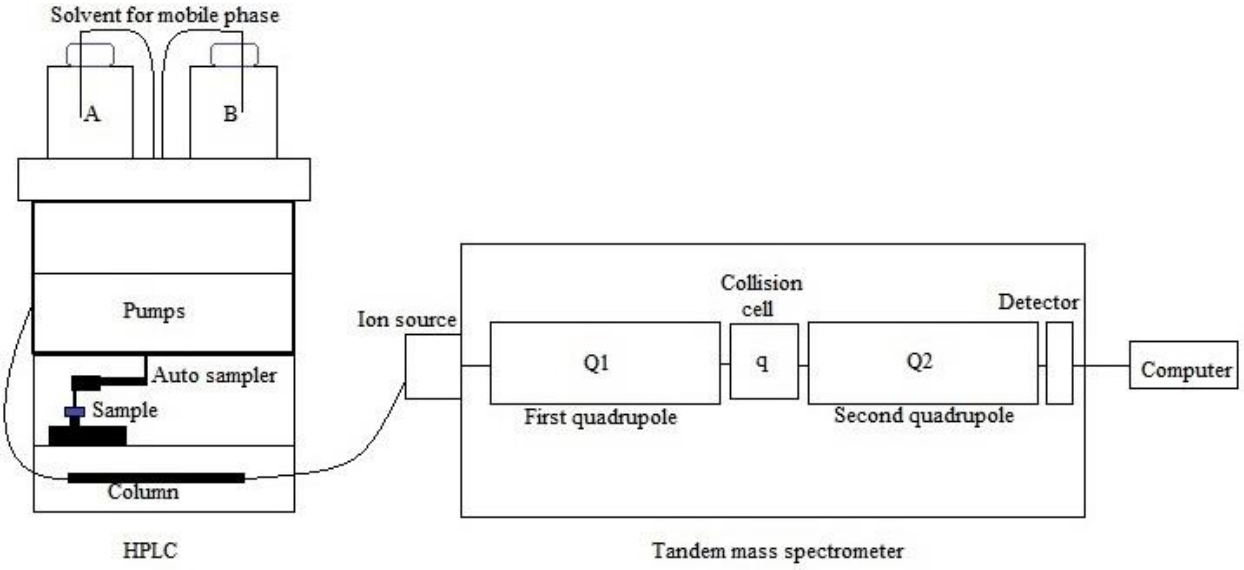


Figure 6: Schematic drawing of HPLC-tandem-MS.

Detection and quantification are two central concepts in analytical chemistry, which is often obtained by separation in a chromatographic system. High pressure liquid chromatography (HPLC) is a technique used for separating compounds by distributing it between two phases. The detection is often carried out by ultraviolet (UV), refraction index (RI) or mass spectrometer (MS). The two phases in a HPLC are a mobile liquid phase (often a gradient between organic solvent and water) and a stationary phase. The mobile phase is pumped through a column packed with a stationary phase. A sample of different compounds injected into the mobile phase, will have different interactions with the stationary phase and mobile phase depending on the chemical properties of the compound. This will give the various compounds different retentions on the column, and thus separation will occur.

By linking a HPLC to MS (Figure 6), the separated compounds can be ionised and detected by its mass to charge (*m/z*) ratio. Commonly used ion sources in LC-MS are; Electrospray ionisation (ESI), Atmospheric pressure chemical ionisation (APCI) and Atmospheric pressure photo ionisation (APPI). These ion sources are operating under atmospheric pressure, before transferring ions into vacuum in the MS. The ESI (Figure 7) is a soft ionisation technique, which gives the opportunity to have less fragmentation in the ion source (compared to electron ionisation used for GC) and transfers the ions from a solution into a gaseous state. The separated compounds from the HPLC-column are transferred by a capillary into the ESI. As

the flow is pumped through a nebulizing needle at ground potential, it enters a semi-cylindrical electrode at a high potential. This creates a strong electrical field that charges the surface on the liquid, which forms a spray of charged droplets, also known as the Taylor cone. The Taylor cone produces smaller and smaller droplets because of disintegration from Columbic repulsion which eventually produces gaseous molecular ions (mainly $[M+H]^+$ or $[M-H]^-$, but adducts (sodium, ammonium and potassium in positive mode and chloride, nitrate and acetate in negative mode) can also occur[41].

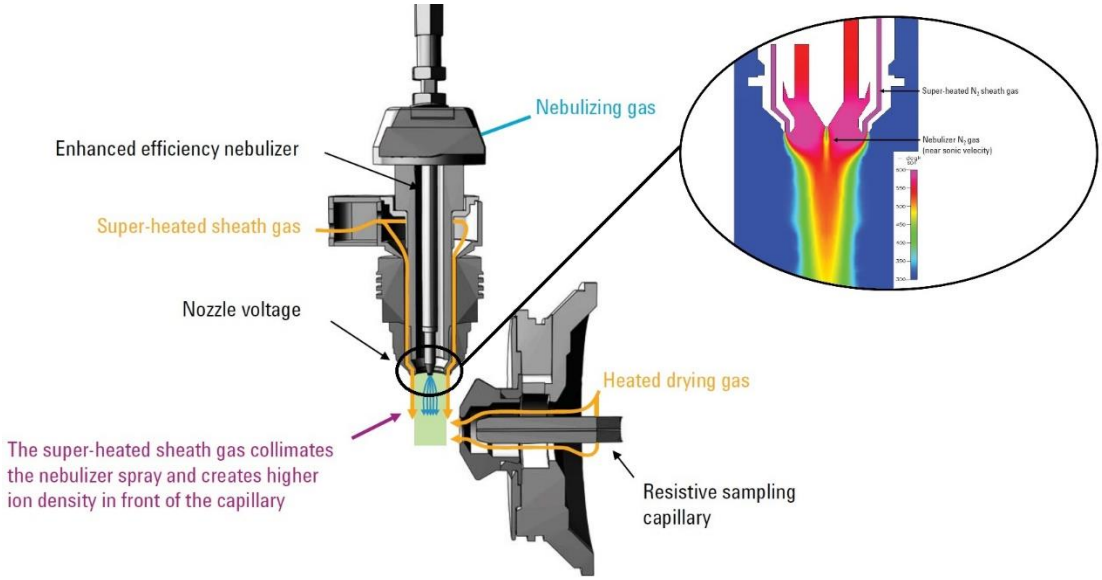


Figure 7: Illustration of an atmospheric pressure ionisation jet stream ESI adapted from Agilent [42].

There are many different types of mass analysers. A triple quadrupole (QqQ) consists of two sections of four circular poles that creates an oscillating magnetic field by pairwise (poles being 180º of each other) applying radiofrequency and direct-current. At specific values of these voltages, the mass analyser is able to filter away unwanted ions and let stable oscillating ions, depending on *m/z* ratio, pass the quadrupole[43]. Between the two sections of quadrupoles, fragmentation is carried out by collision induced dissociation (CID) a collision cell. By colliding the ions with an inert gas (often argon, helium or nitrogen) without making new products, the internal energy to the analyte increases and the weakest bond will break. A typical fragmentation site occurs on the bond between a carbon and a functional group (alfa carbon). The most abundant fragments, also called the product ions, can be used to identify one compound from another with the same mass due to different fragmentation patterns. There are several ways to use the tandem MS for monitoring molecules; Full scan, selected ion

monitoring, product ion scan, parent ion scan and multiple reaction monitoring (MRM) are some of them. The MRM method is carried out by setting the first quadrupole at a fixed mass and only the molecular ion with the specific mass is able to pass this quadrupole. By colliding the molecular ion in the collision cell, the molecular ion produces product ions that passes the second quadrupole and is detected. These transitions are called MRM transitions and the most abundant are selected. This method reduces the background noise because of fewer compounds in the detector, and thus increases the sensitivity. By having multiple MRM transitions for one compound (often 2), the probability of another compound with the same mass having the same product ions is very small. The transitions with the highest responses, are used for identification and quantification. A detector often used in MS is an electron multiplier (Figure 8) where the ions are transformed into signals by dynodes[44]. The first dynode convert the ions into electrons. The electrons are amplified by a cascade effect when hitting the next dynodes, which produces a current that is equivalent to the abundance of the ions. The electron multiplier consists of either a discrete or a continuous dynode. The advantage of using electron multipliers is that they can scan for positive and negative ions in the same scan, it is a very sensitive detector with a fast response, but is has shorter lifetime than other detectors.

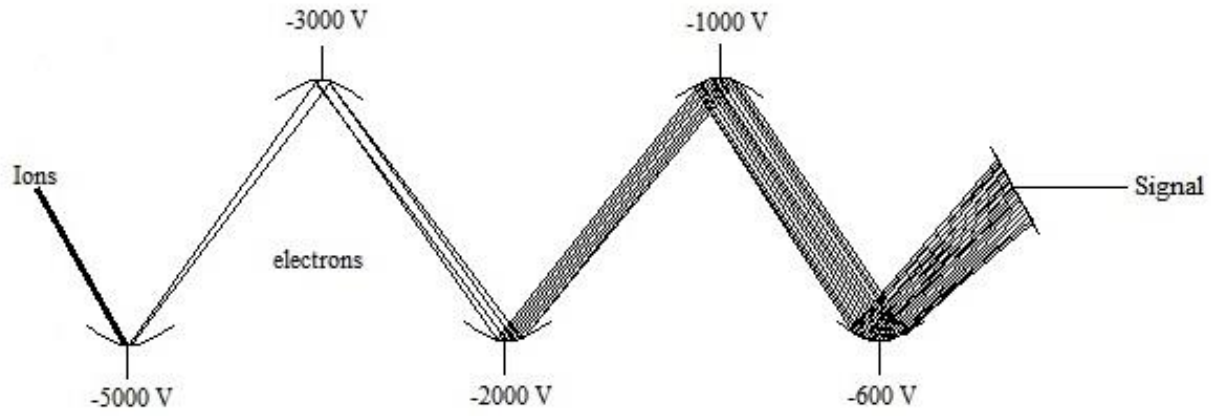


Figure 8: Illustration of the principle of an electron multiplier adapted from[38].

1.6 Quality control and validation

Measuring techniques with high selectivity and sensitivity are necessary in trace analysis in order to confidently identify compounds in samples. This can be obtained by having a good quality control system both in the method and analysis. In order to ensure quality control, one should always include identification and quantification criteria, reference material, blind/blank samples, control charts, spiked samples and duplicates. Another important application, is to have systems which include suitable environments, requirements for reagents, calibrated measurement equipment, "smart" working routines, and contamination controls. Validation criteria's are necessary to follow when developing a new method or using a developed method from another laboratory.

1.7 Criteria for identification and quantification

1.7.1 Identification

A compound can be identified according to its mass and the retention time (RT). The retention time is defined as the time it takes for a compound to travel through a column and is set by a combination of the mobile phases, flow rate and the properties of the column. When working with complex matrixes, the retention times can often be altered and not directly comparable to a standard solution. A matrix matched sample spiked with a standard solution or an isotopic labelled standard added in the same sample can be used to monitor this problem.

1.7.2 Limit of detection and limit of quantification

For every chromatographic peak it is necessary to be able to distinguish the peak from the baseline, which is the signal to noise ratio (S/N). In matrix blank samples (matrix matched samples without target compound), a signal at the retention time of the target analyte can be produced and is considered as the noise level. The general rule for limit of detection (LOD) and limit of quantification (LOQ), is that the S/N ratio should be 3 and 10 (Figure 9) respectively. The detection limits can be calculated in different ways. One method is to use a blank sample and multiply the signal at the respective RT with 3 and 10 and calculate the LOD and LOQ. This can also be done by diluting a standard solution until a signal lower than 3 and 10 in S/N ratio and must be regarded as an instrument limit of detection/quantification.

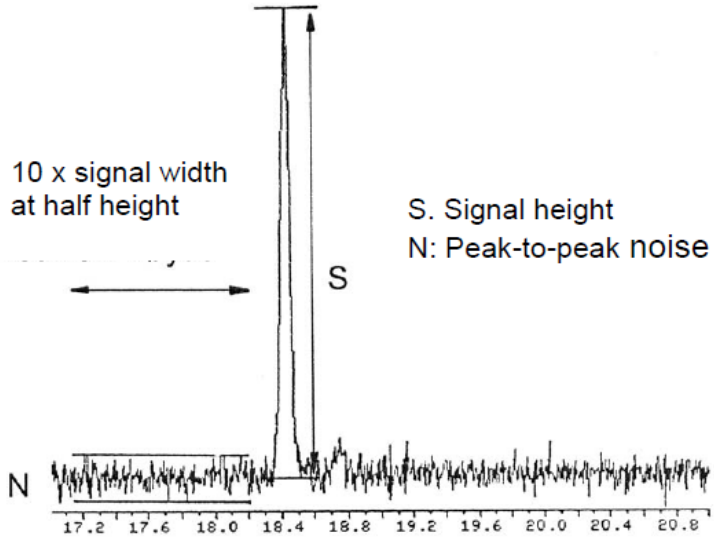


Figure 9: Illustration of the determination of the S/N ratio [45]

1.7.3 Quantification

Matrix interferences and losses during the sample preparation can never be completely excluded. Quantification is carried out by an internal standard (ISTD), a method that is highly preferred. An ISTD is a compound that is added to the sample at a specific stage in the sample preparation. If added as the first step in the preparation, it will automatically correct for loss of analytes during the sample preparation, and disturbances due to matrix effects. Compounds used as ISTD should have chemical properties similar to the analytes, and should not be occurring naturally in the sample. Isotopic-labelled compounds are ideal as they are identical in physical and chemical behaviour, but differ in mass. Deuterated compounds are widely used as they are more available and cheaper than ^{13}C- , ^{15}N-, and ^{18}O-labeled analytes. Deuterated compounds can sometimes be problematic as they can be transferred into chlorinated solvents, or replaced on aromatic rings[45]. The quantification and identification can be carried out by adding an external matrix matched calibration curve of the analytes together with ISTD. By plotting the relative responses (RR) (the area of the analyte over the area of the ISTD) to concentration, the concentration in an unknown sample can be calculated (Equation 1).

$$RR = \frac{\text{Area of analyte}}{\text{Area of ISTD}} \qquad Y = ax + b$$

Equation 1: Y = RR: Relative Responses between target compound and ISTD, x: Calculated concentration, a: Slope of the matrix matched calibration curve, b: Intercept of the matrix matched calibration curve

1.7.4 Recovery

Recovery is a measure of loss of analytes due to matrix effects, steps in the sample preparation and ion suppression. In this study, it is calculated for total recovery (R^T), recovery of the LC-MS method (R^{MS}) and recovery for the sample preparation (R^{PO}) with the following equations:

$$R^T = \frac{\text{Area of spiked sample} - \text{Area of matrix blank}}{\text{Area of spiked solution}} \; x\,100$$

$$R^{MS} = \frac{\text{Area of spiked matrix blank} - \text{Area of matrix blank}}{\text{Area of spiked solution}} \; x\,100$$

$$R^{PO} = \frac{\text{Area of spiked sample - Area of matrix blank}}{\text{Area of spiked matrix blank}} \; x\,100$$

Equation 2: Equations for calculation of the recovery rates, R^T, R^{MS}, R^{PO}

A spiked sample is prepared by using the matrix blank material, and adding a known concentration as the first step of the sample preparation. This sample is prepared the same way as the real samples. The spiked matrix blank, is prepared by adding a known concentration to a matrix blank as the last step before injection on the LC-MS/MS. A spiked solution is made by adding a known concentration in the solvent used in the LC-method. By using samples spiked at the same concentration, the recovery can be calculated for the analytes and ISTD. A disadvantage of using this method for calculating the recovery is that it is not sample specific, and the assumption of identical recoveries for every sample must be made.

1.8 General quality assurance

Purity and contamination control are two important factors when working with trace analysis. The reference compounds and solvents used need to be at a high purity level (≥ 98 %) in order to be able to have satisfactory levels of certainty in the method.

When developing a method, or performing a validated method it is beneficial to prevent unnecessary work. By planning and developing a good standard operation procedure (SOP) combined with good laboratory skills and habits, the chance of doing it correctly the first time increases significantly.

1.8.1 Contamination control and control samples

It is imperative to have good cleaning routines, as well as good routines in the lab in order to prevent contamination. The equipment needs to be free of contaminants that can interfere with the analysis, and not give false positives or unwanted matrix effects. Hence, one should always soak equipment used for reference material in soap water overnight in a separate container, before cleaning it in a dishwasher without soap. All of the equipment should have adequate cleaning by a dishwasher, and be flushed with solvent used in the method, to rinse out possible soap remnants or other contaminants. Injection of a blank sample during the analysis (pure water, pure solvent or a mix) should be run after a high concentration injection and after every tenth injection. This is to control possible carry-over or possible contamination in the system. A matrix blank is prepared and stored the same way as the samples and contain a “zero sampling material”. The matrix blank should not contain the analytes, but if it is not accessible, a blank value has to be defined and calculated with the measuring result. The matrix blank can also be used to measure recovery of analytes in the method as discussed in 1.7.4. A reagent blank is produced by switching the matrix with water or the solvent used in the method, and preparing it as a real sample. This will monitor if the reagents are contaminated.

2 Experimental

Figure 10: Map of Tromsøya and Breivika adapted from Kartdata © 2017 Google.com

2.1 Description of the study area and sampling site

Tromsø is a city in the northern part of Norway. The main part of the city is located on Tromsøya which is an island between the main land and Kvaløya. Breivika is a district located east of the northern part of the island, which includes UiT The Arctic University of Norway and UNN The University Hospital. Breivika STP has its outlet approximately 100 m from the shore. The sampling site was chosen based on its proximity to the outlet of effluent water from the STP, and availability to reach the water by foot. The sampling location (1) and location of the STPs outlet (2) are seen in Figure 10.

2.2 Collection of samples

All of the samples were collected during the field work conducted in October 2016 in Tromsø. The different locations are listed in Table 4.

Table 4: Information of the different locations of sampling spots

Location	Sampling date	Coordinates (google)	Sample matrix	Location name
Storesand teltplass*	28.08.16 07.11.16	59°01'31.4"N , 11°01'00.8"E	Seawater, 10 L Seawater 30 L	R2
Folehavna fort*	29.09.16	59°02'37.6"N , 10°16'23.2"E	Seawater, 30 L	R3
Breivika sampling location	05.10 – 11.10 2017	69°40'21.6"N, 18°58'42.5"E	Seawater, 42 L	1
Breivika outlet	18.10.16	69°40'24.2"N 18°59'02.7"E	Seawater, 6L Sediments, 500 mg Fish (Saithe, Shorthorn sculpin, Atlantic cod)	2
North of Breivika	06.10.17	69°41'13.6"N 19°00'33.0"E	Seawater, 6L	A
Reference location north of Tromsøya	20.10.16	69°46'02.2"N 19°04'17.7"E	Seawater, 6 L Fish (Saithe, Atlantic cod)	R1

*** Not collected in Tromsø.** Locations of collected seawater for the optimisation of the method for seawater previous to the analysis

2.2.1 Seawater samples

Seawater samples were collected on the same spot at sampling location 1 (Figure 10) around 09:00 every day (except the first day, sampled at 16:00) over a 7-day period. The samples were collected from a location on the main land near the outlet of sewage effluent from Breivika region. Pre-cleaned water bottles (2 x 2.5 L and 1 L) were rinsed with seawater three times before they were lowered by hand to approximately 10 cm below the water surface. This procedure was repeated for all the water sampling during the 7-day period.

Seawater was also collected by boat and was carried out by using a Niskin Water sampler (2.8 L) that was lowered to approximately 1 m above the seabed, where the closing mechanism was triggered. The samples were collected near the outlet location (location 2) and at a location far away from the city outlets (location R1).

Seawater for the matrix matched calibration curve (MMCC) were collected at Folehavna Fort (location R3) and Storesand teltplass (location R2).

2.2.2 Fish liver samples

Atlantic Cod (*Gadus morhua*), Saithe (*Pollachius virens*) and Shorthorn Sculpin (*Myoxocephalus scorpius*) were caught around Tromsøya from a boat with a fishing rod. The Shorthorn Sculpin was caught near the location of the outlet from Breivika STP. The fish were dissected and the liver was wrapped in aluminium foil in Tromsø and shipped on ice to Adamstuen in Oslo.

2.2.3 Sediment samples

A Van Veen Grab (250 cm^2) was lowered from the boat to the seabed at location 2. Sediment was sampled in a container and shipped on ice to Adamstuen in Oslo.

2.3 Sample preparation

For complete information and description of solvents, instrument, and sample preparation see appendix Table 10, Table 11, Table 9, SOP1, SOP2 and SOP3, respectively.

2.3.1 Seawater

A multi-compound method based on two similar studies[46, 47] were used for the quantitative trace analysis in recipient seawater. The water samples were extracted on SPE cartridges in Noruts laboratory in Tromsø, and eluted at NMBU in Ås.

The seawater samples were filtered with Quantitative filter paper, 454, 150 mm diameter, 12-15 µm particle retention using a Buchner funnel and vacuum filter flask coupled to a water aspirator pump, or a vacuum pump, depending on availability. The pH was adjusted to 7 by adding 37 % HCl (30 µl), and 4 mL of 5 g/L Na_2EDTA was added as a chelating agent to 1 L sample. The sample was spiked with 50 µL of 10 µg/mL ISTD mix (Caffeine $^{13}C_3$, Carbamazepine d10, metoprolol d7, Sulfadoxin d3 and Trimethoprim d3). An Oasis MCX cartridge (60 cc, 150 mg, Waters) was

Figure 11: The seawater extraction setup

conditioned with 6 mL acetonitrile (ACN) followed by 6 mL H_2O, before the sample was applied by tubing and cartridge adaptors (Figure 11).

The extraction was carried out on a SPE manifold under vacuum at a steady flowrate of approximately 1-3 drops per second. The cartridge was washed with 2 x 4 mL 2 % FA in H_2O and dried with air flowing through the column. The elution was done by applying 8 mL ACN followed by 8 mL 5 % NH_3OH in ACN to the cartridge. The eluate was evaporated until dryness at 35 ˚C under a flow of nitrogen. The dry residue was reconstituted in 500 µL 20 % ACN in H_2O, before centrifugation through a Spin-X filter and loaded into a HPLC-vial ready for analysis.

2.3.2 Fish liver

The sample preparation for fish liver was carried out at faculty of MatInf, Oslo in February 2017.

The fish liver was homogenised with an Ultra-Turrax and 1 g was weighed into a 12 mL glass centrifuge tube, and spiked with 10 µL of 10 µg/mL ISTD mix. To the sample was added 5 mL acidified ACN (5 % FA in ACN) and vortexed for 2 min followed by centrifugation for 5 min at 3000 rpm. The supernatant was transferred to an Enhanced Matrix Removal (EMR) – Lipid tube (Agilent Technologies) containing lipid removal salts and 5 mL ammonium acetate buffer (5 mM) was added and vortexed immediately after. The sample was put on a mechanical shaker for 2 min before centrifugation for 5 min at 4500 rpm. The supernatant was transferred to a EMR - Lipid polish tube (Agilent Technologies) containing 2 g salts (1:4 NaCl:$MgSO_4$) and vortexed for 2 min before centrifugation for 5 min at 4500 rpm. The upper ACN layer (5 mL) was transferred to a clean glass tube and evaporated until dryness under a stream of pressurized air at 35 ºC. The sample was reconstituted in 1000 µL 20 % ACN in H_2O, before centrifugation through a Spin-X filter and loaded into a HPLC-vial ready for analysis.

2.3.3 Sediment

The sample preparation of the ASE method was carried out at the laboratories of NILU, Kjeller in March 2017.

Extraction cells were assembled and packed according to Figure 5. Sediment samples were weighed (1 g) and homogenised with 2 g of a 1:1 mixture of Dionex ASE Prep diatomaceous earth (DE) dispersant and Dionex ASE prep moisture absorbing polymer (MAP) and 0.5 g Florisil. ISTD mix (10 µg/ml) was added to the samples by adding 20 µl of ISTD mix into 1 mL of 1:1 Hexane, Acetone, and then added to the homogenised sediment sample. The packed extraction cells were loaded into the ASE instrument. The pressure and temperature was set to 1500 psi and 100 ˚C, and the cycle was set to four times at; 10 min static, 5 min heat, and 60 sec purging. After the ASE, the solvent in the extracted sample was changed by evaporation on a Turbovap at 37 ºC to dryness. The samples were reconstituted in 1000 µl 20 % ACN in H_2O. The samples were added in 10 mL of H_2O and loaded on a MCX SPE cartridge that was conditioned the same way as the method for seawater samples. The MCX cartridge was washed with 6 mL of 2% FA in H_2O and eluted with 5 mL ACN followed by 5 mL of 5 % NH_3OH ACN. The samples were evaporated till dryness and reconstituted in 1 mL 20% ACN in H_2O, before centrifugation through a Spin-X filter and loaded into a HPLC-vial ready for analysis.

2.4 Analysis

2.4.1 Liquid Chromatography – Mass Spectrometry

The instrumental analysis was carried out at MatInf, Oslo. The instruments used for this analysis were LC-MS/MS Agilent 1200 HPLC system and Agilent 6460 triple quadrupole system. The HPLC system used an Agilent Zorbax Eclipse Plus C_{18} RRHD (1.8 µm 2.1 x 100 mm) column with Agilent UHPLC fast guard Eclipse C_{18} (1.8 µm 2.1 x 5 mm) guard column. Mobile phase [A] was 0.1 % formic acid in water and mobile phase [B] was ACN. The flow rate of the MP was 0.4 mL/min and an injection volume of 10 µL was used. A linear gradient of mobile phase [B] was used, going from 2 % to 100 % at 0 - 6 min followed by 100 % [B] for 3 min and back to 98 % of [A] and 2 % [B] for re-equilibration of the column for the rest of the run (total 15 min). The MS detection was achieved by using an Agilent jet stream electrospray ionisation (AJS ESI) source with Dynamic multiple reaction monitoring (MRM). All of the target analytes were optimized individually on the following parameters: 1) The

fragmentor was adjusted by finding the correct molecular ion (either M+1 or M-1) and scanning it in SIM mode at different fragmentor values. The chromatogram was inspected visually and the fragmentor value with the highest peak was selected. 2) The product ions were selected by its abundance in a product ion scan. The collision energy was set at different values for each product ion, and the values with the highest chromatographic peaks were selected. 3) The cell acceleration voltage was optimized for every MRM transition in a MRM scan. The highest chromatographic peak produced at a specific voltage was selected. This was carried out by injecting a standard solution of analyte (100 ng/mL or 10 µg/mL) free of matrix, prepared fresh from the stock solution. Ethinyl estradiol and Estrone had very poor sensitivity and were not found in the MS scan by injecting a high concentration standard solution (10 µg/mL), and were excluded from the method. All of the optimized parameters for each analyte are found in Table 8.

2.4.2 Data analysis and quantification

All of the MRM chromatograms were processed by using "Agilent MassHunter Qualitative Analysis" and "Agilent MassHunter Quantitative Analysis (for QQQ) software. The chromatograms were automatically integrated and were visually inspected and manually adjusted if necessary. The identification of analytes in a sample was done by visual comparison of RT of the MRM transitions in the matrix matched samples spiked with standards solution. The samples were quantified by using isotopic labelled analytes (ISTD) and a MMCC. The MMCC were constructed with nine calibration levels for the seawater samples (1 – 3000 ng/L) and eight levels for the fish and sediment samples (1 – 500 ng/g). For some of the compounds, the highest and/or the lowest calibration points were excluded to get a better coefficient of regression (R^2) $\geq$ 0.990.

Recovery was calculated in every sample as mentioned in 1.7.4 and the precision was found by preparing six matrix matched parallel samples spiked at the same level.

3 Results and discussion

The fish liver samples and sediment samples had no chromatographic peaks within the set parameters for identification and quantification.

The results of the seawater samples are presented as the average concentration of parallels taken from location 1 every day for a week. The presentation of the concentrations are divided into two graphs (Figure 14 and Figure 15) because of large differences in concentration. The tidal currents during the time of sampling of the seawater at location 1 is illustrated in Figure 12. The samples taken the Wednesday the 5th and Tuesday the 11th of October were all sampled at high tide which can influence the concentrations.

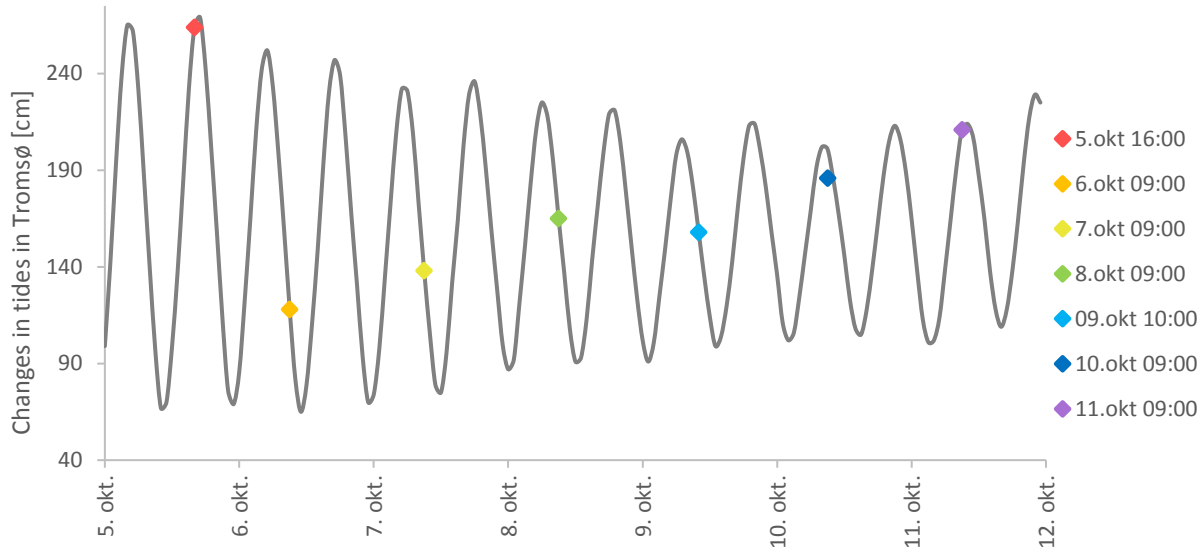


Figure 12: Overview of the tide at the time of sampling at location 1 (adapted from Kartverket.no)

APAP, CAF, CBZ and MPL were found in the seawater samples in concentrations within the calibration curve. Identification and quantification within the set validation parameters are further discussed in the following chapters.

3.1 Identification and quantification

ATN, DEET and TMP were identified in the seawater samples but were calculated below the calibration curve. They also had chromatographic peaks in the solvent blanks and thus were found to not be qualified for quantification. Signals of MET in solvent blank and matrix blank was a continuous problem throughout the validation process and the analysis combined with low RT (< 1 min). MET was found to not be qualified for identification or quantification.

The identification was performed by ion transitions and by their RT (Figure 13). Calibration curves were generated for assessing the performance of the method and calculating the concentrations combined with the ISTD. With a MMCC, only the analytes with a minimum of 5 calibration points and $R^2 \geq 0.990$ were accepted to be quantified. The MRM transitions selected for quantification of the identified compounds were APAP - 152 → 110, CAF - 195 → 110, CBZ - 237 → 194 and MPL - 268.3 → 116.2. CAF was the only compound that was identified at the other sampling locations in Tromsø (Figure 16). CAF has been identified in the North Sea far from potential contamination sources[48]. The results in this study demonstrate similar indication by being found in locations thought of no not be contaminated by emissions. The concentration of CAF in location R1 is very low compared to location 1 (Figure 16, Figure 14), which is expected due to dilution and long distance from emission sources.

Table 5: The MRM transitions, linear rage, assigned ISTD and RT of the quantified analytes

	MRM	Linear range [ng/L]	ISTD	RT (min)
APAP	152 -> 110	25 - 3000	198.2 -> 140.2	2,7
APAP	152 -> 65.1	25 - 3000	198.2 -> 112	2,7
CAF	195 -> 138	25 - 3000	198.2 -> 140.2	3
CAF	195 -> 110	25 - 3000	198.2 -> 112	3
CBZ	237 -> 194	1 - 500	247.1 -> 204.1	4,5
CBZ	237 -> 179	1 - 500	247.1 -> 187.1	4,5
MPL	268,3 -> 191	1 - 3000	275.3 -> 191	3,3
MPL	268,3 -> 116.2	1 - 1000	275.3 -> 121	3,3
MPL	268,3 -> 98.1	5 - 1000	275.3 -> 105.2	3,3
MPL	268,3 -> 74.1	1 - 1000	275.3 -> 105.2	3,3

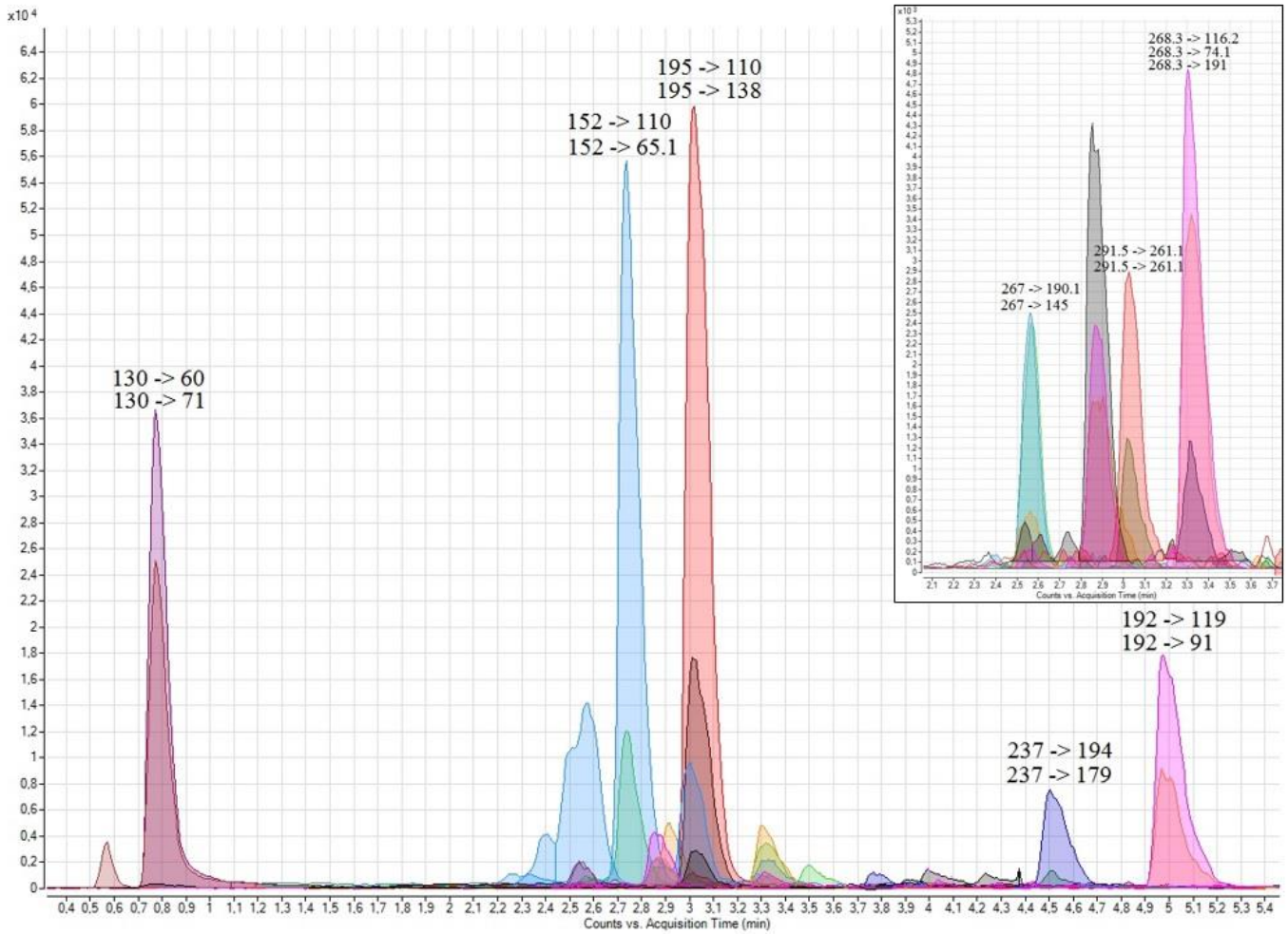


Figure 13: Chromatogram of the chromatographic peaks in the seawater samples, MET (130), APAP (152), CAF (195), CBZ (237), DEET (192), ATN (267), 291.5 (TMP), MPL (268.3).

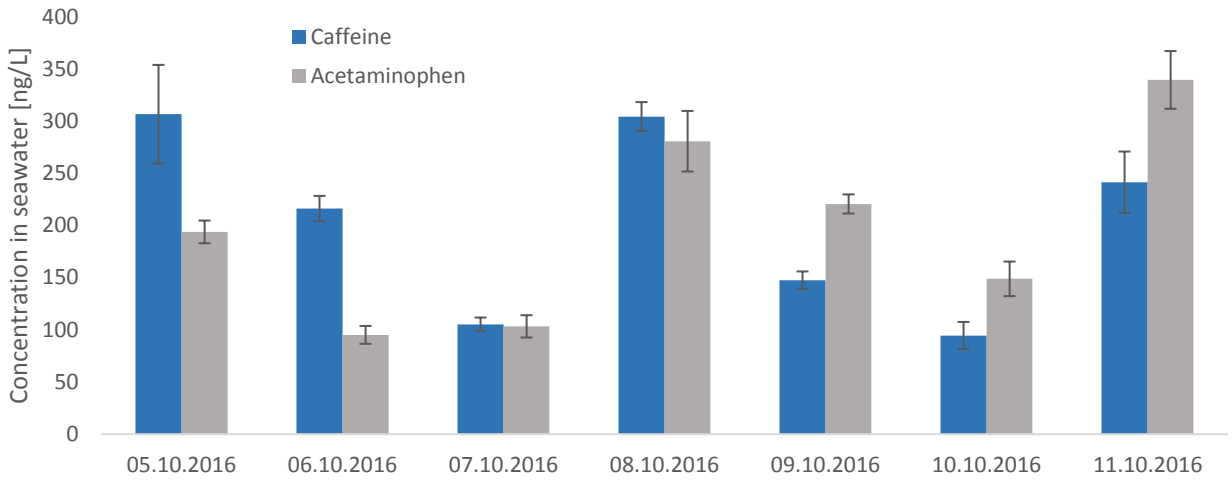


Figure 14: The high concentrations of calculated PPCPs in seawater from location 1

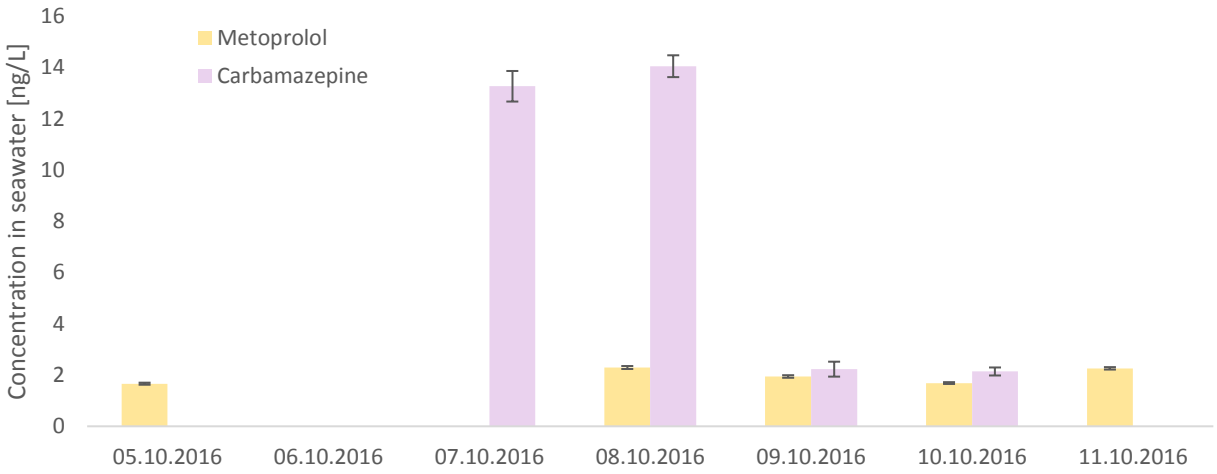


Figure 15: The low concentrations of calculated PPCPs in seawater from location 1

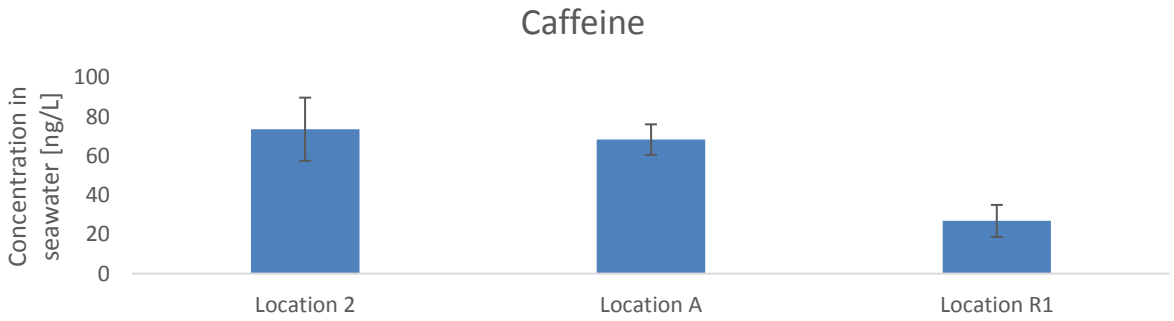


Figure 16: Concentration of Caffeine at the three other locations

Table 6: Results of the samples from location 1

		Metoprolol [ng/L]	Carbamazepine [ng/L]	Caffeine [ng/L]	Acetaminophen [ng/L]
Wednesday 05.10.2016	Average	1,66 ± 0,04	ND*	306,69 ± 47	193,88 ± 11
	n	6		4	6
Thursday 06.10.2016	Average	ND*	ND*	216,33 ± 12	95,18 ± 8,6
	n			6	6
Friday 07.10.2016	Average	ND*	13,27 ± 0,59	105,27 ± 6,6	103,35 ± 11
	n		5	4	6
Saturday 08.10.2016	Average	2,29 ± 0,06	14,05 ± 0,43	304,46 ± 14	280,80 ± 29
	n	6	3	5	6
Sunday 09.10.2016	Average	1,94 ± 0,05	2,23 ± 0,29	147,64 ± 8,4	220,69 ± 9,2
	n	6	5	6	6
Monday 10.10.2016	Average	1,69 ± 0,04	2,14 ± 0,16	94,58 ± 13	148,93 ± 17
	n	6	6	6	6
Tuesday 11.10.2016	Average	2,26 ± 0,05	ND*	241,56 ± 29	339,57± 28
	n	6		5	6

* No chromatographic peak detected

These results (Table 6) have comparable levels of concentration as in the studies conducted in the same area [5, 31]. It also showed that there are daily differences in the area around Breivika STP, and that APAP and CAF dominated the findings. The concentrations of APAP and CAF are highest on Tuesday (11.10), Wednesday (05.10) and Saturday (08.10). The Wednesday and Tuesday samples were collected at high tide and the others were collected as the tide was going down. Consumption during the weekend would be expected to be higher than the rest of the week, but no there is no clear conclusion regarding this. CBZ and MPL were found in the lower range of the calibration curve, which can be expected as a result of lower consumption compared to APAP and CAF.

3.2 Validation of the results

The MRM transition selected for calculating the concentration of APAP was 152 -> 110 because of only 4 calibration points (without matrix blank) in the other transition (152 -> 65.1). The quantified transitions for CAF, CBZ and MPL were selected based on the steepest slope and highest intensity of chromatographic peaks. The RT and integration for all of the chromatic peaks were visually inspected in MassHunter and compared to matrix match spiked samples.

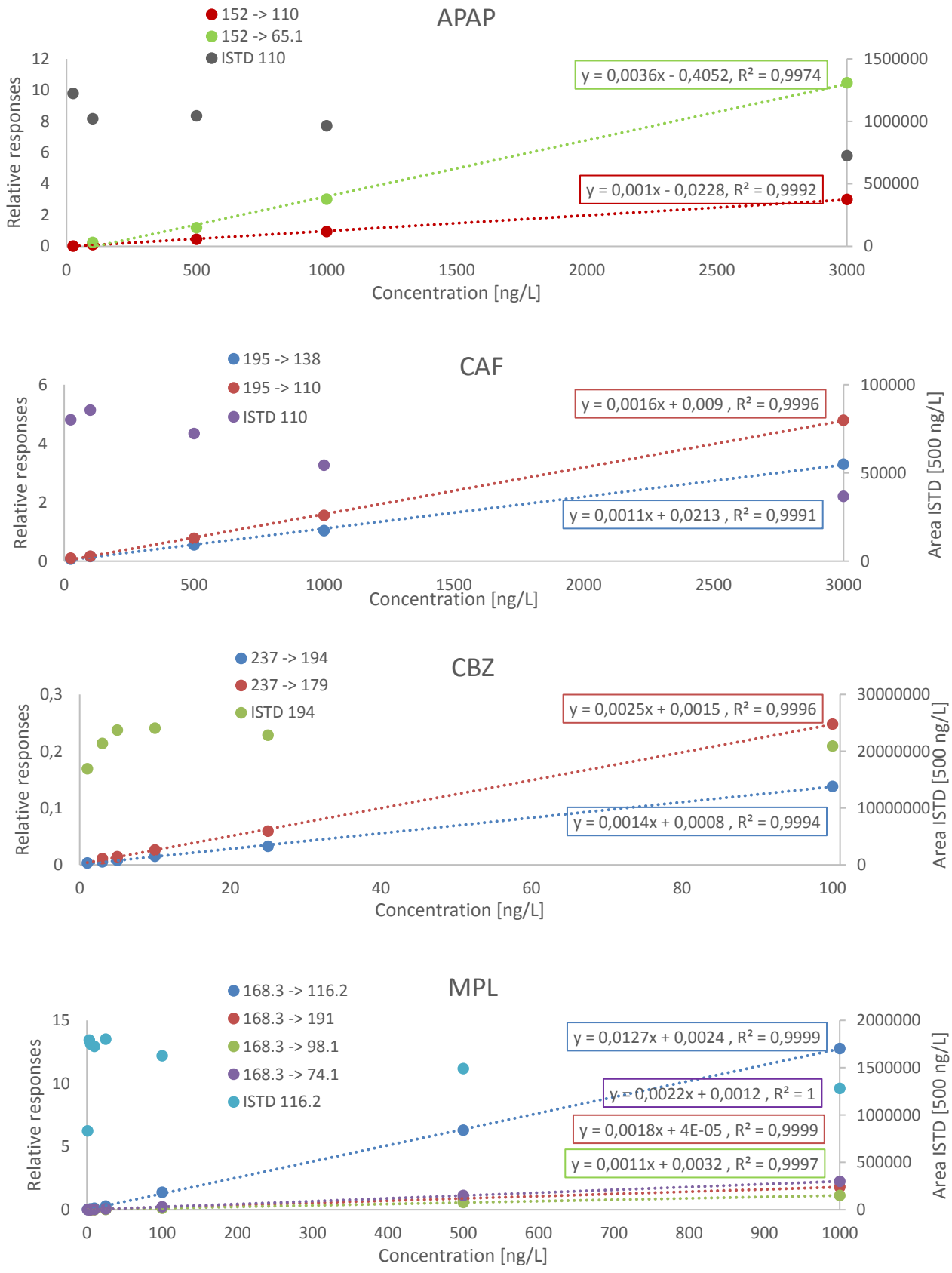


Figure 17: MMCC of the analytes found in the samples from location 1

3.2.1 Recovery and precision results

The recovery of the results (Table 7) indicate satisfactory rates (≥ 50 %) for CBZ and MPL. APAP has low rates for R^T (25 %) and R^{PO} (27%). The ISTD used for quantification of APAP was Caffeine $^{13}C_3$, combined with low R^T gives a high uncertainty in the reported concentrations in Table 6. An assumption of identical behaviour of APAP and Caffeine $^{13}C_3$ in the sample preparation and the LC-MS/MS is proposed. The high R^{MS} (94 %) for APAP in indicates that the ion is not influenced by signal suppression or signal enhancement. CAF has low R^T (45 %) but has analogue ISTD, which will compensate for loss of analyte during the sample preparation and possible matrix effects. The R^{MS} and R^{PO} for CAF is within the accepted limits (> 50%). CBZ and MPL have recovery rates (R^T, R^{MS}, R^{PO}) within the accepted limits.

The precision was calculated as the relative standard deviation (RSD) from the analyte peak area of six parallel samples. The precision ranges between 3 % and 13 % for the different compounds and is within the accepted range (< 16 %) for every transition.

Table 7: Recovery and precision rates of the quantifying ion and qualifier ion(s)

Analytes	MRM transition	R^T [%]	R^{MS} [%]	R^{PO} [%]	RSD* [%]
APAP	152 -> 65.1	25	94	27	9
APAP	152 ->110	25	94	27	8
CAF	195 -> 110	45	73	50	10
CAF	195 -> 138	44	66	58	6
CBZ	237 -> 194	62	67	92	13
CBZ	237 -> 179	64	71	91	13
MPL	268.3 -> 191	93	89	104	3
MPL	268.3 -> 116.2	94	89	105	3
MPL	268.3 -> 98.1	80	76	86	4
MPL	268.3 -> 74.1	95	89	107	4

* RSD is calculated from six parallels (n=6)

The recovery rates of the sample preparation for the water samples, were calculated from the equations discussed in 1.7.3. Samples of seawater collected at Storesand teltplass, in Hvaler, Norway were used for calculating the recovery rates. This will not be sample specific and thus not directly comparable with the actual recovery rate in the water samples prepared in Tromsø.

3.2.2 Blank signals

The chromatographic peaks in blank samples (solvent and matrix) is illustrated in Figure 18. The contribution of signals of DEET in the matrix blank are of unknown origin. DEET is a compound that has been identified in several different studies[47-50], which can indicate presence of DEET in remote locations from contamination sources similar to CAF. Signals of DEET in the solvent blank indicates that a contamination in the LC-MS/MS system is present. ATN, MPL and TMP blank signals are only present in the solvent blanks, which indicate a carry-over problem. Due to large differences in the RT of DEET in matrix blank and the solvent blank also indicates that the contribution of matrix effects are high.

MET has a relatively short RT (< 1 min), which contributes to a high uncertainty when identifying and quantifying the analyte because of possible signals from the dead volume of the column. Signals of MET were produced throughout the analysis process, which strengthen the possibility of problems with the short RT.

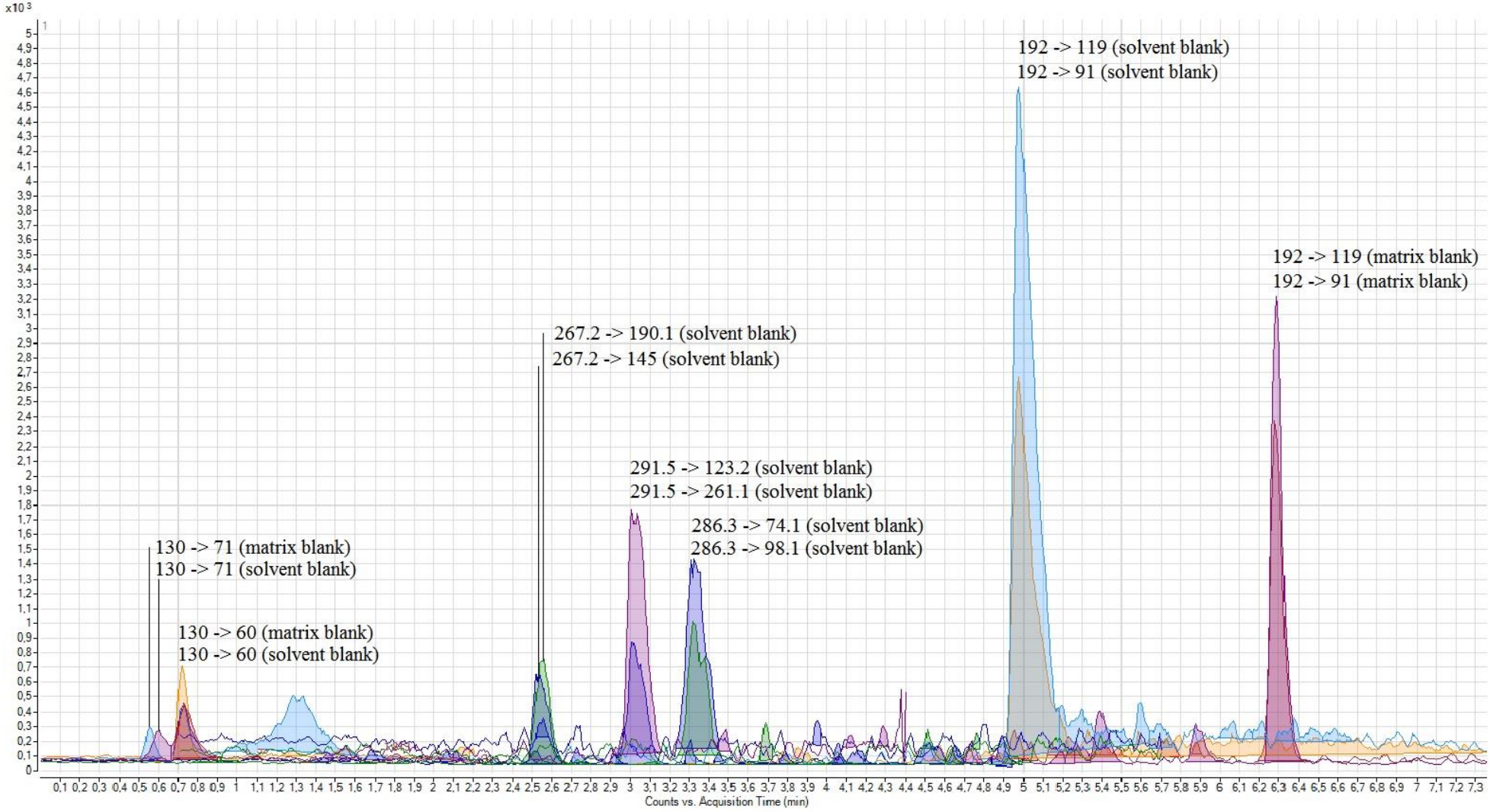


Figure 18: Chromatograms of solvent blanks and matrix blanks MET (130), DEET (192), ATN (267), 291.5 (TMP), MPL (268.3)

4 Conclusions

The sample preparation for seawater gave the best results regarding identification, linearity and recovery of the analytes. These are expected results because of a simple matrix, fewer steps and less components in the sample preparation. The ASE method uses a lot of filtering and packing material in the extraction cell, which can retain analytes and add disturbances into the matrix that can interfere with the analytes. Pharmaceuticals are not persistent and will, as mentioned in the introduction, be metabolised and exerted within a short period of time. Therefore, it is not expected to find high concentrations of pharmaceuticals in wild fish. The sediment samples could have been sampled up stream of the sewage outlet, and thus not affected of the sewage effluent. ASA, CEP, IBU and SCA were not detected in any of the methods, thus not compatible within the set linearity range or lost completely during the sample preparation. Analytes compatible with all three methods were AMT, CPN, MPL, SFD and TMP in the set linearity range and with five calibration levels. Further optimization need to be conducted in both of the methods in order to identify the analytes in environmental samples. Good results were found for the seawater method with only five analytes not found, and is concluded to be suitable for this analysis.

Studies conducted on PPCPs in the arctic environment and the general interests for pollutants is increasing[51-53]. Since PPCPs are not considered to have persistent properties, it is not expected to find high concentrations in the aquatic environment. Even though the concentrations of PPCPs found in this study are relatively low, and are considered to not be harmful, the potential exposure of multiple compounds in low concentration over time, can be a reason of concern. Possible synergetic, antagonistic or allosteric effects can occur as a result of combined effects when exposed to a variety of compounds.

The cleaning processes of Breivika STP is not specific for removal of PPCPs, and thus the expected exposure of adjacent aquatic environment needs to be considered.

The location by the shore in Breivika for sampling the water samples was proven to be a good spot for sampling. This location had higher concentrations than the samples collected near the outlet 30 m under water. An explanation for these findings could be correlated to under water currents in the wrong direction and that the sampling was done up stream of the outlet. Another reason can be that the water discharged from the STP is freshwater. Freshwater is lighter than

seawater and consequently the discharge from the outlet will favour distribution in the surface water, and not in the sea bed.

Since there were variations in the concentrations of PPCPs found in seawater during one week, a seasonal variation is expected because of differences in consumption and degradation rates during the year. A recording of seasonal variation would be interesting in order to be able to regulate and have legislations on emissions. Especially during a period of potential higher releases and thus higher presence of PPCPs in the aquatic environment.

5 Future perspectives

The metabolites of the analytes were not investigated in this study. Several studies conducted on the parent compound and its metabolites, gives a better picture of the total contribution to the environment where the parent compound is partly or completely degraded into metabolites. IBU was proven to be more abundant of the CX-IBU metabolite in receiving waters in Tromsø[5]. UV- and microbial degradation in the receiving environment of sewage effluent, especially in the arctic environment, should be investigated in order to estimate the contribution to longer half-life.

Even though the sediment- and fish liver samples were negative, the recovery rates and linearity rates are poor and further optimization of the method needs to be conducted in order to confirm presence or not. It is recommended to increase the sample volumes in every method because of low concentrations.

Monitoring the annual occurrence of PPCPs in the environment should be conducted in Tromsø in order to strengthen the knowledge of distribution and emission rates in cities in the Arctic. It is important to prevent possible hazardous and adverse effects, before accidents happens similar to the incident in Pakistan[28].

Other relevant PPCPs are recommended to be looked into, and possibly include them in the method if they are compatible. Some of the compounds in this study were not compatible and can be excluded or switched with other compounds.

Further monitoring of the Arctic environment is desirable, in order to strengthen knowledge and prevent potential harmful exposure to aquatic biota and further distribution of PPCPs.

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Appendix

Contents

MS-parameters

Table 8: MS-parameters for target compounds

Target Compound	Precursor Ion	Product Ion 1	Product Ion 2	Product Ion 3	Product Ion 4	Fragmentor (V)	Collision Energy (V)	Cell Accelerator (V)	Retention Time (min)	Retention Window	Polarity
Acetaminophen	152	110	65.1			90	15, 35	4	2,7	3	Positive
Amitriptyline	278	105	91			135	20, 30	4	5,7	3	Positive
Amlodipine	409.1	293.9	237.8			100	10, 15	6, 3	5,5	3	Positive
Atenolol	267.2	190.1	145			135	20, 30	4	2	3	Positive
Atorvastatin	559.2	440.1	292.1	250		135	20, 40, 50	6, 2, 3	7	3	Positive
Caffeine	195	138	110			135	20, 30	4	3,8	3	Positive
Caffeine $^{13}C_3$	198.2	140.2	112			100	20	3, 4	3,8	3	Positive
Carbamazepine	237	194	179			135	15, 35	4	5,8	3	Positive
Carbamazepine d_{10}	247.1	204.1	187.1			100	20, 40	5	5,8	3	Positive
Cephalexin	348	174	158	106		135	15, 5, 20	4	3,9	3	Positive
Chlorphenamine	275	230	167			135	10, 30	4	4.9	3	Positive
Ciprofloxacin	332	288	245			110	20, 30	4	4	3	Positive
Diclofenac	294	250	214			60	5, 20	4	7.2	3	Negative
Diethyltoluamide	192	119	91			135	20, 30	4	6.4	3	Positive
Fluoxetine	310	148	117			135	10, 20	4	6,2	4	Positive
Ibuprofen	205	161				60	5	4	7.3	3	Negative
Losartan	423.2	404.9	377	207		100	10, 10, 30	4, 3, 5	6,1	3	Positive
Metformin	130	71	60			80	20, 10	4	0.7	3	Positive
Metoprolol	268.3	191	116.2	98.1	74.1	100	20	5, 3, 6, 7	4,3	3	Positive
Metoprolol d_7	275.3	191	121	105.2		100	20	5, 3, 4	4,3	3	Positive
Metronidazole	172	128	82			135	10, 25	4	2.7	3	Positive
Penicillin G	335.2	288.9	128.1	91.1		133	30, 30, 70	2	4,1	3	Positive
Prednisolone	361.1	325.1	146.7	90.8		85	10, 30, 70	1, 2, 6	5,4	3	Positive
Ranitidine	315	170	130			110	10, 20	4	4	3	Positive
Simvastatin	419	225	199			135	20, 5	4	8,5	3	Positive
Sulfadoxin	311	156.1	108	92.1		100	20, 30, 30	2, 5, 5	5	3	Positive
Sulfadoxin d_3	314.1	156	108	92.1		100	20, 30, 30	1, 4, 5	5	3	Positive
Sulfamethoxazole	254	156	108	92		135	10, 20, 30	4	5.4	3	Positive
Trimethoprim	291.5	261.1	123.2			135	25	4	3,9	3	Positive
Trimethoprim d_9	300.3	264	234.1	122.9		100	30	2, 3, 3	3,9	3	Positive
Warfarin	307.2	161	117.1			80	15, 30	4	6.9	3	Negative

Chemicals, instruments, materials and standards

Table 9: List of instruments and further information

Product	Name/description	Producer	Supplier	Cat#
HPLC	Agilent 1200 series with auto sampler, binary pump and thermostatted column compartment	Agilent Technologies, Santa Clara, CA, USA	Matriks AS, Oslo, Norway	G1367C, G1312B, G1316B
MS	6460 series triple quadrupole LC/MS	Agilent Technologies, Santa Clara, CA, USA	Matriks AS, Oslo, Norway	G6460A
Software	MassHunter, Quantitative analysis for QQQ, Versjon B.07.00/Build 7.0.457.0	Agilent Technologies, Santa Clara, CA, USA	Matriks AS, Oslo, Norway	
Software	MassHunter, Qualititative analysis for QQQ, Versjon B.06.00/Build 6.0.633.10	Agilent Technologies, Santa Clara, CA, USA	Matriks AS, Oslo, Norway	
ASE 200	Accelerated Solvent extractor, DIONEX	Dionex corp. Sunnyvale CA 94086, USA		
Evaporator	TurboVap® II	Caliper Life Sciences		
Evaporator	Reacti-Vap III™ Evaporator	Thermo Scientific, Waltham, MA, USA	VWR International AS, Oslo, Norge	
Homogeniser	IKA T18 basic Ultra Turrax	IKA®-Werke GmbH & Co. KG Staufen, Tyskland		
Grab	Van Veen Grab 250 cm^2	KC Denmark AS Research equipment		
Shaker	VXR basic Vibrax	IKA® Werke GmbH & Co, KG. Staufen, Tyskland	Sigma Aldrich, Oslo, Norge	
Centrifuge	Rotanta, 50 mL	Andreas Hettih GmbH & Co. KG, Tuttlingen, Tyskland	Dipl.ing. Houm AS, Oslo, Norge	
Table centrifuge	Micro 1207 – 230 V. 2 mL	VWR International, Radnor, PA, USA	VWR International AS, Oslo, Norge	
Vortex	MS 3 basic	IKA-Werke GmbH & Co, KG. Wilmington, N.C, USA	Sigma Aldrich, Oslo, Norge	
Water sampler	Niskin Water sampler 2.8 L	KC Denmark AS Research equipment		

Table 10: List of chemicals used in this study

Name	Quality	Purity ≥ %	CAS#	Producer	Supplier	Size
Acetone	PESTINORM®	99.7	67-64-1	VWR Chemicals	BDH Prolabo®	2.5 L
Acetonitrile	LC-MS CHROMASOLV®	99.9	75-05-8			2.5 L
Ammonium hydroxide solution	ACS reagent	25 - 28	1336-21-6	Sigma Aldrich, St. Louis, USA	Sigma Aldrich, Oslo, Norge	1 L
Diatomaceous earth	Dionex™ ASE™ Prep DE			ThermoFisher Scientific	ThermoFisher Scientific	1 Kg
Formic acid	ACS reagent	96	64-18-6	Sigma Aldrich, Seelze Tyskland	Sigma Aldrich, Oslo, Norge	1 L
Methanol	HiPerSolv CHROMANORM®	99.8	67-56-1	VWR International AS, Oslo, Norge	VWR International AS, Oslo, Norge	2.5 L
Moisture absorbing polymer	Dionex™ ASE™ Prep MAP			ThermoFisher Scientific	ThermoFisher Scientific	200 g
Milli-Q water	>18mΩ, Type 1			Merck Millipore, Billerica, Massachusetts, USA	Merck Life Science AS, Oslo, Norway	
Na_2EDTA		99,0-101.0 (titration)	6381-92-6	Sigma Aldrich, St. Louis, USA	Sigma Aldrich, Oslo, Norge	
n-Hexane	PESTINORM®	99		VWR Chemicals	BDH Prolabo®	2.5 L
Nitrogen	5.0	99,998	7727-37-9	AGA, Oslo, Norge	AGA, Oslo, Norge	
QuEChERS Enhanced Matrix Removal - Lipid				Agilent Technologies	Agilent Technologies	

Table 11: Reference material used for stock solutions

Reference material	Purity	CAS		Producer	Supplier
Acetaminophen	98.0-102.0%	103-90-2	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Acetylsalicylic acid	98, 1 ± 0,5	50-78-2	Powder	Chiron AS, Trondheim, Norge	Chiron AS, Trondheim, Norge
Amitriptyline hydrochloride	≥98% (TLC)	549-18-8	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Amlodipine	99.5 %	88150-42-9	Powder	Chiron AS, Trondheim, Norge	Chiron AS, Trondheim, Norge
Atenolol	≥98%	29122-68-7	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Atorvastatin	98 %	134523-03-8	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Caffeine	Reagent plus	58-08-2	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Caffeine 3-^{13}C	99.0 %	78972-66-9	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich. Seelze, Tyskland
Carbamazepine	99.0 %	298-46-4	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Carbamazepine-d_{10}	99.9 %	132183-78-9	Powder	Chiron AS, Trondheim, Norge	Chiron AS, Trondheim, Norge
(±)-Chlorpheniramine maleate salt	99 %	113-92-8	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Cephalexin	99.7 %	15686-71-2	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Ciprofloxacin		85721-33-1	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
DEET	≥ 98.8 %	134-62-3	Liquid	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Diclofenac sodium salt		15307-79-6	Powder		
Fluoxetine hydrochloride	99 %	56296-78-7	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Ethinylestradiol		57-63-6	Powder		
Estrone	≥ 99 %	53-16-7	Powder		
Ibuprofen	> 98,0	15687-27-1	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Losartan potassium	99.5 %	124750-99-8	Powder	Chiron AS, Trondheim, Norge	Chiron AS, Trondheim, Norge
Metformin hydrochloride		1115-70-4	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Metoprolol free base	99.5 %	51384-51-1	Powder	Chiron AS, Trondheim, Norge	Chiron AS, Trondheim, Norge
Metoprolol-d_7 Hydrochloride	99, 0 ± 0,5	1219798-61-4	Ampoule	Chiron AS, Trondheim, Norge	Chiron AS, Trondheim, Norge
Metronidazole	99.8 %	443-48-1	Powder	Sigma Aldrich. Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Penicillin G potassium salt	Bio reagent	69-57-8	Powder	Sigma Aldrich, Shanghai, Kina	Sigma Aldrich, Oslo, Norge
Prednisolone	98.9 %	50-24-8	Powder	Fluka analytical, Sigma Aldrich, Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Ranitidine hydrochloride		66357-59-3	Powder	Sigma Aldrich, Oslo, Norge	Sigma Aldrich, Oslo, Norge
Trimethoprim crystallized	≥99.0% (HPLC)	738-70-5	Powder	Sigma Aldrich, Oslo, Norge	Sigma Aldrich, Oslo, Norge
Trimethoprim-d_9	≥ 99.9 %	1189460-62-5	Powder	Sigma Aldrich, Oslo, Norge	Sigma Aldrich, Oslo, Norge
Simvastatin	99.5 %	79902-63-9	Powder	Chiron AS, Trondheim, Norge	Chiron AS, Trondheim, Norge
Sulfadoxine	99.8 %	2447-57-6 , 738-70-5	Powder	Fluka analytical, Sigma Aldrich, Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Sulfadoxine-d_3	≥ 99.9 %	1262770-70-6	Powder	Sigma Aldrich, Seelze, Tyskland	Sigma Aldrich, Oslo, Norge
Sulfamethoxazole	> 99.0	723-46-6	Powder	Sigma Aldrich, Oslo, Norge	Sigma Aldrich, Oslo, Norge
Warfarin		81-81-2	Powder	Fluka analytical, Sigma Aldrich, Seelze, Tyskland	Sigma Aldrich, Oslo, Norge

Raw data

Table 12: Calculated concentrations from MassHunter

		Metoprolol 116	Carbamazepine 174	Caffeine 110	Acetaminophen 110
Monday	1	1,694986	2,3896638	111,3696	129,96893
Monday	2	1,6612548	2,0958653	77,382787	158,45728
Monday	3	1,7311965	2,0360555	101,01164	164,02972
Monday	4	1,6789063	2,2668853	103,88087	167,92039
Monday	5	1,7205927	1,9845708	83,576632	140,25717
Monday	6	1,6376261	2,0623143	90,284621	132,95909
Tuesday	1	2,2427177		222,78602	322,11387
Tuesday	2	2,263782		293,57453	295,13922
Tuesday	3	2,3359247		230,6237	362,90521
Tuesday	4	2,2217926		226,74498	359,00449
Tuesday	5	2,2094144		234,04984	334,24526
Tuesday	6	2,2956574		197,49042	364,00616
Wednesday	1	1,7162216		577,00672	184,12497
Wednesday	2	1,6387334		238,67565	178,19863
Wednesday	3	1,6651863		315,88801	195,74796
Wednesday	4	1,61209		246,42221	204,58513
Wednesday	5	1,7024946		361,2265	195,2776
Wednesday	6	1,6389268		303,21759	205,32091
Thursday	1			215,6752	106,75417
Thursday	2			226,12624	89,468859
Thursday	3			228,57553	88,077372
Thursday	4			198,76139	87,258406
Thursday	5			205,27688	95,044064
Thursday	6			223,53893	104,49837
Friday	1		13,592266	162,90984	91,982489
Friday	2		13,541945	111,96183	104,99196
Friday	3		13,055866	165,4464	101,43379
Friday	4		13,821929	105,1418	122,94971
Friday	5		12,324048	107,63459	96,113755
Friday	6			96,360006	102,63331
Saturday	1	2,2957293	13,558891	294,68584	238,55055
Saturday	2	2,3761884		304,97837	300,68254
Saturday	3	2,1980287		232,19974	277,26037
Saturday	4	2,3131545	14,304969	311,72921	288,26293
Saturday	5	2,3041072	14,287055	287,88858	260,02179
Saturday	6	2,2754529		323,02838	320,04301
Sunday	1	1,9089092	1,9575962	140,90673	221,28629
Sunday	2	2,0149665	2,0078688	148,57943	215,85477
Sunday	3	1,8788687	2,5874977	141,46628	213,65011
Sunday	4	1,9685518	2,0989336	163,46585	210,59867
Sunday	5	1,970522	2,4975685	147,63023	228,48199
Sunday	6	1,9184136		143,77524	234,23869

Table 13: Results of the external matrix matched calibration curves of the analytes

	MRM	Water samples			Sediment samples			Liver samples		
		Linear range [ng/L]	ISTD	RT (min)	Linear range [ng/g]	ISTD	RT (min)	Linear range [ng/g]	ISTD	RT (min)
APAP	152 -> 110	25 - 3000	198.2 -> 140.2	2,7	Not linear	198.2 -> 140.2	2,6	Not linear	198.2 -> 140.2	2,7
APAP	152 -> 65.1	25 - 3000	198.2 -> 112	2,7	Not linear	198.2 -> 112	2,6	Not linear	198.2 -> 140.2	2,7
ASA	179,1 -> 137	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
ASA	179,1 -> 93	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
AMT	278 -> 105	1 - 100	247.1 -> 204.1	4,2	1 - 500	247.1 -> 187.1	5,6	5 - 500	247.1 -> 204.1	5,6
AMT	278 -> 91	1 - 100	247.1 -> 204.1	4,2	5 - 500	247.1 -> 187.1	5,6	1 - 500	247.1 -> 204.1	5,6
ADP	409,1 -> 293.9	Not linear	198.2 -> 112	4,1	Not linear	247.1 -> 187.1	5,4	n.a.	n.a.	n.a.
ADP	409,1 -> 237.8	Not linear	198.2 -> 112	4,1	Not linear	247.1 -> 187.1	5,4	n.a.	n.a.	n.a.
ATN	267,2 -> 190.1	1 - 100	275.3 -> 191	2,5	n.a.	n.a.	n.a.	5 - 200	275.3 -> 191	2,4
ATN	267,2 -> 145	3 - 100	275.3 -> 121	2,5	n.a.	n.a.	n.a.	1 - 500	275.3 -> 121	2,4
AVS	559,2 -> 440.1	1 - 3000	275.3 -> 121	5,5	n.a.	n.a.	n.a.	Not linear	198.2 -> 112	6,9
AVS	559,2 -> 292.1	10 - 3000	275.3 -> 121	5,5	n.a.	n.a.	n.a.	Not linear	198.2 -> 112	6,9
AVS	559,2 -> 250	3 - 3000	275.3 -> 121	5,5	n.a.	n.a.	n.a.	Not linear	198.2 -> 112	6,9
CAF	195 -> 138	25 - 3000	198.2 -> 140.2	3	20 - 500	198.2 -> 140.2	3,8	10 - 500	198.2 -> 140.2	3,9
CAF	195 -> 110	Not linear*	198.2 -> 112	3	Not linear	198.2 -> 112	3,8	20 - 500	198.2 -> 112	3,9
CBZ	237 -> 194	1 - 500	247.1 -> 204.1	4,5	1 - 500	247.1 -> 204.1	5,8	1 - 200	247.1 -> 204.1	5,8
CBZ	237 -> 179	5 - 500	247.1 -> 187.1	4,5	5 - 500	247.1 -> 187.1	5,8	5 - 200	247.1 -> 187.1	5,8
CEP	348 -> 174	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CEP	348 -> 158	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CEP	348 -> 106	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CPN	275 -> 230	1 -100	275.3 -> 105.2	3,4	5 - 500	275.3 -> 105.2	4,5	1 - 500	275.3 -> 105.2	4,5
CPN	275 -> 167	1 -100	275.3 -> 105.2	3,4	10 - 500	275.3 -> 105.2	4,5	5 - 100	275.3 -> 105.2	4,5
CIP	332 -> 288	Not linear	275.3 -> 105.2	3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CIP	332 -> 245	Not linear	275.3 -> 105.2	3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
DCF	294 -> 250	1 - 3000	198.2 -> 140.2	5,8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
DCF	294 -> 214	25 - 3000	198.2 -> 140.2	5,8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
DCF +	296 -> 250	25 - 3000	198.2 -> 140.2	5,8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
DCF +	296 -> 215	25 - 3000	198.2 -> 140.2	5,8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
DCF +	296 -> 214	1 - 3000	247.1 -> 204.1	5,8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
DEET	192 -> 119	1 - 100	247.1 -> 204.1	4,9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
DEET	192 -> 91	1 - 100	247.1 -> 204.1	4,9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
FLX	310 -> 148	25 - 3000	198.2 -> 140.2	4,3	Not linear	300.3 -> 264	6,9	Not linear	247.1 -> 204.1	5,6
FLX	310 -> 117	Not linear	247.1 -> 204.1	4,3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
IBP	205 -> 161	Not linear	247.1 -> 204.1	5,9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
IBP+	207,1 -> 160	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
IBP+	207,1 -> 118.9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
LAT	423,2 -> 404.9	Not linear	247.1 -> 204.1	4,7	n.a.	n.a.	n.a.	Not linear	198.2 -> 140.2	6
LAT	423,2 -> 337	25 - 3000	198.2 -> 140.2	4,7	n.a.	n.a.	n.a.	Not linear	198.2 -> 140.2	6
LAT	423,2 -> 207	Not linear	247.1 -> 204.1	4,7	n.a.	n.a.	n.a.	Not linear	198.2 -> 140.2	6
MET	130 -> 71	Not linear	198.2 -> 140.2	0,7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MET	130 -> 60	Not linear	198.2 -> 140.2	0,7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MPL	268,3 -> 191	3 - 3000	275.3 -> 191	3,3	5 - 500	275.3 -> 191	4,3	5 - 200	275.3 -> 191	4,3
MPL	268,3 -> 116.2	1 - 1000	275.3 -> 121	3,3	1 - 500	275.3 -> 121	4,3	1 - 500	275.3 -> 121	4,3

MPL	268,3 -> 98.1	5 - 1000	275.3 -> 105.2	3,3	20 - 500	275.3 -> 105.2	4,3	5 - 200	275.3 -> 105.2	4,3
MPL	268,3 -> 74.1	1 - 1000	275.3 -> 105.2	3,3	10 - 500	275.3 -> 105.2	4,3	5 - 200	275.3 -> 105.2	4,3
MTZ	172 -> 128	Not linear	198.2 -> 140.2	2,7	n.a.	n.a.	2,6	Not linear	300.3 -> 234.1	2,5
MTZ	172 -> 82	Not linear	198.2 -> 140.2	2,7	n.a.	n.a.	2,6	5 - 500	300.3 -> 234.1	2,5
PEN G	335.2 -> 288.9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PEN G	335.2 -> 128.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PEN G	335.2 -> 91.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PNS	361,1 -> 325.1	Not linear	198.2 -> 140.2	4,1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PNS	361,1 -> 146.7	Not linear	198.2 -> 140.2	4,1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
RAN	315 -> 170	1 - 100	198.2 -> 140.2	2,5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
RAN	315 -> 130	1 - 100	198.2 -> 140.2	2,5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SCA	137 -> 93	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SCA	137 -> 65	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SIV	419 -> 225	25 - 1000	198.2 -> 112	6,9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SIV	419 -> 199	5 - 3000	198.2 -> 112	6,9	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SFD	311 -> 165.1	1 - 500	314.1 -> 156	3,9	5 - 500	314.1 -> 156	5	5 - 500	314.1 -> 156	5
SFD	311 -> 108	1 - 500	314.1 -> 108	3,9	10 - 500	314.1 -> 108	5	25 - 500	314.1 -> 108	5
SFD	311 -> 92.1	1 - 500	314.1 -> 92.1	3,9	10 - 500	314.1 -> 92.1	5	Not linear	314.1 -> 92.1	5
SMX	254 -> 156	Not linear	314.1 -> 156	4	Not linear	314.1 -> 156	5,1	n.a.	n.a.	n.a.
SMX	254 -> 108	Not linear	314.1 -> 156	4	20 - 500	314.1 -> 108	5,1	n.a.	n.a.	n.a.
SMX	254 -> 92	10 - 3000	314.1 -> 156	4	20 - 500	314.1 -> 92.1	5,1	n.a.	n.a.	n.a.
TMP	291,5 -> 261.1	1 - 100	300.3 -> 264	3	20 - 500	300.3 -> 264	3,9	1 - 500	300.3 -> 264	3,9
TMP	291,5 -> 123.2	1 - 100	300.3 -> 122,9	3	20 - 500	300.3 -> 122,9	3,9	5 - 500	300.3 -> 122,9	3,9
WAR	307,2 -> 161	1 - 1000	198.2 -> 140.2	5,4	Not linear	198.2 -> 140.2	6,8	Not linear	198.2 -> 140.2	6,8
WAR	307,2 -> 117.1	10 - 3000	198.2 -> 140.2	5,4	Not linear	198.2 -> 140.2	6,8	Not linear	198.2 -> 140.2	6,8

Table 14: Recovery of all the methods, the recoveries marked in yellow is not linear

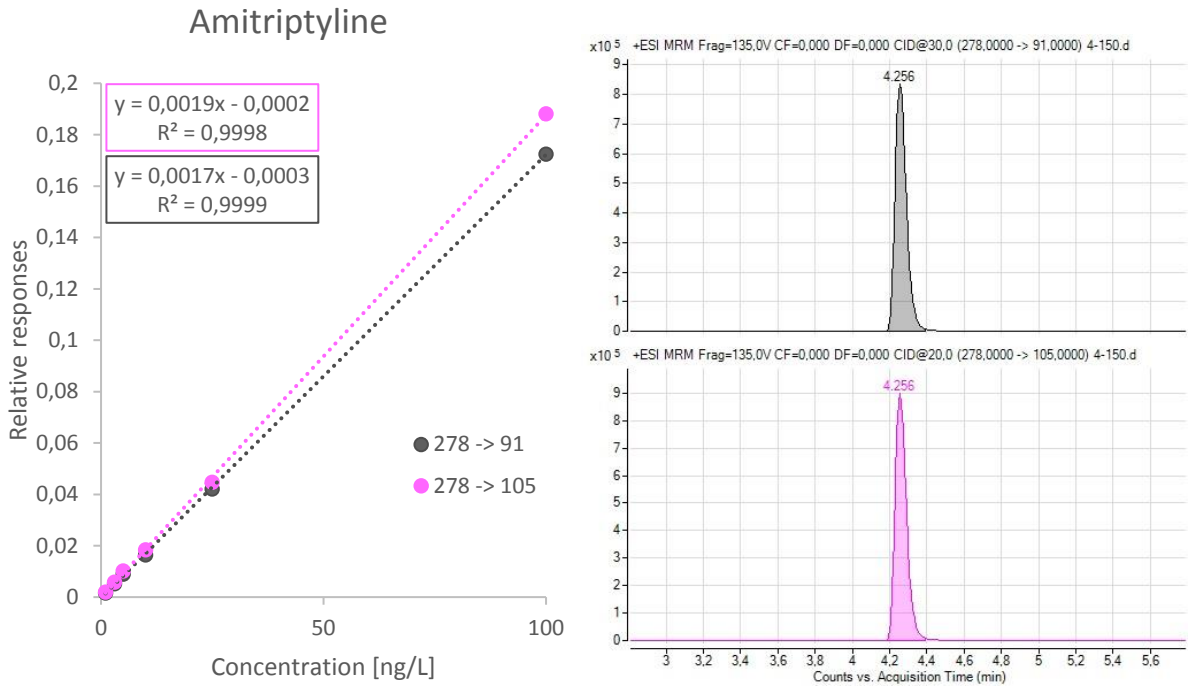
Analytes	Seawater			Sediment			Fish liver		
	R^T	R^{MS}	R^{PO}	R^T	R^{MS}	R^{PO}	R^T	R^{MS}	R^{PO}
APAP 65.1	25 %	94 %	27 %	65,1	2 %	65 %	2 %	39 %	7 %
APAP 110	25 %	94 %	27 %	2 %	63 %	2 %	3 %	40 %	7 %
AMT 91	95 %	112 %	84 %	32 %	83 %	38 %	43 %	64 %	67 %
AMT 105	93 %	110 %	85 %	32 %	82 %	39 %	42 %	62 %	67 %
ADP 293.9	74 %	133 %	55 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
ADP 237.8	75 %	129 %	58 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
ATN 190.1	84 %	95 %	88 %	n.a.	n.a.	n.a.	76 %	114 %	66 %
ATN 145	85 %	96 %	88 %	n.a.	n.a.	n.a.	78 %	116 %	67 %
AVS 250	10 %	56 %	17 %	n.a.	n.a.	n.a.	2 %	35 %	6 %
AVS440.1	10 %	56 %	19 %	n.a.	n.a.	n.a.	2 %	38 %	6 %
AVS 292.1	10 %	50 %	19 %	n.a.	n.a.	n.a.	2 %	35 %	5 %
CAF 110	45 %	73 %	50 %	8 %	24 %	30 %	14 %	25 %	57 %
CAF 138	44 %	66 %	58 %	7 %	24 %	26 %	18 %	26 %	70 %
CAF $^{13}C_3$ 112	26 %	57 %	33 %	n.a.	n.a.	n.a.	2 %	38 %	6 %
CAF $^{13}C_3$ 140.2	45 %	68 %	66 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CBZ 194	62 %	67 %	92 %	54 %	68 %	80 %	35 %	63 %	55 %
CBZ 179	645 %	71 %	91 %	55 %	70 %	79 %	38 %	73 %	53 %
CBZ d_{10} 174.1	69 %	73 %	95 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CBZ d_{10} 187.1	72 %	78 %	92 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CBZ d_{10} 204.1	68 %	72 %	94 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CPN 230	69 %	79 %	88 %	19 %	68 %	28 %	20 %	57 %	36 %
CPN 167	84 %	92 %	91 %	20 %	75 %	26 %	25 %	68 %	37 %
DEET 91	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	50 %	71 %	69 %
DEET 119	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
FLX 148	67 %	81 %	83 %	36 %	73 %	50 %	28 %	40 %	69 %
FLX 117	n.a	n.a	n.a	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
LAT 377	43 %	78 %	55 %	n.a.	n.a.	n.a.	20 %	50 %	40 %
LAT 404.9	41 %	73 %	55 %	n.a.	n.a.	n.a.	19 %	45 %	41 %
LAT 207	46 &	79 %	58 %	n.a.	n.a.	n.a.	20 %	54 %	38 %
MET 60	1 %	72 %	2 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MET 71	1 %	66 %	2 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MPL 191	93 %	89 %	104 %	21 %	38 %	56 %	26 %	36 %	73 %
MPL 116.2	94 %	89 %	105 %	21 %	39 %	53 %	27 %	37 %	71 %
MPL 98.1	80 %	76 %	86 %	20 %	38 %	53 %	27 %	37 %	72 %
MPL 74.1	95 %	89 %	107 %	21 %	40 %	54 %	27 %	38 %	71 %
MPL d_7 191	100 %	95 %	105 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MPL d_7 121	101 %	95 %	105 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MPL d_7 105.2	103 %	97 %	106 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MNZ 82	18 %	92 %	19 %	n.a.	n.a.	n.a.	16 %	29 %	53 %
MNZ 128	17 %	91 %	19 %	n.a.	n.a.	n.a.	15 %	29 %	53 %
PNS 325.1	38 %	47 %	79 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PNS 146.7	37 %	47 %	76 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
RAN 130	43 %	101 %	43 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
RAN 170	43 %	101 %	42 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SCA93	5 %	71 %	5 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SCA65	n.a	n.a	n.a	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SIV 199	57 %	81 %	69 %	1 %	77 %	1 %	n.a.	n.a.	n.a.
SIV 225	53 %	78 %	68 %	4 %	79 %	5 %	n.a.	n.a.	n.a.
SFD 156.1	26 %	69 %	38 %	n.a.	n.a.	n.a.	1 %	3 %	53 %
SFD 92.1	26 %	69 %	38 %	n.a.	n.a.	n.a.	1 %	3 %	54 %
SFD 108	27 %	71 %	37 %	n.a.	n.a.	n.a.	2 %	3 %	57 %
SFD d_3 92.1	26 %	70 %	37 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SFD d_3 108	26 %	72 %	36 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SFD d_3 156	24 %	64 %	38 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SMX 156	7 %	43 %	17 %	14 %	4 %	350 %	n.a.	n.a.	n.a.
SMX 108	7 %	50 %	15 %	16 %	63 %	25 %	n.a.	n.a.	n.a.
SMX 92	8 %	50 %	17 %	15 %	61 %	25 %	n.a.	n.a.	n.a.
TMP 261.1	72 %	80 %	90 %	5 %	40 %	11 %	25 %	34 %	73 %
TMP 123.2	71 %	77 %	92 %	5 %	40 %	11 %	25 %	35 %	71 %
TMP d_9 264	73 %	74 %	99 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
TMP d_9 234.1	71 %	75 %	95 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
TMP d_9 122.9	74 %	77 %	95 %	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
WAR 161	75 %	79 %	93 %	32 %	80 %	40 %	105 %	235 %	45 %
WAR 117.1	66 %	75 %	76 %	40 %	78 %	51 %	99 %	225 %	44 %

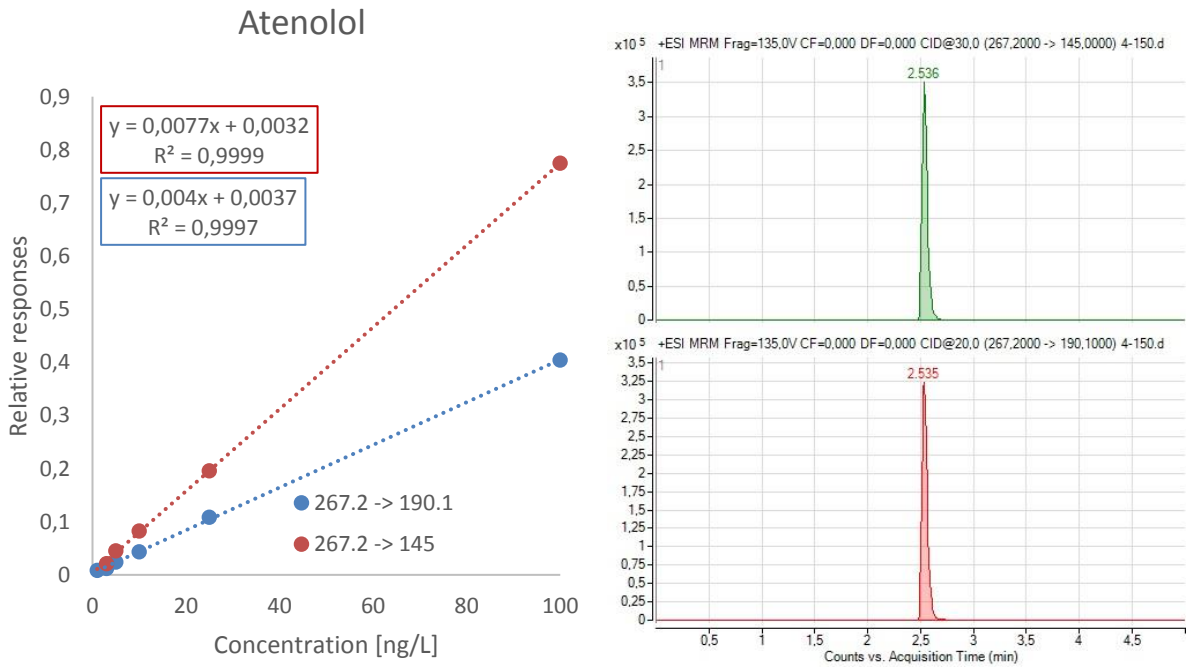
Table 15: Raw data of the calculation of recovery

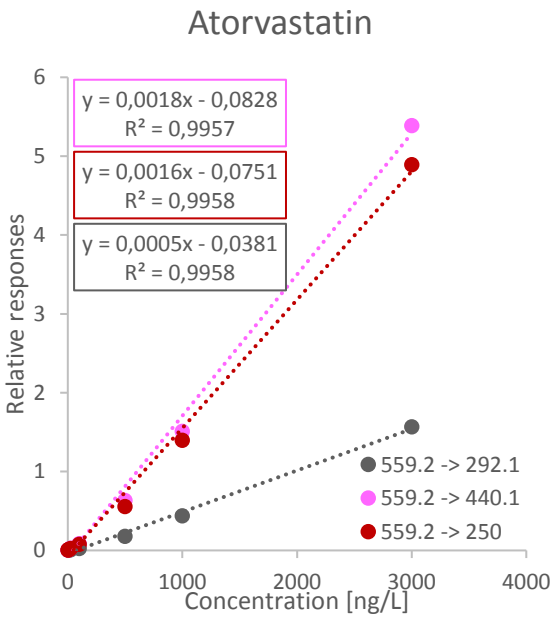
*** average of 6 parallels	Seawater				Sediment				Fish liver			
	LM 100	MB100***	MB	100	LM100	MB100	MB	100	LM100	MB100***	B	100
APAP 110	1537314	1447626	5075	395801,6	1285233,05	805308,2	803,7088	16288,84	1579575	630153,2	489,5282	43599,24
APAP 65.1	619599	582822,5	2539	157954	596138,6	386497,8	232,8429	9458,539	811497	318770,9	137,9756	23198,88
AMT 105	2319836	2546906	2860	2168313	2008318	1654185	1467,62	639495,3	2025246	1265874	424,0277	850562,7
AMT 91	2801625	3151342	3715	2666484	2435521	2033777	1924,765	774740,6	2448876	1576956	1117,78	1050547
ADP 293.9	116728	156195,5	1142	87436,8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
ADP 237.8	233038	301228	171	175777,2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
ATN 190.1	1047333	992965	399	876340,6	n.a.	n.a.	n.a.	n.a.	860406,4	983309,3	60,82342	652973,9
ATN 145	1647644	1578314	471	1396457	n.a.	n.a.	n.a.	n.a.	1205906	1398595	79,87971	942607,9
AVS440.1	314493	175831,5	198	30253	n.a.	n.a.	n.a.	n.a.	298814,5	112828	17,39374	7149,324
AVS 292.1	101328	50834	69	9769,8	n.a.	n.a.	n.a.	n.a.	63227,85	22263,82	13,95605	1074,651
AVS 250	314493	175831,5	198	30253	n.a.	n.a.	n.a.	n.a.	197342	69562,78	66,31378	4544,482
CAF 138	129030	96062,5	10455	66601,8	141618,3	36090,27	1580,875	10866,5	135379,9	35262,06	43,22844	24606,38
CAF 110	17846	16090	3122	11209,8	23185,03	6153,35	617,9614	2456,472	22163,33	5608,965	178,01	3375,359
CAF $^{13}C_3$ 112	21518	17069	4907	10472,2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CAF $^{13}C_3$ 140.2	193160	131275	717	86941,4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CBZ 194	8329734	5606948	25745	5164440	5393600,78	3659300,18	3951,50857	2928082,75	5066091	3172083	1641,362	1756066
CBZ 179	1138776	809528,5	3582	740749	636125,188	444388,602	148,799638	351659,136	553228,8	403186,9	512,3893	212863,6
CBZ d_{10} 174.1	715422	521080,5	791	497957	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CBZ d_{10} 187.1	588669	460396,5	631	425388,8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CBZ d_{10} 204.1	8384877	6004443	3142	5666557	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CPN 230	7435777	5884390	4618	5159334	4778515,15	3242693,55	1933,27647	894105,161	5660109	3218865	1243,978	1147014
CPN 167	1208875	1116216	594	1014972	860363,708	644024,977	14,294250 2	169551,849	981160,3	670016,4	191,8875	249994
DEET 91	3221	20039	9997	9589	n.a.	n.a.	n.a.	n.a.	24579,02	17933,19	407,1805	12746,11
DEET 119	3946	28154	13083	12907,2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
FLX 148	104038	84074,5	146	69990,2	41848,67	30468,67	57,18244	15265,44	88644,03	35943,78	60,138688	24901,93
LAT 404.9	426091	320444,5	10287	185628,8	n.a.	n.a.	n.a.	n.a.	604120,9	273062,1	0	112713,3
LAT 377	317236	246593,5	371	136572,4	n.a.	n.a.	n.a.	n.a.	342369,2	170874,8	188,2089	67921,79
LAT 207	910133	715570,5	473	415817,8	n.a.	n.a.	n.a.	n.a.	822747,9	441541	164,1273	166909,3
MET 60	4473329	3208760	2253	54995,25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MET 71	2730901	1806863	5026	32471	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MPL 191	229286	204098	583	213238,2	504670	190093	22,00056	106888,7	513708,6	183501,4	74,70074	134669,9
MPL 116.2	721125	641184,5	710	676104,4	1701393	666719,6	10,12221	353834,1	1689827	633940,2	406,6929	452122,9
MPL 98.1	382502	353953,5	64435	368608,4	798386,9	304863,8	1163,963	161704,8	795709	293304,2	528,5907	212237,7
MPL 74.1	612467	543373,5	448	581359	1401834	556804	66,44181	301412,7	1395404	525676,1	122,5439	373388
MPL d_7 191	213811	203499	953	215067,6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MPL d_7 121	114669	110177	1595	117495	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

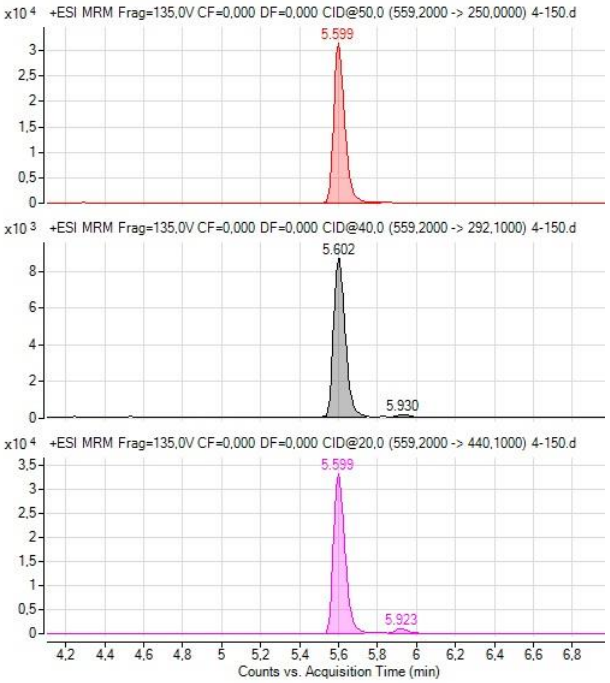
MPL d_7 105.2	400696	389621,5	2260	413755,4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
MNZ 128	379598	347128	243	66021,2	n.a.	n.a.	n.a.	n.a.	284097,3	81817,1	129,122	43493,34
MNZ 82	213193	195644	323	37679,2	n.a.	n.a.	n.a.	n.a.	174573,8	51683,49	552,2769	28067,01
PNS 325.1	90135	43256	867	35002,4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PNS 146.7	57352	28008,5	989	22156,6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
RAN 130	1912904	1932270	298	826003	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
RAN 170	157620	160174,5	247	67472	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SCA93	5161	4469	780	1023,6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SCA65	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SIV 225	96685	75774,5	247	51644,8	59355,2	47169,72	316,4293	2780,446	n.a.	n.a.	n.a.	n.a.
SIV 199	96685	75774,5	247	51644,8	86630,46	67125,48	20,28591	674,9101	n.a.	n.a.	n.a.	n.a.
SFD 156.1	4233355	2910072	1318	1113453	4517950	3104192	8203,086	1176130	3866063	100840,3	327,1937	53799,96
SFD 108	1256613	898081,5	662	336850,2	1271351	884544,8	1929,286	328097,5	1017752	28583,68	474,0321	16717,46
SFD 92.1	1355730	937554	220	358401,2	1376983	949760,3	453,3727	344686,5	1180525	32242,75	234,6244	17711,4
SFD d_3 92.1	1362028	952559	193	351788	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SFD d_3 108	1237673	886925	363	320140,4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SFD d_3 156	4042693	2595065	514	975182,4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SMX 156	359891	156611,5	187	26372,2	144744,1	5675,054	212,717	20061,54	n.a.	n.a.	n.a.	n.a.
SMX 108	169359	85231	447	13062,2	91008,89	57894,99	175,3693	14931,1	n.a.	n.a.	n.a.	n.a.
SMX 92	268529	134371	473	22792,2	155590,4	95207,41	112,4104	23845,02	n.a.	n.a.	n.a.	n.a.
TMP 261.1	956352	761524,5	333	686421,6	987687,2	391818,9	181,9104	45127,06	883761,4	301125,6	36,30618	220474,6
TMP 123.2	1471796	1131150	1196	1046812	1479092	593150,8	566,2151	67204,81	1294042	459692,5	411,6124	324892,9
TMP d_9 264	659680	490706,5	335	483778,8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
TMP d_9 234.1	790374	593307,5	83	563467	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
TMP d_9 122.9	989211	766477	2051	731138,4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
WAR 161	13913	11151,5	121	10517,6	16716,76	13454,99	2,736421	5320,093	15120,15	35499,15	34,138881	15887,55
WAR 117.1	2214	1913,5	254	1716,6	3048,954	2378,418	10,21031	1234,636	2662,067	6007,563	10,76033	2641,052

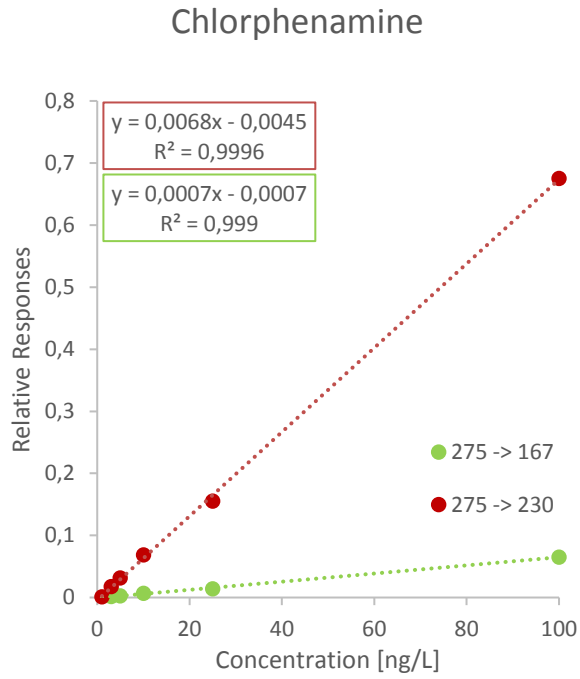
Calibration curves and chromatograms for seawater samples

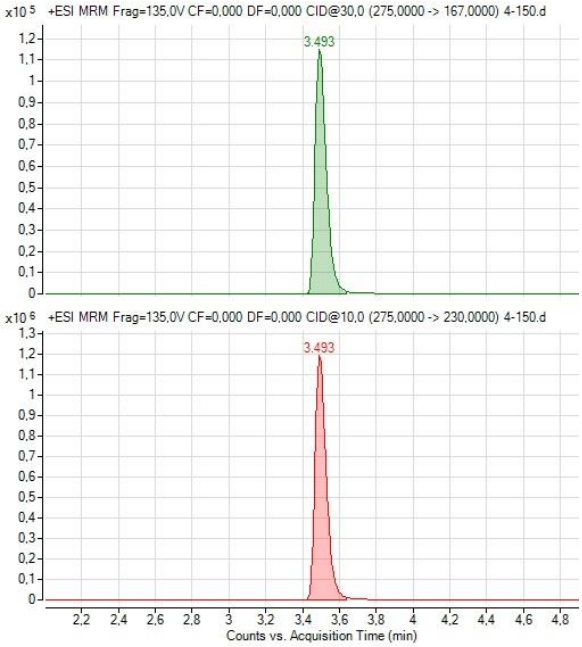


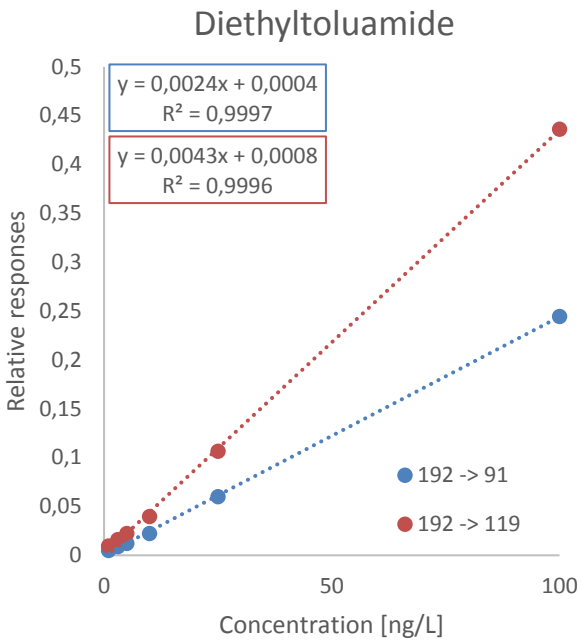


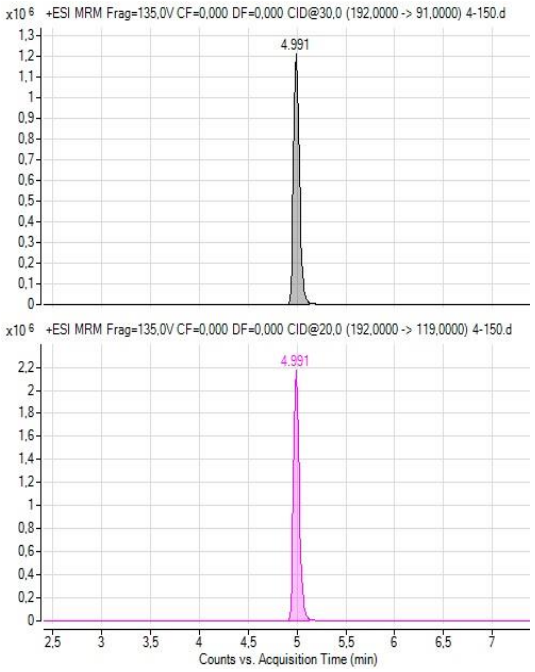

Atorvastatin
y = 0,0018x - 0,0828
R² = 0,9957
y = 0,0016x - 0,0751
R² = 0,9958
y = 0,0005x - 0,0381
R² = 0,9958
559.2 -> 292.1
559.2 -> 440.1
559.2 -> 250
Relative responses
Concentration [ng/L]

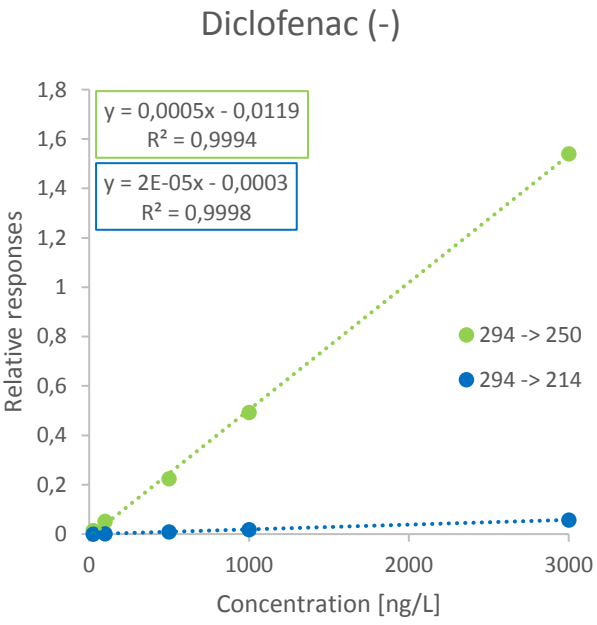

+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@50,0 (559,2000 -> 250,0000) 4-150.d
5.599
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@40,0 (559,2000 -> 292,1000) 4-150.d
5.602
5.930
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@20,0 (559,2000 -> 440,1000) 4-150.d
5.599
5.923
Counts vs. Acquisition Time (min)

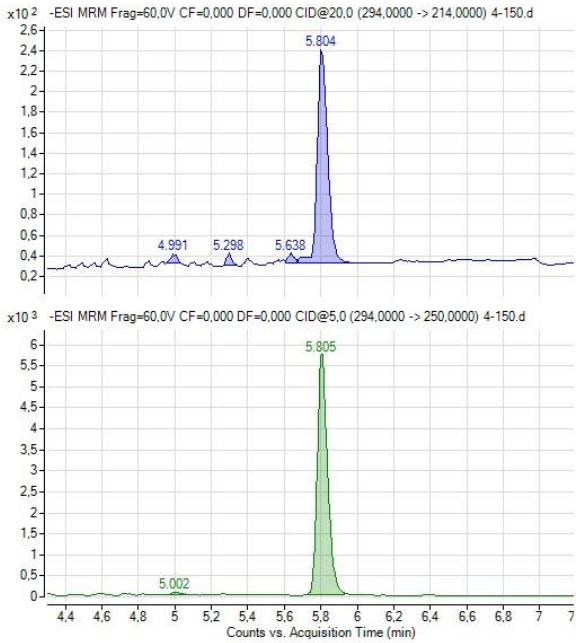

Chlorphenamine
y = 0,0068x - 0,0045
R² = 0,9996
y = 0,0007x - 0,0007
R² = 0,999
275 -> 167
275 -> 230
Relative Responses
Concentration [ng/L]

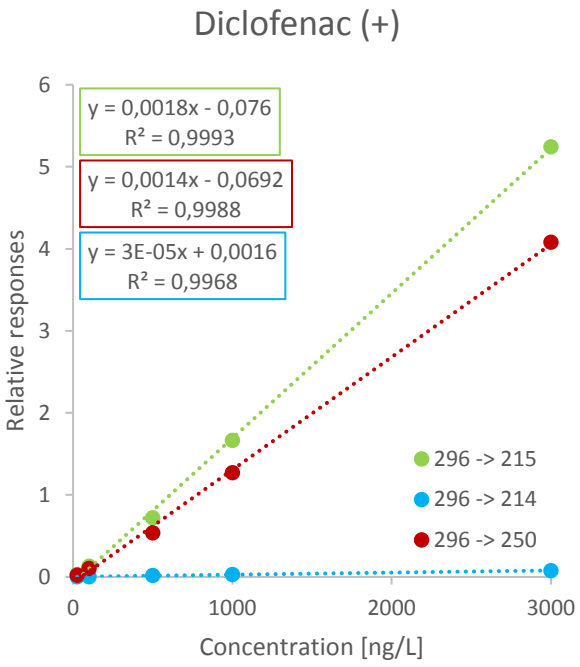

+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@30,0 (275,0000 -> 167,0000) 4-150.d
3.493
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@10,0 (275,0000 -> 230,0000) 4-150.d
3.493
Counts vs. Acquisition Time (min)

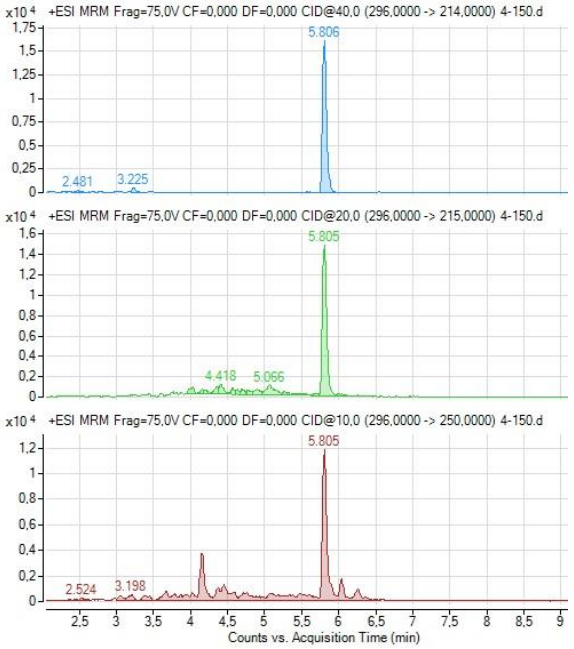

Diethyltoluamide
y = 0,0024x + 0,0004
R² = 0,9997
y = 0,0043x + 0,0008
R² = 0,9996
192 -> 91
192 -> 119
Relative responses
Concentration [ng/L]

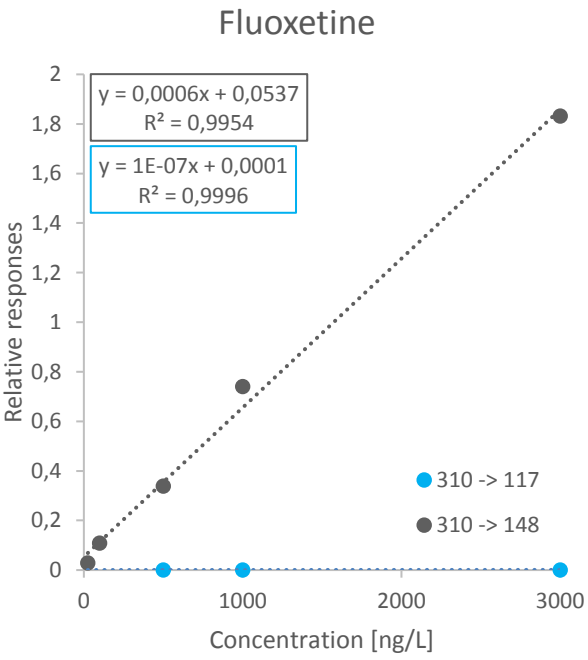

+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@30,0 (192,0000 -> 91,0000) 4-150.d
4.991
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@20,0 (192,0000 -> 119,0000) 4-150.d
4.991
Counts vs. Acquisition Time (min)

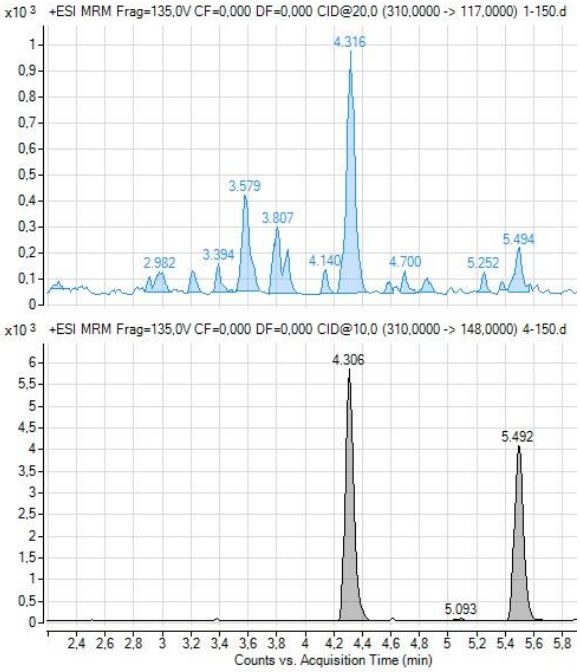

Diclofenac (-)
y = 0,0005x - 0,0119
R² = 0,9994
y = 2E-05x - 0,0003
R² = 0,9998
294 -> 250
294 -> 214
Relative responses
Concentration [ng/L]

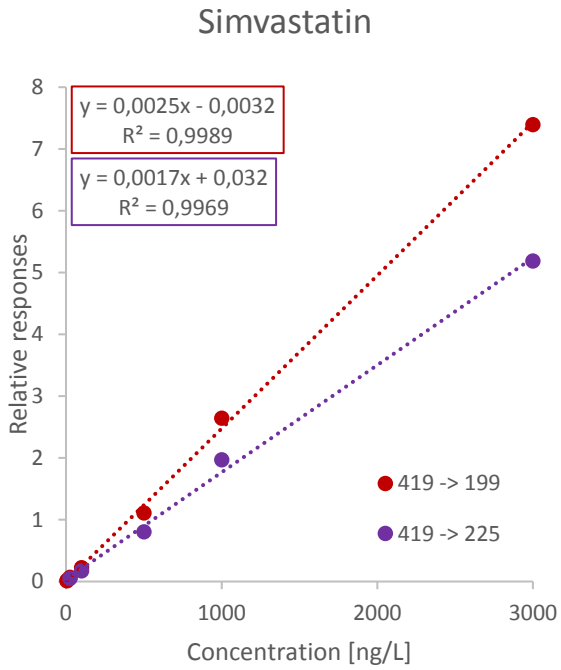

-ESI MRM Frag=60,0V CF=0,000 DF=0,000 CID@20,0 (294,0000 -> 214,0000) 4-150.d
5.804
4.991
5.298
5.638
-ESI MRM Frag=60,0V CF=0,000 DF=0,000 CID@5,0 (294,0000 -> 250,0000) 4-150.d
5.805
5.002
Counts vs. Acquisition Time (min)

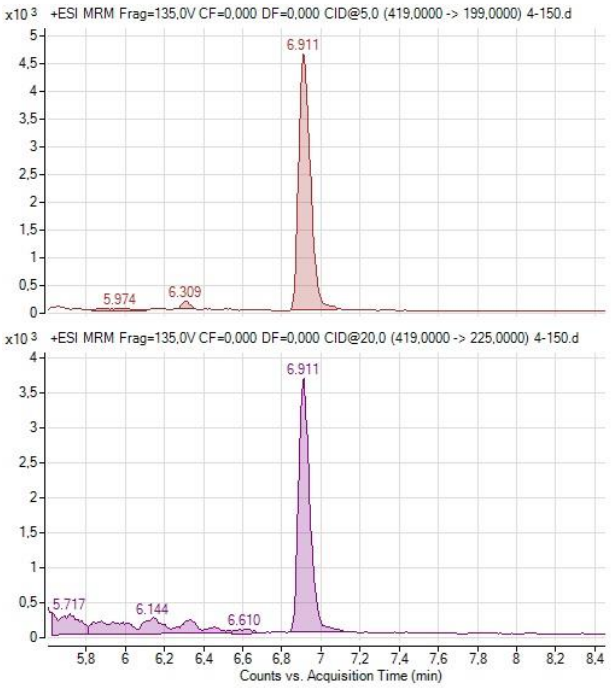

Diclofenac (+)
y = 0,0018x - 0,076
R² = 0,9993
y = 0,0014x - 0,0692
R² = 0,9988
y = 3E-05x + 0,0016
R² = 0,9968
296 -> 215
296 -> 214
296 -> 250
Relative responses
Concentration [ng/L]

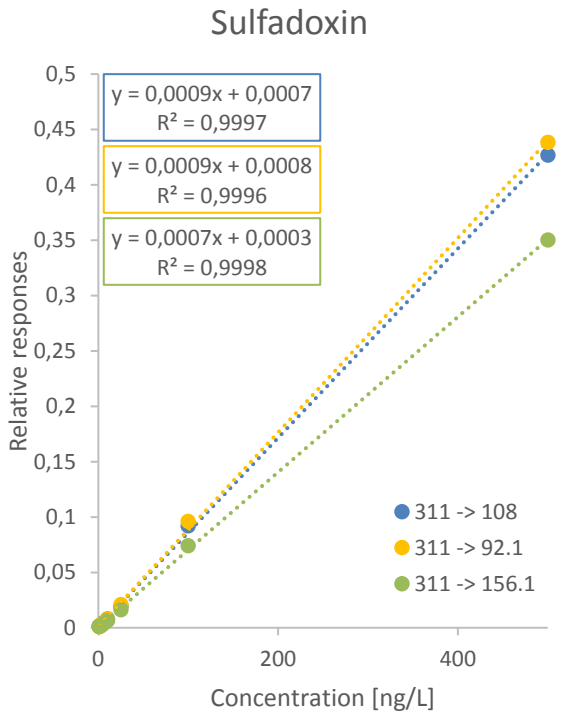

+ESI MRM Frag=75,0V CF=0,000 DF=0,000 CID@40,0 (296,0000 -> 214,0000) 4-150.d
+ESI MRM Frag=75,0V CF=0,000 DF=0,000 CID@20,0 (296,0000 -> 215,0000) 4-150.d
+ESI MRM Frag=75,0V CF=0,000 DF=0,000 CID@10,0 (296,0000 -> 250,0000) 4-150.d
Counts vs. Acquisition Time (min)

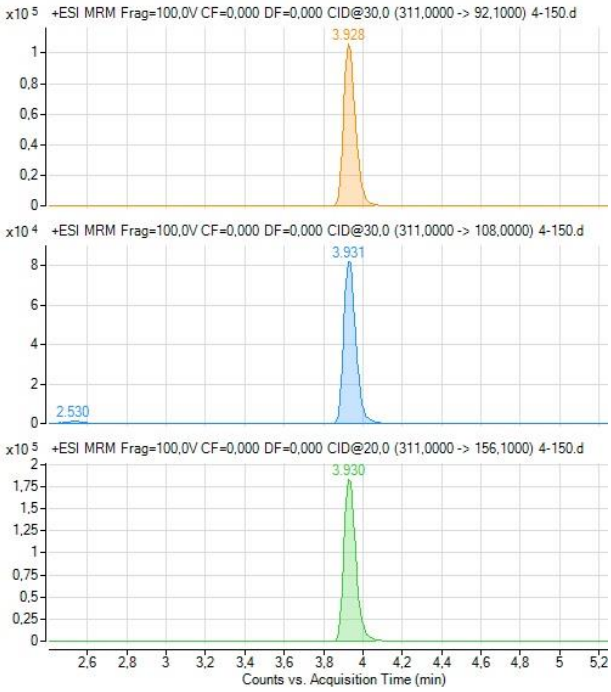

Fluoxetine
y = 0,0006x + 0,0537
R² = 0,9954
y = 1E-07x + 0,0001
R² = 0,9996
310 -> 117
310 -> 148
Relative responses
Concentration [ng/L]

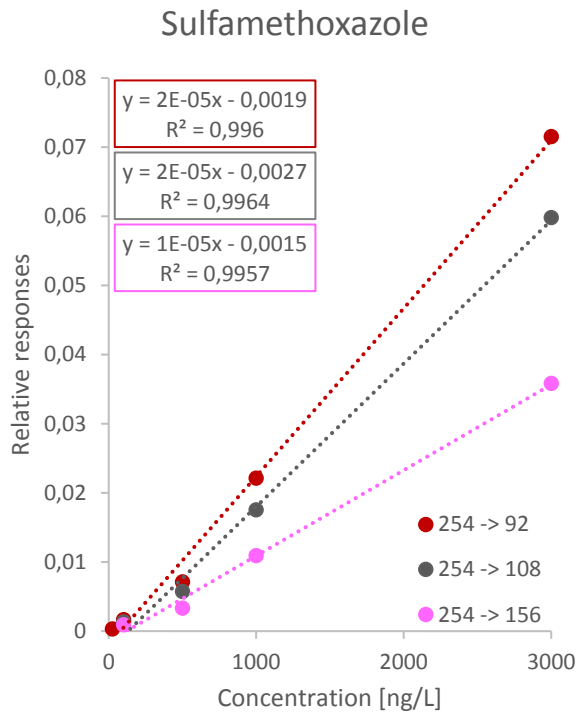

+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@20,0 (310,0000 -> 117,0000) 1-150.d
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@10,0 (310,0000 -> 148,0000) 4-150.d
Counts vs. Acquisition Time (min)

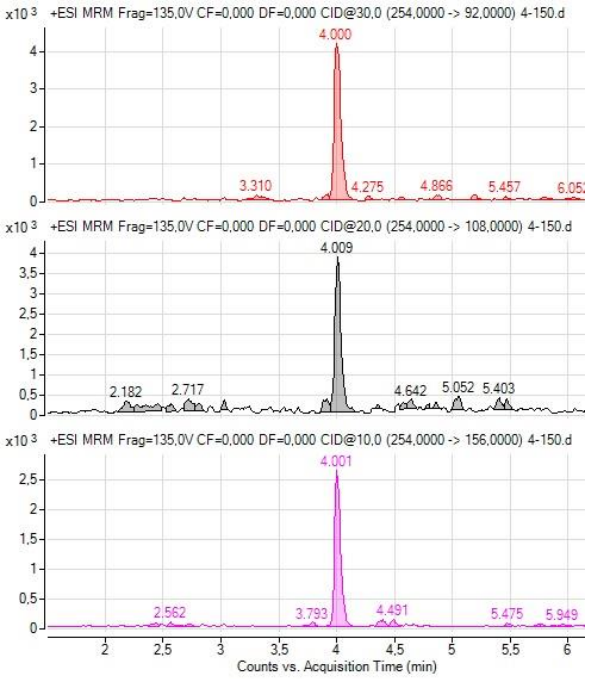

Simvastatin
y = 0,0025x - 0,0032
R² = 0,9989
y = 0,0017x + 0,032
R² = 0,9969
Relative responses
Concentration [ng/L]
419 -> 199
419 -> 225

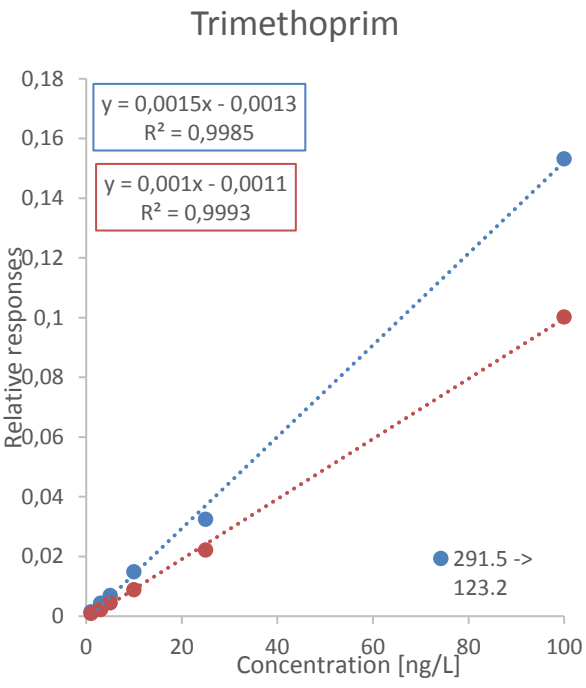

x10 3 +ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@5,0 (419,0000 -> 199,0000) 4-150.d
6.911
5.974
6.309
x10 3 +ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@20,0 (419,0000 -> 225,0000) 4-150.d
6.911
5.717
6.144
6.610
Counts vs. Acquisition Time (min)

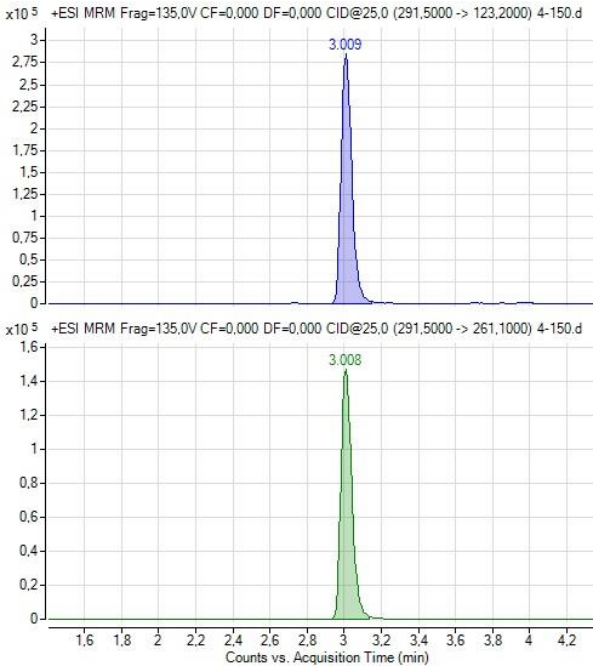

Sulfadoxin
y = 0,0009x + 0,0007
R² = 0,9997
y = 0,0009x + 0,0008
R² = 0,9996
y = 0,0007x + 0,0003
R² = 0,9998
Relative responses
Concentration [ng/L]
311 -> 108
311 -> 92.1
311 -> 156.1

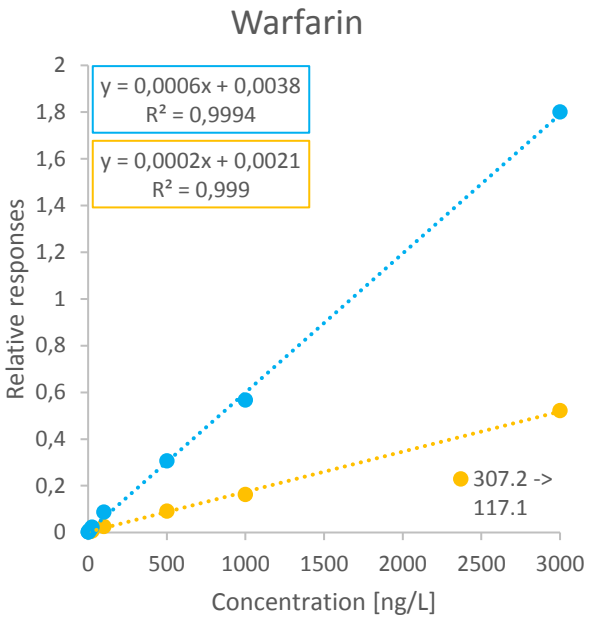

x10 5 +ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@30,0 (311,0000 -> 92,1000) 4-150.d
3.928
x10 4 +ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@30,0 (311,0000 -> 108,0000) 4-150.d
3.931
2.530
x10 5 +ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@20,0 (311,0000 -> 156,1000) 4-150.d
3.930
Counts vs. Acquisition Time (min)

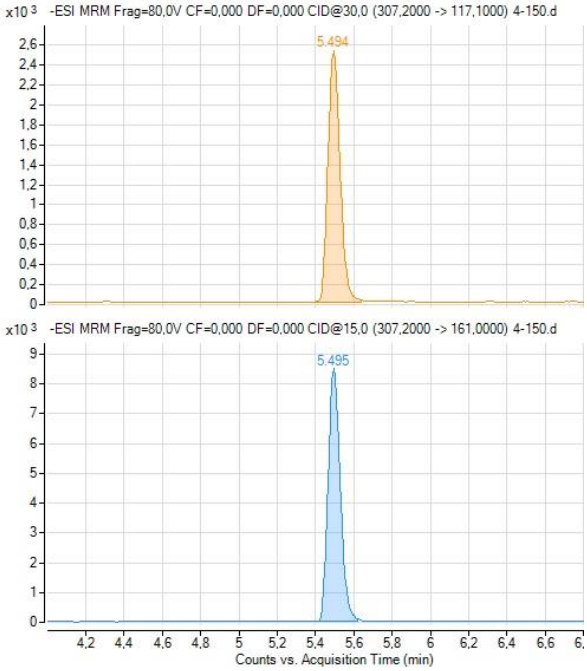

Sulfamethoxazole
y = 2E-05x - 0,0019
R² = 0,996
y = 2E-05x - 0,0027
R² = 0,9964
y = 1E-05x - 0,0015
R² = 0,9957
254 -> 92
254 -> 108
254 -> 156
Relative responses
Concentration [ng/L]


+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@30,0 (254,0000 -> 92,0000) 4-150.d
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@20,0 (254,0000 -> 108,0000) 4-150.d
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@10,0 (254,0000 -> 156,0000) 4-150.d
Counts vs. Acquisition Time (min)

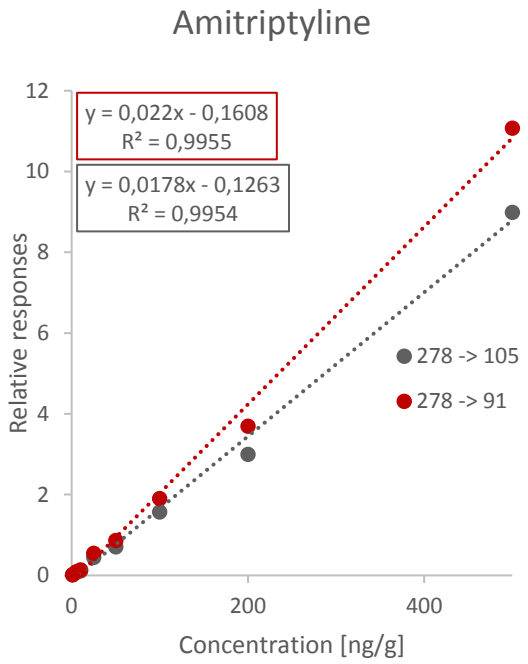

Trimethoprim
y = 0,0015x - 0,0013
R² = 0,9985
y = 0,001x - 0,0011
R² = 0,9993
291.5 -> 123.2
Relative responses
Concentration [ng/L]

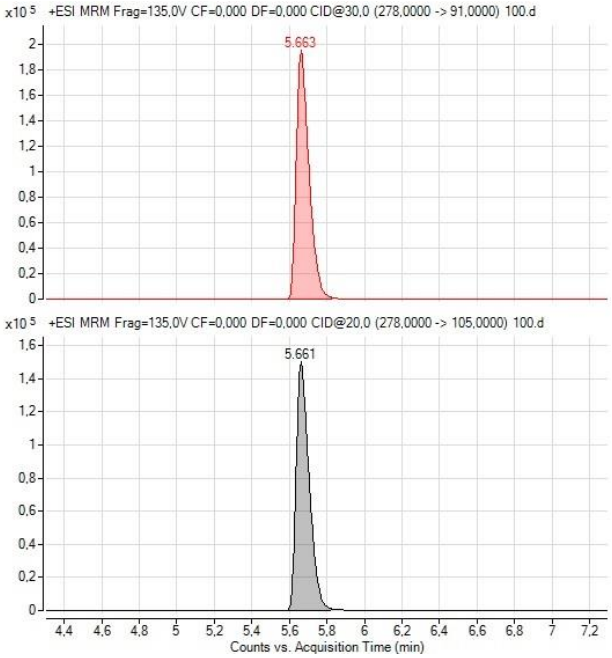

+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@25,0 (291,5000 -> 123,2000) 4-150.d
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@25,0 (291,5000 -> 261,1000) 4-150.d
Counts vs. Acquisition Time (min)

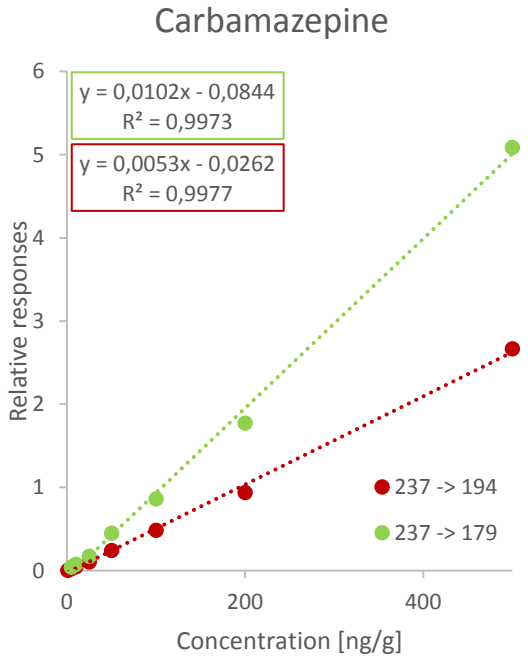

Warfarin
y = 0,0006x + 0,0038
R² = 0,9994
y = 0,0002x + 0,0021
R² = 0,999
307.2 -> 117.1
Relative responses
Concentration [ng/L]
0
0,2
0,4
0,6
0,8
1
1,2
1,4
1,6
1,8
2
0
500
1000
1500
2000
2500
3000

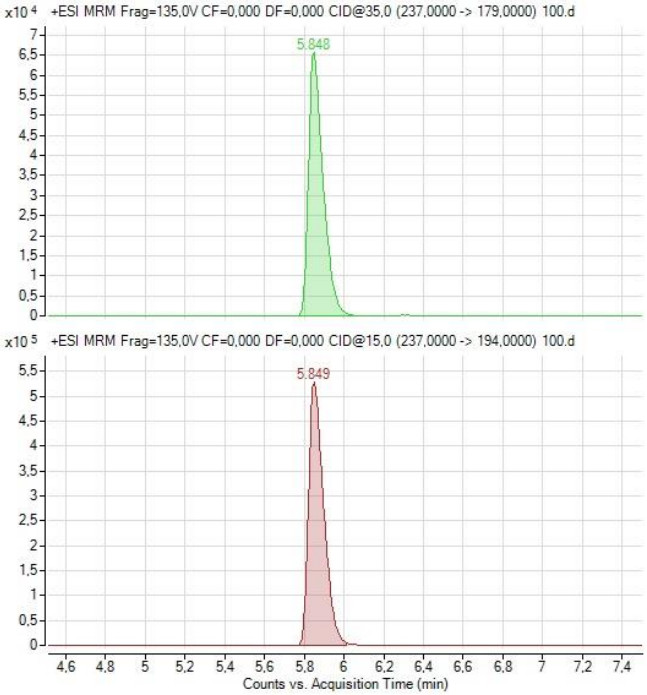

x10 3 -ESI MRM Frag=80,0V CF=0,000 DF=0,000 CID@30,0 (307,2000 -> 117,1000) 4-150.d
5.494
x10 3 -ESI MRM Frag=80,0V CF=0,000 DF=0,000 CID@15,0 (307,2000 -> 161,0000) 4-150.d
5.495
Counts vs. Acquisition Time (min)

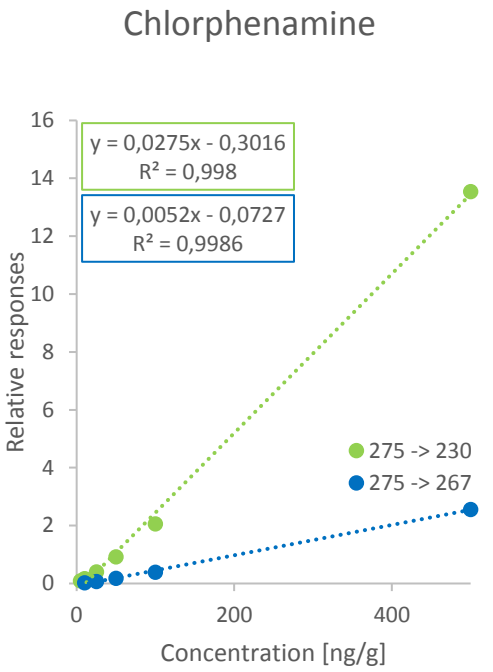
Calibration curves and chromatograms of sediment samples

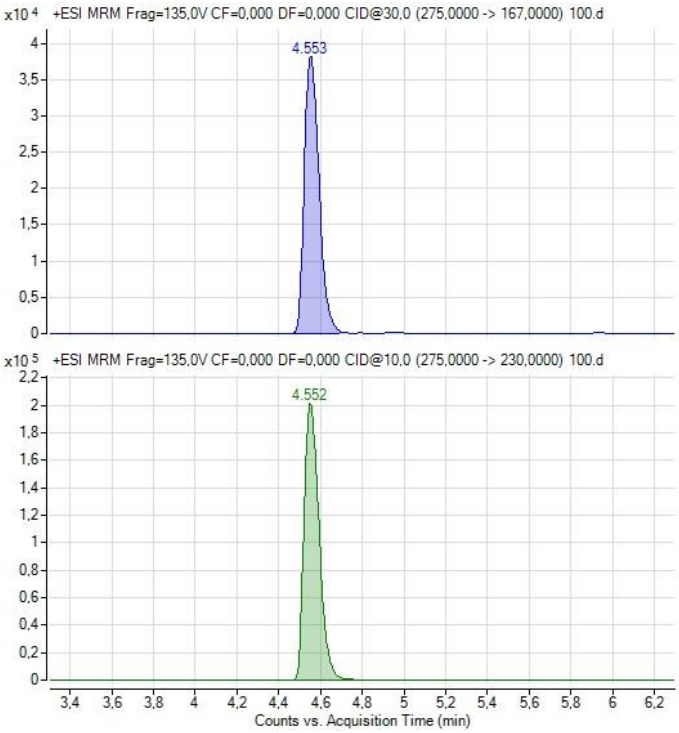


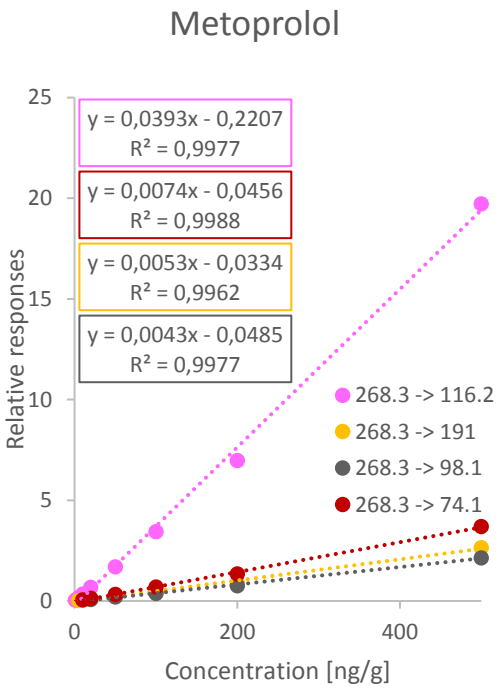


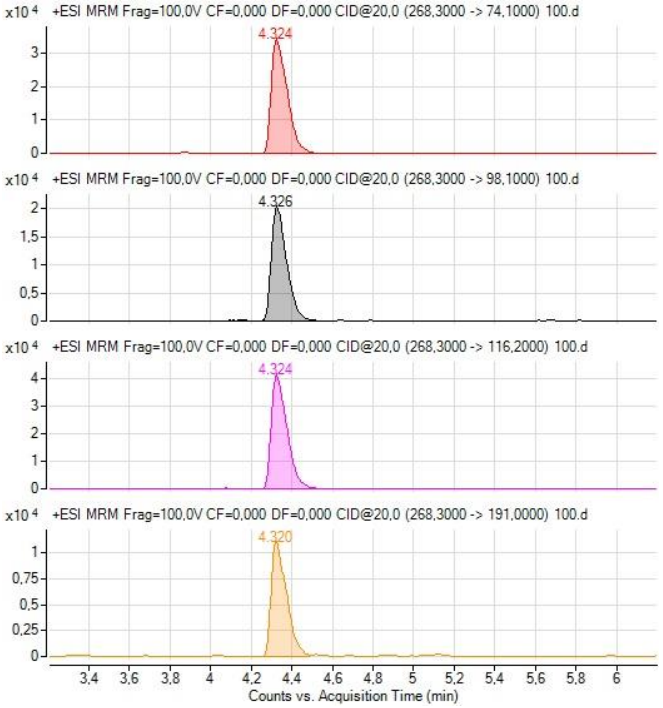


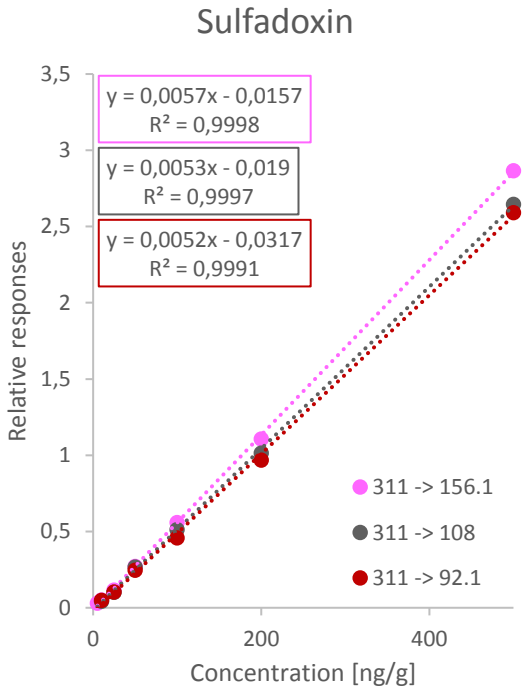


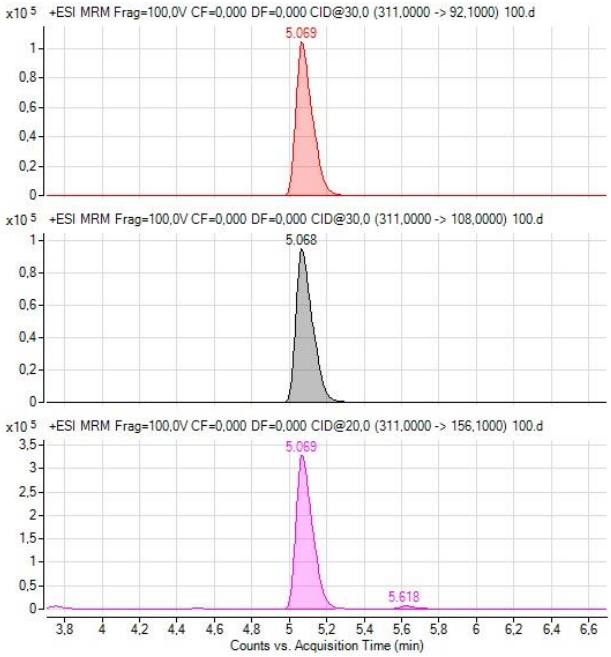

Chlorphenamine
y = 0,0275x - 0,3016
R² = 0,998
y = 0,0052x - 0,0727
R² = 0,9986
275 -> 230
275 -> 267
Relative responses
Concentration [ng/g]
0
2
4
6
8
10
12
14
16
0
200
400

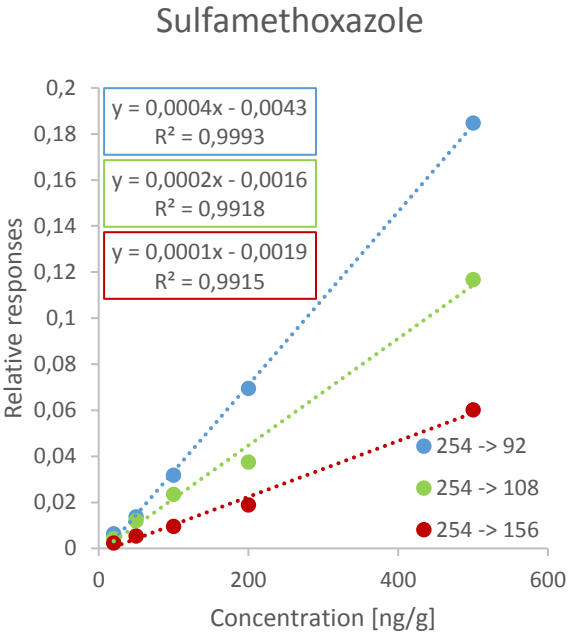

x10 4 +ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@30,0 (275,0000 -> 167,0000) 100.d
4.553
x10 5 +ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@10,0 (275,0000 -> 230,0000) 100.d
4.552
Counts vs. Acquisition Time (min)

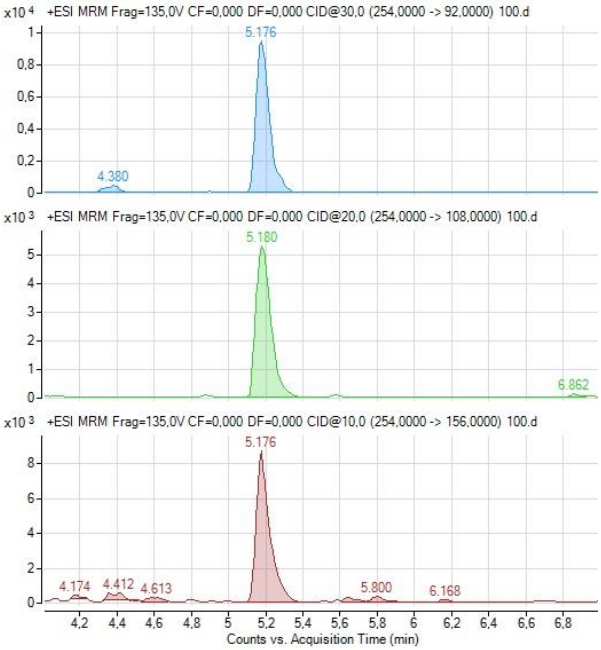

Metoprolol
y = 0,0393x - 0,2207
R² = 0,9977
y = 0,0074x - 0,0456
R² = 0,9988
y = 0,0053x - 0,0334
R² = 0,9962
y = 0,0043x - 0,0485
R² = 0,9977
268.3 -> 116.2
268.3 -> 191
268.3 -> 98.1
268.3 -> 74.1
Relative responses
Concentration [ng/g]
0
5
10
15
20
25
0
200
400

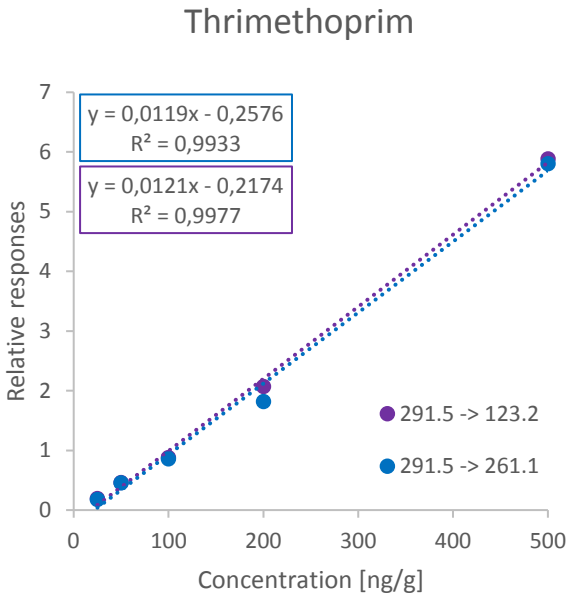

x10 4 +ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@20,0 (268,3000 -> 74,1000) 100.d
4.324
x10 4 +ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@20,0 (268,3000 -> 98,1000) 100.d
4.326
x10 4 +ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@20,0 (268,3000 -> 116,2000) 100.d
4.324
x10 4 +ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@20,0 (268,3000 -> 191,0000) 100.d
4.320
Counts vs. Acquisition Time (min)

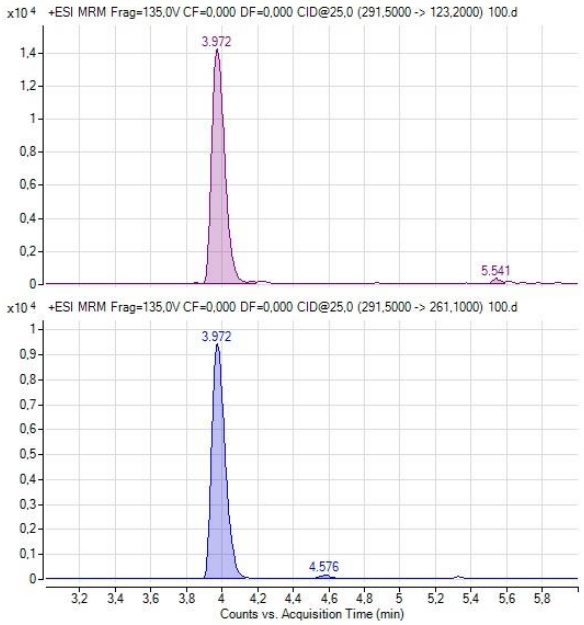

Sulfadoxin
y = 0,0057x - 0,0157
R² = 0,9998
y = 0,0053x - 0,019
R² = 0,9997
y = 0,0052x - 0,0317
R² = 0,9991
311 -> 156.1
311 -> 108
311 -> 92.1
Relative responses
Concentration [ng/g]


+ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@30,0 (311,0000 -> 92,1000) 100.d
5.069
+ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@30,0 (311,0000 -> 108,0000) 100.d
5.068
+ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@20,0 (311,0000 -> 156,1000) 100.d
5.069
5.618
Counts vs. Acquisition Time (min)

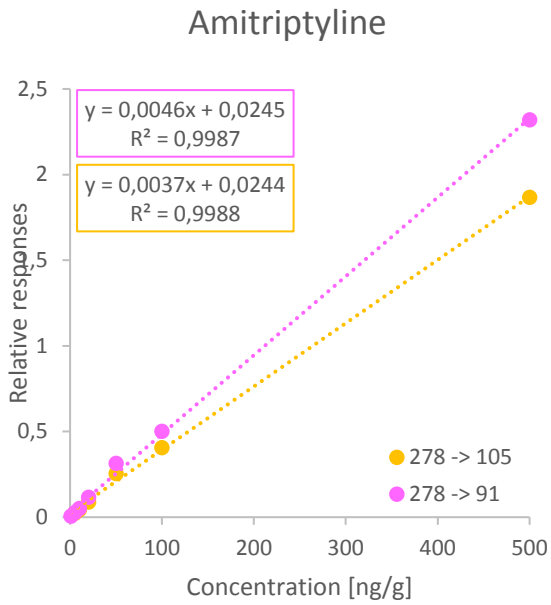

Sulfamethoxazole
y = 0,0004x - 0,0043
R² = 0,9993
y = 0,0002x - 0,0016
R² = 0,9918
y = 0,0001x - 0,0019
R² = 0,9915
254 -> 92
254 -> 108
254 -> 156
Relative responses
Concentration [ng/g]

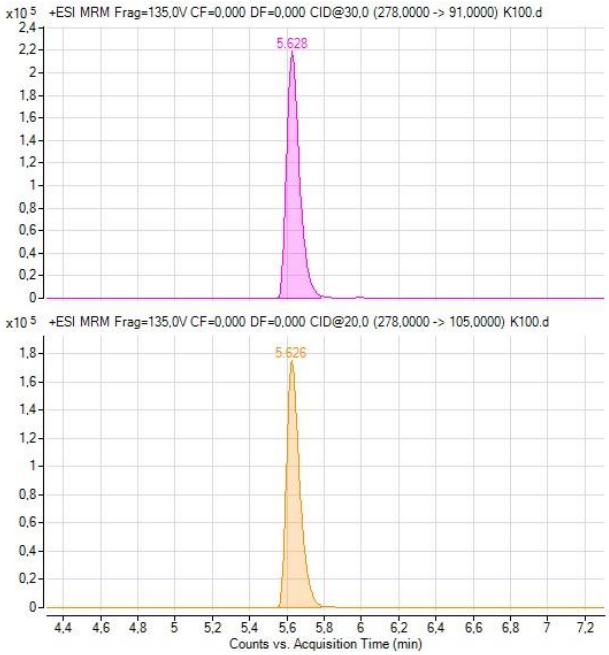

+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@30,0 (254,0000 -> 92,0000) 100.d
5.176
4.380
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@20,0 (254,0000 -> 108,0000) 100.d
5.180
6.862
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@10,0 (254,0000 -> 156,0000) 100.d
5.176
4.174
4.412
4.613
5.800
6.168
Counts vs. Acquisition Time (min)

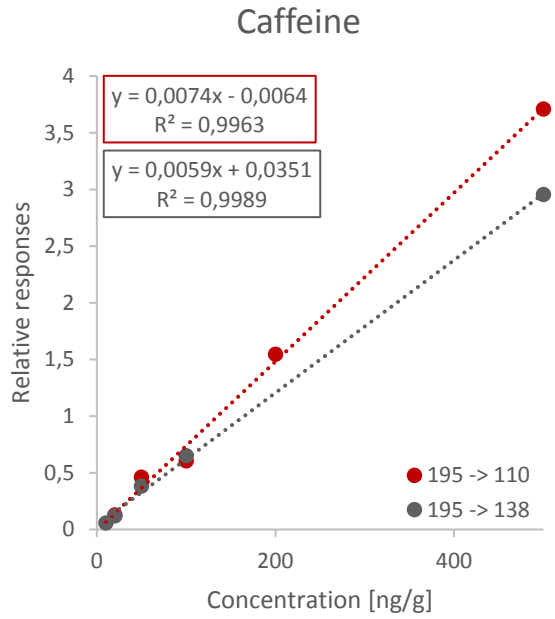

Thrimethoprim
y = 0,0119x - 0,2576
R² = 0,9933
y = 0,0121x - 0,2174
R² = 0,9977
291.5 -> 123.2
291.5 -> 261.1
Relative responses
Concentration [ng/g]
0
1
2
3
4
5
6
7
0
100
200
300
400
500

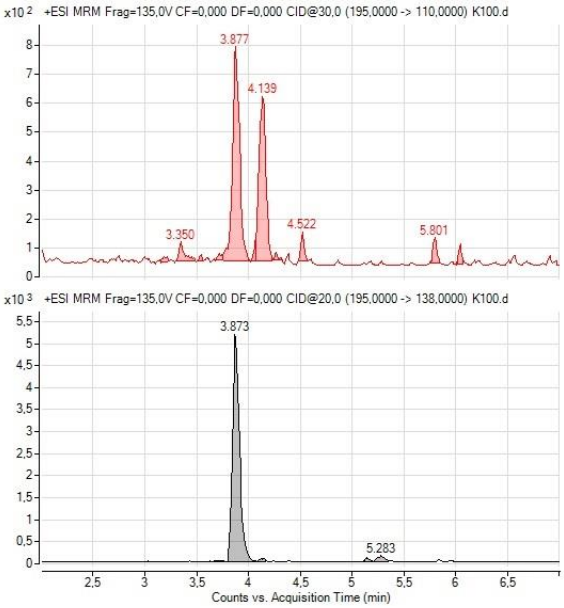

x10 4 +ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@25,0 (291,5000 -> 123,2000) 100.d
3.972
5.541
x10 4 +ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@25,0 (291,5000 -> 261,1000) 100.d
3.972
4.576
Counts vs. Acquisition Time (min)

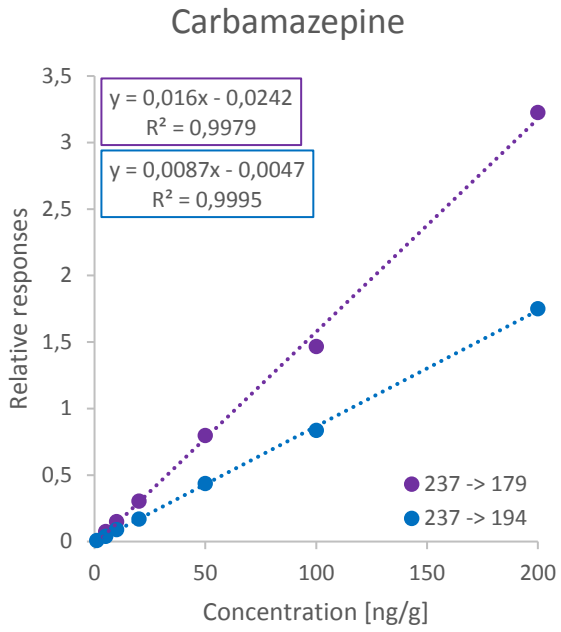
Calibration curves and chromatograms of fish liver

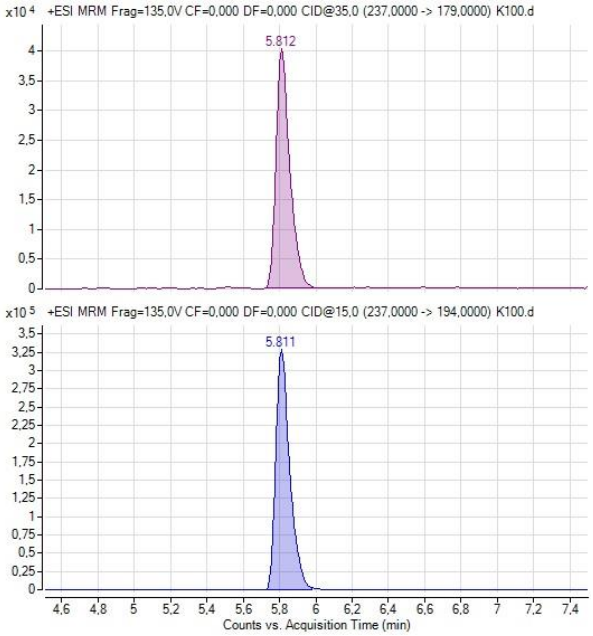


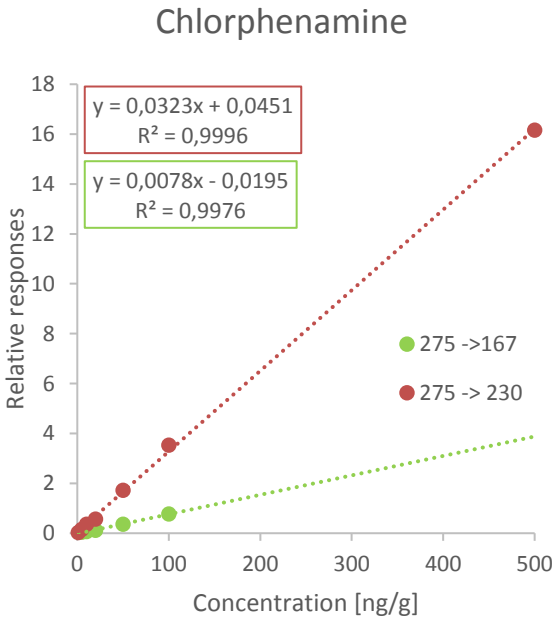


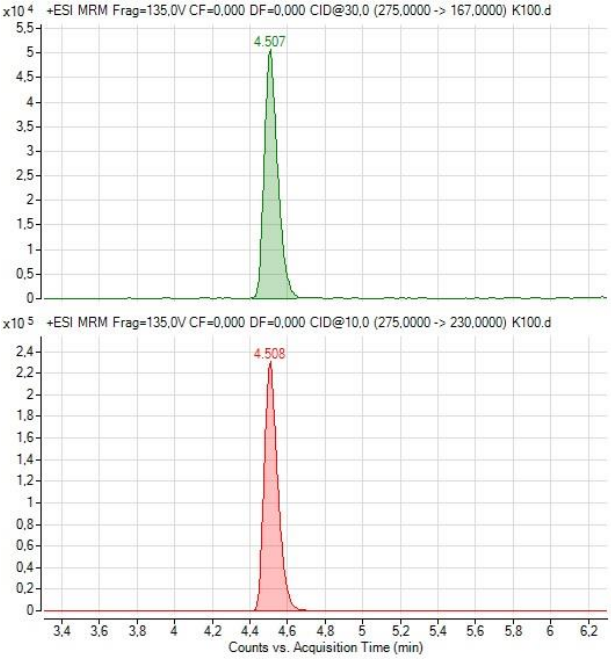


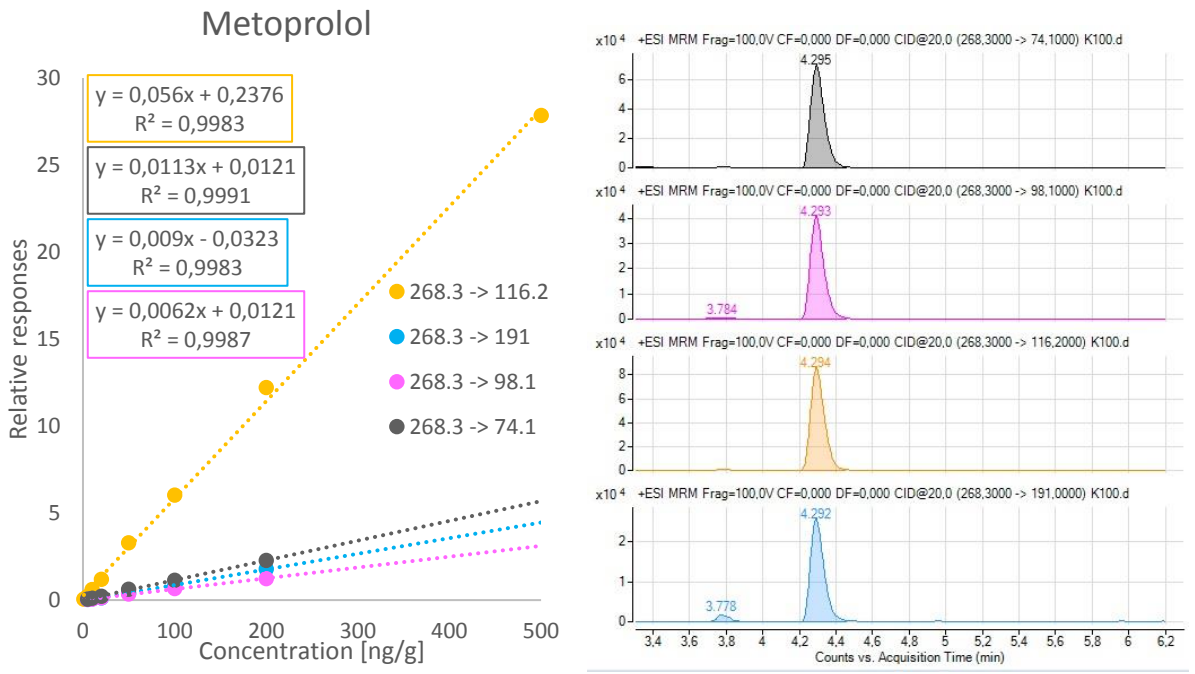


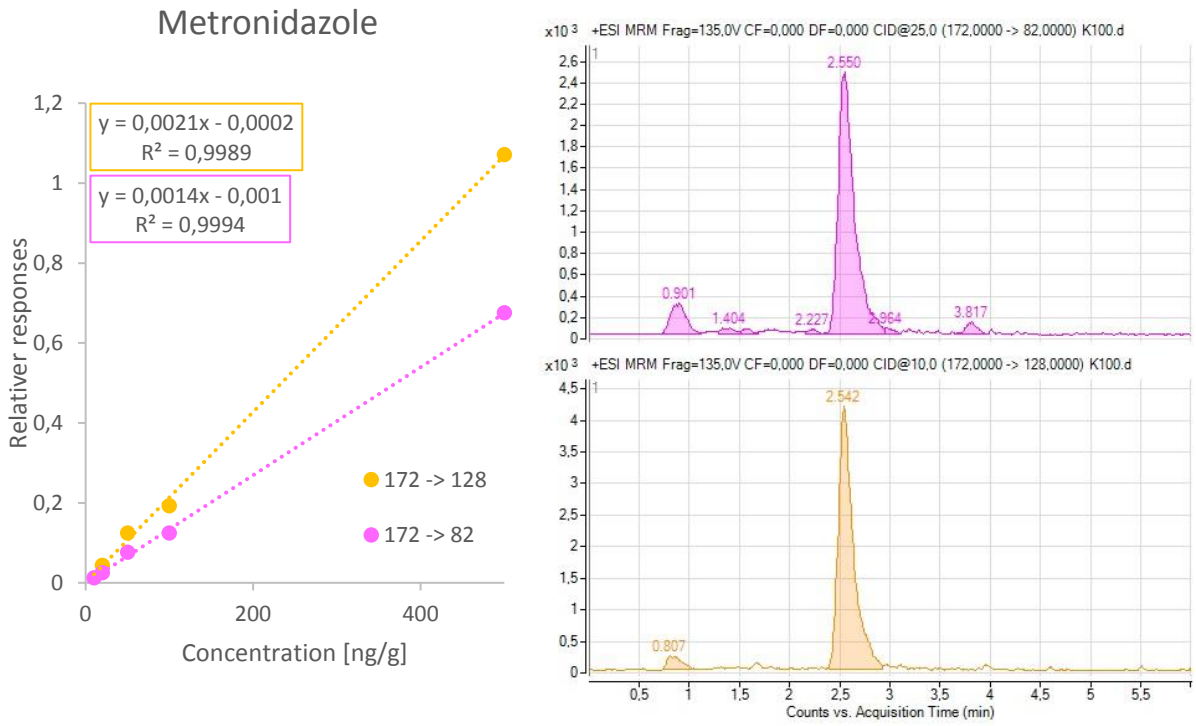

Carbamazepine
y = 0,016x - 0,0242
R² = 0,9979
y = 0,0087x - 0,0047
R² = 0,9995
Relative responses
Concentration [ng/g]
237 -> 179
237 -> 194

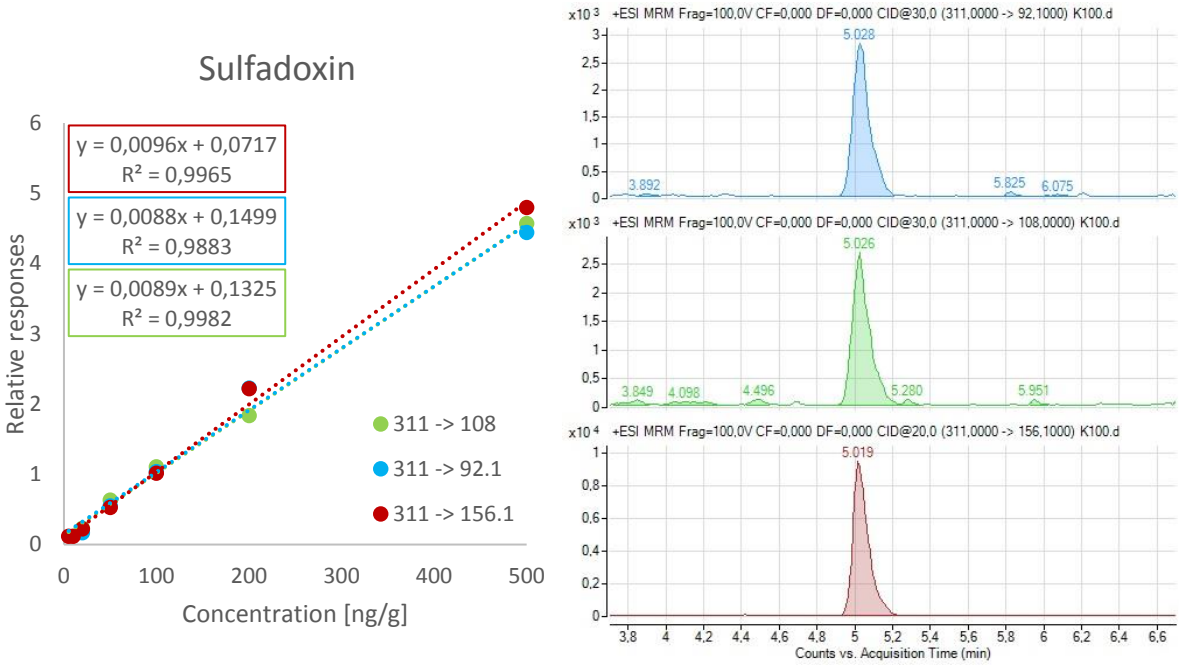

+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@35,0 (237,0000 -> 179,0000) K100.d
5.812
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@15,0 (237,0000 -> 194,0000) K100.d
5.811
Counts vs. Acquisition Time (min)

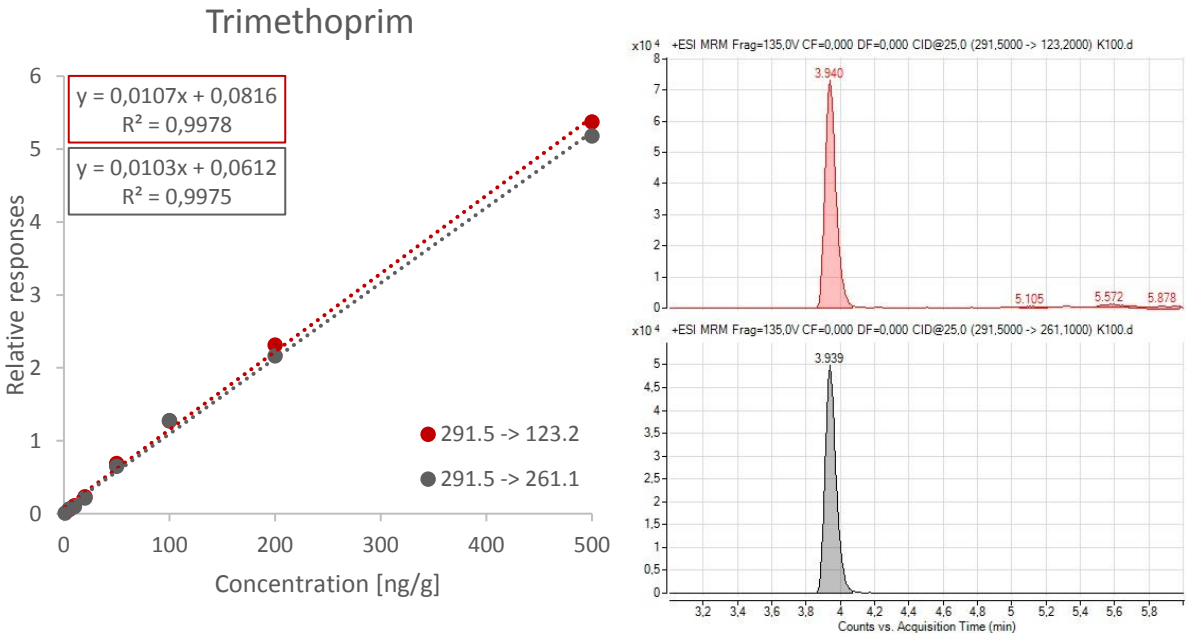

Chlorphenamine
y = 0,0323x + 0,0451
R² = 0,9996
y = 0,0078x - 0,0195
R² = 0,9976
Relative responses
Concentration [ng/g]
275 ->167
275 -> 230


+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@30,0 (275,0000 -> 167,0000) K100.d
4.507
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@10,0 (275,0000 -> 230,0000) K100.d
4.508
Counts vs. Acquisition Time (min)


Metoprolol
y = 0,056x + 0,2376
R² = 0,9983
y = 0,0113x + 0,0121
R² = 0,9991
y = 0,009x - 0,0323
R² = 0,9983
y = 0,0062x + 0,0121
R² = 0,9987
268.3 -> 116.2
268.3 -> 191
268.3 -> 98.1
268.3 -> 74.1
Relative responses
Concentration [ng/g]
+ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@20,0 (268,3000 -> 74,1000) K100.d
4.295
+ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@20,0 (268,3000 -> 98,1000) K100.d
4.293
3.784
+ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@20,0 (268,3000 -> 116,2000) K100.d
4.294
+ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@20,0 (268,3000 -> 191,0000) K100.d
4.292
3.778
Counts vs. Acquisition Time (min)


Metronidazole
y = 0,0021x - 0,0002
R² = 0,9989
y = 0,0014x - 0,001
R² = 0,9994
172 -> 128
172 -> 82
Relativer responses
Concentration [ng/g]
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@25,0 (172,0000 -> 82,0000) K100.d
2.550
0.901
1.404
2.227
2.964
3.817
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@10,0 (172,0000 -> 128,0000) K100.d
2.542
0.807
Counts vs. Acquisition Time (min)


Sulfadoxin
y = 0,0096x + 0,0717
R² = 0,9965
y = 0,0088x + 0,1499
R² = 0,9883
y = 0,0089x + 0,1325
R² = 0,9982
311 -> 108
311 -> 92.1
311 -> 156.1
Relative responses
Concentration [ng/g]
+ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@30,0 (311,0000 -> 92,1000) K100.d
+ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@30,0 (311,0000 -> 108,0000) K100.d
+ESI MRM Frag=100,0V CF=0,000 DF=0,000 CID@20,0 (311,0000 -> 156,1000) K100.d
Counts vs. Acquisition Time (min)


Trimethoprim
y = 0,0107x + 0,0816
R² = 0,9978
y = 0,0103x + 0,0612
R² = 0,9975
291.5 -> 123.2
291.5 -> 261.1
Relative responses
Concentration [ng/g]
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@25,0 (291,5000 -> 123,2000) K100.d
+ESI MRM Frag=135,0V CF=0,000 DF=0,000 CID@25,0 (291,5000 -> 261,1000) K100.d
Counts vs. Acquisition Time (min)

SOP 1: Seawater

Standard Operation Procedure for Pharmaceuticals and Personal Care Products in Seawater

1. ANALYSIS PRINCIPLE

1.1. The analytes were extracted with Solid Phase Extraction and analysed with LC-MS/MS.

2. MATERIALS

2.1. Glassware and disposables

2.1.1. Volumetric flask
2.1.2. SPE manifold
2.1.3. Buchner funnel
2.1.4. Vacuum filter flask
2.1.5. Whatman glass microfiber filter, GF/C grade, 1.2 µm pore size
2.1.6. Oasis MCX 6 cc Vac Cartridge 150 mg Sorbent per Cartridge, 60 µm Particle Size
2.1.7. HPLC-vials

2.2. Instruments

2.2.1. Analytical weight
2.2.2. Automat pipette
2.2.3. Evaporator
2.2.4. LC-MS/MS

2.3. Other equipment

2.3.1. Sep-Pak® Reservoir adaptor
2.3.2. Tubing, 0.149 in. (3.78 mm) O.D x 0.119 in. (3 mm) I.D x 25 ft (7.6 m) Length , PTFE
2.3.3. 10 L Container, Vacuum proof
2.3.4. Vortex mixer
2.3.5. Costar Spin-X

3. CHEMICALS AND REAGENTS

3.1. Pure Chemicals

3.1.1. Formic acid, HPLC-quality
3.1.2. Acetonitrile, HPLC-quality
3.1.3. Methanol, HPLC-quality
3.1.4. Ammonium hydroxide
3.1.5. Na_2EDTA
3.1.6. Concentrated hydrochloric acid

3.2. Solutions

3.2.1. 20 % Acetonitrile in water
3.2.2. 5 % NH_4OH in ACN
3.2.3. 5.0 g/L Na2EDTA
3.2.4. 1% formic acid in water
3.2.5. 0.3 M potassium hydroxide aqueous solution

3.3. Native standards

3.3.1. Acetaminophen
3.3.2. Acetylsalicylic acid
3.3.3. Amitriptyline
3.3.4. Amlodipine
3.3.5. Atenolol
3.3.6. Atorvastatin
3.3.7. Caffeine
3.3.8. Carbamazepine
3.3.9. Cephalexin
3.3.10. Chlorphenamine
3.3.11. Ciprofloxacin
3.3.12. Diclofenac
3.3.13. Diethyltoluamide
3.3.14. Fluoxetine
3.3.15. Ibuprofen
3.3.16. Losartan
3.3.17. Metformin
3.3.18. Metoprolol
3.3.19. Metronidazole
3.3.20. Penicillin G
3.3.21. Prednisolone
3.3.22. Ranitidine
3.3.23. Salicylic acid
3.3.24. Simvastatin
3.3.25. Sulfamethoxazole
3.3.26. Trimethoprim
3.3.27. Warfarin

3.4. Internal standard and recovery standard

3.4.1. Caffeine $^{13}C_3$
3.4.2. Carbamazepine-d_{10}
3.4.3. Metoprolol-d_7
3.4.4. Sulfadoxin-d_3
3.4.5. Trimethoprim-d_9

3.5. Standard Solutions

3.5.1. 1 mg/mL in methanol stock solutions were made for every standard (native, ISTD), except Ciprofloxacin 1 mg/mL was prepared in 0.3 M KOH aqueous solution and 1 mg/mL.
3.5.2. 10 µg/mL internal standards (ISTDs) mixture (20 % ACN) were prepared from 3.5.1.
3.5.3. 10 µg/mL native mixture (20 % ACN) were prepared from 3.5.1.
3.5.4. 100 ng/mL native mixture (20 % ACN) were prepared from 3.5.3.
3.5.5. All of the standard solutions were made by dilution in a volumetric flask no lower than 10 mL with 20 % ACN in water.

4. SAMPLE PREPARATION

4.1.1. Filter 1000 mL sample with micro fibre filter using a Büchner funnel and vacuum filter flask, 1 filter per 1000 mL.
4.1.2. Adjust the pH to 7.0 using concentrated hydrochloric acid
4.1.3. Add 50 µL internal standard (3.5.2)
4.1.4. Add 4 mL of solution 3.2.3
4.1.5. Condition SPE cartridge with 6 mL with ACN followed by 6 mL distilled water
4.1.6. Connect SPE cartridge to the sample bottles via adaptors, consisting of PTFE plugs and PTFE tubing (2.3.1, 2.3.2)
4.1.7. Pass the water sample through SPE cartridge by applying vacuum to the manifold, assuring a flow rate of approximately 1 – 3 mL/min (1- 3 drops per second).
4.1.8. Wash cartridge with 2x 4 mL 2 % formic acid.
4.1.9. Dry SPE cartridge under a gentle vacuum (850 mbar).
4.1.10. Elute with 2 x 4 mL of ACN followed by 2x 4 mL of 5 % NH_4OH in ACN in glass tubes.
4.1.11. Evaporate eluate to dryness under a stream of N_2 at 35 °C.
4.1.12. Add 500 µL of 20 % ACN.
4.1.13. Mix the sample using vortex mixing
4.1.14. Filter through a 0.2 µm micro centrifuge filter (Spin-X).

4.2. Calibration curve and control samples

4.2.1. The Matrix Matched Calibration Curve in made by adding standard in blind matrix.

4.2.2. The concentration level must be considered with respect to witch samples that are analysed

Table 4.2.2 – Added to matrix in different concentration levels (1000 mL seawater)

Cons. ng/mL	**1**	**3**	**5**	**10**	**25**	**100**	**500**	**1000**	**3000**
3.5.3 (µL)						10	50	100	300
3.5.4 (µL)	10	30	50	100	250				
3.5.2 (µL)	50	50	50	50	50	50	50	50	50

5. ANALYSIS CONDITIONS

5.1. LC-conditions

Column:
Pre-column:
Mobile phase A: 0.1 % formic acid in water
Mobile phase B: ACN 100%
Flow: 0.4 ml/min
Injection volume: 10 µL
Autos ampler temp: 8 °C
Wash solution: Water: MeOH (1:1)
Column temp: 25 °C
Analyse time: 15 min

5.2. Pump program LC

Time min	Mobile phase A %	Mobile phase B %
0.00	98.0	2.0
6.00	0.00	100.0
9.00	0.00	100.0
10.10	98.0	2.0
15.00	98.0	2.0

6. ANALYSIS QUALITY AND DATA TREATMENT

6.1. Accept of analyse series

6.1.1. Before starting the analyse series a MS-standard must be injected at least two times to check the sensitivity of the instrument set up.

6.1.2. There should be control blank sample for every 10 injection and after an injection of a high concentrated standard

6.1.3. The sample series is rejected if the regression coefficient of one or more calibration curves is lower than 0.990

6.2. Quantification

Processing of results and quantification is processed with Masshunter Quantitative Analysis. A matrix based calibration curve (4.2.2) is used to quantification of the assay results. The concentration is plotted to the area of the analyte/area of the internal standard. The resulting calibration curve is used to calculate the concentration of analyte in the samples.

7. CONTRIBUTION TO UNSERTAINTY

Action	Contribution to uncertainty
Weight / pipetting	*
Adding of standard and internal standard	***
Homogenisation and extraction	**
Evaporation and reconstitution of samples	***
Transfer to HPLC-vials	*
Equilibration of HPLC-column and ion source	*
Dead volume in HPLC-set up	**

* = Small contribution to uncertainty
** = Medium contribution to uncertainty
*** = Large contribution to uncertainty

SOP 2: Sediments

Standard Operation Procedure for Pharmaceuticals and Personal Care Products in sediments

1. ANALYSIS PRINCIPLE

1.1. The analytes were extracted with an Accelerated Solvent Extraction (ASE) based method and analysed with LC-MS/MS.

2. MATERIALS

2.1. Glassware, disposables and other equipment

2.1.1. Dionex ASE Prep diatomaceous earth (DE)
2.1.2. Dionex ASE Prep Moisture Absorbing Polymer (MAP)
2.1.3. Florisil
2.1.4. Glass pipette
2.1.5. Pasteur pipettes
2.1.6. HPLC-vials
2.1.7. Costar Spin-X

2.2. Instruments

2.2.1. Analytical weight
2.2.2. Automat pipette
2.2.3. Reacti vap III
2.2.4. TurboVap II
2.2.5. Extraction cells (22 mL)
2.2.6. ASE 200, DIONEX
2.2.7. Vortex mixer
2.2.8. LC-MS/MS

3. CHEMICALS AND REAGENTS

3.1. Pure Chemicals

3.1.1. n-Hexane, HPLC-quality
3.1.2. Acetone, HPLC-quality
3.1.3. Methanol, HPLC-quality
3.1.4. Acetonitrile, HPLC-quality
3.1.5. Ammonia solution (NH_3OH)
3.1.6. Formic acid HPLC-quality

3.2. Solutions

3.2.1. 20 % Acetonitrile (ACN) in water
3.2.2. 1:1 n-Hexane Acetone
3.2.3. 5 % NH_3OH in ACN
3.2.4. 2 % FA in water
3.2.5. 0.1 % FA in water

3.3. Native standards

3.3.1. Acetaminophen
3.3.2. Acetylsalicylic acid
3.3.3. Amitriptyline
3.3.4. Amlodipine
3.3.5. Atenolol
3.3.6. Atorvastatin
3.3.7. Caffeine
3.3.8. Carbamazepine
3.3.9. Cephalexin
3.3.10. Chlorphenamine
3.3.11. Ciprofloxacin
3.3.12. Diclofenac
3.3.13. Diethyltoluamide
3.3.14. Fluoxetine
3.3.15. Ibuprofen
3.3.16. Losartan
3.3.17. Metformin
3.3.18. Metoprolol
3.3.19. Metronidazole
3.3.20. Penicillin G
3.3.21. Prednisolone
3.3.22. Ranitidine
3.3.23. Salicylic acid
3.3.24. Simvastatin
3.3.25. Sulfadoxin
3.3.26. Sulfamethoxazole
3.3.27. Trimethoprim
3.3.28. Warfarin

3.4. Internal standard and recovery standard

3.4.1. Caffeine $^{13}C_3$
3.4.2. Carbamazepine-d_{10}
3.4.3. Metoprolol-d_7
3.4.4. Sulfadoxin-d_3
3.4.5. Trimethoprim-d_9

3.5. Standard Solutions

3.5.1. Stock solutions of 1 mg/mL in methanol were made for every standard (native, ISTD), except Ciprofloxacin 1 mg/mL was prepared in 0.3 M KOH aqueous solution and 1 mg/mL.
3.5.2. Internal standards (ISTDs) mixture of 10 µg/mL (20 % ACN) were prepared from 2.7.1.
3.5.3. Native mixture of 10 µg/mL (20 % ACN) were prepared from 2.7.1.
3.5.4. Native mixture of 1 µg/mL (20 % ACN) were prepared from 2.7.3.
3.5.5. Native mixture of 100 ng/mL (20 % ACN) were prepared from 2.7.3.
3.5.6. All of the standard solutions were made by dilution in a volumetric flask no lower than 10 mL with 20 % ACN in water.

4. SAMPLE PREPARATION

4.1.1. Weigh 1 g homogenised sediment liver.
4.1.2. Homogenise the sediment with 2 g 1:1 DE & MAP and 0.5 g Florisil.
4.1.3. Pack the extraction cell with 2 filters followed by 3 g Florisil and a filter.
4.1.4. Add 1 g of 1:1 DE and MAP followed by a filter.
4.1.5. Add the homogenised sediment and 1:1 DE & MAP to the extraction cell and spike with 20 µL ISTD (2.7.2).
4.1.6. Add a filter and close the extraction cell.
4.1.7. Apply the extraction cell in an ASE 200 Dionex and set the temperature to 100 ºC, 1500 psi, 5 min heat, 10 min static 80 % flush, 60 sec purge and 4 cycles.
4.1.8. The solvent used for the extraction in a 1:1 n-Hexane & Acetone.
4.1.9. Transfer the extracted sample to a Turbo Vap vial and evaporate the sample to dryness.
4.1.10. Reconstitute the sample in 1000 µL 20 % AVN in water and dilute it in 10 mL water.
4.1.11. Condition SPE cartridge with 6 mL with ACN followed by 6 mL distilled water.
4.1.12. Pass the diluted sample through a conditioned SPE cartridge.
4.1.13. Wash cartridge with 2x 4 mL 2 % formic acid.
4.1.14. Dry SPE cartridge under a gentle vacuum (850 mbar).
4.1.15. Elute with 5 of ACN followed by 5 mL of 5 % NH_4OH in ACN in glass tubes.
4.1.16. Evaporate eluate to dryness under a stream of N_2 at 35 ˚C.
4.1.17. Add 1000 µL of 20 % ACN.
4.1.18. Mix the sample using a vortex.
4.1.19. Filter through a 0.2 µm micro centrifuge filter (Spin-X).

4.2. Calibration curve and control samples

4.2.1. The Matrix Matched Calibration Curve in made by adding standard in blind matrix.

Table 4.2.2 – Added to matrix in different concentration levels (1 g sediment)

Cons. ng/mL	**1**	**5**	**10**	**20**	**50**	**100**	**200**	**500**
2.7.5 (µL)	10	50	100					
2.7.4 (µL)				20	50	100	200	
2.7.3 (µL)								50
2.7.2 (µL)	20	20	20	20	20	20	20	20

5. ANALYSIS CONDITIONS

5.1. LC-conditions

Column:
Pre-column:
Mobile phase A: 0.1 % formic acid in water
Mobile phase B: ACN 100%
Flow: 0.4 mL/min
Injection volume: 10 µL
Autos ampler temp: 8 °C
Wash solution: Water: MeOH (1:1)
Column temp: 25 °C
Analyse time: 15 min

5.2. Pump program LC

Time min	Mobile phase A %	Mobile phase B %
0.00	98.0	2.0
6.00	0.00	100.0
9.00	0.00	100.0
10.10	98.0	2.0
15.00	98.0	2.0

6. ANALYSIS QUALITY AND DATA TREATMENT

6.1. Accept of analyse series

6.1.1. Before starting the analyse series a MS-standard must be injected at least two times to check the sensitivity of the instrument set up.

6.1.2. There should be at least one control sample for every 10 sample and after an injection of a high concentration standard

6.1.3. The sample series is rejected if the regression coefficient of one or more calibration curves is lower than 0.990

6.2. Quantification

Processing of results and quantification is processed with Masshunter Quantitative Analysis. A matrix based calibration curve (4.2.2) is used to quantification of the assay results. The concentration in plotted to the area of the analyte/area of the internal standard. The resulting calibration curve is used to calculate the concentration of analyte in the samples.

7. CONTRIBUTION TO UNSERTAINTY

Action	Contribution to uncertainty
Weight / pipetting	*
Adding of standard and internal standard	***
Homogenisation and extraction	**
Evaporation and reconstitution of samples	***
Transfer to HPLC-vials	*
Equilibration of HPLC-column and ion source	*
Dead volume in HPLC-set up	**

* = Small contribution to uncertainty
** = Medium contribution to uncertainty
*** = Large contribution to uncertainty

SOP 3: Fish liver

Standard Operation Procedure for Pharmaceuticals and Personal Care Products in Fish Liver

1. ANALYSIS PRINCIPLE

1.1. The analytes were extracted with QuEChERS based method and analysed with LC-MS/MS.

2. MATERIALS

2.1. Glassware, disposables and other equipment

2.1.1. Enhanced Matrix Removal (EMR) - Lipid tube
2.1.2. EMR - Lipid polish tube (2g salts (1:4 NaCl:$MgSO_4$))
2.1.3. Tubes for centrifugation
2.1.4. Pasteur pipettes
2.1.5. HPLC-vials
2.1.6. Costar Spin-X

2.2. Instruments

2.2.1. Analytical weight
2.2.2. Automat pipette
2.2.3. Ultra-Turrax
2.2.4. Vortex mixer
2.2.5. Mechanical shaker
2.2.6. Reacti vap III
2.2.7. LC-MS/MS

3. CHEMICALS AND REAGENTS

3.1. Pure Chemicals

3.1.1. Formic acid, HPLC-quality
3.1.2. Acetonitrile, HPLC-quality
3.1.3. Methanol, HPLC-quality

3.2. Solutions

3.2.1. 20 % Acetonitrile (ACN) in water
3.2.2. 5 % Formic acid in ACN
3.2.3. 0.1% formic acid (FA) in water
3.2.4. 5 mM Ammonium Acetate Buffer (AAB) in water

3.3. Native standards

3.3.1. Acetaminophen
3.3.2. Acetylsalicylic acid
3.3.3. Amitriptyline
3.3.4. Amlodipine
3.3.5. Atenolol
3.3.6. Atorvastatin
3.3.7. Caffeine
3.3.8. Carbamazepine
3.3.9. Cephalexin
3.3.10. Chlorphenamine
3.3.11. Ciprofloxacin
3.3.12. Diclofenac
3.3.13. Diethyltoluamide
3.3.14. Fluoxetine
3.3.15. Ibuprofen
3.3.16. Losartan
3.3.17. Metformin
3.3.18. Metoprolol
3.3.19. Metronidazole
3.3.20. Penicillin G
3.3.21. Prednisolone
3.3.22. Ranitidine
3.3.23. Salicylic acid
3.3.24. Simvastatin
3.3.25. Sulfadoxin
3.3.26. Sulfamethoxazole
3.3.27. Trimethoprim
3.3.28. Warfarin

3.4. Internal standard and recovery standard

3.4.1. Caffeine $^{13}C_3$
3.4.2. Carbamazepine-d_{10}
3.4.3. Metoprolol-d_7
3.4.4. Sulfadoxin-d_3
3.4.5. Trimethoprim-d_9

3.5. Standard Solutions

3.5.1. Stock solutions of 1 mg/mL in methanol were made for every standard (native, ISTD).
3.5.2. Internal standards (ISTDs) mixture of 10 µg/mL (20 % ACN) were prepared from 2.7.1.
3.5.3. Native mixture of 10 µg/mL (20 % ACN) were prepared from 2.7.1.
3.5.4. Native mixture of 1 µg/mL (20 % ACN) were prepared from 2.7.3.
3.5.5. Native mixture of 100 ng/mL (20 % ACN) were prepared from 2.7.3.
3.5.6. All of the standard solutions were made by dilution in a volumetric flask no lower than 10 mL with 20 % ACN in water.

4. SAMPLE PREPARATION

4.1.1. Weigh 1 g homogenised cod liver into glass centrifuge tube.
4.1.2. Spike sample with internal standard (10 µl of 2.7.2)
4.1.3. Add 5 mL acidified ACN (2.4.2)
4.1.4. Mix samples on a mechanical shaker for 2 min
4.1.5. Centrifuge sample for 5 min on 3000 rpm
4.1.6. Add 5 mL AAB to a 15 mL lipid tube (2.1.1)
4.1.7. Transfer 5 mL of supernatant to lipid tube
4.1.8. Vortex lipid tube immediately and shake for 2 min (2.2.5)
4.1.9. Centrifuge lipid tube for 5 min at 4500 rpm
4.1.10. Transfer 5 mL of supernatant to a 15 mL lipid polish tube (2.1.2)
4.1.11. Vortex sample for 2 min
4.1.12. Centrifuge sample for 5 min at 4500 rpm
4.1.13. Transfer supernatant to a glass tube
4.1.14. Evaporate sample to dryness at 35 ℃.
4.1.15. Add 1000 µL of 20 % ACN.
4.1.16. Mix the sample using vortex mixing
4.1.17. Filter through a 0.2 µm micro centrifuge filter (Spin-X).

4.2. Calibration curve and control samples

4.2.1. The Matrix Matched Calibration Curve in made by adding standard in blind matrix.

Table 4.2.2 – Added to matrix in different concentration levels (1 g fish liver)

Cons. ng/mL	**1**	**5**	**10**	**20**	**50**	**100**	**200**	**500**
2.7.5 (µL)	10	50						
2.7.4 (µL)			10	20	50			
2.7.3 (µL)						10	20	50
2.7.1 (µL)	10	10	10	10	10	10	10	10

5. ANALYSIS CONDITIONS

5.1. LC-conditions

Column:
Pre-column:
Mobile phase A: 0.1 % formic acid in water
Mobile phase B: ACN 100%
Flow: 0.4 mL/min
Injection volume: 10 µL
Autos ampler temp: 8 °C
Wash solution: Water: MeOH (1:1)
Column temp: 25 °C
Analyse time: 15 min

5.2. Pump program LC

Time min	Mobile phase A %	Mobile phase B %
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6.00	0.00	100.0
9.00	0.00	100.0
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15.00	98.0	2.0

6. ANALYSIS QUALITY AND DATA TREATMENT

6.1. Accept of analyse series

6.1.1. Before starting the analyse series a MS-standard must be injected at least two times to check the sensitivity of the instrument set up.

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6.1.3. The sample series is rejected if the regression coefficient of one or more calibration curves is lower than 0.990

6.2. Quantification

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7. CONTRIBUTION TO UNSERTAINTY

Action	Contribution to uncertainty
Weight / pipetting	*
Adding of standard and internal standard	***
Homogenisation and extraction	**
Evaporation and reconstitution of samples	***
Transfer to HPLC-vials	*
Equilibration of HPLC-column and ion source	*
Dead volume in HPLC-set up	**

* = Small contribution to uncertainty
** = Medium contribution to uncertainty
*** = Large contribution to uncertainty

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