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MASTER THESIS

Determination of emerging pollutants in sewage sludge with LC-QTOF-MS

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Προσδιορισμός Αναδυόμενων Ρύπων σε Ενεργό Ιλύ με LC-QTOF-MS

ΝΙΚΟΛΟΠΟΥΛΟΥ ΒΑΡΒΑΡΑ

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ABSTRACT

Modern sanitary processes result in large volumes of human waste, as well as domestic and industrial sewage, being collected and treated at common collection points, wastewater treatment plants. A wide range of organic compounds is used in modern societies which end up to the WWTPs and consequently to the liquid effluent and sewage sludge. For this reason, it is critical to evaluate the presence and risk of emerging pollutants in such samples.

This thesis reports the target screening of emerging pollutants in sewage sludge samples collected in 2015 from three WWTPs from Nigeria (Iponri, Alausa, Ijaiye). An ultrasound assisted extraction was used for the sample preparation and the exracts were analyzed with reversed-phase liquid-chromatography coupled to quadrupole-time-of-flight mass spectrometry (RP)LC-QTOF-MS and the data were acquired through broad-band Collision Induced Dissociation (bbCID) mode, which provided information on parent and fragment ions without pre-selection of analytes in one run. Validation was performed based on representative compounds of the wide-scope screening method. A database of approximately 2500 compounds was used for target screening and the detection was based on retention time, mass accuracy, isotopic pattern and fragmentation products.

Following the aforementioned procedures, 182 compounds were identified including pharmaceutical drugs, pesticides, illicit drugs, industrial chemicals and other classes of compounds. The target screening revealed the profile of each WWTP and the differences between the analytes after the stage of digestion and the secondary treatment.

SUBJECT AREA: Environmental Analytical Chemistry

KEYWORDS: Emerging Pollutants, Sewage Sludge, Target Screening, LC-QTOF-MS, Nigeria

ΠΕΡΙΛΗΨΗ

Οι σύγχρονες υγειονομικές διαδικασίες έχουν ως αποτέλεσμα μεγάλους όγκους ανθρώπινων αποβλήτων, καθώς και αστικών και βιομηχανικών αποβλήτων, τα οποία συλλέγονται και επεξεργάζονται σε κοινά σημεία συλλογής, τα Κέντρα Επεξεργασίας Λυμάτων. Ένα μεγάλο εύρος οργανικών ενώσεων χρησιμοποιείται στις σύγχρονες κοινωνίες οι οποίες καταλήγουν στα ΚΕΛ και ως συνέπεια στα υγρά απόβλητα και ενεργό ιλύ. Για αυτό το λόγο, είναι κρίσιμο να αξιολογηθεί η παρουσία και η επικινδυνότητα των αναδυόμενων ρύπων σε τέτοιου είδους δείγματα.

Σε αυτή την διπλωματική εργασία περιγράφεται η στοχευμένη σάρωση αναδυόμενων ρύπων σε δείγματα ενεργού ιλύος, τα οποία συλλέχθηκαν το 2015 από τρία ΚΕΛ από την Νιγηρία (Iponri, Alausa, Ijaiye). Χρησιμοποιήθηκε εκχύλιση με τη βοήθεια υπερήχων για την προκατεργασία δείγματος και τα εκχυλίσματα αναλύθηκαν με υγροχρωματογραφία αντίστροφης φάσης συζευγμένη με φαρματομετρία μάζας τετραπόλου-χρόνου πτήσης (RP)LC-QTOF-MS και η λήψη των δεδομένων πραγματοποιήθηκε με τη λειτουργία bbCID η οποία παρείχε πληροφορίες για τα πρόδρομα ιόντα και τα θραύσματα, χωρίς προεπιλογή των αναλυτών και με μία ανάλυση. Πραγματοποιήθηκε επικύρωση χρησιμοποιώντας αντιπροσωπευτικές ενώσεις της εφαρμοζόμενης ευρείας μεθόδου σάρωσης. Μια βάση δεδομένων περίπου 2500 ενώσεων χρησιμοποιήθηκε για τη στοχευμένη ανάλυση και η ανίχνευση βασίστηκε στο χρόνο ανάκτησης, στην ακρίβεια μάζας, στο ισοτοπικό προφίλ και στα προϊόντα θραυσματοποίησης.

Σύμφωνα με τις προαναφερθείσες διαδικασίες, 182 ενώσεις ταυτοποιήθηκαν συμπεριλαμβανομένων φαρμακευτικών ενώσεων, φυτοπροστατευτικών φαρμάκων, ναρκωτικά, χημικά που χρησιμοποιούνται στη βιομηχανία και άλλες κλάσεις ενώσεων. Η στοχευμένη ανάλυση αποκάλυψε το προφίλ για κάθε ΚΕΛ και τις διαφορές μεταξύ των αναλυτών μετά τα στάδια της χώνευσης και της δευτερογενούς επεξεργασίας.

ΘΕΜΑΤΙΚΗ ΠΕΡΙΟΧΗ: Περιβαλλοντική Αναλυτική Χημεία

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ: Αναδυόμενοι Ρύποι, Ενεργός Ιλύς, Στοχευμένη Σάρωση, LC-QTOF-MS, Νιγηρία

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PREFACE

This master thesis was performed at the laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens under the supervision of Professor Nikolaos S. Thomaidis.

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CHAPTER 1 - Emerging Pollutants

1.1 Introduction

Modern sanitary processes result in large volumes of human waste, as well as domestic and industrial sewage, being collected and treated at common collection points, wastewater treatment plants. WWTPs produce aqueous effluents for discharge back into the environment and sewage sludge. Sewage sludge is usually further treated before use as fertilizers and soil amendments or disposed as waste. Sewage sludge can be defined as the solid residue after the treatment of wastewater [1].

A wide range of organic compounds is used in modern societies which end up to the WWTPs and consequently to the liquid effluent and sewage sludge. For this reason, it is critical to evaluate the presence and risk of emerging pollutants in such samples.

1.2 WWTPs – Sewage sludge production

Wastewater influent from domestic and some industrial sources undergoes preliminary, primary, secondary and in some cases tertiary treatment before sewage sludge is produced and the final effluent is discharged [1].

Initially, the influent is screened in order to remove large objects. Solids are settled out in primary and/or secondary settling tanks. They undergo further treatment: thickening, stabilization, conditioning and de-watering. The solid materials are flocculated from the water (thickening) via gravity or dissolved air flotation. Stabilization is achieved through the use of anaerobic or aerobic digesters. Anaerobic digestion reduces the volatile solid content and pathogens. The digested sewage sludge (~2% solids) is conditioned by the addition of inorganic (ferric chloride, lime) or organic (polymers) chemicals and finally, de-watered to produce the final sewage sludge product [1].

In some cases, de-watered sludge is sent to compost operations, where it is composted under aerobic conditions with greenwaste or other bulking agents, or to heat-drying facilities. The final product can be used as fertilizer or fuel [1].

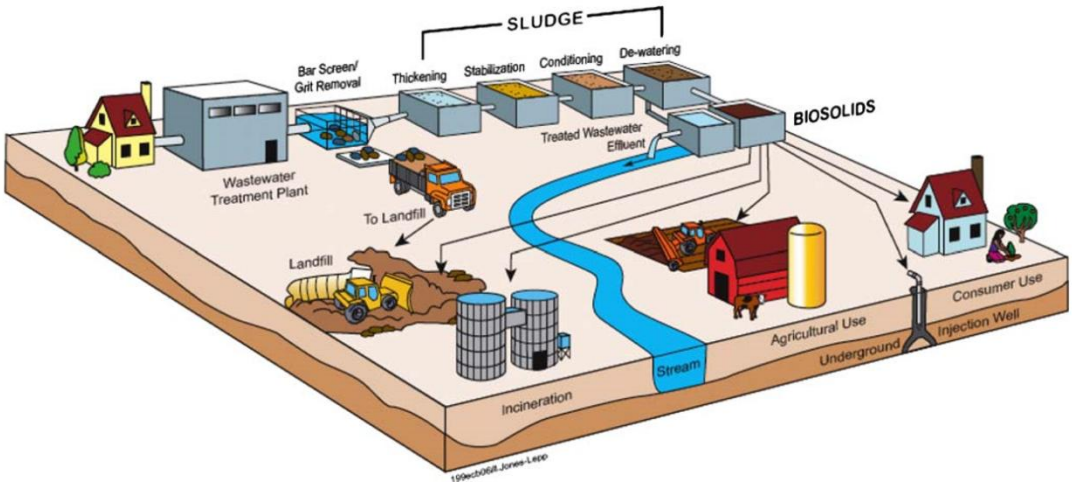


Figure 1: Production and distribution of biosolids [1]

1.3 Classification of EPs

“Contaminants of Emerging Concern” are naturally occurring, manufactured or manmade chemicals or materials which have now been discovered or are suspected present in various environmental compartments and whose toxicity or persistence are likely to significantly alter the metabolism of a living being [2].

"Emerging Pollutants" can be defined as pollutants that are currently not included in routine monitoring programs at the European level and which may be candidates for future regulation, depending on research on their (eco)toxicity, potential health effects and public perception and on monitoring data regarding their occurrence in the various environmental compartments [3].

The term “emerging pollutants” includes a wide range of compounds and a further classification is needed. From a broader perspective, one could extend the focus on [2]:

1. “True or really new” emerging pollutants, new compounds that were not previously known or that just recently appeared in the scientific literature;
2. Pollutants of emerging interest, which were known to exist but for which the environmental pollution issues were not fully realized or apprehended;
3. “Old / traditional” pollutants, which were known to exist and new information is jostling our understanding of environmental and human health risks related to such legacy pollutants.

More “true or really new” EPs include many more types of pollutants such as pharmaceuticals, pesticides, PCPs, plasticizers, hormones, flame retardants, nanoparticles, etc.

1.3.1 Pharmaceuticals & Veterinaries

Pharmaceuticals are considered very important emerging pollutants because due to their wide spread presence in the aquatic environment, there are concerns about possible effects, both on wildlife and humans. A major concern for pharmaceuticals also includes the development of bacterial resistance to antibiotics.

Pharmaceuticals represent a wide variety of compounds and classes of compounds with a variety of structures and function groups and physicochemical properties. It is estimated that approximately 3000 different substances are used worldwide as pharmaceutical ingredients including analgesics, antibiotics, antidiabetics, β-blockers, contraceptives, lipid regulators and antidepressants, although, only a small percent of these compounds has been investigated in environmental studies [4].

1.3.2 Steroids

Natural endogenous, like 17β-estradiol, estrone, estriol, as well as synthetic steroids, like 17α-ethinylestradiol, mestranol, are excreted by humans and WWTP effluent is the primary source of synthetic steroids entering the environment. Steroids are excreted from the human body as inactive polar conjugates, although in sewage influent and effluent are present in the free or active form. This happens as they undergo chemical or enzymatic dissociation from the bacteria in the sludge and they reform into active steroids [5].

Free estrogens, unlike excreted conjugated forms (less toxic and more polar), are more likely to accumulate in sewage sludge due to their moderate to high hydrophobic properties (log Kow=3-5) [6].

1.3.3 Illicit drugs – drugs of abuse

The use of illicit drugs is increasing worldwide with notable references cocaine, heroin, amphetamine-like stimulants and other drugs. Not only parent compounds, but also their metabolites should be determined for forensic sciences and environmental studies [4].

Some classes of drugs of abuse, such as cannabinoids, are highly hydrophobic, with log Kow=5-7.6, so despite their high metabolization before excretion and due to their high consumption, cannabinoids can be found bound to sewage sludge [6].

1.3.4 Flame retardants

Flame retardants are organic or inorganic compounds that are added to industrial polymers used in plastics, textiles, electronic circuitry, building insulation, furniture etc. in order to prevent or retard the spread of fire. These compounds are additives that are not bonded with the material, which leads to their release to the environment during production, lifetime or destruction of the product. Brominated flame retardants are mostly used and they tend to persist in the environment, accumulate in biota and threaten human health. Additionally, organophosphates are used as flame retardants such as organophosphate esters, phosphonates and phosphinates [7].

The compounds in the subcategory of PBDEs have low vapour pressures (4.69×10^{-5} - 6.59×10^{-6} Pa) and are highly lipophilic with log Kow values ranging from 5.9-10 [5].

1.3.5 Perfluorinated chemicals – PFCs

Perfluorinated chemicals are a family of compounds that are used to make products resistant to heat, oil, stains, grease and water. Common applications include nonstick cookware, breathable membranes for clothing, stain-resistant carpets and fabrics, components of fire foam, surfactants and in other industrial applications such as aerospace, automotive, construction, chemical processing and electronics.

The two most common categories of PFCs are perfluoroalkyl sulphonates and perfluoroalkyl carboxulates. They tend to be persistent and dispersed in the environment because of their chemical structure that makes them resistant to degradation (C-F bond) [5].

1.3.6 Benzotriazoles

1H-benzotriazoles are complexing agents that are used as anticorrosives in coolants or anti-freezing liquids, and for silver protection in dish washing liquids. [4] Others, like 2-mercaptobenzothiazole and 2-hydroxybenzothiazole, are used as accelerators for the polymerization of sulfur with rubber (vulcanization) [5].

They are water-soluble, resistant to degradation and are only partially removed in wastewater treatment [4]. The main removal mechanisms are biological and chemical. Benzotriazoles have a degree of aquatic toxicity and they have been used as fungicide, herbicide and anti-algal agents [5].

1.3.7 Surfactants

Common detergents consist of non polar carbon chains and polar active groups such as R–COOH and $R–NH_4^+$. In order to achieve better effectiveness in lower values of pH, the $R–SO_3^-$ group is used and for biodegradable surfactants the linear alkylbenzene sulfonate ($R\text{-}Ph\text{-}SO_3^-$) is used [8].

Almost 25% of surfactants entering a WWTP via influent are removed during primary treatment. Due to restricted metabolic pathways, most common surfactants are not degradable under anaerobic conditions and sludge after the anaerobic digestion process is rich in surfactants [6].

1.3.8 Personal care products

Personal care products include a variety of compounds used in fragrances, soaps, lotions, toothpastes and sunscreens. These compounds attracted increasing interest in recent years due to their large consumption and potentially harmful effects to the environment. It is proofed that they are persistent, bioactive and have the potential for accumulation [7].

Typical examples are synthetic musks that are used as inexpensive substitutes for natural musks in domestic and industrial products such as detergent, shampoo, cosmetics, perfume, food and cigarette additives. Additionally, triclosan and triclocarban are widely used as antimicrobial agents in PCPs such as shampoos, soaps, deodorants, cosmetics, skin-care lotions and creams, mouth rinses and toothpastes. Domestic use is the major source of triclosan and triclocarban to WWTPs [5]. Furthermore, ultraviolet filters have high log Kow (5-8) and low biodegradability that their sorption onto sludge needs to be taken account in the process for their removal from the water column in WWTPs [6].

1.3.9 Pesticides – Insecticides – Herbicides

Pesticides are classified based on their active groups [8]:

1. Organochlorinated compounds (active group –C–Cl)
    i. Chlorophenols, e.g. DDT, Methoxychlor
    ii. Chlorodienes, e.g. Aldrin, Dieldrin
2. Organophosphates (R–[O or S]–P–[OCH_3 or OCH_2CH_3])
3. Carbamates (active group >N–C–O), e.g. Sevin/Carbaryl, Baygon
4. Other classes of compounds (pyrethrins, ureas, nicotinoids, triazines)

1.3.10 Nanoparticles

Nanoparticles are defined as particles with at least one dimension less than 100 nm. Their small size gives them greater relative surface areas than the conventional materials. They can be further divided between carbon-based (e.g. carbon nanotubes, fullerenes), metal-based (e.g. metal oxides or quantum dots) and organic nanoparticles (polymers and dendrimers) [2] [7].

1.3.11 Plasticizers

Plasticizers such as bisphenol A and phthalates are additives that are used to increase flexibility or plasticity and are recognized as endocrine disruptors. Some plastisizers have already been banned or strictly regulated. Apart from plasticizers, their metabolites are also present in the environment and in WWTPs [2].

1.3.12 Industrial chemicals

Industrial chemicals are discharged from industrial or commercial establishments and they are produced during the manufacturing process. Industrial waste may include residues from materials that have not been used. Urban waste is not included in industrial waste [9].

1.3.13 Endocrinal disruptors

Steroid sex hormones, pharmaceuticals and PCPs, illicit drugs, flame retardants and PFCs are considered emerging pollutants of particular concern as many of them display endocrine-disrupting properties. The main points of collection and release of

these compounds into the environment are wastewater treatment plants, where they enter via domestic, hospital sewages and industrial discharges [6].

1.3.14 WWTPs' By-products

Treatment by-products are created when water treatment (drinking or wastewater) is generating new products from the reaction of the reagents with the components of the matrix or when reactions of the target contaminations are incomplete and some by-products are generated that may have residual toxicity.

Treatment can focus either on simple biological or complex chemical processes such as oxidation with chlorination or permanganate. For example, chlorination can generate haloacetic acids or trihalomethanes. The use of ozone may reduce or eliminate such by-products but ozone itself is so reactive that produces transformation products of antidepressant drugs or natural steroids [2].

1.4 Legislation

Environmental quality criteria are intimately linked to emerging pollutants. As a new compound begins to cause concerns, data accumulate on its environmental chemistry, ecotoxicity and human toxicity, as well as its epidemiology. This eventually results in government action to establish guidelines or criteria to ensure adequate protection. In a similar sequence, compounds that are already regulated are often re-evaluated with the addition of new data [2].

The European Union, taking into account the risk of emerging pollutants, creates Directives in order to protect the environment and human health. For example, the Directive 2010/757/EU is dedicated to POPs which is a subcategory of EPs and includes pesticides, pharmaceuticals, industrial chemicals and other compounds. POPs are resistant to chemical, biological and photo-degradation, which leads to bioaccumulation [10].

Table 1: POPs - Directive 2010/757/EU [10]

Substance	CAS No.	EU No.	Exemption (intermediate use) or specification
Tetrabromodiphenyl ether	not applicable	not applicable	X
Pentabromodiphenyl ether	not applicable	not applicable	X
Hexabromodiphenyl ether	not applicable	not applicable	X
Heptabromodiphenyl ether	not applicable	not applicable	X
Perfluorooctane sulfonic acid and its derivatives (PFOS)	not applicable	not applicable	X
DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane)	50-29-3	200-024-3	—
Chlordane	57-74-9	200-349-0	—
Hexachlorocyclohexanes, including lindane	58-89-9	200-401-2	—
	319-84-6	206-270-8	—
	319-85-7	206-271-3	—
	608-73-1	210-168-9	—
Dieldrin	60-57-1	200-484-5	—
Endrin	72-20-8	200-775-7	—
Heptachlor	76-44-8	200-962-3	—
Hexachlorobenzene	118-74-1	200-273-9	—
Chlordecone	143-50-0	205-601-3	—
Aldrin	309-00-2	206-215-8	—
Pentachlorobenzene	608-93-5	210-172-5	—
Polychlorinated Biphenyls (PCB)	1336-36-3 and others	215-648-1 and others	X
Mirex	2385-85-5	219-196-6	—
Toxaphene	8001-35-2	232-283-3	—
Hexabromobiphenyl	36355-01-8	252-994-2	—

* X: use is allowed under conditions

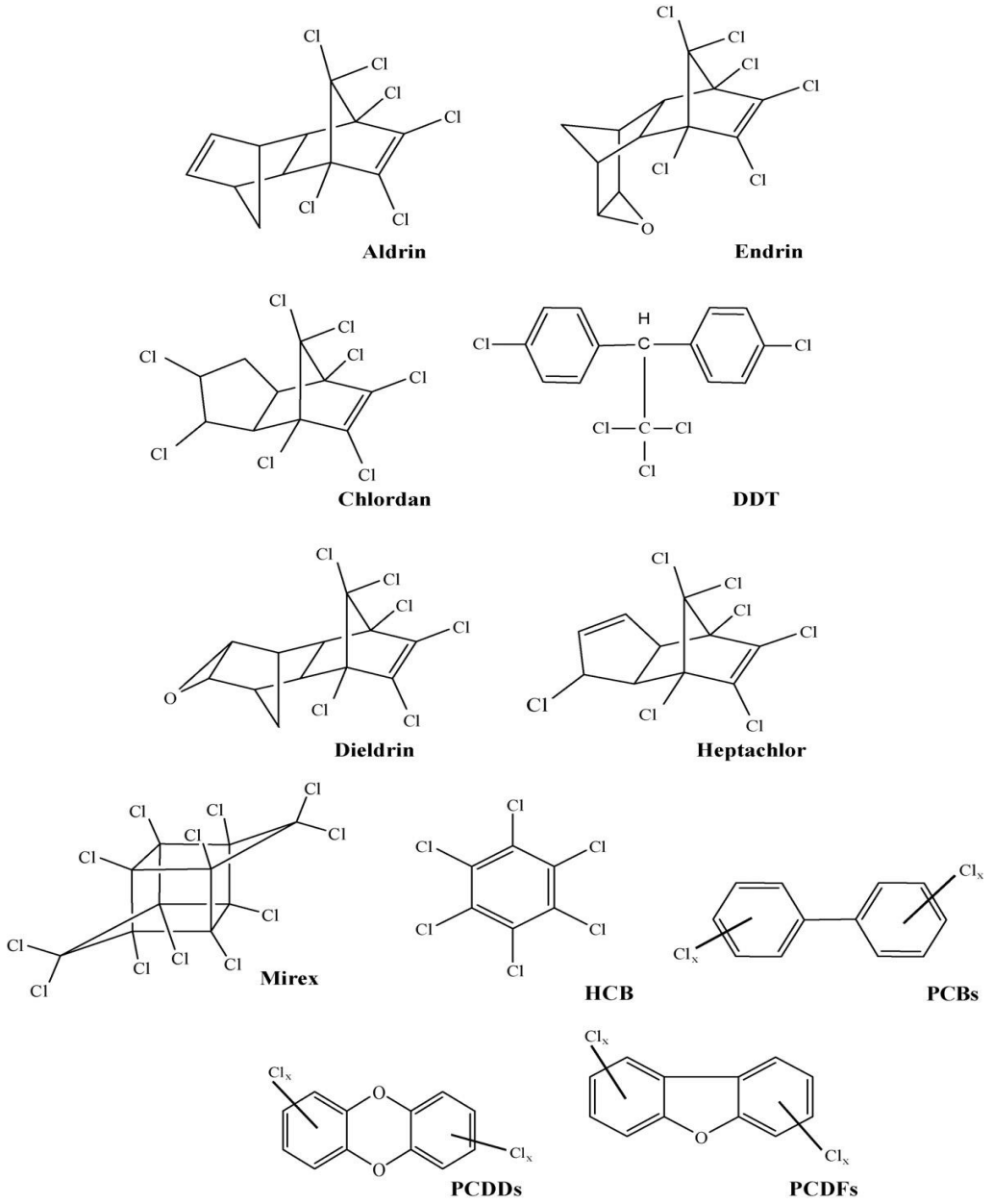


Figure 2: Characteristic POPs

Furthermore, the Directive 2013/39/EU includes a list of priority substances, indicates which of them are priority hazardous substances and establishes EQSs for various types of surface waters and biota Annex I [11].

Table 2: Priority substances - Directive 2013/39/EU [11]

No.	CAS number	EU number	Name of priority substance	Priority hazardous substance
1.	15972-60-8	240-110-8	Alachlor	
2.	120-12-7	204-371-1	Anthracene	X
3.	1912-24-9	217-617-8	Atrazine	
4.	71-43-2	200-753-7	Benzene	
5.	not applicable	not applicable	Brominated diphenylethers	X
6.	7440-43-9	231-152-8	Cadmium and its compounds	X
7.	85535-84-8	287-476-5	Chloroalkanes, C10-13	X
8.	470-90-6	207-432-0	Chlorfenvinphos	
9.	2921-88-2	220-864-4	Chlorpyrifos (Chlorpyrifos-ethyl)	
10.	107-06-2	203-458-1	1,2-dichloroethane	
11.	75-09-2	200-838-9	Dichloromethane	
12.	117-81-7	204-211-0	Di(2-ethylhexyl)phthalate (DEHP)	X
13.	330-54-1	206-354-4	Diuron	
14.	115-29-7	204-079-4	Endosulfan	X
15.	206-44-0	205-912-4	Fluoranthene	
16.	118-74-1	204-273-9	Hexachlorobenzene	X
17.	87-68-3	201-765-5	Hexachlorobutadiene	X
18.	608-73-1	210-168-9	Hexachlorocyclohexane	X
19.	34123-59-6	251-835-4	Isoproturon	
20.	7439-92-1	231-100-4	Lead and its compounds	
21.	7439-97-6	231-106-7	Mercury and its compounds	X
22.	91-20-3	202-049-5	Naphthalene	
23.	7440-02-0	231-111-4	Nickel and its compounds	
24.	not applicable	not applicable	Nonylphenols	X
25.	not applicable	not applicable	Octylphenols	
26.	608-93-5	210-172-0	Pentachlorobenzene	X
27.	87-86-5	201-778-6	Pentachlorophenol	
28.	not applicable	not applicable	Polyaromatic hydrocarbons (PAH)	X
29.	122-34-9	204-535-2	Simazine	
30.	not applicable	not applicable	Tributyltin compounds	X
31.	12002-48-1	234-413-4	Trichlorobenzenes	
32.	67-66-3	200-663-8	Trichloromethane (chloroform)	
33.	1582-09-8	216-428-8	Trifluralin	X
34.	115-32-2	204-082-0	Dicofol	X
35.	1763-23-1	217-179-8	Perfluorooctane sulfonic acid and its derivatives (PFOS)	X
36.	124495-18-7	not applicable	Quinoxyfen	X
37.	not applicable	not applicable	Dioxins and dioxin-like compounds	X
38.	74070-46-5	277-704-1	Aclonifen	
39.	42576-02-3	255-894-7	Bifenox	
40.	28159-98-0	248-872-3	Cybutryne	
41.	52315-07-8	257-842-9	Cypermethrin	

42.	62-73-7	200-547-7	Dichlorvos	
43.	not applicable	not applicable	Hexabromocyclododecanes (HBCDD)	X
44.	76-44-8 / 1024-57-3	200-962-3 / 213-831-0	Heptachlor and heptachlor epoxide	X
45.	886-50-0	212-950-5	Terbutryn	

Finally, in 2014 the EPA has developed a list of 126 Priority Pollutants, shown below, can also be found at 40 CFR Part 423 [12]. These are not the only pollutants regulated in Clean Water Act programs. The list is an important starting point for EPA to consider, for example, in developing national discharge standards (such as Effluent Guidelines) or in national permitting programs (such as NPDES).

Table 3: The list of 126 Priority Pollutants developed by the EPA [12]

No.	Substance	No.	Substance	No.	Substance
1.	Acenaphthene	44.	Methylene chloride	87.	Trichloroethylene
2.	Acrolein	45.	Methyl chloride	88.	Vinyl chloride
3.	Acrylonitrile	46.	Methyl bromide	89.	Aldrin
4.	Benzene	47.	Bromoform	90.	Dieldrin
5.	Benzidine	48.	Dichlorobromomethane	91.	Chlordane
6.	Carbon tetrachloride	49.	(Removed)	92.	4,4-DDT
7.	Chlorobenzene	50.	(Removed)	93.	4,4-DDE
8.	1,2,4-trichlorobenzene	51.	Chlorodibromomethane	94.	4,4-DDD
9.	Hexachlorobenzene	52.	Hexachlorobutadiene	95.	Alpha-endosulfan
10.	1,2-dichloroethane	53.	Hexachlorocyclopentadiene	96.	Beta-endosulfan
11.	1,1,1-trichloreothane	54.	Isophorone	97.	Endosulfan sulfate
12.	Hexachloroethane	55.	Naphthalene	98.	Endrin
13.	1,1-dichloroethane	56.	Nitrobenzene	99.	Endrin aldehyde
14.	1,1,2-trichloroethane	57.	2-nitrophenol	100.	Heptachlor
15.	1,1,2,2-tetrachloroethane	58.	4-nitrophenol	101.	Heptachlor epoxide
16.	Chloroethane	59.	2,4-dinitrophenol	102.	Alpha-BHC
17.	(Removed)	60.	4,6-dinitro-o-cresol	103.	Beta-BHC
18.	Bis(2-chloroethyl) ether	61.	N-nitrosodimethylamine	104.	Gamma-BHC
19.	2-chloroethyl vinyl ethers	62.	N-nitrosodiphenylamine	105.	Delta-BHC
20.	2-chloronaphthalene	63.	N-nitrosodi-n-propylamine	106.	PCB-1242 (Arochlor 1242)
21.	2,4,6-trichlorophenol	64.	Pentachlorophenol	107.	PCB-1254 (Arochlor 1254)
22.	Parachlorometa cresol	65.	Phenol	108.	PCB-1221 (Arochlor 1221)
23.	Chloroform	66.	Bis(2-ethylhexyl) phthalate	109.	PCB-1232 (Arochlor 1232)
24.	2-chlorophenol	67.	Butyl benzyl phthalate	110.	PCB-1248 (Arochlor 1248)
25.	1,2-dichlorobenzene	68.	Di-n-butyl phthalate	111.	PCB-1260 (Arochlor 1260)
26.	1,3-dichlorobenzene	69.	Di-n-octyl phthalate	112.	PCB-1016 (Arochlor 1016)
27.	1,4-dichlorobenzene	70.	Diethyl phthalate	113.	Toxaphene
28.	3,3-dichlorobenzidine	71.	Dimethyl phthalate	114.	Antimony
29.	1,1-dichloroethylene	72.	Benzo (a) anthracene	115.	Arsenic
30.	1,2-trans-dichloroethylene	73.	Benzo (a) pyrene	116.	Asbestos
31.	2,4-dichlorophenol	74.	Benzo (b) fluoranthene	117.	Beryllium
32.	1,2-dichloropropane	75.	Benzo (k) fluoranthene	118.	Cadmium
33.	1,3-dichloropropylene	76.	Chrysene	119.	Chromium
34.	2,4-dimethylphenol	77.	Acenaphthylene	120.	Copper
35.	2,4-dinitrotoluene	78.	Anthracene	121.	Cyanide, Total
36.	2,6-dinitrotoluene	79.	Benzo (g,h,i) perylene	122.	Lead
37.	1,2-diphenylhydrazine	80.	Fluorene	123.	Mercury
38.	Ethylbenzene	81.	Phenanthrene	124.	Nickel
39.	Fluoranthene	82.	Dibenzo(,h) anthracene	125.	Selenium
40.	4-chlorophenyl phenyl ether	83.	Indeno (1,2,3-cd) pyrene	126.	Silver
41.	4-bromophenyl phenyl ether	84.	Pyrene	127.	Thallium
42.	Bis(2-chloroisopropyl) ether	85.	Tetrachloroethylene	128.	Zinc
43.	Bis(2-chloroethoxy) methane	86.	Toluene	129.	2,3,7,8-TCDD

1.5 Occurrence of Emerging Pollutants in Sewage Sludge in Nigeria

Most work on Nigeria has focused on heavy metal pollution and the presence of emerging pollutants in Nigeria was only recently studied. In 2013, Sindiku et al. focused their study on per- and poly-fluoroalkyl substances. It was the first time that PFCAs and PFSAs were determined in sewage sludge from WWTPs in Nigeria and the detected compounds were: PFBS, PFHxS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA and PFOS being the most dominant. The results are shown in Table 4.

PFOS concentration was ranging from 101 pg/g to 540 pg/g and it was detected in every sludge sample analyzed. The highest value of PFOS corresponds to a hospital WWTP, because of its presence in specific medical equipment. Although compared to the concentrations of PFOS worldwide, such levels are quite low which indicates its limited use. No industry releases revealed high levels of PFSAs, which was achieved either by appropriate chemical policy or by consumption patterns, which do not favor expensive consumer articles that in their production require the use of those compounds [13].

In 2016, Olarinmoye et al. focused their study on pharmaceutical substances. Nine pharmaceuticals were detected: diclofenac, carbamazepine, erythromycin, bezafibrate, clarithromycin, ibuprofen, metoprolol, propranolol, sulfamethoxazole and trimethoprim, and the results are shown in Table 5. The concentration (d.w.) of diclofenac was up to 1140 μg/Kg and it was positive in every sample (10 different WWTPs).

The maximum concentrations measured, in this project in Nigeria, of 19 pharmaceutical substances were compared with their maximum concentrations worldwide. Although 18 of 19 analytes had very low concentrations in comparison with the worldwide maximum, diclofenac was the only analyte that had the highest concentration ever published. The high values of diclofenac are attributed to the presence of many pharmaceutical production facilities [14].

Table 4: Concentrations (pg/g) of perfluoroalkyl substances in sludge samples from selected wastewater treatment plants in Nigeria. [13]

WWTP	**PFBS**	**PFHxS**	**PFOS**	**PFHxA**	**PFHpA**	**PFOA**	**PFNA**	**PFDA**	**PFUnDA**	**PFDoDA**
Brewery	48.9	20	382.4	174.9	<LOQ	416.3	129.3	596.5	160.9	<LOQ
WEMABOD	41.3	<LOQ	327.5	<LOQ	<LOQ	44.4	52.7	281.2	48.4	283.0
Dairy	14.1	42	>MLQ	<LOQ	<LOQ	146.7	38.0	42.9	<LOQ	<LOQ
AGBARA	19.5	<LOQ	294.5	245.8	<LOQ	56.9	44.0	279.0	53.6	195.2
Food	<LOQ	<LOQ	183.4	<LOQ	<LOQ	26.6	<LOQ	<LOQ	<LOQ	<LOQ
Ikeja	<LOQ	<LOQ	240.3	<LOQ	<LOQ	41.5	10.2	63.2	63.9	<LOQ
Abesan	<LOQ	<LOQ	276.1	<LOQ	<LOQ	18.9	<LOQ	38.6	39.6	<LOQ
Iponri	137.8	<LOQ	100.7	<LOQ	<LOQ	30.9	<LOQ	22.5	<LOQ	<LOQ
Oke Afa	<LOQ	<LOQ	109.9	<LOQ	<LOQ	34.6	10.8	28.9	15.7	<LOQ
UCH	<LOQ	<LOQ	539.6	<LOQ	14.2	81.2	<LOQ	94.9	103.2	<LOQ

Table 5: The analyses of ten sewage sludge samples from Nigeria, Concentrations (µg/kg) [14]

Pharmaceutical substance	Brewery	WEMABOD	Dairy	AGBARA	Food	Ikeja	Abesan	Iponri	Oke Afa	UCH
	Industrial	Industrial	Industrial	Industrial	Industrial	Domestic	Domestic	Domestic	Domestic	Hospital
Bezafibrate	<10	<10	<10	<10	<10	**11**	<10	<10	<10	<10
Carbamazepine	<10	<10	**49**	**23**	**24**	**39**	<30	<10	<10	**71**
Diclofenac	**30**	**38**	**395**	**1100**	**169**	**635**	**119**	<10	**71**	**111**
Erythromycin	<10	<10	<10	<10	<10	**39**	**147**	<10	**38**	**49**
Ibuprofen	**360**	<100	<100	**210**	<100	<100	<100	<100	<100	<60
Metoprolol	<10	**<10**	<10	<10	<10	<10	<10	<10	<10	<30
Propranolol	<10	<10	**33**	<10	<10	**25**	<10	<10	<10	<10
Sulfamethoxazole	<10	<10	<10	**11**	<10	<10	<10	<10	<10	<10
Trimethoprim	<10	<10	<10	**31**	<10	**19**	<10	<10	<10	**38**

CHAPTER 2 – Basic Principles

2.1 Introduction

Sewage sludge is a challenging matrix because of its non-uniform composition. The concentrations of pollutants present vary depending on the nature of inputs to the WWTP. Additionally, sewage sludge contains a number of other components that are potential interferences in analyzing the pollutants of interest, including naturally occurring materials and materials that may be added during the process (e.g. surfactants, ferric chloride, polymeric colloids or lime). These components can manifest themselves as interferences at all stages of the analytical process from sample preparation, so it is crucial to remove them from the extracts using established clean-up procedures [6].

2.2 Categories – sample preparation and separation-detection

2.2.1 Sample preparation

In recent, many advanced analytical methods have been developed for the simultaneous determination of emerging pollutants of various classes. Extraction methods have usually been based on USE, MAE, or the more advanced PLE that provides great recoveries, while saving time and organic solvents. The most effective clean-up of extracts of sewage sludge samples has proven to be SPE with various sorbents such as C8 or C18 silica gel, alumina or Florisil® [6]. QuEChERS is a method that is gaining popularity and it is based on extraction with acetonitrile [15] [16] [17]. Other methods that are more rarely used are SFE, Soxhlet, DLLME, SBSE and MSPD [18] [19] [15].

2.2.2 Separation and detection

Most current analytical methods for separation and detection of emerging pollutants in sludge use GC-MS or GC-MS^2 (interfaced to CI or EI) and (RP)LC-MS or (RP)LC-MS^2 (interfaced to APCI or ESI). For the more polar and non-volatile compounds, a derivatization step is required because they are not compatible with GC [6] [19].

2.3 Analytical techniques

2.3.1 (RP)-UHPLC

Ultra-HPLC uses the same separation methodology as HPLC, using short columns with small diameter particles for the stationary phase, usually less than 2 μm, providing higher separation speed and improved sensitivity as well as resolution. The vast majority of UHPLC separations have been carried out with C18 columns in the classic reversed phase mode [20]. Gradient elution programs are preferred for better and faster separations. For the improvement of the ionization, small percentages of formic or acetic acid are added in the mobile phase [21].

2.3.2 LC-MS coupling

LC-MS is a sophisticated hyphenation of analytical techniques that is mostly used for the determination of non volatile compounds (no need for a derivatization step), polar and heat sensitive compounds that cannot be determined with GC-MS.

The problems that occur are the incompatibility because of the large volume of solvents of LC and the vacuum that is required for the MS, the incompatibility with non volatile buffers of the mobile phase and the problematic ionization of non volatile and heat sensitive compounds.

All the above can be resolved with the use of short columns with small diameter (using small diameter particles for the stationary phase) and allowing only a small fraction of the eluent to reach the ion source, using volatile buffers (HCOOH / CH_3COOH / triethylamine) and using new ion sources such as ESI, APCI, MALDI [22].

2.3.3 HRMS

The environmental issue of emerging pollutants is tied to the analysis of wastewater samples using LC-MS/MS and TOF-MS techniques. Because of the high complexity of sewage sludge samples, high-resolving power techniques are needed to provide additional structural information [23].

LC coupled to TOF-MS has the resolving power that enables mass-measurement accuracy for small molecules, charge-state identification of multiply-charged ions and

greater differentiation of isobaric species (two different compounds with the same integer mass but different elemental compositions and therefore, different exact masses). The TOF-MS instrument represents a powerful tool for identifying non-target compounds in complex environmental matrices because of three important characteristics [23]:

1. The ability to collect data across a wide range without a decrease in sensitivity;
2. The possibility of resolving interferences away from signals of interest with high resolving power;
3. The achievement of mass-measurement accuracy adequate for the estimation of elemental composition.

The Q-TOF-MS combines the simplicity of a quadruple MS with ultra-high efficiency of a TOF analyzer. The TOF side achieves simultaneous detection of ions across full-scan mass range at all times (sensitivity) [23]. The Q-TOF-MS configuration is considered a high-resolving power instrument capable of 20,000-80,000 FWHM and mass accuracy less than 2 ppm [24].

Product-ion spectra can be obtained with either data dependent acquisition or data independent acquisition modes, where the instrument automatically switches after a full-scan mode acquisition to a product-ion scan mode as the second scan event in the scan cycles [25].

2.3.3.1 DIA

With this acquisition mode, there is no pre-selection of the precursor ion. Full-scan spectra at two different collision energies are obtained in one injection. This acquisition provides simultaneously accurate mass data of parent compounds and fragment ions in a single run using two alternate scans, one at low and one at high collision energy. By applying low energy (LE) in the collision cell, no fragmentation is performed. A full-scan spectrum is obtained that provides information for the parent ion (the protonated [positive mode] or de-protonated [negative mode] molecule) and, in some cases, adduct ions and in-source fragments. Applying high energy (HE) in the collision cell, fragmentation is performed and a spectrum similar

to MS/MS experiments is obtained. This approach is called all-ions MS/MS, MS^E or bbCID, according to the QTOF manufacturer [24].

2.3.3.2 DDA

In this acquisition mode, there is at first a full scan which is defined as the survey scan and data are processed "on-the-fly" to determine the candidates of interest based on predefined selection criteria, such as intensity threshold or suspect inclusion list. If the selection criteria are met, MS/MS analysis is then triggered and MS/MS scans (data-dependent) take place [26] [24]. With this acquisition mode, clean spectra with structural information are obtained in one injection. However, on QTOF instruments, if the number of candidates of interest is big, the number of scans is decreased, so there are less data points that affect the detectability of the chromatographic peak [24].

2.4 Data treatment – Approach in HRMS screening

The main approaches for post-acquisition data evaluation are target, suspect and non-target screening as proposed by Krauss et al. [26] and shown in Figure 5.

2.4.1 Target screening

Target screening is based on the presence of reference standards. An in-house developed database is used for the screening of a large number of compounds. The information included in the database is based on the analysis of the available reference standards. The reference standard is necessary for comparison of the retention time, the MS spectrum profile (precursor ion, adducts, in-source fragments), as well as the MS/MS spectrum (fragment ions and ion ratios) [27]. Quantification can be performed in full-scan mode, but requires greater effort than in LRMS methods where Single Reaction Monitoring mode is used [26].

2.4.2 Suspect screening

In contrast to target screening, suspect screening approach does not rely on reference standards for confirmation. For a large number of potential environmental contaminants there are not available reference standards, especially for transformation products. Compound-specific information for suspects (a list of suspect compounds that are possible to be found in specific samples) is available, such as molecular

formula and structure. The screening is based only on the exact m/z of the expected ions, which, in case of the ESI source, are usually the pseudomolecular ions $[M+H]^+$ and $[M-H]^-$, except for some compounds which exclusively show adduct formation. Molecular formula and structure are known, so this information can be efficiently used in the identification and confirmation process [26]. Absence from blank samples, mass accuracy, isotopic pattern, retention time prediction, ionization efficiency and information on fragment ions reported in the literature are parameters that can facilitate tentative identification of suspect candidates [28].

2.4.3 Non-target screening

Non-target screening starts without any a priori information on the compounds to be detected. These unknown compounds are actually new, unexpected or not searched ones in specific samples. Identification is challenging as more than one formula and structure can be assigned to the exact mass of interest. Except for the elucidation of unknowns, non-target screening is used for the identification of metabolites and transformation products, arising from in vivo and in vitro experiments, in-silico modeling and degradation laboratory studies [24] [25]. In this case, the number of chemically meaningful structures, which can be assigned to an unknown peak, is limited to structures that show a close relationship with the parent compound and also, an adequate control sample or time series is available [26].

2.5 Confidence in identification procedure

2.5.1 Confidence in target screening

According to the guideline 2002/657/EC, a minimum of 4 identification points is required, for the confirmation of the analytes for HRMS instruments with resolution higher than 10,000. It states that the precursor ion earns 2 points and the transition products earn 2.5 points [29]. However, mass accuracy and mass resolution are crucial for the identification and should be taken account [26]. Bletsou et al. proposed an identification points system for HRMS analysis in order to take full advantage of the capabilities of HRMS instruments [27].

2.5.2 Confidence in suspect and non target screening

Schymanski et al. proposed a system of levels of confidence. As it can be seen in Figure 3, Level 1 corresponds to the confirmed structure verified by reference standard, Level 2 corresponds to a probable structure based on literature or diagnostic data, Level 3 corresponds to tentative candidates with possible structure, Level 4 corresponds to an unequivocal formula and Level 5 to an exact mass [30].

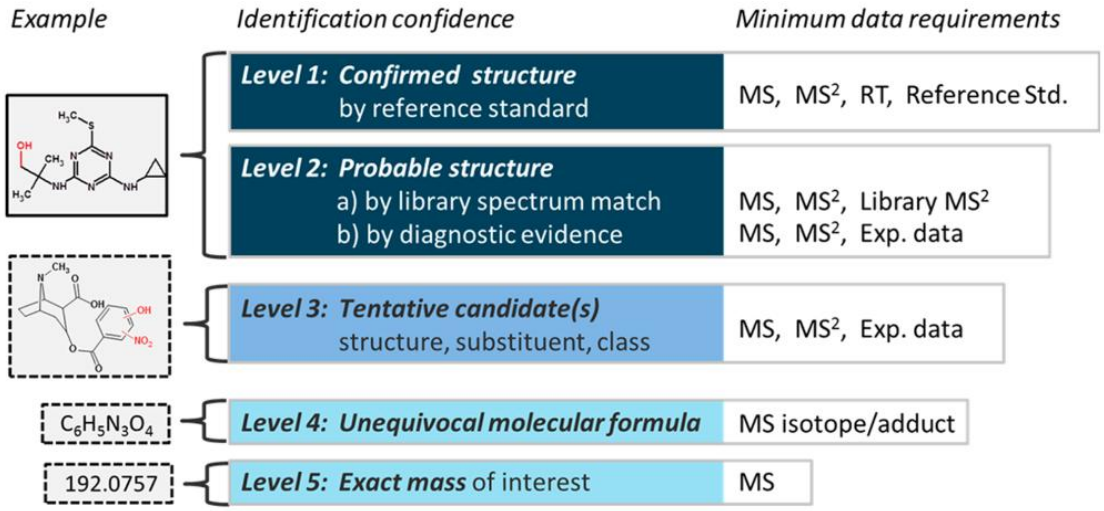


Figure 3: Identification confidence levels [30]

Non-target screening starts from level 5 and suspect screening from level 3 and if sufficient MS (exact mass, isotope, adduct), MS/MS and experimental information is available, they can gain in confidence (green arrows) through to Level 2 (library match and/or diagnostic fragments) and even Level 1 after purchase of the corresponding standard for identifications. Target screening starts by definition from level 1 [31].

Although, if the evidence of the sample and the evidence of the reference standard (target) or the tentative candidate (suspect) do not match, then the component associated with the target or suspect should become a 'non-target of interest' and 'downgrade' to level 5 (red arrows). If the HRMS data and Rt matches, but the MS/MS is not available due to low intensity, it still belongs to the target group although it should be reported with fewer identification points [31]. Generally, in both suspect and non-target screening, reference standards are required for unambiguous confirmation.

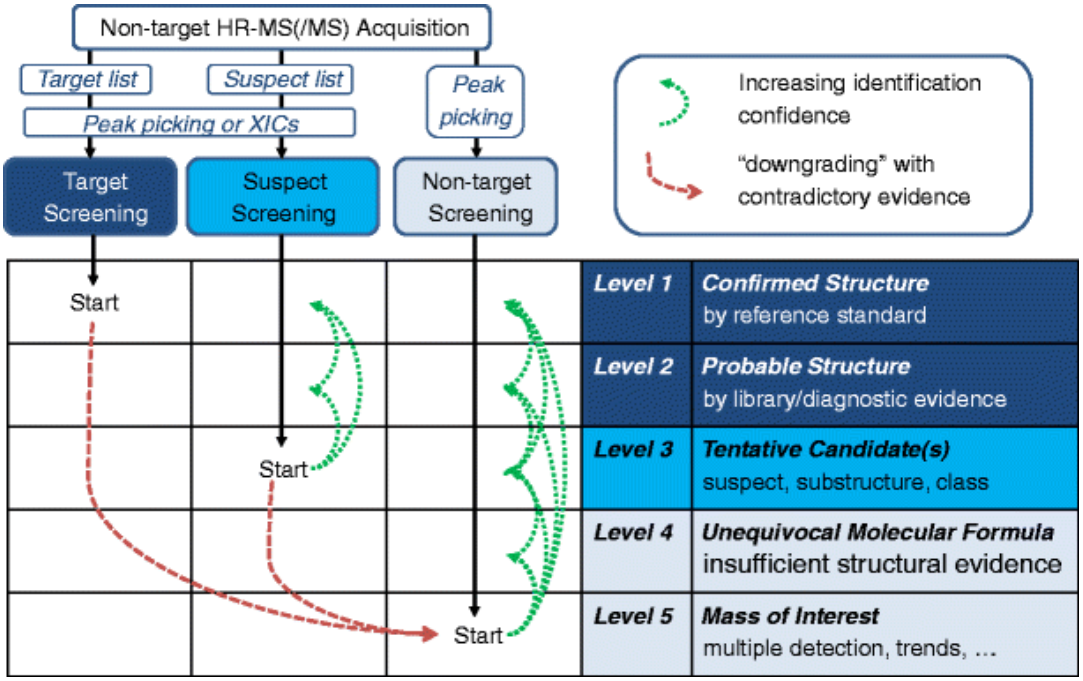


Figure 4: Matrix of identification approach versus identification confidence [31]

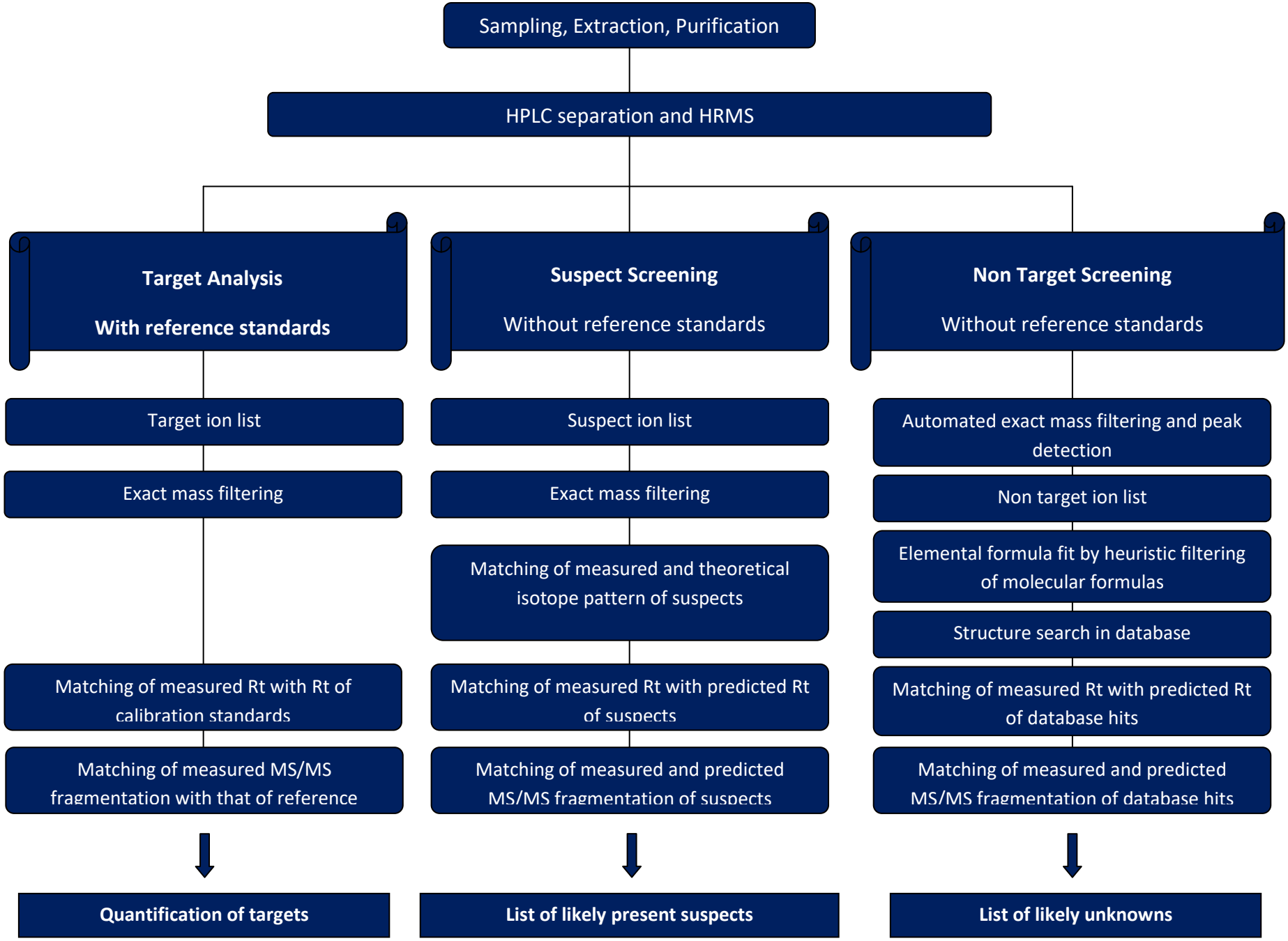


Figure 5: Systematic workflow for target, suspect and non-target screening by LC-HRMS/MS [26]

CHAPTER 3 – Scope

A broad range of organic compounds is used in modern society. From the moment that they are discharged through industrial, domestic and agricultural wastes and end up in sewer systems, they are transferred to the liquid and the solid products of wastewater treatment. For that reason, the evaluation of appearance of emerging pollutants in sewage sludge samples is necessary, taking into consideration that sewage sludge is recycled.

UHPLC-HRMS allows the wide-scope screening of EPs, their metabolites and transformation products with an acquisition of accurate-mass full spectrum data. These data can be used for target, suspect and non-target screening, as well as retrospective screening, years after the treatment of samples without additional analysis of them.

The scope of this study is the wide-scope target screening of emerging pollutants in sewage sludge from three wastewater treatment plants in Nigeria that were collected in 2015. For the determination of a broad range of compounds, a generic sample preparation was used as well as a data independent acquisition by UHPLC-HRMS, where with one injection and no pre-selection of analytes, information were obtain for both parent compounds and fragment ions. The quantitative approach can show the levels of the emerging pollutants that are released into the environment after the wastewater treatment plant. In this study, we hope, also, to see the difference of the levels of emerging pollutants after the stage of digestion and after the secondary treatment.

CHAPTER 4 – Materials and Methods

4.1 Chemicals and Materials

All the solvents for the LC-QTOF-MS analysis and sample preparation were UHPLC-MS grade. Methanol was purchased from Merck (Darmstadt, Germany) and the eluent additives ammonium formate, ammonium acetate and formic acid 99% were purchased from Fluka (Buchs, Switzerland). Sodium hydroxide monohydrate (NaOH) for trace analysis ≥99.9995% was purchased from Fluka (Buchs, Switzerland) for the external calibration standard. Ultrapure water was provided by a Milli-Q purification apparatus (Millipore Direct-Q UV, Bedford, MA, USA). Regenerated cellulose syringe filters (RC) of 15 mm diameter and 0.2 μm pore size were obtained from Phenomenex (Torrance, CA, USA) and the Certified Reference Material (BCR-143R) sewage sludge amended soil from the European Commission – Community Bureau of Reference.

Regarding the internal standards that were used for the analysis and the method validation, Cocaine- D3, Codeine-D6, Diazepam-D5, Ketamine-D4, Morphine-D3 were purchased from LGC Promochem (Molsheim, France). Sulfadiazine-D4, Sulfadimethoxin-D4, Diuron-D6 were purchased from Toronto Research Chemicals (Toronto, Canada). Bisphenol A-D16 was purchased from Sigma-Aldrich (Sheboygan Falls, WI, U.S.). Flunixin-D3 was donated by the Veterinary Drug Residues Laboratory of the State General Laboratory of Cyprus, while Amphetamine-D6, Atorvastatin-D5, Cetirizine-D8, Citalopram-D6, Lamotrigine-^{13}C3D3, Metformin-D6, Metronidazole-D4, Ranitidine-D6, Ritonavir-D6, Saccharin-^{13}C6, Valsartan-^{13}C5^{15}N, Venlafaxine-D6 were kindly offered from Eawag (aquatic research institute, Zurich, Switzerland). All pharmaceutical standards were of high purity grade (>90%) and were purchased from Sigma-Aldrich (Athens, Greece) and LGC Promochem (Molsheim, France) with the exception of sulfadoxine and sulfaclozine, which were dkindly donated by the National Laboratory of Residue Analysis of Food of Animal Origin of the Hellenic Ministry of Rural Development and Food. Regarding the psycotropic illicit drugs, all analytes were of high purity (98%) and solutions or solids were obtained from LGC Promochem (Molsheim, France). Solutions of pesticides were kindly offered from Eawag (aquatic research institute, Zurich, Switzerland).

4.2 Sampling and Storage

4.2.1 Sampling

Treated sewage sludge samples were collected in 2015 from three WWTPs of Lagos in Nigeria. In Table 6 are shown the details for the WWTPs.

Table 6: Characterisitcs of the wastewater treatment plants studied in Nigeria

Wastewater treatment plant	Location	Type of wastewater treated	Process type	Type of treatment	Capacity of sludge production (m^3/day)	Receiving water body for effluent	Sludge disposal
Iponri	Lagos	Domestic	Activated sludge	Primary, Secondary	3,600	Odo-Iyaalaro (river)	Landfill, Agricultural application
Alausa	Lagos	Domestic	Activated sludge	Primary, Secondary, followed by disinfection (chlorination)	1,440	Odo-Iyaalaro (river)	Agricultural application
Ijaiye	Lagos	Hospital	Membrane bioreactor/ anaerobic digestion	Primary, secondary, followed by disinfection (chlorination)	n.a.	Odo-Iyaalaro (river)	Agricultural application

The samples that were collected were 5 for each WWTP (after the secondary trearment), in 5 consecutive days, and 4 extra samples, in 4 consecutive days, from the Ijaiye WWTP after the stage of digestion. Overall, the final number of samples was 19.

4.2.2 Storage

After sampling, freeze-dried sewage sludge samples were kept in amber glass bottles and wrapped with aluminum foil. Finally, they were stored in the dark at -20^{0}C until analysis.

4.3 Sample preparation

Sample preparation was carried out using the protocol developed by Gago-Ferrero et al. [32]. 0.1 g freeze-dried sewage sludge was placed in plastic centrifuge tube (15 mL), then spiked with an internal standard mix and kept in contact for 30 min. The sample was extracted with 2 mL MeOH-Milli-Q water (FA 0.5%, 0.1% EDTA),

50:50 (v/v), by vortex (1 min), followed by ultrasonic extraction for 15 min at 50 ℃ (Ultrasons H-D, Selecta, Abrera, Barcelona, Spain). The extract was centrifuged for 10 min at 4000 rpm (Rotofix 32, Hettich, Tuttlingen, Germany) and the supernatant was collected in a glass tube. This procedure was repeated two more times with a total of 6 ml supernatant. The total extract was evaporated under a gentle nitrogen stream at 40 ^{o}C to dryness and then reconstituted to 0.5 mL with a final proportion of MeOH-Milli-Q water, 1:1 (v/v). Finally, the extract was filtered through a 0.2 μm regenerated cellulose (RC) filters (15 mm, 0.2 μm, Phenomenex, Torrance, California, USA), transferred to a glass vial and it was ready for injection in the chromatographic system. The Milli-Q water used throughout the sample preparation was provided by the apparatus Millipore Direct-Q 3 UV (Bedford, Massachusetts, USA).

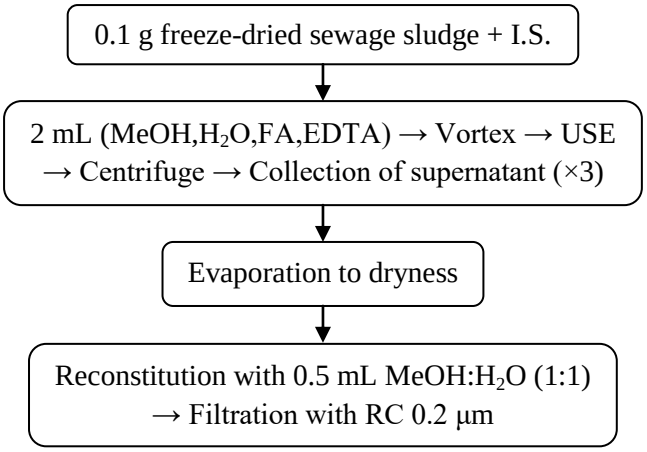


Figure 6: Sample preparation steps

4.4 Instrumentation [27]

The screening analysis was performed using an ultrahigh-performance liquid chromatography (Dionex UltiMate 3000 RSLC, Thermo Fisher Scientific, Germany) interfaced to an (ESI)-Q-TOF mass spectrometer (Maxis Impact, Bruker Daltonics, Bremen, Germany) with the source operating in positive and negative mode.

The UHPLC apparatus consists of a solvent rack degasser, a binary pump with solvent selection valve (HPG-3400), an auto-sampler and a column. For the chromatographic separation an Acclaim RSLC 120 C18 column (2.2 μm, 120 Å, 2.1 x 100 mm, Dionex Bonded Silica Products, Thermo Scientific, Germany) was used with a guard column of the same packaging material (ACQUITY UPLC BEH C18 1.7 μm, VanGuard Pre-Column, Waters, Ireland), thermostated at 30℃.

Figure 7: UHPLC-QTOF-MS, Maxis Impact, Bruker Daltonics [33]

Pesticide Screener by Bruker

In our analysis, two separate reversed-phase chromatographic runs were performed for positive and negative ESI mode.

The composition of the mobile phase, for the reversed phase liquid chromatography, in Positive Ionization mode is (A): 90% H_2O, 10% MeOH, 5 mM ammonium formate, 0.01% formic acid and (B): MeOH, 5 mM ammonium formate, 0.01% formic acid. For Negative Ionization mode the mobile phase is (A): 90% H_2O, 10% MeOH, 5 mM ammonium acetate and (B): MeOH, 5 mM ammonium acetate.

Each chromatogram lasts 16 min with a gradient elution program which is the same for both ionization modes and in the last 4 min the column is re-equilibrated for the next injection. The program is presented in the Table 7. The injection volume is set to 5 μL.

Table 7: Gradient elution program

Time (min)	Flow rate (mL/min)	%A	%B
0.0	0.200	99.0	1.0
0.1		99.0	1.0
1.0	0.200		
3.0		61.0	39.0
14.0	0.400	0.1	99.9
16.0	0.480	0.1	99.9
16.1	0.480	99.0	1.0
19.0	0.480	99.0	1.0
19.1	0.200	99.0	1.0
20.0	0.200	99.0	1.0

The operating parameters of the electrospray ionization interface in both ionization modes are presented in the Table 8.

Table 8: Electrospray ionization operating parameters

Ionization mode	**Positive**	**Negative**
Capillary voltage	500 V	500 V
End plate offset	2500 V	3000 V
Nebulizer (N_2)	2.0 bar	2.0 bar
Drying gas (N_2)	8.0 L/min	8.0 L/min
Dry temperature	200 °C	200 °C

External calibration of the Q-TOF-MS is performed in the beginning of every analysis with a solution of sodium formate: sodium formate 10 mM, H_2O:2-propanol (50:50). In the beginning of each run, in the segment 0.1-0.25 min of the chromatogram, calibrant solution is injected for internal calibration.

The Q-TOF MS system was set to bbCID mode (broadband collision-induced dissociation) in which spectra is recorded over the range of m/z 50-1000 with a frequency/scan rate of 2 Hz. Two different collision energies are applied to the collision cell providing both MS and MS/MS spectra in alternate scan events. Using low collision energy (4.0 eV) MS spectra is provided, while using high collision energy (25.0 eV) fragmentation is taking place in the collision cell providing MS/MS spectra.

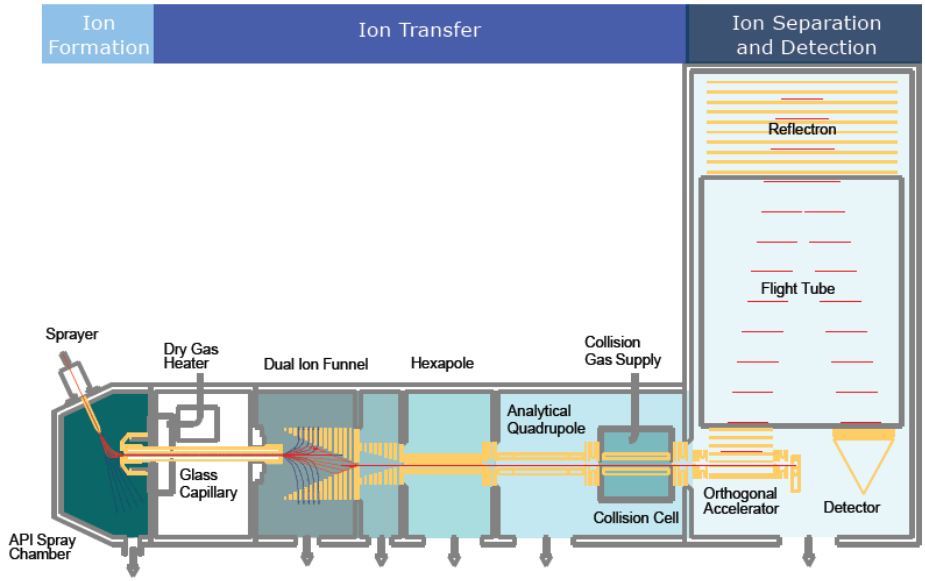


Figure 8: Schematic representation of Q-TOF-MS [33]

The software used for data analysis was TASQ 1.0 & 1.4, DataAnalysis 4.3 and Compass Isotope Pattern (Bruker Daltonics, Bremen, Germany).

4.5 Method validation

For method validation, there are no clear guidelines for wide-scope methods including high number of compounds. The validation dataset was chosen in order to guarantee representativeness (retention time, m/z ratio, classification).

A representative validation dataset of 274 compounds (pharmaceutical and veterinary drugs, illicit drugs, industrial chemicals, pesticides, etc) was used in order to evaluate linearity, accuracy, matrix effects and detectability of the screening method. The compounds of the validation dataset are shown in Table 9 including its CAS Number, molecular formula, ESI mode of ionization, pseudomolecular ion, retention time and some of their fragments in positive/negative ESI mode.

The Certified Reference Material (BCR-143R) sewage sludge amended soil from the European Commission – Community Bureau of Reference was used for method validation. Although the CRM was certified for metals and not for emerging pollutants, the presence of emerging pollutants was evaluated and positive results were obtained for 23 of the 274 analytes.

The CRM sample was spiked at a concentration of 200 ng/g d.w. and a sample (Iponri 4) was used for the spiked samples and the matrix matched standard solution. Both the CRM sample and the CRM spiked sample run 3 times, in the beginning, in the middle and in the end of the sequence, in order to evaluate %RSDr instrumental for quality control.

4.5.1 Performance criteria

In order to evaluate the performance of the method, linearity, accuracy and matrix effect was taken into account. Linearity was studied in standard solutions and spiked sewage sludge samples (50 ng/g, 100 ng/g, 200 ng/g). Method recovery was calculated by dividing the peak area of the spiked sample by the matrix matched standard solution (100 ng/g) multiplied with the appropriate factor (0.5, 1, 2). Finally, matrix factor was calculated by dividing the peak area of the matrix matched standard

solution (100 ng/g) by the peak area of the standard solution (100 ng/g). Matrix effect was evaluated by the equation: %ME=(MF-1)×100.

4.5.1.1 Linearity

Linearity was studied in standard solutions and spiked samples for the compounds of the validation dataset. The linear range was studied at 3 concentration levels, ranging from 50 ng/g to 200 ng/g. The linear range, the slope (b) and the correlation coefficients (r^2) of the calibration curve and the standard addition curve (spiked) are presented in the Table 11.

4.5.1.2 Recovery / Accuracy

Accuracy was assessed with recovery experiments. Method recovery was studied using standard solutions, spiked samples and matrix matched standard solution. The initial samples were analyzed for determination of the analytes of the validation dataset and if the sample already contained the analyte, its peak area was subtracted from the peak area of the spiked sample and the peak area of the matrix-matched sample. Recovery experiments were performed at 3 concentration levels, ranging from 50 ng/g to 200 ng/g.

4.5.1.3 Matrix effect

After the calculation of the matrix factor by dividing the peak area of matrix-matched samples by the peak area of the standard solutions, matrix effect was assessed by the equation: %Matrix Effect = (Matrix Factor – 1) × 100. Matrix effect was studied in 1 concentration level (100 ng/g) and the results are presented in Table 12. The results are presented as a percentage of signal enhancement (ME > 0) or signal suppression (ME < 0).

4.5.1.4 Detectability

The method limit of quantification (MLOQ) was calculated using the standard addition curve and the limit of detection (MLOD) is by definition LOD = LOQ / 3.

Table 9: Validation dataset

No.	Compound	CAS number	Molecular formula	ESI ionization	[M+H]	[M-H]	Ret. time	Fragment 1	Fragment 2	Fragment 3
1	2-4-Dinitrophenol	(51-28-5)	C6H4N2O5	Negative		183.0036	4.5	109.0174	123.0085	95.0146
2	2-Aminobenzimidazole	(934-32-7)	C7H7N3	Positive	134.0713		3.74	134,0716	135,0746	
3	2-Aminoheptan	(123-82-0)	C7H17N	Positive	116.1434		5.01	no fragmentation		
				Negative		114.1277	4.41	no fragmentation		
4	3.5.6-Trichloro-2-pyridinol	(6515-38-4)	C5H2Cl3N1O1	Positive	197.9275		8.96	no fragmentation		
				Negative		195.9118	7.87	no fragmentation		
5	9-OH-Risperidone (Paliperidone)	(144598-75-4)	C23H27F1N4O3	Positive	427.2140		5.33	207.1128		
6	Acephate	(30560-19-1)	C4H10NO3PS	Positive	184.0192		3.11	142.9926		
				Negative		182.0035	3.09	no fragmentation		
7	Adenosine	(58-61-7)	C10H13N5O4	Positive	268.1040		3.14	119.0340	136.0610	
				Negative		266.0884	3.84	107.0358	134.0467	266.0890
8	Albendazole	(54965-21-8)	C12H15N3O2S	Positive	266.0958		9.19	191.0148	234.0696	
9	Albendazole sulfone	(75184-71-3)	C12H15N3O4S	Positive	298.0856		5.86	224.0129	266.0608	298.087
10	Allopurinol	(315-30-0)	C5H4N4O	Positive	137.0458		2.19	137.0461		
				Negative		135.0301	3.04	no fragmentation		
11	Alprazolam	(28981-97-7)	C17H13N4Cl1	Positive	309.0902		8.36	274.1213	281.0714	
12	Ambroxol	(18683-91-5)	C13H18Br2N2O	Positive	376.9859		6.04	116.107	261.8861	
13	Amisulpride	(71675-85-9)	C17H27N3O4S1	Positive	370.1795		3.88	112.1121	242.0482	
				Negative		368.1639	5.08	no fragmentation		
14	Amitriptyline	(50-48-6)	C20H23N1	Positive	278.1903		8.23	218.109	233.1325	117.0699
15	Amlodipine	(88150-42-9)	C20H25N2O5Cl1	Positive	409.1525		8.36	208.0606	238.0632	294.0898
16	Amphetamine	(300-62-9)	C9H13N	Positive	136.1121		4.16	91.0542		
17	Ampicillin	(69-53-4)	C16H19N3O4S	Positive	350.1169		4.21	79.0564	91.0566	
18	Anilofos	(64249-01-0)	C13H19ClNO3PS2	Positive	368.0305		10.84	198.9647	170.9698	124.9821
				Negative		366.0149	10.81	no fragmentation		
19	Aspartame	(22839-47-0)	C14H18N2O5	Positive	295.1288		5.01	120.0797	180.1011	200.0702
				Negative		293.1132	4.98	no fragmentation		
20	Atazanavir	(198904-31-3)	C38H52N6O7	Positive	705.3970		10.91	168.0814	335.1989	705.4037
				Negative		703.3814	12.36	155.0808	639.3199	671.3480

21	Atenolol	(29122-68-7)	C14H22N2O3	Positive	267.1703		3.09	225.1234	190.0863	145.0648
22	Atenolol acid (Metoprolol acid)	(37350-58-6)	C15H25N1O3	Positive	268.1907		4.93	191.1067	98.0964	74.06
23	Atorvastatin	(134523-00-5)	C33H35N2O5F1	Positive	559.2603		9.96	440.2232		
24	Atrazine	(1912-24-9)	C8H14ClN5	Positive	216.1011		8.16	174.0541		
25	Atrazine-Desethyl	(6190-65-4)	C6H10ClN5	Positive	188.0697		5.73	146.0228	104.0010	
				Negative		186.0541	5.71	no fragmentation		
26	Atrazine-Desisopropyl	(1007-28-9)	C5H8ClN5	Positive	174.0541		4.75	104.0010	96.0556	
				Negative		172.0384	4.74	no fragmentation		
27	Azinphos-Eth	(2642-71-9)	C12H16N3O3PS2	Positive	346.0443		10.17	160.0505		
28	Azithromycin	(83905-01-5)	C38H72N2O12	Positive	749.5158		5.98	591.4215	158.1176	116.107
29	Benserazide	(14919-77-8)	C10H15N3O5	Positive	258.1084		1.38	no fragmentation		
30	Benzoic acid	(65-85-0)	C7H6O2	Negative		121.0284	2.88	no fragmentation		
31	Benzothiazole (BTH)	(95-16-9)	C7H5NS	Positive	136.0215		6.88	no fragmentation		
32	Benzotriazole (BTR)	(95-14-7)	C6H5N3	Positive	120.0556		4.76	120.0546	65.0380	
33	Benzotriazole-4-Me	(29878-31-7)	C7H7N3	Positive	134.0713		5.83	51.0220	53.0375	66.0454
34	Benzoylecgonine (BECG)	(519-09-5)	C16H19N1O4	Positive	290.1387		4.68	105.0335	168.1019	
35	Biotin (Vitamin B7)	(58-85-5)	C10H16N2O3S	Positive	245.0954		4.44	227.0849	199.09	
36	Bisphenol A (BPA)	(80-05-7)	C15H16O2	Negative		227.1067	8.49	no fragmentation		
37	Bromazepam	(1812-30-2)	C14H10N3O1Br1	Positive	316.0080		7.28	288.0131		
38	Bromohexine	(3572-43-8)	C14H20N2Br2	Positive	375.0066		10.01	114.1277	261.8861	
39	Buprenorphine (BN)	(52485-79-7)	C29H41N1O4	Positive	468.3108		7.96	468.3114		
40	Caffeine	(58-08-2)	C8H10N4O2	Positive	195.0877		4.23	163.074	138.0662	
41	Cannabidiol	(13956-29-1)	C21H30O2	Positive	315.2319		12.7	93.0699	193.1223	
				Negative		313.2162	12.42	no fragmentation		
42	Capecitabine	(154361-50-9)	C15H22FN3O6	Positive	360.1565		7.59	130.0399	174.0294	244.1088
				Negative		358.1409	8.63	no fragmentation		
43	Capsaicin	(404-86-4)	C18H27NO3	Positive	306.2064		9.83	137.0597	122.0362	69.0699
44	Carbamazepin	(298-46-4)	C15H12N2O1	Positive	237.1022		7.36	194.0964		
45	Carprofen	(53716-49-7)	C15H12ClNO2	Negative		272.0473	8.94	no fragmentation		
46	Cathine	(492-39-7)	C9H13NO	Positive	152.1070		3.68	134.0964	117.0699	115.0542
47	Cefaclor	(53994-73-3)	C15H14ClN3O4S	Positive	368.0466		4.09	no fragmentation		
48	Cefadroxil	(66592-87-8)	C16H17N3O5S	Positive	364.0962		2.84	no fragmentation		
49	Cefalexine	(15686-71-2)	C16H17N3O4S	Positive	348.1013		4.08	68.0489	118.0653	

50	Cefalonium	(5575-21-3)	C20H18N4O5S2	Positive	459.0791		4.03	no fragmentation		
51	Cefazoline	(25953-19-9)	C14H14N8O4S3	Positive	455.0373		4.16	156.0127		
52	Cefoperazone	(62893-19-0)	C25H27N9O8S2	Positive	646.1497		4.36	no fragmentation		
53	Cefquinome	(84957-30-2)	C23H24N6O5S2	Positive	529.1322		3.76	no fragmentation		
54	Chloramphenicol	(56-75-7)	C11H12Cl2N2O5	Negative		321.0040	5.74	176.0371		
55	Chlorbufam	(1967-16-4)	C11H10ClNO2	Positive	224.0473		9.27	no fragmentation		
56	Chlordiazepoxide	(58-25-3)	C16H14ClN3O	Positive	300.0898		8.79	57.0447	227.0496	282.0793
57	Chlorpheniramine	(132-22-9)	C16H19N2Cl1	Positive	275.1310		6.25	230.0731	167.0730	118.0651
58	Chlorpromazine	(50-53-3)	C17H19N2Cl1S1	Positive	319.1030		8.89	246.0139	58.0651	86.0964
59	Cimaterol	(54239-37-1)	C12H17N3O	Positive	220.1444		2.99	116.0493	143.0603	160.0869
60	Cimetidine	(51481-61-9)	C10H16N6S1	Positive	253.1230		3.24	159.0699	99.0665	117.0481
61	Ciprofloxacin	(85721-33-1)	C17H18FN3O3	Positive	332.1405		4.42	231.0564	314.1299	
62	Citalopram	(59729-33-8)	C20H21N2O1F1	Positive	325.1711		6.59	166.0651	234.0714	116.0495
63	Clarithromycin	(81103-11-9)	C38H69NO13	Positive	748.4842		9.18	158.1164	590.3894	591.3924
64	Clazuril	(101831-36-1)	C17H10Cl2N4O2	Negative		371.0097	9.42	no fragmentation		
65	Clenbuterol	(37148-27-9)	C12H18Cl2N2O	Positive	277.0869		4.76	203.0137	132.0682	
66	Clobazam	(22316-47-8)	C16H13N2O2Cl1	Positive	301.0738		8.08	259.0633		
67	Clofibric acid	(882-09-7)	C10H11ClO3	Negative		213.0313	6.54	no fragmentation		
68	Clonazepam	(1622-61-3)	C15H10N3O3Cl1	Positive	316.0483		7.61	302.0453	270.0554	
69	Clopidogrel Carboxylic Acid	(144457-28-3)	C15H14ClNO2S	Positive	308.0507		5.56	no fragmentation		
				Negative		306.0350	7.86	no fragmentation		
70	Clopidol	(2971-90-6)	C7H7Cl2NO	Positive	191.9977		4.2	no fragmentation		
71	Closantel	(57808-65-8)	C22H14Cl2I2N2O2	Negative		660.8438	12.16	no fragmentation		
72	Clotrimazole	(23593-75-1)	C22H17ClN2	Positive	345.1153		11.19	no fragmentation		
73	Clozapine	(5786-21-0)	C18H19N4Cl1	Positive	327.1371		7.19	227.0371	296.0949	84.0808
74	Clozapine-Nor	(6104-71-8)	C17H17N4Cl1	Positive	313.1215		7.29	296.0949	227.0371	192.0682
75	Cocaine (COC)	(50-36-2)	C17H21N1O4	Positive	304.1543		4.84	150.0913	105.0335	82.0651
76	Codeine (COD)	(76-57-3)	C18H21N1O3	Positive	300.1594		3.4	225.091	243.1016	215.1067
77	Colchicine	(64-86-8)	C22H25N1O6	Positive	400.1755		6.43	310.12	341.1384	358.1649
78	Corticosterone	(50-22-6)	C21H30O4	Positive	347.2217		8.74	97.0648	329.2111	311.2006
				Negative		345.2060	9.91	no fragmentation		
79	Cortisol F	(50-23-7)	C21H30O5	Positive	363.2166		7.86	no fragmentation		
80	Cortisone E	(53-06-5)	C21H28O5	Positive	361.2010		7.41	163.1117		

81	Coumaphos	(56-72-4)	C14H16ClO5PS	Positive	363.0217		11.19	288.9486	306.9591	226.9928
				Negative		361.0061	10.98	no fragmentation		
82	Crotamiton	(483-63-6)	C13H17NO	Positive	204.1383		9.24	no fragmentation		
83	Crotethamide	(6168-76-9)	C12H22N2O2	Positive	227.1754		6.13	no fragmentation		
84	Curcumin (E100)	(458-37-7)	C21H20O6	Negative		367.1176	10	134.0359	149.0596	173.0608
85	Cytarabine	(147-94-4)	C9H13N3O5	Positive	244.0928		1.39	no fragmentation		
86	Danofloxacin	(112398-08-0)	C19H20FN3O3	Positive	358.1561		4.54	358.1585	359.1611	360.1719
87	Dapsone	(80-08-0)	C12H12N2O2S	Positive	249.0692		4.13	74.095	108.0464	156.0116
88	Decoquinate	(18507-89-6)	C24H35NO5	Positive	418.2588		13.43	372.2172	390.2280	418.2595
89	Dexamethazone	(50-02-2)	C22H29O5F1	Positive	393.2072		8.38	no fragmentation		
90	Diaveridine	(5355-16-8)	C13H16N4O2	Positive	261.1346		3.88	217.1084	245.1033	123.0665
91	Diazepam	(439-14-5)	C16H13N2O1Cl1	Positive	285.0789		9.53	228.0575	257.084	222.1152
92	Diazepam-Nor	(1088-11-5)	C15H11N2O1Cl1	Positive	271.0633		9.23	243.0684	208.0995	140.0262
93	Diclazuril	(101831-37-2)	C17H9Cl3N4O2	Negative		404.9707	10.31	no fragmentation		
94	Diclofenac	(15307-86-5)	C14H11Cl2NO2	Positive	296.0240		10.18	214.0418	215.0496	
				Negative		294.0083	9.22	no fragmentation		
95	Didecyldimethylammonium (DADMAC (C10:C10))	(7173-51-5)	C22H48N^1+	Positive	326.3781		12.64	no fragmentation		
96	Difloxacin	(98106-17-3)	C21H19F2N3O3	Positive	400.1467		4.96	400.1503		
97	Diflufenzopyr	(109293-97-2)	C15H12F2N4O3	Positive	335.0950		6.22	162.0665	206.0561	
				Negative		333.0794	5.55	160.0516	204.0415	289.0906
98	Diltiazem	(42399-41-7)	C22H26N2O4S1	Positive	415.1686		7.18	370.1108	178.0321	
99	Dimetridazole	(551-92-8)	C5H7N3O2	Positive	142.0611		4.27	81.0447		
100	Diphenhydramine	(58-73-1)	C17H21N1O1	Positive	256.1696		6.63	167.0855	165.0699	152.0621
101	Disulfoton	(298-04-4)	C8H19O2PS3	Positive	275.0358		11.52	no fragmentation		
102	Duloxetine	(116539-59-4)	C18H19N1O1S1	Positive	298.1260		8.11	124.0341		
103	EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)	(30223-73-5)	C20H23N1	Positive	278.1903		6.38	201.1512	234.1277	249.1512
104	Efavirenz	(154598-52-4)	C14H9ClF3NO2	Positive	316.0347		10.69	no fragmentation		
				Negative		314.0190	12.07	no fragmentation		
105	Emamectin B1a	(119791-41-2)	C49H75NO13	Positive	886.5311		12.4	199.1693		
106	Emamectin B1b	(119791-41-2)	C48H73NO13	Positive	872.5155		12.01	199.1693		
107	EME (Ecgonine methyl ester)	(7143-09-1)	C10H17N1O3	Positive	200.1281		1.38	no fragmentation		

108	Enrofloxacin	(93106-60-6)	C19H22FN3O3	Positive	360.1718		4.64	84.0807	86.097	288.1527
109	Ephedrine	(299-42-3)	C10H15N1O1	Positive	166.1226		3.76	91.0542	115.0542	117.0699
110	Ephedrine-Nor	(14838-15-4)	C9H13NO	Positive	152.1070		3.54	117.0699	134.0964	115.0542
111	Erythromycin	(114-07-8)	C37H67N1O13	Positive	734.4685		8	83.0491	576.3742	
112	Ethopabate	(59-06-3)	C12H15NO4	Positive	238.1074		6.63	136.0387	164.07	178.0495
113	Ethoprophos	(13194-48-4)	C8H19O2PS2	Positive	243.0637		10.16	no fragmentation		
114	Fenbendazole	(43210-67-9)	C15H13N3O2S	Positive	300.0801		10.26	160.0457	268.0541	269.0569
115	Fenfluramine	(458-24-2)	C12H16N1F3	Positive	232.1308		5.99	109.0448	159.0416	
116	Fentanyl	(437-38-7)	C22H28N2O1	Positive	337.2274		6.04	216.1383	188.1434	
117	Fentanyl-Nor	(1609-66-1)	C14H20N2O1	Positive	233.1648		4.68	150.0913	84.0808	
118	Flonicamid	(158062-67-0)	C9H6F3N3O	Positive	230.0536		4.22	203.0427	174.0161	148.0369
				Negative		228.0379	4.24	no fragmentation		
119	Florfenicol	(73231-34-2)	C12H14Cl2FNO4S	Negative		355.9921	4.68	no fragmentation		
120	Fluazuron	(86811-58-7)	C20H10Cl2F5N3O3	Positive	506.0092		12.68	no fragmentation		
				Negative		503.9936	12.58	no fragmentation		
121	Flubendazole	(31430-15-6)	C16H12FN3O3	Positive	314.0935		8.65	282.0669	314.0938	
122	Fludioxonil	(131341-86-1)	C12H6F2N2O2	Negative		247.0314	9.43	no fragmentation		
123	Flumequine	(42835-25-6)	C14H12FNO3	Positive	262.0874		7.46	202.0307	244.078	245.0815
124	Flunitrazepam	(1622-62-4)	C16H12N3O3F1	Positive	314.0935		7.83	268.1006		
125	Flunitrazepam-7-Amino	(34084-50-9)	C16H14N3O1F1	Positive	284.1194		5.41	227.0979		
126	Flunixin	(38677-85-9)	C14H11N2O2F3	Positive	297.0845		9.09	264.0505	279.0740	
				Negative		295.0689	8.34	no fragmentation		
127	Fluoxetine	(54910-89-3)	C17H18N1O1F3	Positive	310.1413		8.6	no fragmentation		
128	Flurazepam	(17617-23-1)	C21H23N3O1Cl1F1	Positive	388.1586		6.61	288.0586	100.1121	315.0695
129	Furosemide	(54-31-9)	C12H11ClN2O5S	Negative		328.9993	5.08	no fragmentation		
130	Gabapentin	(60142-96-3)	C9H17N1O2	Positive	172.1332		3.78	154.1226	137.0961	93.0699
				Negative		170.1176	4.08	no fragmentation		
131	Gamma-Hydroxybutyrate (GHB sod.salt)	(591-81-1)	C4H8O3	Negative		103.0390	1.26	57.0346		
132	Gemfibrozil	(25812-30-0)	C15H22O3	Negative		249.1485	10.86	121.065888		
133	Haloperidol	(52-86-8)	C21H23N1O2Cl1F1	Positive	376.1474		7.18	358.1368	123.0241	165.071
134	Hydrochlorothiazide	(58-93-5)	C7H8ClN3O4S2	Negative	295.9572		3.49	268.9463	204.9844	77.9655
135	Ibuprofen	(15687-27-1)	C13H18O2	Positive	207.1380		10.68	no fragmentation		

136	Imipramine	(50-49-7)	C19H24N2	Positive	281.2012		8.08	208.1121	58.0651	86.0964
137	Indapamine	(26807-65-8)	C16H16N3O3Cl1S1	Positive	366.0674		6.61	132.0808		
				Negative		364.0517	6.78	no fragmentation		
138	Ketamine	(6740-88-1)	C13H16ClNO	Positive	238.0993		4.61	220.0888	179.0622	125.0153
139	Ketoprofen	(22071-15-4)	C16H14O3	Positive	255.1016		8.53	209.0961		
140	Lacosamide	(175481-36-4/ 175481-37-5)	C13H18N2O3	Positive	251.1390		5.09	116.0706	74.06	
141	Levamisol	(14769-73-4)	C11H12N2S	Positive	205.0794		3.68	206.0794		
142	Levetiracetam	(102767-28-2)	C8H14N2O2	Positive	171.1128		3.74	126.0913		
143	Levofloxacin	(100986-85-4)	C18H20FN3O4	Positive	362.1511		4.41	no fragmentation		
144	Lidocaine	(137-58-6)	C14H22N2O1	Positive	235.1805		4.54	86.0964		
145	Lidocaine-Nor	n.a.	C12H18N2O1	Positive	207.1492		4.33	122.0956	132.0796	150.0904
				Negative		205.1335	5.99	no fragmentation		
146	Lincomycin	(154-21-2)	C18H34N2O6S	Positive	407.2210		4.08	126.1278	407.2237	
147	Lorazepam	(846-49-1)	C15H10N2O2Cl2	Positive	321.0192		8.36	275.0137		
148	LSD (Lysergic acid diethylamide)	(50-37-3)	C20H25N3O1	Positive	324.2070		5.48	128.107	74.0964	281.1648
149	Mabuterol	(56341-08-3)	C13H18ClF3N2O	Positive	311.1133		5.28	57.0695	132.0683	168.0447
150	MAM (6-O-Monoacetylmorphine)	(2784-73-8)	C19H21NO4	Positive	328.1543		3.75	268.1332	211.0754	
151	Maprotiline	(10262-69-8)	C20H23N1	Positive	278.1903		8.08	219.1168	250.1590	117.0699
152	Marbofloxacin	(115550-35-1)	C17H19FN4O4	Positive	363.1463		4.18	72.0808	204.0448	320.1064
153	MDA (3,4-methylenedioxy-amphetamine)	(4764-17-4)	C10H13N1O2	Positive	180.1019		4.19	163.0754	133.0648	135.0441
154	MDMA (3,4-methylenedioxy-methamphetamine)	(42542-10-9)	C11H15N1O2	Positive	194.1176		4.18	163.0754	133.0648	135.0441
155	Mebendazole	(31431-39-7)	C16H13N3O3	Positive	296.1030		8.25	264.0765	296.1040	
156	Meclofenamic acid	(644-62-2)	C14H11Cl2NO2	Positive	296.0240		11.27	no fragmentation		
				Negative		294.0083	8.66	no fragmentation		
157	Medazepam	(2898-12-6)	C16H15N2Cl1	Positive	271.0997		10.22	242.0731	207.1043	
158	Mefenamic acid	(61-68-7)	C15H15NO2	Positive	242.1176		11.6	no fragmentation		
				Negative		240.1019	9.29	no fragmentation		
159	Meloxicam	(71125-38-7)	C14H13N3O4S2	Positive	352.0420		7.09	141.0117	115.0324	
				Negative		350.0264	6.41	no fragmentation		
160	Mephedrone	(1189805-46-6)	C11H15N1O1	Positive	178.1226		4.49	145.0886	144.0808	91.0542

161	Metformin	(657-24-9)	C4H11N5	Positive	130.1087		1.39	no fragmentation		
162	Methacrifos	(62610-77-9)	C7H13O5PS	Positive	241.0294		8.16	209.0032	124.9821	109.0049
				Negative		239.0137	5.12	no fragmentation		
163	Methadone	(76-99-3)	C21H27N1O1	Positive	310.2165		8.04	223.1117	57.0335	265.1587
164	Methamidophos	(10265-92-6)	C2H8NO2PS	Positive	142.0086		2.76	no fragmentation		
165	Methamphetamine (MA)	(537-46-2)	C10H15N1	Positive	150.1277		4.21	119.0855	91.0542	
166	Methimazole (Tapazole)	(60-56-0)	C4H6N2S	Positive	115.0324		2.61	no fragmentation		
167	Methomyl	(16752-77-5)	C5H10N2O2S	Positive	163.0536		4.16	106.0321	72.9977	88.0215
168	Metronidazole	(443-48-1)	C6H9N3O3	Positive	172.0717		3.58	98.0475	128.0455	
169	Midazolam	(59467-70-8)	C18H13N3Cl1F1	Positive	326.0855		8.63	244.0324	291.1166	
170	Mirtazapine	(61337-67-5)	C17H19N3	Positive	266.1652		5.29	209.1073	72.0808	195.0917
171	Monocrotophos	(6923-22-4)	C7H14NO5P	Positive	224.0682		4.36	98.06	193.026	127.0155
172	Morantel	(20574-50-9)	C12H16N2S	Positive	221.1107		4.36	111.0275	164.0529	221.1118
173	Morphine	(57-27-2)	C17H19N1O3	Positive	286.1438		2.54	58.0651	229.0859	201.091
174	Moxidectin	(113507-06-5)	C37H53NO8	Positive	640.3844		13.85	no fragmentation		
175	Naproxen	(22204-53-1)	C14H14O3	Positive	231.1016		8.99	185.0961		
176	Nicotinamide (B3 amide)	(98-92-0)	C6H6N2O	Positive	123.0553		2.73	80.0495	96.0444	53.0386
177	Nicotine-Nor	(5746-86-1)	C9H12N2	Positive	149.1073		3.14	117.0566	130.0646	132.0798
178	Niflumic acid	(4394-00-7)	C13H9F3N2O2	Positive	283.0689		9.86	245.0521	265.0583	
				Negative		281.0532	8.34	no fragmentation		
179	Nigericin	(28380-24-7)	C40H68O11	Positive	725.4834		13.94	no fragmentation		
180	Nitrazepam	(146-22-5)	C15H11N3O3	Positive	282.0873		7.79	no fragmentation		
181	Norfloxacin	(70458-96-7)	C16H18FN3O3	Positive	320.1405		4.46	302.1299		
182	Novobiocin	(303-81-1)	C31H36N2O11	Positive	613.2392		11.24	no fragmentation		
183	Ofloxacin	(82419-36-1)	C18H20FN3O4	Positive	362.1511		4.34	344.1405	261.1034	318.1612
184	Olanzapine	(132539-06-1)	C17H20N4S1	Positive	313.1481		4.99	282.1059	213.0481	256.0903
185	Omeprazole	(73590-58-6)	C17H19N3O3S1	Positive	346.1220		7.49	168.1019		
186	Omethoate	(1113-02-6)	C5H12NO4PS	Positive	214.0297		3.35	154.9921	142.9916	182.9875
187	Oxamyl	(23135-22-0)	C7H13N3O3S	Positive	220.0750		3.77	90.055	72.0444	
188	Oxazepam	(604-75-1)	C15H11N2O2Cl1	Positive	287.0582		8.43	241.0527		
189	Oxcarbazepine	(28721-07-5)	C15H12N2O2	Positive	253.0972		6.46	236.0706	208.0757	180.0808
190	Oxfendazole	(53716-50-0)	C15H13N3O3S	Positive	316.0750		6.66	284.0485	316.0755	
191	Oxolinic acid	(14698-29-4)	C13H11NO5	Positive	262.0710		6.03	244.0616	262.0725	

192	Oxprenolol	(6452-71-7)	C15H23N1O3	Positive	266.1751		5.66	116.107	72.0808	
193	Paracetamol	(103-90-2)	C8NH9O2	Positive	152.0706		3.48	110.06		
194	Phenylbutazone	(50-33-9)	C19H20N2O2	Positive	309.1598		8.98	no fragmentation		
				Negative		307.1441	7.46	no fragmentation		
195	Phenytoin	(57-41-0)	C15H12N2O2	Positive	253.0972		7.08	104.0495		
				Negative		251.0815	8.26	no fragmentation		
196	Phosphate-Triphenyl	(115-86-6)	C6H15O4P	Positive	183.0781		6.16	no fragmentation		
197	Phthalate-bis-(2-ethylhexyl) DEHP	(117-81-7)	C24H38O4	Positive	391.2843		15	149.0239	167.0349	
198	Pioglitazone	(111025-46-8)	C19H20N2O3S	Positive	357.1267		9.09	134.0964		
				Negative		355.1111	10.64	no fragmentation		
199	Prazepam	(2955-38-6)	C19H17N2O1Cl1	Positive	325.1102		10.58	140.0262	271.0633	
200	Prednisolone	(50-24-8)	C21H28O5	Positive	361.2010		7.84	147.0804		
				Negative		359.1853	8.92	no fragmentation		
201	Pregabalin	(148553-50-8)	C8H17N1O2	Positive	160.1332		3.88	83.0855	55.0542	
				Negative		158.1176	4.04	no fragmentation		
202	Primidone	(125-33-7)	C12H14N2O2	Positive	219.1128		5.29	162.0913	91.0542	
203	Progesteron	(57-83-0)	C21H30O2	Positive	315.2319		11.22	97.0648		
204	Prometryn	(7287-19-6)	C10H19N5S	Positive	242.1434		10.06	200.0964	85.0509	116.0277
205	Propham	(122-42-9)	C10H13NO2	Positive	180.1019		8.11	138.055		
206	Propranolol	(525-66-6)	C16H21N1O2	Positive	260.1645		6.59	157.0648	155.0855	98.0964
207	Pseudoephedrine	(90-82-4)	C10H15N1O1	Positive	166.1226		3.69	148.1121	117.0699	115.0542
208	Quetiapine	(111974-69-7)	C21H25N3O2S1	Positive	384.1740		7.26	158.1176	221.1073	279.095
209	Ractopamine	(97825-25-7)	C18H23NO3	Positive	302.1751		4.39	91.0544	107.051	121.0653
210	Rafoxanide	(22662-39-1)	C19H11Cl2I2NO3	Negative		623.8122	12.36	no fragmentation		
211	Ranitidine	(66357-35-5)	C13H22N4O3S1	Positive	315.1485		3.14	102.0372	98.0838	176.0488
212	Rifaximin	(80621-81-4)	C43H51N3O11	Positive	786.3596		10.06	787.3671		
213	Risperidone	(106266-06-2)	C23H27N4O2F1	Positive	411.2191		5.88	191.1179		
214	Robenidine	(25875-51-8)	C15H13Cl2N5	Positive	334.0621		10.43	140.0254	142.0223	155.0362
215	Ronidazole	(7681-76-7)	C6H8N4O4	Positive	201.0618		3.55	140.046		
216	Salicylic acid	(69-72-7)	C7H6O3	Negative		137.0233	3.58	93.0346		
217	Sarafloxacin	(98105-99-8)	C20H17F2N3O3	Positive	386.1311		4.83	368.123	386.1346	
218	Sertraline	(79617-96-2)	C17H17N1Cl2	Positive	306.0811		8.94	158.9763	129.0699	275.0389

219	Sertraline-Nor	n.a.	C16H15Cl2N	Positive	292.0654		9.3	158.9753	160.9724	275.0381
220	Simvastatin	(79902-63-9)	C25H38O5	Positive	419.2792		12.64	285.1849	225.1638	
221	Spinosad A (Spinosyn A)	(168316-95-8)	C41H65NO10	Positive	732.4681		11.34	142.1226		
222	Sulfachloropyridazine	(80-32-0)	C10H9ClN4O2S	Positive	285.0208		4.56	120.0562		
223	Sulfaclozine	(102-65-8)	C10H9ClN4O2S	Positive	285.0208		5.45	no fragmentation		
224	Sulfadiazine	(68-35-9)	C10H10N4O2S	Positive	251.0597		3.48	156.0114	96.0556	108.0444
225	Sulfadimethoxine	(122-11-2)	C12H14N4O4S	Positive	311.0809		5.6	156.0762	218.0235	
226	Sulfadimidine (Sulfamethazine)	(57-68-1)	C12H14N4O2S	Positive	311.0809		4.31	122.0716	124.0872	126.0663
227	Sulfadoxine	(2447-57-6)	C12H14N4O4S	Positive	311.0809		4.75	140.0455	92.0495	
228	Sulfaguanidine	(57-67-0)	C7H10N4O2S	Positive	215.0597		1.93	no fragmentation		
229	Sulfamerazine	(127-79-7)	C11H12N4O2S	Positive	265.0754		3.95	110.0713		
230	Sulfameter (Sulfumetin)	(651-06-9)	C11H12N4O3S	Positive	281.0703		4.18	108.0465	156.0116	283.0684
231	Sulfamethizole	(144-82-1)	C9H10N4O2S2	Positive	271.0318		4.21	156.0114	108.0444	
232	Sulfamethoxazole	(723-46-6)	C10H11N3O3S	Positive	254.0594		4.6	156.0114	108.0444	92.0495
233	Sulfamethoxypyridazine	(80-35-3)	C11H12N4O3S	Positive	281.0703		4.38	126.0662	156.0114	108.0444
234	Sulfamonomethoxine	(1220-83-3)	C11H12N4O3S	Positive	281.0703		4.75	no fragmentation		
235	Sulfamoxole	(729-99-7)	C11H13N3O3S	Positive	268.0750		4.11	72.0439	92.0498	108.0459
236	Sulfapyridine	(144-83-2)	C11H11N3O2S	Positive	250.0645		3.8	184.0869		
237	Sulfaquinoxaline	(59-40-5)	C14H12N4O2S	Positive	301.0754		5.81	156.0114		
238	Sulfathiazole	(72-14-0)	C9H9N3O2S2	Positive	256.0209		3.63	156.0114	92.0495	108.0444
239	Sulfisoxazole	(127-69-5)	C11H13N3O3S	Positive	268.0750		4.71	92.0498	120.0559	
240	Sulpiride	(15676-16-1)	C15H23N3O4S1	Positive	342.1482		2.94	214.0169	112.1121	
				Negative		340.1326	4.42	no fragmentation		
241	Telmisartan	(144701-48-4)	C33H30N4O2	Positive	515.2442		10.31	497.2336		
				Negative		513.2285	11.24	469.2333		
242	Temazepam	(846-50-4)	C16H13N2O2Cl1	Positive	301.0738		7.94	no fragmentation		
243	Terbufos	(13071-79-9)	C9H21O2PS3	Positive	289.0514		12.25	no fragmentation		
244	Terbutaline	(23031-25-6)	C12H19N1O3	Positive	226.1438		3.11	152.0706		
				Negative		224.1281	3.48	no fragmentation		
245	Terbuthylazine	(5915-41-3)	C9H16ClN5	Positive	230.1167		9.36	132.0315	138.0764	146.0219
246	Ternidazole	(1077-93-6)	C7H11N3O3	Positive	186.0873		4.21	59.0489		
247	Tetrahydrocannabinol (THC)	(1972-08-3)	C21H30O2	Positive	315.2319		13.97	193.1223		
				Negative		313.2162	13.75	313.2173	191.1078	

248	Tetrahydrocannabinolic acid (THC-COOH)	(56354-06-4)	C21H28O4	Positive	345.2060		12.5	no fragmentation		
				Negative		343.1904	12.02	no fragmentation		
249	Tetrazepam	(10379-14-3)	C16H17N2O1Cl1	Positive	289.1102		10.54	81.0699		
250	Theophylline	(58-55-9)	C7H8N4O2	Positive	181.0720		3.93	124.0505		
251	Thiabendazole	(148-79-8)	C10H7N3S	Positive	202.0433		6.15	175.0324		
				Negative		200.0277	6.52	173.0156		
252	Thiamphenicol	(15318-45-3)	C12H15Cl2NO5S	Negative		353.9964	4.08	no fragmentation		
253	Thiopental	(76-75-5)	C11H18N2O2S	Negative		241.1005	8.63	57.9757		
254	Tiagabine	(115103-54-3)	C20H25N1O2S2	Positive	376.1399		8.43	111.0263	247.061	
255	Tiamullin	(55297-95-5)	C28H47NO4S	Positive	494.3299		7.71	119.0166	163.1127	193.1099
256	Tilmicosin	(108050-54-0)	C46H80N2O13	Positive	869.5733		6.69	174.113		
257	Toltrazuril	(69004-03-1)	C18H14F3N3O4S	Negative		424.0573	10.96	no fragmentation		
258	Tralkoxydim	(87820-88-0)	C20H27NO3	Positive	330.2064		9.84	no fragmentation		
				Negative		328.1907	8.1	no fragmentation		
259	Tramadol	(27203-92-5)	C16H25N1O2	Positive	264.1958		4.88	58.0651		
260	Triamterene	(396-01-0)	C12H11N7	Positive	254.1149		4.68	237.0883		
261	Tributylamine	(102-82-9)	C12H27N	Positive	186.2216		5.28	57.0699	74.0964	130.159
262	Triclabendazole	(68786-66-3)	C14H9Cl3N2OS	Positive	358.9574		11.96	60.0548	358.9596	360.9568
				Negative		356.9417	13.26	196.9581	211.9813	341.9203
263	Triclosan	(3380-34-5)	C12H7Cl3O2	Negative		286.9428	12.02	no fragmentation		
264	Trimethoprim	(738-70-5)	C14H18N4O3	Positive	291.1452		4.06	275.1139	230.1162	123.0679
265	Triptyline-Nor	(72-69-5)	C19H21N1	Positive	264.1747		8.51	91.0542	105.0699	117.0699
266	Tylosin	(1401-69-0)	C46H77NO17	Positive	916.5264		7.88	no fragmentation		
267	Tyramine	(51-67-2)	C8H11N1O1	Positive	138.0913		2.28	95.0491	103.0542	121.0648
268	Ursodeoxycholic acid	(128-13-2)	C24H40O4	Negative		391.2843	11.12	no fragmentation		
269	Valproic acid	(99-66-1)	C8H16O2	Negative		143.1067	7.04	no fragmentation		
270	Valsartan	(137862-53-4)	C24H29N5O3	Positive	436.2343		9.21	no fragmentation		
271	Vedaprofen	(71109-09-6)	C19H22O2	Negative		281.1536	11.24	no fragmentation		
272	Venlafaxine	(93413-69-5)	C17H27N1O2	Positive	278.2115		6.14	147.0804	121.0648	58.0651
273	Venlafaxine-O-Desmethyl (Desvenlafaxine)	(93413-62-8)	C16H25N1O2	Positive	264.1958		4.76	58.0651	107.0491	133.0648
274	Zolpidem	(82626-48-0)	C19H21N3O1	Positive	308.1757		5.8	263.1179	236.1308	235.123

4.6 Target screening

A database of approximately 2500 compounds was used for the target screening of the sewage sludge samples in the positive and negative ESI mode. The database contained precursor ions, retention time, adducts and bbCID MS/MS fragments. This information was acquired from the analysis of the standard solutions, which were available in the laboratory, with the bbCID method, or was part of the manufacturer's database, Bruker's Pesticide Screener, which was built with the same bbCID method.

The raw data were processed with Bruker's TASQ Client 1.0 & 1.4 and DataAnalysis 4.3. The TASQ method in TASQ Client created in all samples the Extracted Ion Chromatogram (EIC) of the precursor ion of the compounds included in the database with a mass error window of ±0.005 Da.

Every peak that was detected for a target compound was evaluated according to some parameters that were set to the method and after manual inspection was performed. The first one was the mass accuracy, which refers to the difference between the accurate mass (measured) and the exact mass (theoretical) and is expressed in mDa or ppm. The second one was the retention time shift, which refers to the difference between the measured retention time and the one that is recorded to the database. The last parameter was the isotopic fitting, which refers to the correlation between the theoretical and the experimental isotopic pattern. Its calculation is based on the standard deviation of the masses and the intensities for all isotopic peaks and is expressed by the mSigma value. Lower mSigma value indicates better isotopic fitting.

The screening parameters that were set to the method in positive ESI mode were an area threshold of 2000 counts and an intensity threshold of 500 counts, where in negative ESI mode were an area threshold of 1000 counts and an intensity threshold of 250 counts. Regarding the mass accuracy, peaks having this value higher than 5 mDa were rejected. Regarding the retention time, peaks having this value higher than 0.4 min were also rejected. The mSigma threshold was set to 200. However, this value was only considered as a positive confirmation and not for rejecting peaks, because strong matrix effects combined with low concentration levels of analytes may affect the isotopic pattern results and give a bad mSigma value, although the compound may be present.

In order to confirm the screening results, bbCID MS/MS fragments were examined, as well as adducts (most common adducts: M+Na, $M+NH_4$) in full scan MS.

Apart from the EIC of the precursor ion of a compound, the TASQ method created with the same mass error window the EICs of its adducts and bbCID MS/MS fragments, so the fitting of their chromatographic profiles were evaluated. Except for TASQ Client, DataAnalysis was used for the inspection and evaluation of the bbCID mass spectra.

For the identification and confirmation of the analytes, the Identification Points (IPs) system that has been proposed for HRMS analysis by Bletsou et al. [27] was used. Precursor ion (mass accuracy) and retention time earn together 2 IPs, while isotopic fitting earns 0.5 IP. Furthermore, each of the in-source and bbCID MS/MS fragments (mass accuracy) earns 2.5 IPs.

4.7 Quantification

Quantification of the analytes was mostly based on spiked samples and isotopically labeled compounds and less on standard solutions. It was performed with comparison of the peak areas in the sample with those in a spiked sample or in a standard solution. The concentrations of the analytes in the spiked sample ranged depending on the estimated concentration of every analyte. The sample that was used as a blank sample was chosen based on the number of analytes present and their peak areas, in order to conclude with the "cleanest" sample (minimum number of analytes with low peak areas).

In cases where no standard solution of the analyte was available, semi-quantification was performed. For the semi-quantification where used compounds that were spiked and had similar structure and function groups. The verification whether the compound was suitable for the semi-quantification of the analyte was achieved by comparing their logP value (similar chromatographic behavior).

The concentrations were calculated including the dilution factor, in this case: 5. Moreover, if there was internal standard (deuterated, ^{15}N or ^{13}C enriched) with similar structure, logP and its recovery was satisfactory, the peak area of the analyte was divided by the area of the corresponding I.S. . In order to avoid false positive errors, the chosen I.S. should have same or better recovery than the analyte, otherwise it was

not used. The recovery of the I.S. was calculated dividing the peak area of the I.S. in the sample by the peak area of the I.S. in the matrix matched standard solution. The average recovery of all internal standards in all samples (19) was approximately 70%.

Table 10: The internal standards used

Internal Standard	Recovery mean (±SD) n=19
Positive ESI mode	
Amphetamine-D6	64 (±17)
Atorvastatin-D5	53 (±12)
Cetirizine-D8	74 (±8.6)
Ciprofloxacin-D8	24 (±9.0)
Citalopram-D6	69 (±6.6)
Cocaine-D3	65 (±18)
Codeine-D6	73 (±16)
Diazepam-D5	81 (±15)
Diuron-D6	85 (±15)
Ketamine-D4	72 (±21)
Lamotrigine-^{13}C3,D3	68 (±22)
Metformin-D6	59 (±19)
Metronidazole-D4	89 (±8.1)
Morphine-D3	64 (±17)
Ritonavir-D6	77 (±11)
Sulfadimethoxine-D4	52 (±8.0)
Valsartan-^{13}C5,^{15}N	45 (±17)
Venlafaxine-D6	86 (±12)
Negative ESI mode	
Bisphenol A-D16	74 (±23)
Flunixin-D3	93 (±24)
Saccharine-^{13}C6	86 (±23)

4.8 Quality Control

In order to evaluate the results, QC charts were used for the internal standards. The analysis of the quality control charts indicates if the process is under control. 21 I.S. were spiked in every sample (19 samples), and the QC charts are listed in Annex II.

This is not the usual QC chart where one sample is analyzed multiple times in order to evaluate the repeatability of the method or the stability of the process. In this case, the QC charts indicates the response of every I.S. during the sequence of injections. LC-QTOF-MS does not have stable sensitivity, even during the same sequence of injections. As it can be seen, instrument response drift is present in the final

injections. In order to correct the results in those samples, it was necessary to correct all the peak areas with a corresponding I.S. .

Additionally, the CRM sample that was spiked at a concentration of 200 ng/g d.w. ran 3 times, in the beginning, in the middle and in the end of the sequence, in order to evaluate instrumental %RSDr for quality control. The results are shown in Annex III. The average %RSDr was 7.2% which is acceptable for a wide scope analysis.

CHAPTER 5 – Results & Discussion

5.1 Validation results

As mentioned in chapter 4.5, for the evaluation of linearity, accuracy, matrix effect and detectability of the screening method, a representative validation dataset of 274 compounds was used.

Regarding linearity, the slope, the intercept and the correlation coefficient (r^2) of the standard solution calibration curve and standard addition calibration curve for each compound are presented in Table 11.

In most cases (92%), the correlation coefficient for standard solution calibration curves was >0.9, which represents good fit to the linear regression model. The correlation coefficient for standard addition calibration curves was >0.9 for the 84% of the cases. For some compounds, the calibration curves could not be calculated due to high intensity in the sample.

The results for recoveries and matrix effect are presented in total in Table 12. In addition, the method limits of detection (MLODs) and the method limits of quantification (MLOQs) that were calculated from the data of the calibration curves are presented in Table 12.

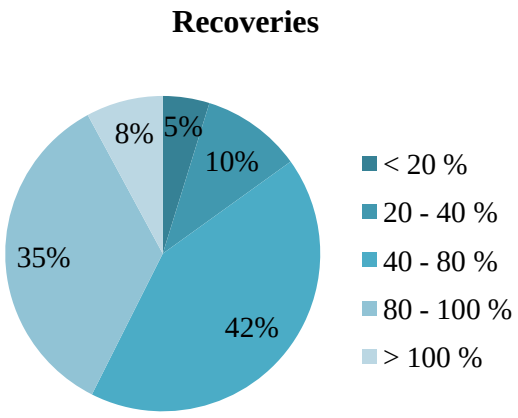


Figure 10: Distribution of recoveries (%) for the validation dataset

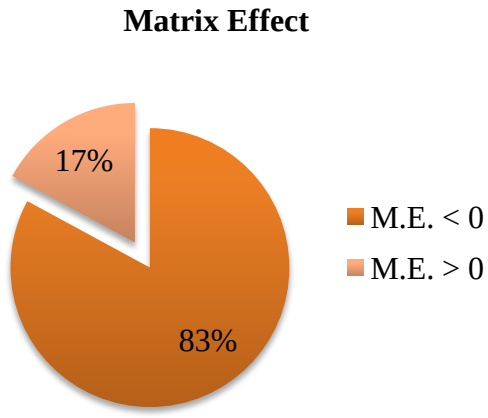


Figure 9: Matrix Effect for the validation dataset

The results from the recovery experiments show that in the most cases recovery was over 70%, which is satisfactory considering that the selected analytes had a wide range of physicochemical properties. The compounds with recovery below 50% were among the most lipophilic ones, almost all of them with log Kow values above 4 such as cannabidiol, closantel, decoquinate, dexamethasone, disulfoton, robenidine and valsartan. The method used is generic in order to obtain information for analytes of various classifications and it is not optimized for specific analytes, as a result recoveries below 50% are acceptable. In the majority of the studied analytes, the signal suppression was ranging from -3 to -195. Only for some analytes, signal enhancement was from 1 to 677.

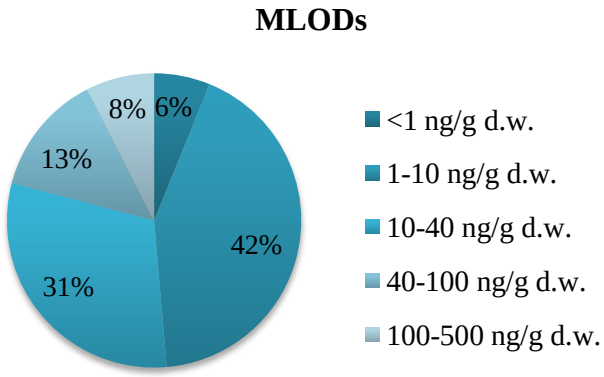


Figure 11: Distribution of MLODs (ng/g d.w.) for the validation dataset

The average MLOD was 30 ng/g, with only few exceptions such as cefaclor, cefadroxil, cefazoline, cefoperazone and chlorbufam, which had MLODs over 300 ng/g, indicating low sensitivity.

Table 11: Linearity: Slope, intercept and correlation coefficient (r^2) of the standard solution calibration curves and standard addition calibration curves ranging from 50-200 ng/g for each compound

		Standard Solution			Spiked Samples		
ESI	**Analyte**	**Range (ng/g)**	**Slope**	**r^2**	**Range (ng/g)**	**Slope**	**r^2**
(+)	3.5.6-Trichloro-2-pyridinol	50-200	7	0.972	50-200	4	0.967
(-)	3.5.6-Trichloro-2-pyridinol	50-200	398	0.999	50-200	640	0.993
(+)	Acephate	50-200	4968	0.942	50-200	3109	0.999
(-)	Acephate	50-200	54	0.999	50-200	50	0.999
(+)	Adenosine	50-200	125	0.995	50-200	606	0.953
(-)	Adenosine	50-200	186	0.998	50-200	25	0.996
(+)	Albendazole	50-200	3195	0.956	50-200	1578	0.997
(+)	Albendazole sulfone	50-200	836	0.903	50-200	639	0.994
(-)	Allopurinol	50-200	29	0.949	50-200	10	0.947
(+)	Alprazolam	50-200	6359	0.962	50-200	3156	0.995
(+)	Ambroxol	50-200	178	0.991	50-200	89	0.873
(+)	Aminobenzimidazole (2-)	50-200	10135	0.999	50-200	9875	0.999
(+)	Aminoheptan (2-)	50-200	901	0.991	50-200	3621	0.999
(+)	Amisulpride	50-200	17688	0.984	50-200	11311	0.998
(-)	Amisulpride	50-200	31	0.973	100-200	36	1
(+)	Amitriptyline	50-200	14284	0.973	50-200	11002	0.998
(+)	Amlodipine	50-200	560	0.865	50-200	432	0.999
(+)	Amphetamine	50-200	5141	0.999	50-200	5578	0.975
(+)	Amphetamine-Methyl (methamphetamine)	50-200	13996	0.979	50-200	12965	0.997
(+)	Ampicillin	50-200	52	0.979	high intensity in the sample		
(+)	Anilofos	50-200	5332	0.981	50-200	5126	0.999
(+)	Aspartame	50-200	281	0.896	50-200	217	0.989
(-)	Aspartame	50-200	67	0.856	50-200	90	0.989
(+)	Atazanavir	50-200	4535	0.997	50-200	3860	0.991
(-)	Atazanavir	50-200	155	0.991	50-200	267	0.999
(+)	Atenolol	50-200	7551	0.951	50-200	6329	0.991
(+)	Atenolol acid (Metoprolol acid)	50-200	223	0.941	high intensity in the sample		
(+)	Atorvastatin	50-200	444	0.967	50-200	280	0.975
(+)	Atrazine	50-200	4324	0.955	50-200	1704	0.939
(+)	Atrazine-desethyl	50-200	2237	0.923	50-200	822	0.998
(+)	Atrazine-desisopropyl	50-200	1515	0.927	50-200	626	0.999
(+)	Azinphos-ethyl	50-200	2210	0.999	50-200	2023	0.993
(+)	Azithromycin	50-200	1341	0.995	50-200	676	0.964
(+)	Benserazide	50-200	87	0.940	50-200	121	0.998
(-)	Benzoic acid	50-200	103	0.998	50-200	64	0.967
(+)	Benzothiazole (BTH)	50-200	57	0.710	high intensity in the sample		
(+)	Benzotriazole (BTR)	50-200	3575	0.966	50-200	2049	0.955
(+)	Benzotriazole -4-Me	50-200	7299	0.919	50-200	7254	0.995
(+)	Benzoylecgonine	50-200	6147	0.943	50-200	4199	0.996
(+)	Biotin	50-200	338	0.922	50-200	162	0.999
(-)	Bisphenol A	50-200	64	0.982	50-200	51	0.999
(+)	Bromazepam	50-200	382	0.977	50-200	253	0.988
(+)	Bromhexine	50-200	1912	0.998	50-200	512	0.703

(+)	Buprenorphine (BN)	50-200	3834	0.977	50-200	2529	1.000
(+)	Caffeine	100-200	138	1.000	50-200	204	0.999
(+)	Cannabidiol	50-200	185	0.981	high intensity in the sample		
(-)	Cannabidiol	50-200	412	0.985	50-200	118	0.989
(+)	Capecitabin	50-200	942	0.999	50-200	745	0.991
(-)	Capecitabin	50-200	202	0.992	50-200	436	0.997
(+)	Capsaicin	50-200	3260	0.911	50-200	2235	0.999
(+)	Carbamazepine	100-400	2473	0.974	100-400	2098	0.996
(-)	Carprofen	100-200	33	1	50-200	54	0.994
(+)	Cathine	50-200	4745	0.968	50-200	5020	0.999
(+)	Cefaclor	50-200	96	0.990	50-200	33	0.915
(+)	Cefadroxil	100-200	58	1	100-200	21	1
(+)	Cefalexin	50-200	128	0.898	50-200	75	0.999
(+)	Cefalonium	100-200	11	1	high intensity in the sample		
(+)	Cefazolin	50-200	84	0.978	high intensity in the sample		
(+)	Cefoperazone	50-200	17	0.995	100-200	8	1
(+)	Cefquinome	50-200	117	0.921	50-200	12	0.987
(-)	Chloramphenicol	50-200	1117	0.992	50-200	1104	0.995
(+)	Chlorbufam	100-200	37	1	100-200	15	1
(+)	Chlordiazepoxide	50-200	2185	0.970	50-200	948	0.995
(+)	Chlorpheniramine	50-200	15499	0.932	50-200	4595	0.998
(+)	Chlorpromazine	100-400	7462	0.973	100-400	4273	0.999
(+)	Cimaterol	50-200	6946	0.983	50-200	4759	0.968
(+)	Cimetidine	50-200	8799	0.942	50-200	4266	0.897
(+)	Ciprofloxacin	50-200	704	0.82	50-200	1519	0.960
(+)	Citalopram	50-200	9766	0.969	50-200	5399	0.991
(+)	Clarithromycin	50-200	11312	0.957	50-200	5114	0.973
(-)	Clazuril	100-200	73	1	50-200	67	0.996
(+)	Clenbuterol	50-200	4555	0.948	50-200	2382	0.992
(+)	Clobazam	50-200	3367	0.964	50-200	2338	0.999
(-)	Clofibric acid	100-200	13	1	50-200	67	0.955
(+)	Clonazepam	50-200	811	0.946	50-200	442	0.999
(+)	Clopidogrel Carbon acid	50-200	883	0.999	50-200	905	0.997
(+)	Clopidol	50-200	562	0.965	50-200	304	0.996
(-)	Closantel	50-200	2744	0.989	high intensity in the sample		
(+)	Clotrimazole	50-200	200	0.978	50-200	446	0.991
(+)	Clozapine	50-200	10275	0.958	50-200	4679	0.997
(+)	Clozapine-Nor	50-200	5615	0.945	50-200	1541	0.999
(+)	Cocaine	50-200	8448	0.984	50-200	5079	0.965
(+)	Codeine	50-200	5790	0.954	50-200	4592	0.999
(+)	Colchicine	50-200	3094	0.945	50-200	2503	0.994
(+)	Corticosterone	50-200	828	0.999	50-200	629	0.979
(-)	Corticosterone	50-200	16	0.955	50-200	36	0.966
(+)	Cortisol F	50-200	369	0.935	50-200	205	0.874
(+)	Cortisone E	50-200	234	0.983	50-200	115	0.913
(+)	Coumaphos	50-200	2526	0.980	50-200	1655	0.996
(+)	Crotamiton	50-200	15119	0.999	50-200	5132	0.999
(+)	Crotethamide	50-200	307	0.837	50-200	399	0.963

(-)	Curcumin (E100)	high intensity in the sample			50-200	2	0.907
(+)	Cytarabin	50-200	159	0.962	50-200	75	0.970
(+)	Danofloxacin	50-200	2095	0.906	50-200	1383	0.991
(+)	Dapsone	50-200	3737	0.959	50-200	569	0.959
(+)	Decoquinate	50-200	9749	0.998	50-200	783	0.986
(+)	Dexamethasone	100-200	46	1	high intensity in the sample		
(+)	Diaveridine	50-200	12957	0.973	50-200	7177	0.998
(+)	Diazepam	50-200	11405	0.931	50-200	9250	0.999
(+)	Diazepam-Nor	50-200	1613	0.963	50-200	745	0.999
(-)	Diclazuril	50-200	232	0.995	50-200	150	0.991
(+)	Diclofenac	50-200	83	0.947	50-200	98	0.991
(-)	Diclofenac	50-200	58	0.919	100-200	71	1
(+)	Didecyldimethylammonium (DADMAC (C10:C10))	50-200	14960	0.999	50-200	1948	0.995
(+)	Difloxacin	50-200	3612	0.958	50-200	919	0.999
(-)	Diflufenzopyr	high intensity in the sample			50-200	12	0.996
(+)	Diltiazem	50-200	6593	0.994	50-200	4272	0.999
(+)	Dimetridazole	50-200	1127	0.964	50-200	898	0.978
(-)	Dinitrophenol-2-4- (DNP)	50-200	2172	0.958	50-200	1394	0.994
(+)	Diphenhydramine	50-200	7967	0.875	50-200	10504	0.955
(+)	Disulfoton	50-200	72	0.838	high intensity in the sample		
(+)	Duloxetine	50-200	2870	0.970	50-200	1557	0.997
(+)	EDDP	50-200	7405	0.964	50-200	5036	0.999
(+)	Efavirenz	50-200	270	0.998	50-200	301	0.964
(-)	Efavirenz	50-200	1966	0.987	50-200	1086	0.991
(+)	Emamectin B1a	50-200	2777	0.991	50-200	1761	0.997
(+)	Emamectin B1b	50-200	119	0.987	50-200	100	0.988
(+)	EME (Ecgonine methyl ester)	50-200	12933	0.980	50-200	1807	0.995
(+)	Enrofloxacin	50-200	4463	0.946	50-200	1130	0.999
(+)	Ephedrine	50-200	10002	0.931	50-200	8060	0.999
(+)	Ephedrine-Nor (Cathin. Phenylpropanolamine)	50-200	4936	0.971	50-200	4995	0.999
(+)	Ephedrine-Pseudo	50-200	6192	0.963	50-200	4813	0.999
(+)	Erythromycin	50-200	5955	0.978	high intensity in the sample		
(+)	Ethopabate	50-200	3402	0.958	50-200	1940	0.987
(+)	Ethoprophos	50-200	14729	0.954	50-200	1568	0.806
(+)	Fenbendazole	50-200	3563	0.972	50-200	1457	0.999
(+)	Fenfluramine	50-200	6081	0.999	50-200	13832	0.996
(+)	Fentanyl	50-200	11764	0.964	50-200	6768	0.994
(+)	Fentanyl-Nor	50-200	6572	0.957	50-200	4327	0.999
(+)	Flonicamid	50-200	560	0.953	50-200	450	0.998
(-)	Flonicamid	50-200	793	0.998	50-200	1159	0.999
(-)	Florfenicol	50-200	936	0.961	50-200	845	0.999
(+)	Fluazuron	50-200	649	0.983	50-200	193	0.997
(-)	Fluazuron	50-200	2058	0.984	50-200	618	0.99
(+)	Flubendazole	50-200	1346	0.945	50-200	728	0.997
(-)	Fludioxonil	50-200	4552	0.981	50-200	5652	0.998
(+)	Flumequine	50-200	6833	0.957	50-200	2959	0.998
(+)	Flunitrazepam	50-200	1915	0.978	50-200	1090	0.999

(+)	Flunitrazepam-7-Amino	50-200	9971	0.949	50-200	1022	0.713
(+)	Flunixin	50-200	7218	0.944	50-200	6363	0.999
(-)	Flunixin	50-200	1383	0.978	50-200	3194	0.997
(+)	Fluoxetine	50-200	14702	0.963	50-200	9667	0.999
(+)	Flurazepam	50-200	5941	0.971	50-200	4058	0.999
(-)	Furosemide	50-200	38	0.907	50-200	81	0.963
(+)	Gabapentin	50-200	842	0.979	50-200	920	0.997
(-)	Gabapentin	50-200	39	0.951	50-200	54	0.999
(-)	Gemfibrozil	50-200	48	0.952	50-200	108	0.997
(-)	GHB (Gamma-Hydroxybutyric acid)	high intensity in the sample			50-200	0,9	0.635
(+)	Haloperidol	100-400	7157	0.957	100-400	4599	0.999
(-)	Hydrochlorothiazide	50-200	61	0.98	50-200	88	0.999
(+)	Ibuprofen	50-200	121	0.857	high intensity in the sample		
(+)	Imipramine	50-200	15099	0.972	50-200	10404	0.998
(+)	Indapamide	50-200	874	0.953	50-200	542	0.997
(-)	Indapamide	50-200	497	0.995	50-200	480	0.999
(+)	Ketamine	50-200	3769	0.999	50-200	6779	0.965
(+)	Ketoprofen	50-200	187	0.510	50-200	401	0.999
(+)	Lacosamide	50-200	6560	0.965	50-200	4615	0.999
(+)	Levamisol	50-200	6982	0.960	50-200	6102	0.999
(+)	Levetiracetam	50-200	777	0.839	50-200	1060	0.963
(+)	Levofloxacin	50-200	1707	0.756	50-200	939	0.997
(+)	Lidocaine	50-200	14887	0.959	50-200	10682	0.999
(+)	Lidocaine-Nor	50-200	9868	0.999	50-200	9136	0.995
(-)	Lidocaine-Nor	50-200	45	0.997	50-200	71	0.999
(+)	Lincomycin	50-200	979	0.979	50-200	1206	0.995
(+)	Lorazepam	50-200	158	0.893	50-200	102	0.982
(+)	LSD	50-200	13311	0.950	50-200	8029	0.999
(+)	Mabuterol	50-200	5627	0.951	50-200	4457	0.992
(+)	MAM (6-O-Monoacetylmorphine)	50-200	5729	0.959	50-200	3278	0.999
(+)	Maprotiline	50-200	9377	0.996	50-200	8433	0.992
(+)	Marbofloxacin	50-200	4283	0.927	50-200	1002	0.988
(+)	MDA	50-200	3933	0.950	50-200	3078	0.995
(+)	MDMA	50-200	6600	0.947	50-200	4394	0.998
(+)	Mebendazole	50-200	1490	0.954	50-200	420	0.980
(+)	Meclofenamic acid	100-200	6	1	100-200	13	1
(-)	Meclofenamic Acid	100-200	13	1	50-200	44	0.989
(+)	Medazepam	50-200	7436	0.949	50-200	5892	0.999
(+)	Mefenamic acid	50-200	35	0.877	high intensity in the sample		
(-)	Mefenamic acid	50-200	136	0.985	50-200	244	0,999
(+)	Meloxicam	50-200	873	0.979	50-200	631	0.992
(-)	Meloxicam	50-200	682	0.978	50-200	1224	0.999
(+)	Mephedrone	50-200	5301	0.973	50-200	9249	0.999
(+)	Metformin	50-200	4434	0.978	50-200	167	0.999
(+)	Methacrifos Peak 2	50-200	2124	0.934	high intensity in the sample		
(+)	Methadone	50-200	12461	0.970	50-200	9342	0.999
(+)	Methamidophos	50-200	4	0.966	50-200	9	0.980
(+)	Methimazole	50-200	379	0.993	high intensity in the sample		

(+)	Methomyl	50-200	1399	0.972	50-200	964	0.998
(+)	Metronidazole	50-200	820	0.929	50-200	620	0.998
(+)	Midazolam	50-200	16523	0.973	50-200	11261	0.999
(+)	Mirtazapine	50-200	10659	0.945	50-200	5186	0.996
(+)	Monocrotophos	50-200	8208	0.957	50-200	5434	0.999
(+)	Morantel	50-200	5467	0.978	50-200	4336	0.999
(+)	Morphine (MOR)	50-200	4640	0.952	50-200	2170	0.999
(+)	Moxidectin	100-200	481	1	high intensity in the sample		
(+)	Naproxen	50-200	106	0.955	100-200	79	1
(+)	Nicotinamide	50-200	1739	0.914	50-200	1360	0.921
(+)	Nicotine-Nor	high intensity in the sample			50-200	80	0.995
(+)	Niflumic acid	50-200	2101	0.953	50-200	1448	0.998
(-)	Niflumic acid	50-200	994	0.991	50-200	2493	0.998
(+)	Nigericin	50-200	16	0.878	50-200	77	0.684
(+)	Nitrazepam	50-200	697	0.979	50-200	197	0.950
(+)	Norfloxacin	50-200	635	0.625	50-200	615	0.996
(+)	Novobiocin	50-200	78	0.998	high intensity in the sample		
(+)	Ofloxacin	50-200	1813	0.948	50-200	1527	0.999
(+)	Olanzapine	50-200	4360	0.969	50-200	1380	0.971
(+)	Omeprazole	50-200	2024	0.971	high intensity in the sample		
(+)	Omethoate	50-200	14765	0.972	50-200	10677	0.999
(+)	Oxamyl	50-200	1508	0.926	50-200	698	0.997
(+)	Oxazepam	50-200	138	0.988	100-200	38	1
(+)	Oxcarbazepine	50-200	1538	0.979	50-200	1089	0.994
(+)	Oxfendazole	50-200	1356	0.974	50-200	654	0.990
(+)	Oxolinic acid	50-200	1089	0.966	50-200	544	0.955
(+)	Oxprenolol	50-200	7047	0.926	50-200	7159	0.999
(+)	Paracetamol	50-200	1177	0.998	50-200	1165	0.915
(+)	Phenylbutazone	50-200	538	0.917	high intensity in the sample		
(-)	Phenylbutazone	50-200	2547	0.994	50-200	378	0.999
(-)	Phenytoin	50-200	76	0.999	50-200	154	0.979
(+)	Phosphate-Triphenyl	50-200	6923	0.944	50-200	4436	0.999
(+)	Phthalate-bis-(2-ethylhexyl). DEHP	50-200	3827	0.808	50-200	4861	0.903
(+)	Pioglitazone	50-200	10346	0.955	50-200	7176	0.998
(-)	Pioglitazone	50-200	316	0.956	50-200	348	0.999
(+)	Prazepam	50-200	7478	0.973	50-200	4938	0.999
(+)	Prednisolone	50-200	610	0.987	50-200	260	0.986
(-)	Prednisolone	50-200	70	0.997	100-200	59	1
(+)	Pregabalin	50-200	360	0.929	50-200	591	0.999
(-)	Pregabalin	50-200	28	0.960	50-200	49	0.981
(+)	Primidone	50-200	586	0.946	50-200	414	0.996
(+)	Progesterone	50-200	2170	0.999	50-200	878	0.996
(+)	Prometryn	50-200	18592	0.962	50-200	15650	0.999
(+)	Propham	50-200	326	0.985	high intensity in the sample		
(+)	Propranolol	50-200	6781	0.968	50-200	5362	0.997
(+)	Quetiapine	50-200	21065	0.964	50-200	12334	0.999
(+)	Ractopamine	50-200	4818	0.955	50-200	4021	0.985
(-)	Rafoxanide	50-200	2699	0.899	high intensity in the sample		

(+)	Ranitidine	50-200	3301	0.998	50-200	543	0.936
(+)	Rifaximin	50-200	2244	0.969	50-200	1062	0.953
(+)	Risperidone	50-200	10007	0.963	50-200	7470	0.980
(+)	Risperidone-9-OH (Paliperidone)	50-200	8473	0.954	100-200	153	1
(+)	Robenidine	50-200	2603	0.980	100-200	167	1
(+)	Ronidazole	50-200	711	0.964	50-200	485	0.998
(-)	Salicylic acid	50-200	313	0.966	50-200	288	0.982
(+)	Sarafloxacin	50-200	1531	0.972	50-200	549	0.996
(+)	Sertraline	50-200	7271	0.926	50-200	4446	0.999
(+)	Sertraline-Nor	50-200	136	0.936	50-200	77	0.982
(+)	Simvastatin	50-200	568	0.999	50-200	271	0.970
(+)	Spinosad A (Spinosyn A)	50-200	6611	0.992	50-200	3926	0.998
(+)	Sulfachloropyridazine	50-200	615	0.953	50-200	123	0.613
(+)	Sulfaclozine	50-200	160	0.807	50-200	61	0.802
(+)	Sulfadiazine	50-200	1745	0.969	50-200	563	0.601
(+)	Sulfadimethoxine	50-200	1845	0.937	50-200	355	0.603
(+)	Sulfadimidine (Sulfamethazine)	50-200	2893	0.987	50-200	684	0.710
(+)	Sulfadoxine	50-200	3503	0.934	50-200	915	0.618
(+)	Sulfaguanidine	50-200	95	0.966	high intensity in the sample		
(+)	Sulfamerazine	50-200	2369	0.93	50-200	564	0.639
(+)	Sulfameter (sulfumetin)	50-200	1040	0.966	50-200	252	0.574
(+)	Sulfamethizole	50-200	562	0.937	50-200	136	0.762
(+)	Sulfamethoxazole	50-200	1369	0.963	50-200	282	0.611
(+)	Sulfamethoxypyridazine	50-200	2364	0.956	50-200	434	0.604
(+)	Sulfamonomethoxine	50-200	667	0.952	50-200	245	0.939
(+)	Sulfamoxole	50-200	1175	0.942	50-200	461	0.756
(+)	Sulfapyridine	50-200	2367	0.963	50-200	544	0.692
(+)	Sulfaquinoxaline	50-200	556	0.912	50-200	107	0.698
(+)	Sulfathiazole	50-200	1053	0.961	50-200	221	0.697
(+)	Sulfisoxazole	50-200	1325	0.976	50-200	321	0.966
(+)	Sulpiride	50-200	11524	0.944	50-200	9224	0.999
(-)	Sulpiride	100-200	22	1	high intensity in the sample		
(+)	Telmisartan	50-200	1715	0.999	50-200	753	0.978
(-)	Telmisartan	100-200	28	1	100-200	6	1
(+)	Temazepam	50-200	3357	0.966	50-200	2334	0.999
(+)	Terbufos	50-200	236	0.950	high intensity in the sample		
(+)	Terbutaline	50-200	1667	0.998	50-200	1340	0.992
(-)	Terbutaline	100-200	14	1	100-200	42	1
(+)	Terbuthylazine	50-200	4655	0.955	50-200	2244	0.974
(+)	Ternidazole	50-200	845	0.961	50-200	539	0.977
(+)	Tetrazepam	50-200	1000	0.972	100-200	135	1
(+)	THC	50-200	460	0.976	high intensity in the sample		
(-)	THC	50-200	464	0.998	50-200	72	0.982
(+)	THC-COOH	50-200	165	0.864	100-200	27	1
(-)	THC-COOH	50-200	102	0.979	50-200	63	0.887
(+)	Theophylline	50-200	199	0.951	50-200	143	0.986
(+)	Thiabendazole	50-200	10384	0.970	50-200	5314	0.997
(-)	Thiabendazole	50-200	25	0.999	50-200	35	0.984

(-)	Thiamphenicol	50-200	395	0.973	50-200	175	0.993
(-)	Thiopental	50-200	612	0.978	50-200	326	0.999
(+)	Tiagabine	50-200	5629	0.973	50-200	3882	0.999
(+)	Tiamulin	50-200	11157	0.969	50-200	6108	0.995
(+)	Tilmicosin	50-200	1963	0.971	50-200	855	0.994
(-)	Toltrazuril	50-200	241	0.981	50-200	227	0.999
(+)	Tralkoxydim	50-200	665	0.959	50-200	200	0.996
(-)	Tralkoxydim	50-200	386	0.984	50-200	267	0.997
(+)	Tramadol	50-200	9102	0.963	50-200	7937	0.998
(+)	Triamterene	50-200	6564	0.948	50-200	4255	0.998
(+)	Tributylamine	50-200	1045	0.619	50-200	12024	0.999
(+)	Triclabendazole	50-200	834	0.974	50-200	192	0.998
(-)	Triclabendazole	50-200	1678	0.973	50-200	540	0.999
(-)	Triclosan	50-200	649	0.921	50-200	555	0.999
(+)	Trimethoprim	50-200	9340	0.979	50-200	7219	0.993
(+)	Triptyline-Nor	50-200	17741	0.924	50-200	11840	0.999
(+)	Tylosin	50-200	566	0.999	100-200	123	1
(+)	Tyramine	50-200	500	0.910	50-200	508	0.956
(-)	Ursodeoxycholic acid	50-200	96	0.991	50-200	177	0.999
(-)	Valproic acid	50-200	47	0.899	50-200	7	0.638
(+)	Valsartan	50-200	307	0.939	high intensity in the sample		
(-)	Vedaprofen	100-200	10	1	high intensity in the sample		
(+)	Venlafaxine	50-200	13080	0.967	50-200	9158	0.999
(+)	Venlafaxine-O-Desmethyl (Desvenlafaxine)	50-200	9494	0.998	50-200	10938	0.996
(+)	Zolpidem	50-200	25961	0.955	50-200	20022	0.998

Table 12: Recoveries, Matrix Effect, MLODs & MLOQs

Compound	Positive ESI						Negative ESI					
	%Recovery			%ME	MLOD ng/g	MLOQ ng/g	%Recovery			%ME	MLOD ng/g	MLOQ ng/g
	50 ng/g	100 ng/g	200 ng/g	100 ng/g			50 ng/g	100 ng/g	200 ng/g	100 ng/g		
3.5.6-Trichloro-2-pyridinol	107	121	96	-57	208.3	625.0	41	63	62	69	5.3	15.9
Acephate	99	93	91	-56	2.9	8.7	85	91	94	-15	50.3	151.0
Adenosine	225	136	58	116	1.2	3.6	56	82	22	132	8.7	26.0
Albendazole	59	67	64	-49	6.9	20.6						
Albendazole sulfone	94	91	79	-48	12.3	36.8						
Allopurinol							64	36	33	56	66.5	199.6
Alprazolam	69	78	73	-51	3.3	10.0						
Ambroxol	27	64	23	40	127.4	382.2						
Aminobenzimidazole (2-)	62	85	93	-35	1.7	5.1						
Aminoheptan (2-)	58	80	88	42	4.6	13.9						
Amisulpride	77	87	85	-44	1.0	3.0		98	109	19	63.2	189.6
Amitriptyline	60	65	72	-29	1.2	3.5						
Amlodipine	101	89	83	159	17.5	52.6						
Amphetamine	43	61	189	-80	0.7	2.2						
Amphetamine-methyl		301	167	-69	0.5	1.4						
Anilofos	69	83	84	-58	2.5	7.5						
Aspartame	117	113	94	156	33.2	99.5	88	111	101	282	32.6	97.8
Atazanavir	52	83	82	-18	4.5	13.4	43	72	87	84	22.5	67.4
Atenolol	95	100	86	-37	1.3	4.0						
Atenolol acid (Metoprolol acid)	203	44	313	-9	80.7	242.0						
Atorvastatin	37	61	54	-22	55.5	166.6						
Atrazine	57	81	62	-55	6.2	18.5						
Atrazine-desethyl	61	62	59	-73	11.5	34.6						
Atrazine-desisopropyl	69	66	61	-69	13.3	40.0						
Azinphos-ethyl	44	74	75	-16	9.5	28.4						
Azithromycin	35	58	49	-12	21.2	63.7						

Benserazide	190	109	84	188	11.8	35.5						
Benzoic acid							50	28	28	48	23,4	70.1
Benzothiazole (BTH)			9	102	96.2	288.7						
Benzotriazole (BTR)	68	76	57	-19	3.7	11.2						
Benzotriazole -4-Me	97	87	94	155	1.3	4.0						
Benzoylecgonine (BECG)			60	-149	0.6	1.7						
Biotin	125	92	78	137	30.8	92.4						
Bisphenol A (BPA)							114	89	73	13	25.4	76.1
Bromazepam	46	48	64	-36	61.1	183.3						
Bromhexine	25	59	26	-12	21.9	65.7						
Buprenorphine (BN)	72	85	89	-47	5.2	15.6						
Caffeine	91	70	62	-25	28.4	85.1						
Cannabidiol			54	-33	113.7	341.2	53	45	52	-54	21.4	64.1
Capecitabin	57	86	84	-29	21.5	64.6	44	79	86	102	12.9	38,7
Capsaicin	99	93	95	176	4.2	12.7						
Carbamazepin	80	90	84	-28	5.0	14.9						
Carprofen							74	65	71	45	44.1	132.3
Cathine	85	88	92	-27	2.2	6.6						
Cefaclor	38	59	43	-20	337.8	1013.4						
Cefadroxil		75	67	-26	367.0	1101.0						
Cefalexine	61	63	68	-46	153.9	461.8						
Cefazoline			35	-26	311.3	933.9						
Cefoperazone		43	36	5	428.0	1284.0						
Cefquinome	38	22	13	35	101.3	303.9						
Chloramphenicol							77	85	78	4	2.3	6.8
Chlorbufam		44	33	93	367.0	1101.0						
Chlordiazepoxide	60	70	66	-52	11.9	35.6						
Chloropromazine	49	54	58	-31	3.0	8.9						
Chlorpheniramine	39	39	37	111	2.0	5.9						
Cimaterol	82	93	73	-31	1.7	5.2						
Cimetidine	57	63	42	-11	1.4	4.3						

Ciprofloxacin	2	3	6	13	19.5	58.5						
Citalopram	64	63	79	-54	2.4	7.3						
Clarithromycin	43	68	59	-48	2.9	8.6						
Clazuril							51	72	73	-8	58.4	175.3
Clenbuterol	91	96	84	-58	3.7	11.2						
Clobazam	83	90	92	-49	4.9	14.6						
Clofibric acid							68	83	64	677	32.4	97.2
Clonazepam	70	72	72	-52	23.4	70.1						
Clopidogrel Carbon acid	55	83	88	-17	19.7	59.0						
Clopidol	107	84	82	-52	23.0	69.0						
Clotrimazole	136	89	59	513	5.5	16.5						
Clozapine	45	50	60	-52	3.1	9.2						
Clozapine-Nor	35	39	41	-61	8.0	23.9						
Cocaine (COC)	96	120	140	-86	0.2	0.7						
Codeine (COD)	79	84	88	-43	2.5	7.5						
Colchicine	105	107	94	-41	3.4	10.2						
Corticosterone	58	89	79	-31	23.2	69.5	86	118	97	54	78.9	236.8
Cortisol F	53	99	69	-56	64.1	192.4						
Cortisone E	99	104	70	-41	50.3	151.0						
Coumaphos	79	88	82	-37	6.3	18.9						
Crotamiton	31	41	43	-47	2.8	8.5						
Crotethamide	31	82	74	-45	69.2	207.5						
Curcumin (E100)							79	40	25		111.3	333.8
Cytarabin	86	76	55	72	66.8	200.4						
DADMAC (C10:C10)	38	43	40	-59	5.6	16.8						
Danofloxacin	30	33	29	42	6.8	20.3						
Dapsone	28	53	17	-63	15.9	47.6						
Decoquinate	36	28	32	-74	11.3	33.8						
Diaveridine	75	86	84	-59	1.6	4.8						
Diazepam	74	89	94	-47	1.5	4.4						
Diazepam-Nor	50	54	54	-45	15.0	44.9						

Diclazuril							61	75	69	-26	19.0	57.1
Diclofenac	117	96	103	-29	87.1	261.2		76	81	10	41.0	123.0
Difloxacin	29	30	32	-44	12.6	37.7						
Diflufenzopyr							28	28	11	20	48.9	146.8
Dimetridazole	69	80	67	-22	10.4	31.3						
Dinitrophenol-2-4							54	52	45	9	1.4	4.1
Diphenhydramine	95	84	59	11	0.5	1.4						
Duloxetine	59	72	71	-47	8.1	24.4						
EDDP	84	91	94	-53	2.3	6.9						
Efavirenz	87	88	66	64	22.0	66.1	16	51	53	200	2.7	8.2
Emamectin B1a	73	88	87	-37	7.1	21.2						
Emamectin B1b	84	76	95	-22	120.3	361.0						
EME	88	88	79	-86	4.7	14.2						
Enrofloxacin	27	27	27	-39	9.1	27.2						
Ephedrine	99	103	107	49	1.4	4.1						
Ephedrine-Nor	85	89	92	-27	2.2	6.7						
Ephedrine-Pseudo	80	85	93	-49	2.6	7.7						
Ethopabate	92	98	83	-52	4.4	13.1						
Ethoprophos	33	31	18	-38	2.2	6.7						
Fenbendazole	65	69	69	-56	7.5	22.4						
Fenfluramine	54	83	85	-25	1.2	3.7						
Fentanyl	53	60	76	-52	2.4	7.1						
Fentanyl-Nor	79	84	87	-51	2.6	7.8						
Flonicamid	93	95	90	-65	21.2	63.5	89	92	90	-46	2.2	6.6
Florfenicol							80	84	86	-25	3.2	9.7
Fluazuron	28	56	63	-69	133.6	400.7	38	44	59	-60	7.1	21.4
Flubendazole	71	75	70	-48	13.3	39.8						
Fludioxonil							84	94	93	-4	0.5	1.5
Flumequine	152	94	74	-69	3.2	9.7						
Flunitrazepam	83	85	89	-51	10.1	30.3						
Flunitrazepam-7-Amino	24	41	24	-69	8.7	26.0						

Flunixin	81	90	91	-37	1.8	5.5	84	90	84	120	0.8	2.3
Fluoxetine	68	76	79	-46	1.2	3.7						
Flurazepam	80	88	95	-52	3.1	9.3						
Furosemide							70	82	65	82	27.0	81.1
Gabapentin	79	86	82	-3	11.4	34.2	46	77	87	12	99.6	298.7
Gemfibrozil							76	82	78	174	23.5	70.5
GHB (Gamma-Hydroxybutyric acid)							32	10	5	-13	56.0	167.9
Haloperidol	60	68	74	-41	1.5	4.4						
Hydrochlorthiazide (NH4)							50	65	76	32	47.9	143.6
Ibuprofen	64	45	2	-5	12.5	37.6						
Imipramine	72	76	86	-44	1.2	3.7						
Indapamine	84	90	84	-48	18.1	54.3	66	69	70	10	5.6	16.8
Ketamine	10	19	41	-195	0.4	1.3						
Ketoprofen	163	112	85	-3	9.1	27.2						
Lacosamide	72	79	87	-49	2.8	8.4						
Levamisol	100	94	91	-33	1.4	4.3						
Levetiracetam	958	106	98	-56	8.9	26.6						
Levofloxacin	186	100	48	199	0.3	0.8						
Lidocaine	77	89	95	-51	1.2	3.6						
Lidocaine-Nor	55	83	84	-32	1.8	5.5	45	82	101	2	94.4	283.3
Lincomycin	118	110	94	-9	6.7	20.1						
Lorazepam	72	81	68	-37	88.8	266.4						
LSD	64	75	79	-53	1.6	4.9						
Mabuterol	96	99	87	-36	1.9	5.8						
MAM (6-O-Monoacetylmorphine)	73	73	77	-111	0.9	2.7						
Maprotiline	47	76	76	-34	2.1	6.4						
Marbofloxacin	26	33	30	-53	11.6	34.8						
MDA	52	74	75	-42	5.1	15.3						
MDMA	103	179	144	-157	0.6	1.8						
Mebendazole	106	39	35	1	4.4	13.2						
Meclofenamic acid		72	61	-27	271.7	815.2	111	100	80	107	35.3	106.0

Medazepam	81	87	91	-45	2.0	6.0						
Mefenamic acid			32	76	148.9	446.6	76	74	76	82	10.3	30.8
Meloxicam	68	78	71	-24	16.4	49.2	77	75	76	72	2.0	6.0
Mephedrone	91	93	95	117	1.1	3.4						
Metformin	11	12	11	-72	50.6	151.8						
Methadone	75	85	91	-42	1.4	4.1						
Methamidophos	74	58	55	-81	253.6	760.7						
Methimazole (Tapazole)			52	-10	30.4	91.2						
Methomyl	86	87	81	-40	9.5	28.6						
Metronidazole	96	90	81	-42	12.6	37.9						
Midazolam	69	79	83	-44	1.1	3.3						
Mirtazapine	48	51	61	-54	2.7	8.0						
Monocrotophos	87	90	90	-52	1.9	5.8						
Morantel	91	87	84	-27	2.1	6.3						
Morphine	82	81	82	-65	4.6	13.9						
Moxidectin			88	-80	144.9	434.6						
Naproxen		93	75	-11	77.3	231.8						
Nicotinamide	149	79	42	128	0.6	1.8						
Nicotine-Nor	109	66	42	61	21.9	65.6						
Niflumic acid	96	84	83	-39	5.7	17.2	84	88	84	133	1.0	3.0
Nigericin		61	87	-98	38.8	116.5						
Nitrazepam	58	64	47	-47	37.2	111.6						
Norfloxacin	15	22	22	34	21.8	65.3						
Novobiocin			68	-29	211.1	633.2						
Ofloxacin	10	1	6	180	4.7	14.0						
Olanzapine	27	30	36	-45	10.9	32.8						
Omethoate	95	96	95	-48	0.9	2.8						
Oxamyl	86	83	75	-43	11.7	35.2						
Oxazepam		72	49	-25	95.6	286.7						
Oxcarbazepine	87	94	85	21	8.7	26.2						
Oxfendazole	77	86	76	-56	14.7	44.2						

Oxolinic acid	81	67	70	-52	15.2	45.7						
Oxprenolol	103	100	94	-37	1.2	3.7						
Paracetamol	100	115	80	1	5.9	17.8						
Phenylbutazone			26	-71	174.8	524.5	3	12	16	-30	38.8	116.5
Phenytoin							95	127	111	46	19.2	57.7
Phosphate-Triphenyl	80	81	81	62	3.1	9.3						
Phthalate-bis-(2-ethylhexyl). DEHP		55	19	-96	1.4	4.1						
Phthalate-Diethyl	89	72	71	-97	2.0	6.0						
Pioglitazone	69	76	85	-46	1.8	5.5	63	74	84	-11	10.4	31.1
Prazepam	71	79	86	-46	2.6	7.9						
Prednisolone	128	115	91	-54	23.2	69.6		110	105	-32	39.1	117.2
Pregabalin	83	82	85	-2	17.5	52.6	64	92	82	30	67.5	202.6
Primidone	80	84	77	-42	22.4	67.3						
Progesterone	85	83	74	-47	9.4	28.2						
Prometryn	77	95	102	-46	0.9	2.8						
Propranolol	84	92	87	-36	2.5	7.5						
Quetiapine	54	65	71	-45	1.1	3.4						
Ractopamine	87	99	85	-34	2.3	7.0						
Ranitidine	8	19	6	71	22.4	67.3						
Rifaximin	53	74	58	-31	10.2	30.5						
Risperidone	1	3	8	-49	15.1	45.3						
Risperidone-9-OH (Paliperidone)		6	2	-60	36.3	108.9						
Robenidine		10	17	-72	124.0	372.1						
Ronidazole	100	90	80	-44	15.0	45.1						
Salicylic acid							113	109	87	-11	5.8	17.4
Sarafloxacin	36	33	37	-40	20.3	60.8						
Sertraline	90	87	85	131	2.0	6.1						
Sertraline-Nor	88	87	69	156	90.8	272.4						
Simvastatin	56	71	58	6	37.8	113.4						
Spinosad A (Spinosyn A)	90	91	100	-51	2.9	8.8						
Sulfachloropyridazine	54	77	37	-56	44.0	131.9						

Sulfaclozine	50	76	47	-58	137.3	411.8						
Sulfadiazine	85	98	43	-49	12.5	37.5						
Sulfadimethoxine	60	78	38	-54	12.2	36.6						
Sulfadimidine	38	66	29	-42	10.6	31.7						
Sulfadoxine	53	79	38	-45	5.9	17.8						
Sulfamerazine	49	69	33	-45	9.5	28.6						
Sulfameter	57	83	42	-47	21.8	65.3						
Sulfamethizole	49	78	33	-58	47.9	143.6						
Sulfamethoxazole	62	82	40	-53	16.9	50.8						
Sulfamethoxypyridazine	45	73	35	-56	14.9	44.8						
Sulfamonomethoxine	29	61	20	-26	41.0	123.0						
Sulfamoxole	44	71	30	-38	14.6	43.9						
Sulfapyridine	48	69	32	-41	10.1	30.2						
Sulfaquinoxaline	60	72	39	-60	45.1	135.4						
Sulfathiazole	50	71	32	-48	24.6	73.8						
Sulfisoxazole	59	84	28	-50	18.6	55.8						
Sulpiride	80	92	94	-49	1.3	4.0						
Telmisartan	51	54	44	-7	10.3	30.8		77	44	50	62.3	186.8
Temazepam	85	89	92	-49	4.8	14.3						
Terbutaline	105	98	82	-14	5.2	15.7		81	94	73	83.3	250.0
Terbuthylazine	56	78	67	-53	5.4	16.2						
Ternidazole	94	93	73	-42	12.8	38.3						
Tetrazepam		15	3	-74	40.0	120.0						
THC			43	-78	51.6	154.8	45	38	48	-69	39.0	117.1
THC-COOH		67	85	-158	49.5	148.6	-1088	234	130	-64	12.7	38.0
Theophylline	75	49	44	7	31.7	95.2						
Thiabendazole	72	71	79	-54	2.1	6.4	64	75	64	48	70.1	210.3
Thiamphenicol							67	84	79	-59	17.5	52.5
Thiopental							80	74	75	-45	7.0	21.1
Tiagabine	73	79	87	-46	3.2	9.6						
Tiamullin	79	89	82	-51	1.7	5.1						

Tilmicosin	72	85	80	-58	13.3	39.9						
Toltrazuril							76	89	94	-25	14.4	43.1
Tralkoxydim	59	49	49	-74	39.3	117.9	35	43	52	-51	15.4	46.2
Tramadol	90	93	88	-29	1.2	3.6						
Triamterene	78	80	75	-43	2.2	6.6						
Tributylamine	65	69	69	49	0.9	2.7						
Triclabendazole	55	53	49	-63	45.2	135.7	41	50	52	-56	6.3	18.9
Triclosan							4	25	36	131	47.0	141.0
Trimethoprim	102	99	85	-38	1.2	3.5						
Triptyline-Nor	99	90	87	119	0.7	2.1						
Tyramine	168	91	70	111	4.5	13.4						
Ursodeoxycholic acid							59	84	94	37	25.2	75.6
Valproic acid							43	27	15	-92	17.6	52.7
Vedaprofen									68	98	125.0	375.0
Venlafaxine	83	92	100	-55	1.4	4.2						
Venlafaxine-O-Desmethyl (Desvenlafaxine)	62	92	96	-30	1.6	4.7						
Zolpidem	76	83	95	-50	0.7	2.0						

5.2 Target screening results

As shown in Table 14[1], 182 compounds were identified and the concentrations of the analytes range from 1 ng/g d.w. (PFOS) to 239 μg/g d.w. (Ofloxacin). The proposed system with identification points by Bletsou et al. for HRMS analysis was used in order to evaluate the level of confidence for the identification of each analyte [27].

Table 13: Proposed Identification Point system in HRMS analysis by Bletsou et al. [27]

Requirements	Identification Points earned
Retention time + Precursor ion (mass accuracy)	2.0
Isotopic fitting (abundances and accuracy of M+1, M+2,…)	0.5
Fragment ions (mass accuracy)	2.5

112 pharmaceutical compounds, 21 pesticides / herbicides / insecticides, 13 metabolites, 8 illicit and psychoactive drugs and other classes of compounds were identified. Regarding the pesticides, the most abundant were Pyrethrin: Jasmolin I, Trinexapac-ethyl and Flurochloridone, as for the surfactants the most abundant was Nonylphenol di-ethoxylates. The majority of the compounds identified were pharmaceutical compounds of various classes and uses, with tha most abundant being the antibiotics Ofloxacin and Ciprofloxacin.

It is interesting that metabolites of pesticides, pharmaceutical compounds, illicit drugs and industrial chemicals were present as shown in Table 14. There are cases, such as Atrazine-desisopropyl, that the parent compound (Atrazine) is not present and cases such as Clopidogrel Carbon acid that the parent compound (Clopidogrel) is present.

As mentioned in chapter 4.2.1, samples from 2 different stages of the WWTP in Ijaiye were collected. There are four different cases that show the differences of the two stages. First of all, the concentration of the analyte after the secondary treatment is higher than the stage of digestion. For example, Azinphos-ethyl and Pyrethrin: Jasmolin I have two times higher concentration after the secondary treatment. That is probably happening because the conditions of the secondary treatment favor the desorption of the analytes (e.g. temperature, air disturbance). Secondly, the

[1] Total quantitative results for the 19 samples are shown in Table 17 (Annex IV)

concentration of the analyte after the secondary treatment is lower than the the stage of digestion. For example, Befunolol and Benzethonium have lower concentration after the secondary treatment. This indicates that the treatment "removes" a part of the analytes. Thirdly, there is a concentration of the analyte after the secondary treatment although at the stage of digestion was absent. For example, Nonylphenol, Tyramine and Bisphenol A belong in this category. And finally, there is a concentration of the analyte after the digestion although at the stage of secondary treatment was absent. In this case belong Amlodipine, Diflufenzopyr and Ephedrine which indicates high removal rates for the analytes from the treatment.

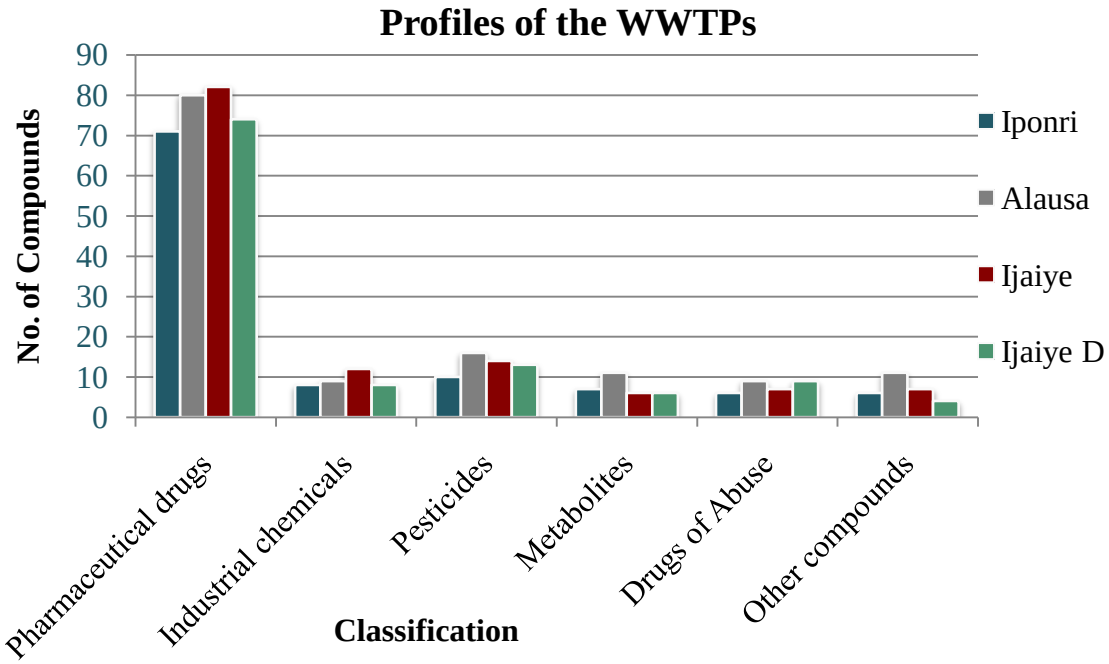


Figure 12: Comparison of the profiles of the WWTPs

It is worth mentioning that each WWTP has a unique profile. The highest number of compounds were found at the Alausa WWTP (with 136 analytes), followed by the Ijaiye WWTP (with 128 analytes) and finally the Iponri WWTP (with 108 analytes). The Ijaiye WWTP samples were "contaminated" mostly by pharmaceutical compounds, taking into consideration that it is a Hospital WWTP. The Alausa WWTP samples (domestic discharges) contained pharmaceutical compounds, but there were also present surfactants in high concentrations, pesticides and illicit drugs. Finally, the Iponri WWTP samples (domestic discharges) had a similar profile to the Alausa WWTP, but the concentrations of the analytes were lower.

Table 14: Mean concentractions (ng/g d.w.) of the analytes of every WWTP – Total: 19 samples

No of samples	IPs	Polarity	Analyte	Compound group	Iponri	Alausa	Ijaiye	Ijaiye D
18	2.5	+	Azinphos-ethyl	Acaricide, insecticide	116	359	7644	2203
5	≥5	+	Chlorpyriphos	Acaricide, insecticide		117		
18	2.5	-	Fluoxymesterone met.	Anabolic agent	6383	8882	7657	8439
18	2.5	-	Mesterolone	Anabolic agent	5419	6730	20371	21228
12	2.5	-	Methenolone	Anabolic agent	1397	1083	736	829
1	2.5	+	Testosterone benzoate	Androgen, Anabolic agent	858			
18	2.5	-	Triclosan	Antiifective agent, Disinfectant	387	7506	590	537
19	≥5	+	Adenine	Biochemical comp., Pharmaceutical drug	6540	14720	16279	17914
1	≥5	+	Apophedrin (Phenylethanolamine)	Biochemical compound	214			
7	2.5	-	Nonylphenol (4-NP)	Degradation product of surfactant Nonoxynol-9, Contraceptive agent		5724	336	
3	2	+	E1 (1-3-5(10)-estratrien-3-ol-17-one estrone)	Estrogen/Hormone		1608	1612	
1	2	+	E1-4-OH (4-hydroxyestrone)	Estrogen/Hormone		1474		
11	2.5	+	Phosphate-Triphenyl	Flame retardant, plasticizer		22	204	125
11	2.5	-	PFOS	Fluorosurfactant	4		1	2
2	≥5	-	Curcumin (E100)	Food additive, dye		741		
19	2.5	+	Galaxolide	Fragrance	58	140	80	53
4	4.5	+	Carbendazim	Fungicide, Nematicide, Antibacterial drug		16		
15	2.5	+	Anilofos	Herbicide, Pesticide	5	12	240	83
5	2.5	+	Diflufenzopyr	Herbicide, Pesticide	13			26
5	4.5	+	Diuron	Herbicide, Pesticide		107		
9	2	+	Fluometuron	Herbicide, pesticide			83	68
9	2	+	Flurochloridone	Herbicide, pesticide			867	850
8	2.5	-	Chloroxuron (Chloroxifenidim)	Herbicide, pesticide	84		115	62
16	2.5	+	DNOC (4.6-dinitro-o-cresol)	Herbicide, Pesticide, Insecticide	73	69	94	125
2	2.5	+	Progesterone	Hormone		199		
1	2.5	-	Corticosterone	Hormone		8058		
19	4.5	+	Methoprene	Hormone, Insecticide	1526	5438	3492	2480

1	4.5	+	Benzylpiperazine	Illicit drug		78		
8	≥5	+	MDA	Illicit drug			221	752
12	2	+	MDAI	Illicit drug		683	162	216
2	2	+	Amphetamine-P-Hydroxy-methyl	Illicit drug, stimulant		20		67
18	2.5	-	Benzenesulfonate-4-hydroxy	Industrial chemicals	29	36	28	39
15	2.5	-	Nonylphenoxy-acetic acid (4-)	Industrial chemicals	53	3434	104	100
2	2	+	Pyrethrins: Cinerin I	Insecticide		4744		
13	2.5	+	Pyrethrins: Jasmolin I	Insecticide	2043	39607	6807	2308
10	2.5	+	Atrazine-desisopropyl	Metabolite of atrazine	110	279		
8	2.5	-	3.5.6-Trichloro-2-pyridinol	Metabolite of chlorpyriphos (& -methyl)	49	720	29	
8	2.5	+	Clopidogrel Carbon acid	Metabolite of clopidogrel			168	332
9	2.5	-	Pyrimidinol	Metabolite of diazinon	80	101		
19	≥5	+	Galaxolidone	Metabolite of galaxolide	1761	566	335	245
8	4.5	+	Lidocaine-Nor	Metabolite of lidocaine			22	23
2	2	+	Pholedrine	Metabolite of methamphetamine		21		69
17	2.5	-	Androsterone-19-nor	Metabolite of nandrolone	872	1233	1154	930
10	2	+	Nicotine-Nor	Metabolite of nicotine, Pesticide, insecticide	61	40		
1	2.5	+	Sertraline-Nor	Metabolite of sestraline		301		
5	2.5	+	THC-COOH	Metabolite of THC		5719		
19	4.5	+	Tramadol-O-Desmethyl	Metabolite of tramadol	61	77	54	67
4	2	+	Tramadol-O-Desmethylnor	Metabolite of tramadol		94		
6	≥5	+	Tyramine	Naturally occuring compound	518	3660	1304	
16	4.5	+	Aminobenzimidazole (2-)	Pesticide	20	100	23	20
18	2.5	+	Trinexapac-ethyl	Pesticide	151	94	4703	6614
5	2.5	-	Fuberidazole	Pesticide, fungicide		32	27	16
6	≥5	-	Bisphenol A	Pesticide, fungicide / Plasticizer		2488	2667	
5	4.5	+	Terbutryn	Pesticide, herbicide		22		
3	2.5	+	Tralkoxydim	Pesticide, herbicide		572	297	
9	2.5	+	Flonicamid	Pesticide, insecticide	108	93		88
1	2.5	+	Crotamiton	Pesticide/ Pharmaceutical drug			111	
19	4.5	+	Adenosine	Pharmaceutical drug	932	1535	670	353

19	≥5	+	Allopurinol	Pharmaceutical drug	382	541	417	302
7	≥5	+	Amiloride	Pharmaceutical drug		31	9	
5	2.5	+	Aminoglutethimide	Pharmaceutical drug		61		
7	≥5	+	Amlodipine	Pharmaceutical drug		88		90
9	4.5	+	Baclofen	Pharmaceutical drug			155	169
19	4.5	+	Befunolol	Pharmaceutical drug	445	460	475	683
7	4.5	+	Capsaicin	Pharmaceutical drug	45		86	91
19	≥5	+	Chlorpheniramine	Pharmaceutical drug	127	200	421	342
19	≥5	+	Cimetidine	Pharmaceutical drug	290	283	229	224
8	≥5	+	Clopidogrel	Pharmaceutical drug			197	205
19	≥5	+	Cyproheptadine	Pharmaceutical drug	155	168	86	99
19	≥5	+	Desloratadine	Pharmaceutical drug	76	161	162	236
19	2.5	+	DADMAC	Pharmaceutical drug	39	2297	156	30
17	2	+	Diosgenin	Pharmaceutical drug	1496	2553	1306	1397
6	2.5	+	Diprophylline	Pharmaceutical drug			676	633
6	2.5	+	Domperidone	Pharmaceutical drug			1144	643
1	≥5	+	Ephedrine	Pharmaceutical drug				140
1	2.5	+	Fenfluramine	Pharmaceutical drug	28			
9	4.5	+	Formoterol	Pharmaceutical drug			103	88
5	4.5	+	Isoxsuprine	Pharmaceutical drug			8	
14	≥5	+	Labetalol	Pharmaceutical drug	31	117	2804	2268
10	≥5	+	Lacosamide	Pharmaceutical drug	58	54	116	90
11	≥5	+	Loratadine	Pharmaceutical drug	108	89	2534	4189
1	≥5	+	MeOT (5-)	Pharmaceutical drug		624		
2	≥5	+	Metformin	Pharmaceutical drug			1778	
11	≥5	+	Metoclopramide	Pharmaceutical drug	47		136	118
18	≥5	+	Miconazole	Pharmaceutical drug	82	27	132	185
6	2.5	+	Obidoxime	Pharmaceutical drug	840		16054	
9	2	+	Oxitropium	Pharmaceutical drug			282	247
9	2.5	+	Papaverine	Pharmaceutical drug			92	113
14	≥5	+	Paracetamol	Pharmaceutical drug	182	606	830	823

8	2	+	Perindopril	Pharmaceutical drug	880	122		
15	≥5	+	Promethazine	Pharmaceutical drug	229	553	6919	7462
17	4.5	+	Propranolol	Pharmaceutical drug	24	38	49	51
19	4.5	+	Rabeprazole	Pharmaceutical drug	12	19	45	62
5	2.5	+	Simvastatin	Pharmaceutical drug		1651		
19	2.5	+	Telmisartan	Pharmaceutical drug	199	153	739	392
11	2.5	-	Gemfibrozil	Pharmaceutical drug	412	626	524	289
18	2.5	-	Ursodeoxycholic acid	Pharmaceutical drug	555	3677	619	408
19	4.5	+	Tramadol	Pharmaceutical drug, analgesic opioid	19	68	59	177
8	4.5	+	Carbamazepine	Pharmaceutical drug, analgesic, anticonvulsant, psycoactive drug	86	41		
19	2	+	Salicylamide	Pharmaceutical drug, analgesic, antiinflammatory	58	85	126	151
18	4.5	-	Salicylic acid	Pharmaceutical drug, analgesic, antiinflammatory, antipyretic	52887	47850	50399	50245
11	4.5	+	Moxifloxacin	Pharmaceutical drug, antibacterial		116	416	694
19	≥5	+	Ciprofloxacin	Pharmaceutical drug, antibiotic	24612	64502	113996	202659
3	4.5	+	Lincomycin	Pharmaceutical drug, antibiotic		122		
9	≥5	+	Nalidixic acid	Pharmaceutical drug, antibiotic			117	130
2	2.5	+	Nigericin	Pharmaceutical drug, antibiotic				1952
19	≥5	+	Ofloxacin	Pharmaceutical drug, antibiotic	17331	21136	130172	238865
9	2.5	-	Chloramphenicol	Pharmaceutical drug, antibiotic			86	88
19	≥5	+	Norfloxacin	Pharmaceutical drug, antibiotic, antiinfective agent	469	1643	235	286
19	≥5	+	Sulfadimidine (Sulfamethazine)	Pharmaceutical drug, antibiotic, antiinfective agent	279	926	1403	1678
5	2	+	Cycloheximide	Pharmaceutical drug, antibiotic, fungicide		80		
1	2.5	-	Topotecan	Pharmaceutical drug, anticancer				396
9	2.5	+	Altretamine	Pharmaceutical drug, anti-cancer	535	49	47	42
2	≥5	+	Levetiracetam	Pharmaceutical drug, anticonvulsant	133	817		
1	2.5	+	Vigabatrin	Pharmaceutical drug, anticonvulsant		3657		
19	≥5	+	Amitriptyline	Pharmaceutical drug, antidepressant	141	82	71	85

10	4.5	+	Harman	Pharmaceutical drug, antidepressant	271	643		
2	2.5	+	Harmine	Pharmaceutical drug, antidepressant	64	123		
1	≥5	+	Protriptyline	Pharmaceutical drug, antidepressant		13		
6	≥5	+	Sertraline	Pharmaceutical drug, antidepressant	58	6302		
4	≥5	+	Triptyline-Nor	Pharmaceutical drug, antidepressant, Metabolite of amitryptiline	21			
19	2	+	Trapidil	Pharmaceutical drug, antidote	96	517	264	268
6	≥5	+	Chlorpromazine	Pharmaceutical drug, antiemetic, psychoactive drug	39		60	
19	2.5	+	Clotrimazole	Pharmaceutical drug, antifungal, antiinfective agent	419	198	614	652
1	2.5	+	Atazanavir	Pharmaceutical drug, anti-HIV (antivirus) agent		75		
19	≥5	+	Lopinavir	Pharmaceutical drug, anti-HIV (antivirus) agent	238	96	254	204
3	≥5	+	Diclofenac	Pharmaceutical drug, antiiflammatory drug			723	276
19	2.5	+	Ibuprofen	Pharmaceutical drug, antiiflammatory drug, analgesic	404	419	276	200
15	≥5	+	Benzododecinium	Pharmaceutical drug, anti-infective agent / Disinfectant	1398	46447	2306	
19	2.5	+	Triclocarban	Pharmaceutical drug, antiinfective agent, Disinfectant	56	21	75	109
4	2.5	-	Celecoxib	Pharmaceutical drug, antiinflammatory	4			
15	4.5	-	Salicylic acid-5-Amino	Pharmaceutical drug, antiinflammatory	264	299	312	308
5	2.5	-	Nabumetone	Pharmaceutical drug, antiinflammatory, analgesic		1839	1370	
3	2.5	-	Meclofenamic Acid	Pharmaceutical drug, antiinflammatory, antipyretic			501	203
19	4.5	+	Mefloquine	Pharmaceutical drug, antimalarial	144	99	48	55
19	≥5	+	Proguanil	Pharmaceutical drug, antimalarial	958	849	1794	2243
19	≥5	+	Pyrimethamine	Pharmaceutical drug, antimalarial	148	164	217	288
7	4.5	+	Quinidine	Pharmaceutical drug, antimalarial		205		138
12	4.5	+	Quinine	Pharmaceutical drug, antimalarial	64	201	362	115

9	≥5	+	Sulfadoxine	Pharmaceutical drug, antimalarial		24	59	53
3	≥5	+	Trimethoprim	Pharmaceutical drug, antimalarial			57	
19	≥5	+	Chloroquine	Pharmaceutical drug, antimalarial, antirheumatic drug	3987	4675	5266	6810
16	≥5	+	Chloroquine-Hydroxy	Pharmaceutical drug, antimalarial, antirheumatic drug	100	81	77	101
3	2.5	+	Melatonin	Pharmaceutical drug, antioxidant		445		
19	2.5	+	Benserazide	Pharmaceutical drug, antiparkinson drug	5489	13259	6432	5344
19	≥5	+	Diphenhydramine	Pharmaceutical drug, antiparkinson drug	427	522	352	338
19	≥5	+	Orphenadrine	Pharmaceutical drug, antiparkinson drug	78	58	107	143
10	2.5	+	Clozapine	Pharmaceutical drug, antipsychotic agent	119		38	
5	≥5	+	Haloperidol	Pharmaceutical drug, antipsychotic agent	138			
5	2.5	+	Quetiapine	Pharmaceutical drug, antipsychotic agent	12			
19	2.5	+	Efavirenz	Pharmaceutical drug, antiviral agent	300	473	1985	2867
6	4.5	+	Ritonavir	Pharmaceutical drug, antiviral agent			42	26
4	2	+	Cytarabin	Pharmaceutical drug, antiviral agent, anticancer drug		3666		
18	≥5	+	Norethisterone acetate	Pharmaceutical drug, contraceptive	1487	5970	1388	1127
18	2.5	-	Norethandrolone met.	Pharmaceutical drug, contraceptive	1520	4092	9434	4166
6	2.5	+	Butoxycaine	Pharmaceutical drug, local anaesthetic	6	72	1092	
9	4.5	+	Lidocaine	Pharmaceutical drug, local anaesthetic			24	27
3	4.5	+	Mebeverine	Pharmaceutical drug, local anaesthetic, antispasmodic		221	116	
12	≥5	+	Azithromycin	Pharmaceutical drug, macrolide antibiotic	348	906	150	201
4	2.5	+	Aminoheptan (2-)	Pharmaceutical drug, nasal decongestant, stimulant		124		
19	≥5	+	Mebendazole	Pharmaceutical drug, nematicide	78	497	138	88
1	2.5	+	Hydrocodone	Pharmaceutical drug, opioid analgesic		16		
13	4.5	+	Morphine-Dihydro	Pharmaceutical drug, opioid analgesic	36	58	234	211
1	4.5	+	Nalbuphine	Pharmaceutical drug, opioid analgesic		105		
15	≥5	+	Pentazocine	Pharmaceutical drug, opioid analgesic	99	169	3169	4011
1	2.5	+	MePPP	Pharmaceutical drug, stimulant	1066			

5	4.5	+	Phentermine	Pharmaceutical drug, stimulant			194	
1	≥5	+	Aminorex	Pharmaceutical drug, stimulant, anorectic (withdrawal: 1968)			83	
17	≥5	+	Albendazole	Pharmaceutical drug, Veterinary drug, Antiparasitic	201	367	74	216
4	2.5	+	Phosphate-triethyl	Plasticizer	49	60	29	
3	≥5	+	Phthalate-Diethyl	Plasticizer		673	197	
10	≥5	+	Phthalate-bis-(2-ethylhexyl). DEHP	Plasticizer	331	675	18	
13	4.5	-	THC	Psychoactive drug	151	304	321	296
4	4.5	+	5-(2-Aminopropyl)indole (5-API)	Psychoactive drug - NPS				342
19	≥5	+	Phenethylamine (2-)	Psychotropic drug	125	207	202	122
1	2.5	+	Seneciphylline-N-oxide	Stimulant		515		
19	≥5	+	Nonylphenol di-ethoxylates (NP2EO)	Surfactant	241	24865	668	234
4	2.5	+	Octylphenol monoethoxylates (OP1EO)	Surfactant		101	85	
14	4.5	+	Nonylphenol mon-ethoxylates (NP1EO)	Surfactant, Contraceptive agent	1659		999	1087
8	4.5	+	Benzethonium	Surfactant, anti-infective agent			88	103
19	2	+	Anatabine	Tobacco ingredient	133	240	126	91
19	≥5	+	Nicotinamide	Vitamin	5964	16672	4072	2866

CHAPTER 6 – Conclusions

HRMS proves to be an amazing technique for the screening of emerging pollutants in environmental chemistry. Target screening is the first step of the workflow for a holistic approach, since detection and identification of many analytes is feasible.

The wide-scope target screening of emerging pollutants was performed in sewage sludge samples from three wastewater treatment plants in Nigeria that were collected in 2015. For the determination of a broad range of compounds, a generic sample preparation was used, as well as a data independent acquisition by UHPLC-HRMS, where with one injection and no pre-selection of analytes, information were obtain for both parent compounds and fragment ions. An analytical methodology for target screening of approximately 2500 emerging contaminants was applied. Retention time combined with accurate mass measurements and fragmentation were used for the screening and identification of the analytes based on an optimized evaluation procedure. The quantitative approach shows the levels of the emerging pollutants that are released into the environment after the wastewater treatment plant. In this study, the difference of the levels of emerging pollutants after the stage of digestion and after the secondary treatment was shown, as well as the different profiles of the three WWTPs.

It is the first time that such extensive target screeing is performed for sewage sludge from Nigeria. 182 compounds were identified and quantified, provided also with IPs and classification. A variety of classes of compounds were identified, such as pharmaceutical drugs, illicit drugs, pesticides, industrial chemicals, hormones, naturally occurring compounds and metabolites of pesticides, pharmaceutical compounds, illicit drugs and industrial chemicals.

ABBREVIATIONS - ACRONYMS

APCI	Atmospheric Pressure Chemical Ionization
CI	Chemical Ionization
DDA	Data Dependent Acquisition
DIA	Data Independent Acquisition
DLLME	Dispersive Liquid-Liquid Microextraction
d.w.	Dry Weight
CAS Number	Chemical Abstracts Service
CEC	Contaminants Of Emerging Concern
EI	Electron Impact
EPA	Environmental Protection Agency U.S.
EPs	Emerging Pollutants
EQSs	Environmental Quality Standards
ESI	Electronspray Ionization
EU Number	European Inventory Of Existing Commercial Substances (EINECS) Or European List Of Notified Chemical Substances (ELINCS)
GC	Gas Chromatography
GC-MS/MS	Gas Chromatography Coupled To Tandem Mass Spectrometry
HRMS	High Resolution Mass Spectrometry
IPs	Identification Points
QC	Quality Control
QuEChERS	Quick Easy Cheap Effective Rugged And Safe
LC	Liquid Chromatography
LC-MS/MS	Liquid Chromatography Coupled To Tandem Mass Spectrometry
NPDES	National Pollutant Discharge Elimination

	System
MAE	Microwave Assisted Extraction
MALDI	Matrix Assisted Laser Desorption Ionization
MS	Mass Spectrometry
MSPD	Matrix Solid Phase Dispersion
PCP	Personal Care Product
PFBS	Perfluoro-N-Butane Sulfonate
PFCAs	Perfluorinated Carboxylic Acids
PFCs	Perfluorinated Compounds
PFDA	Perfluoro-N-Decanoate
PFDoDA	Perfluoro-N-Dodecanoate
PFHpA	Perfluoro-N-Heptanoate
PFHxS	Perfluro-N-Hexane Sulfonate
PFHxA	Perfluoro-N-Hexanoate
PFNA	Perfluoro-N-Nonanoate
PFOA	Perfluoro-N-Octanoate
PFOS	Perfluorooctane Sulfonate
PFSAs	Perfluoroalkane Sulfonates
PFUnDA	Perfluoro-N-Undecanoate
PLE	Pressurized Liquid Extraction
POPs	Persistent Organic Pollutants
RP	Reversed Phase
Rt	Retention Time
SBSE	Stir Bar Sorptive Extraction
SFE	Supercritical Fluid Extraction
SPE	Solid Phase Extraction
TOF-MS	Time Of Flight Mass Spectrometry
UHPLC	Ultra High Performance Liquid Chromatography
USE	Ultrasonic Extraction
WWTPs	Wastewater Treatment Plants

ANNEX I

Table 15: Directive 2013/39/EU - includes a list of priority substances and establishes EQSs for various types of surface waters and biota [11]

No.	Name of substance	CAS number	AA[2]-EQS	AA-EQS	MAC[3]-EQS	MAC-EQS	EQS
			Inland surface waters	Other surface waters	Inland surface waters	Other surface waters[4]	Biota[5]
1.	Alachlor	15972-60-8	0.3	0.3	0.7	0.7	
2.	Anthracene	120-12-7	0.1	0.1	0.1	0.1	
3.	Atrazine	1912-24-9	0.6	0.6	2	2	
4.	Benzene	71-43-2	10	8	50	50	
5.	Brominated diphenylethers	32534-81-9			0.14	0.014	0.0085
6.	Cadmium and its compounds (depending on water hardness classes)	7440-43-9	≤ 0.08 (Class 1)	0.2	≤ 0.45 (Class 1)	≤ 0.45 (Class 1)	
			0.08 (Class 2)		0.45 (Class 2)	0.45 (Class 2)	
			0.09 (Class 3)		0.6 (Class 3)	0.6 (Class 3)	
			0.15 (Class 4)		0.9 (Class 4)	0.9 (Class 4)	
			0.25 (Class 5)		1.5 (Class 5)	1.5 (Class 5)	
6a.	Carbon-tetrachloride	56-23-5	12	12	not applicable	not applicable	
7.	C10-13 Chloroalkanes	85535-84-8	0.4	0.4	1.4	1.4	
8.	Chlorfenvinphos	470-90-6	0.1	0.1	0.3	0.3	
9.	Chlorpyrifos (Chlorpyrifos-ethyl)	2921-88-2	0.03	0.03	0.1	0.1	
9a.	Cyclodiene pesticides:						
	Aldrin	309-00-2	Σ = 0.01	Σ = 0.005	not applicable	not applicable	
	Dieldrin	60-57-1					
	Endrin	72-20-8					
	Isodrin	465-73-6					
9b.	DDT total	not applicable	0.025	0.025	not applicable	not applicable	

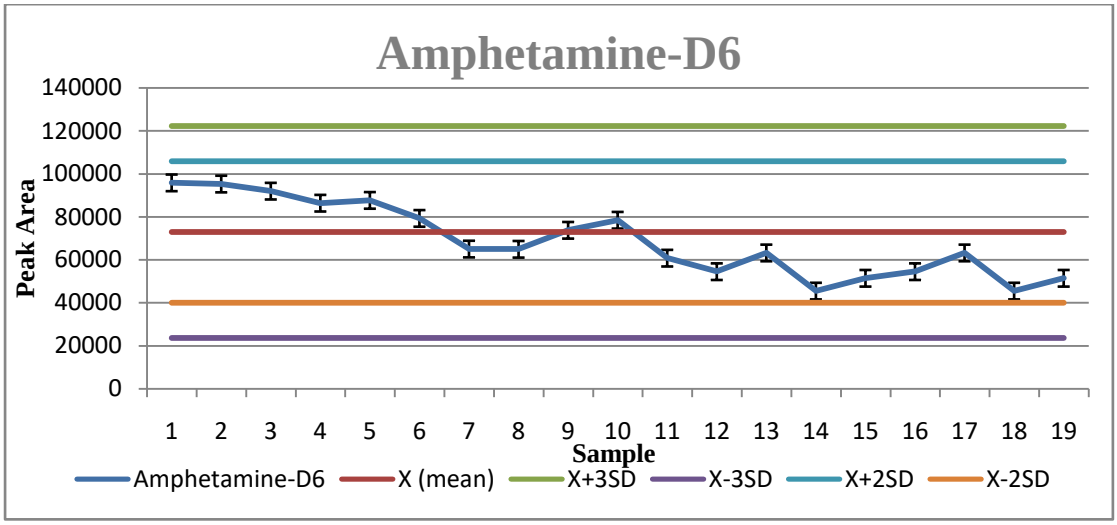
[2] AA: annual average
[3] MAC: maximum allowed concentration
[4] Unit: µg/L (waters)
[5] Unit: µg/kg wet weight (biota)

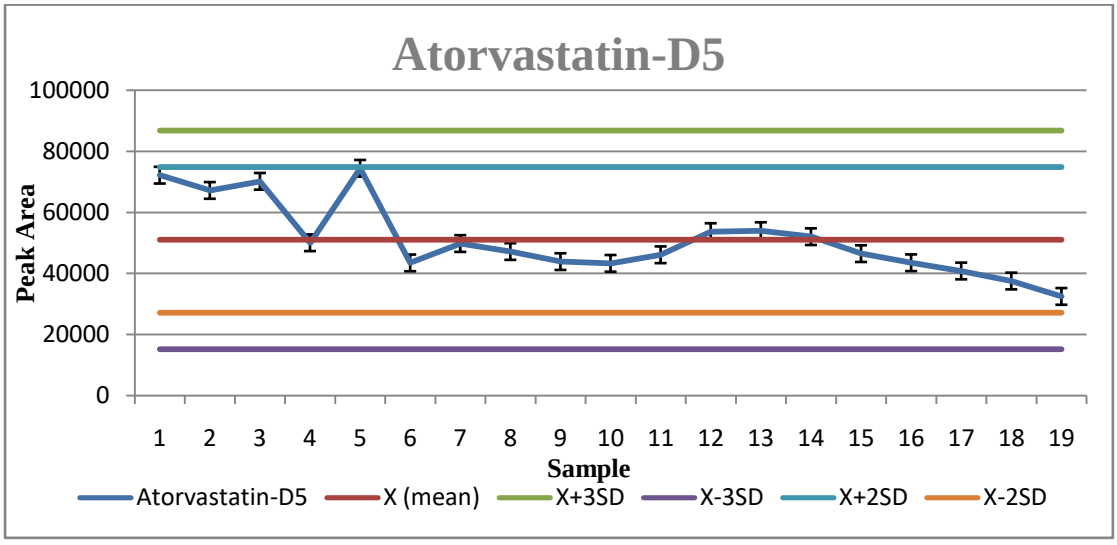
	para-para-DDT	50-29-3	0.01	0.01	not applicable	not applicable	
10.	1,2-Dichloroethane	107-06-2	10	10	not applicable	not applicable	
11.	Dichloromethane	75-09-2	20	20	not applicable	not applicable	
12.	Di(2-ethylhexyl)-phthalate (DEHP)	117-81-7	1.3	1,3	not applicable	not applicable	
13.	Diuron	330-54-1	0.2	0.2	1.8	1.8	
14.	Endosulfan	115-29-7	0.005	0,0005	0.01	0.004	
15.	Fluoranthene	206-44-0	0.0063	0.0063	0.12	0.12	30
16.	Hexachloro-benzene	118-74-1			0.05	0.05	10
17.	Hexachloro-butadiene	87-68-3			0.6	0.6	55
18.	Hexachloro-cyclohexane	608-73-1	0.02	0.002	0.04	0.02	
19.	Isoproturon	34123-59-6	0.3	0.3	1	1	
20.	Lead and its compounds	7439-92-1	1.2	1.3	14	14	
21.	Mercury and its compounds	7439-97-6			0.07	0.07	20
22.	Naphthalene	91-20-3	2	2	130	130	
23.	Nickel and its compounds	7440-02-0	4	8.6	34	34	
24.	Nonylphenols (4-Nonylphenol)	84852-15-3	0.3	0.3	2	2	
25.	Octylphenols ((4-(1,1′,3,3′-tetramethylbutyl)-phenol))	140-66-9	0.1	0.01	not applicable	not applicable	
26.	Pentachloro-benzene	608-93-5	0.007	0.0007	not applicable	not applicable	
27.	Pentachloro-phenol	87-86-5	0.4	0.4	1	1	
28.	Polyaromatic hydrocarbons (PAH)	not applicable	not applicable	not applicable	not applicable	not applicable	
	Benzo(a)pyrene	50-32-8	1.7×10^{-4}	1.7×10^{-4}	0.27	0.027	5
	Benzo(b)fluor-anthene	205-99-2	*Dir 2013/39/EC	*Dir 2013/39/EC	0.017	0.017	*Dir 2013/39/EC
	Benzo(k)fluor-anthene	207-08-9	*Dir 2013/39/EC	*Dir 2013/39/EC	0.017	0.017	*Dir 2013/39/EC
	Benzo(g,h,i)-perylene	191-24-2	*Dir 2013/39/EC	*Dir 2013/39/EC	8.2×10^{-3}	8.2×10^{-4}	*Dir 2013/39/EC
	Indeno(1,2,3-cd)-pyrene	193-39-5	*Dir 2013/39/EC	*Dir 2013/39/EC	not applicable	not applicable	*Dir 2013/39/EC
29.	Simazine	122-34-9	1	1	4	4	
29a.	Tetrachloro-ethylene	127-18-4	10	10	not applicable	not applicable	
29b.	Trichloro-ethylene	79-01-6	10	10	not applicable	not applicable	
30.	Tributyltin compounds (Tributyltin-cation)	36643-28-4	0.0002	0.0002	0.0015	0.0015	

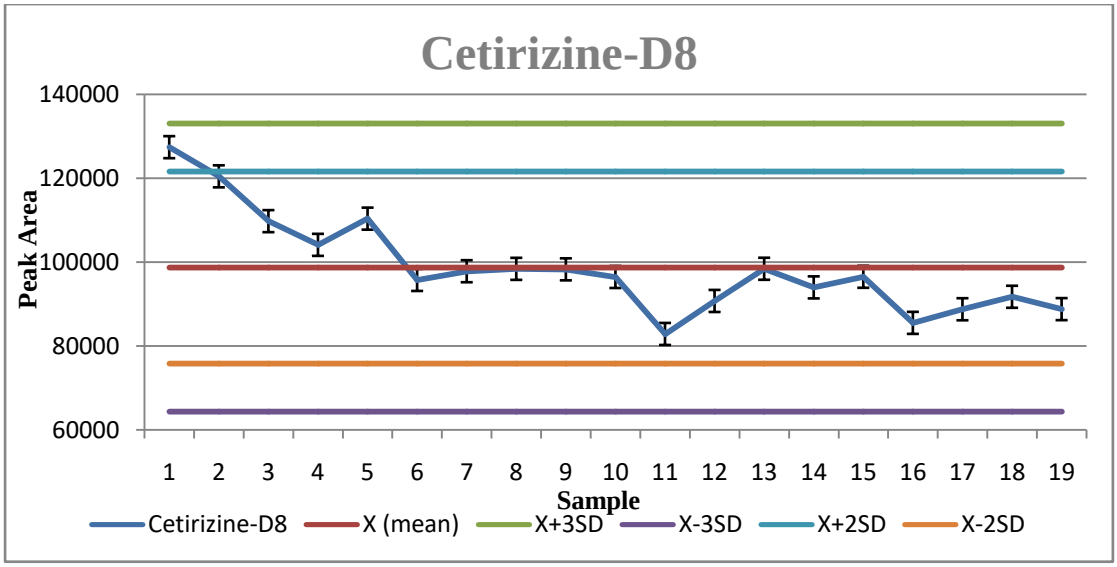
31.	Trichloro-benzenes	12002-48-1	0.4	0.4	not applicable	not applicable	
32.	Trichloro-methane	67-66-3	2.5	2.5	not applicable	not applicable	
33.	Trifluralin	1582-09-8	0.03	0.03	not applicable	not applicable	
34.	Dicofol	115-32-2	1.3×10^{-3}	3.2×10^{-5}	not applicable	not applicable	33
35.	Perfluorooctane sulfonic acid and its derivatives (PFOS)	1763-23-1	6.5×10^{-4}	1.3×10^{-4}	36	7.2	9.1
36.	Quinoxyfen	124495-18-7	0.15	0.015	2.7	0.54	
37.	Dioxins and dioxin-like compounds	*Dir 2000/60/EC			not applicable	not applicable	Sum of PCDD+PCDF+PCB-DL 0,0065 $\mu g.kg^{-1}$ TEQ
38.	Aclonifen	74070-46-5	0.12	0.012	0.12	0.012	
39.	Bifenox	42576-02-3	0.012	0.0012	0.04	0.004	
40.	Cybutryne	28159-98-0	0.0025	0.0025	0.016	0.016	
41.	Cypermethrin	52315-07-8	8×10^{-5}	8×10^{-6}	6×10^{-4}	6×10^{-5}	
42.	Dichlorvos	62-73-7	6×10^{-4}	6×10^{-5}	7×10^{-4}	7×10^{-5}	
43.	Hexabromocyclododecane (HBCDD)	*Dir 2000/60/EC	0.0016	0.0008	0.5	0.05	167
44.	Heptachlor and heptachlor epoxide	76-44-8/ 1024-57-3	2×10^{-7}	1×10^{-8}	3×10^{-4}	3×10^{-5}	6.7×10^{-3}
45.	Terbutryn	886-50-0	0.065	0.0065	0.34	0.034	

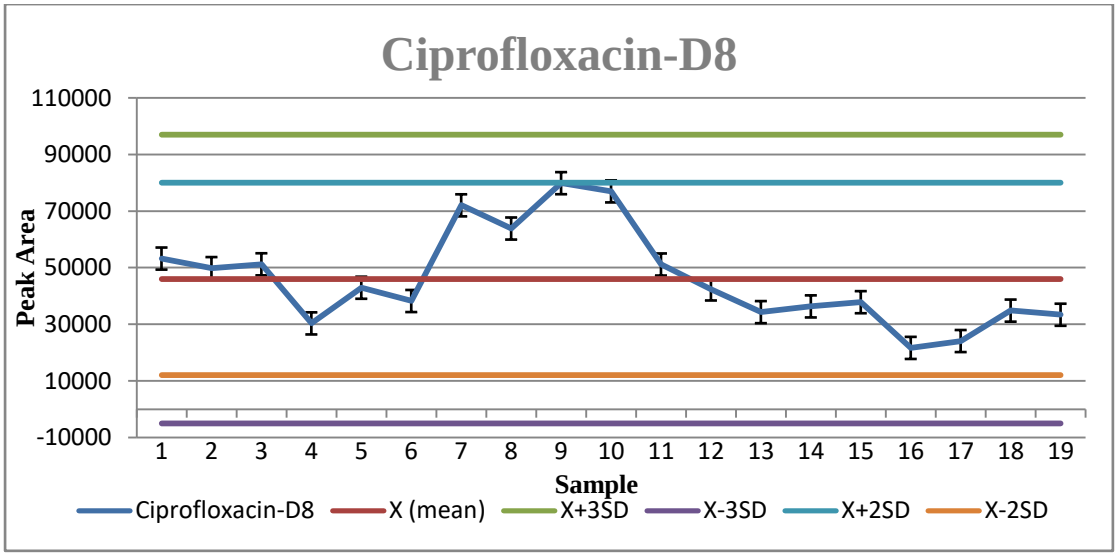
ANNEX II

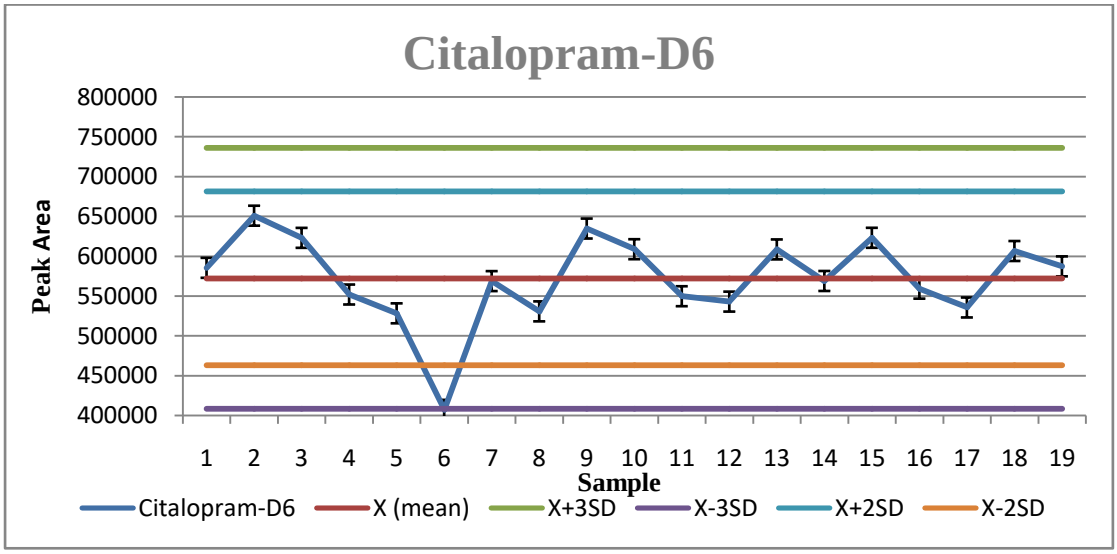
QC Charts – Positive ESI

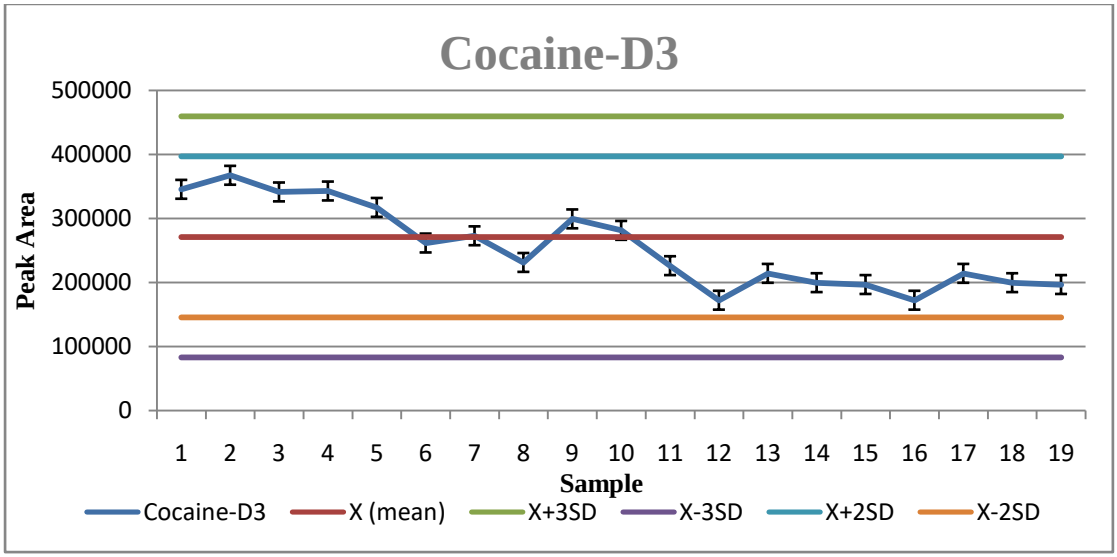







Ciprofloxacin-D8
110000
90000
70000
50000
30000
10000
-10000
Peak Area
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Ciprofloxacin-D8
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD


Citalopram-D6
800000
750000
700000
650000
600000
550000
500000
450000
400000
Peak Area
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Citalopram-D6
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD


Cocaine-D3
500000
400000
300000
200000
100000
0
Peak Area
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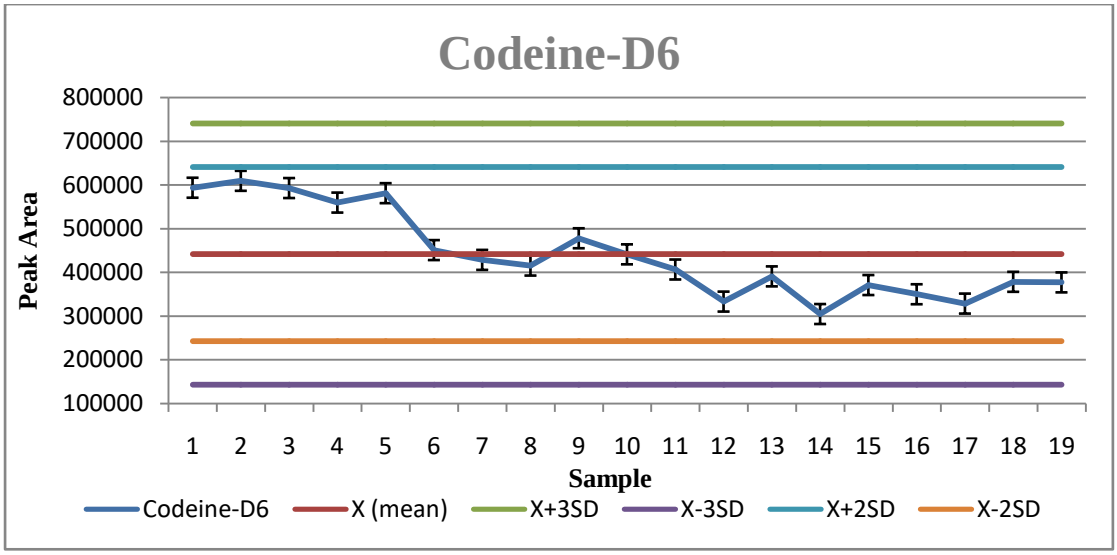
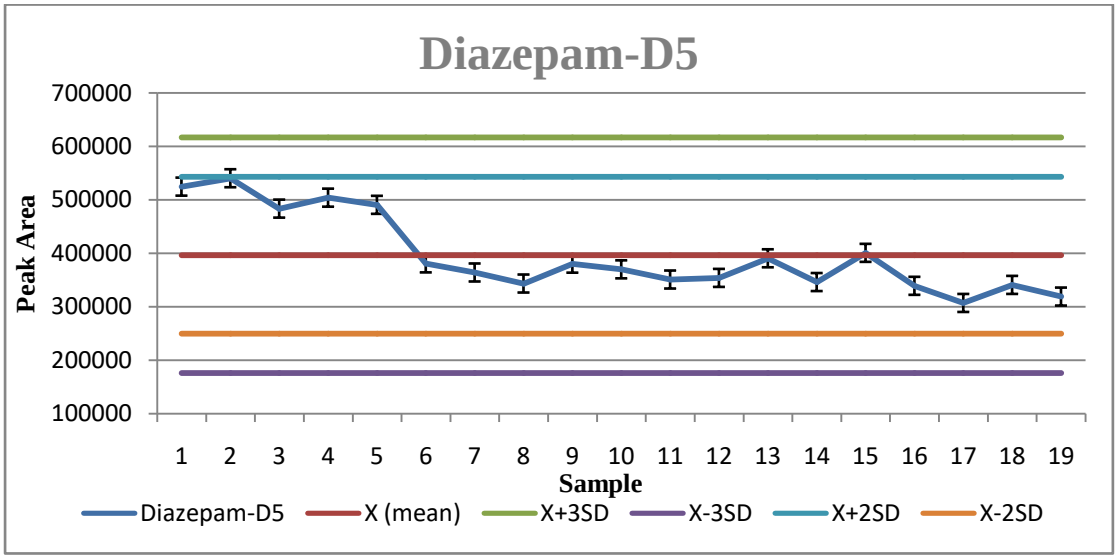
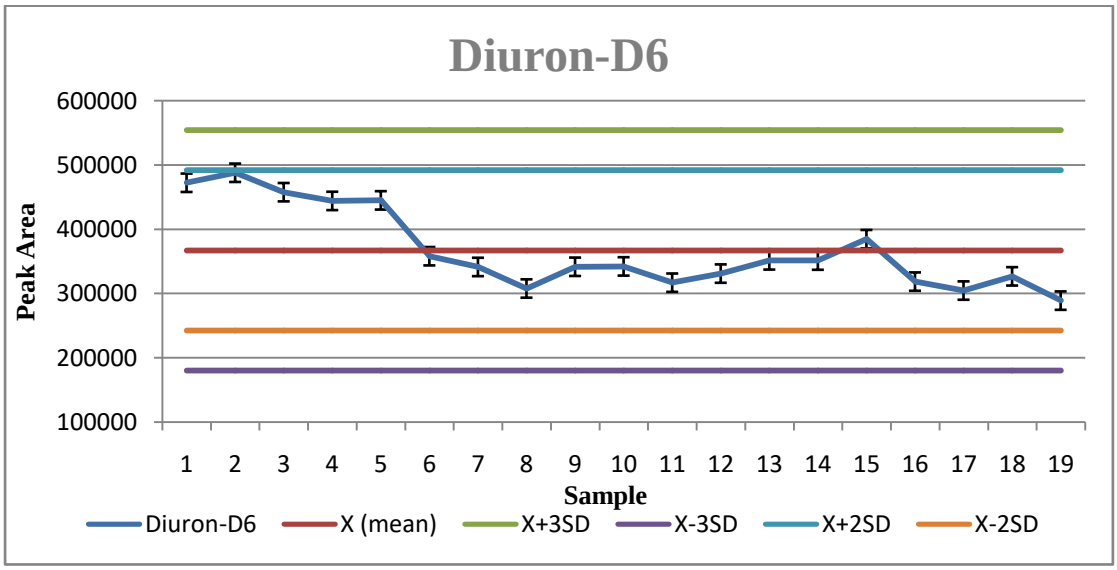
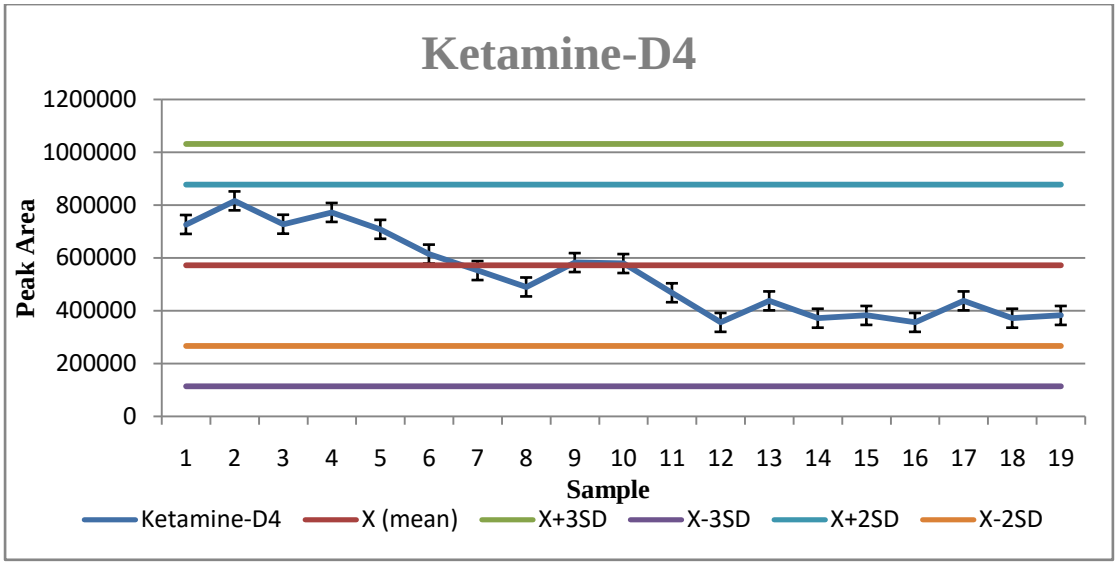
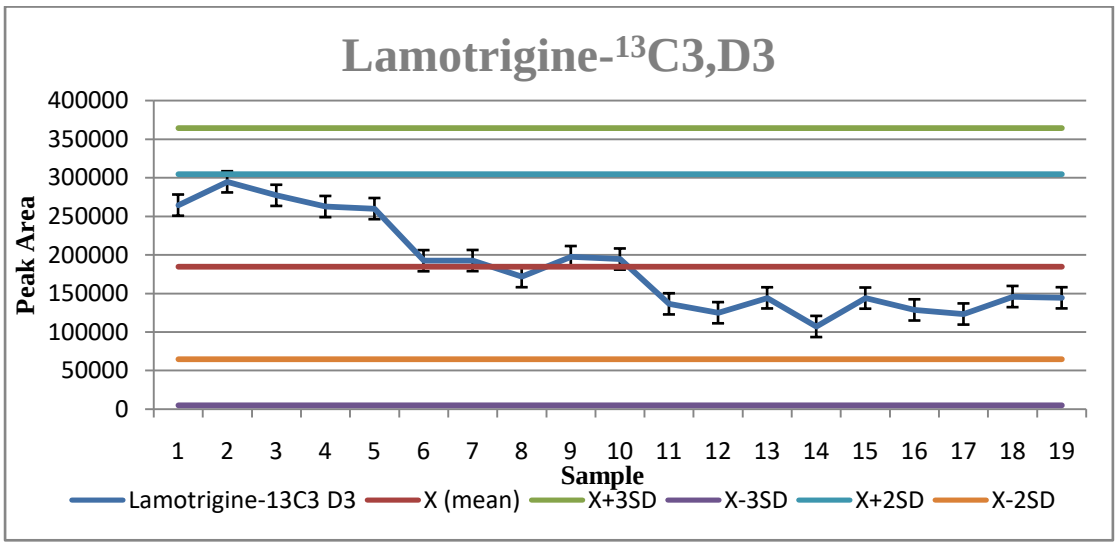
Sample
Cocaine-D3
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD

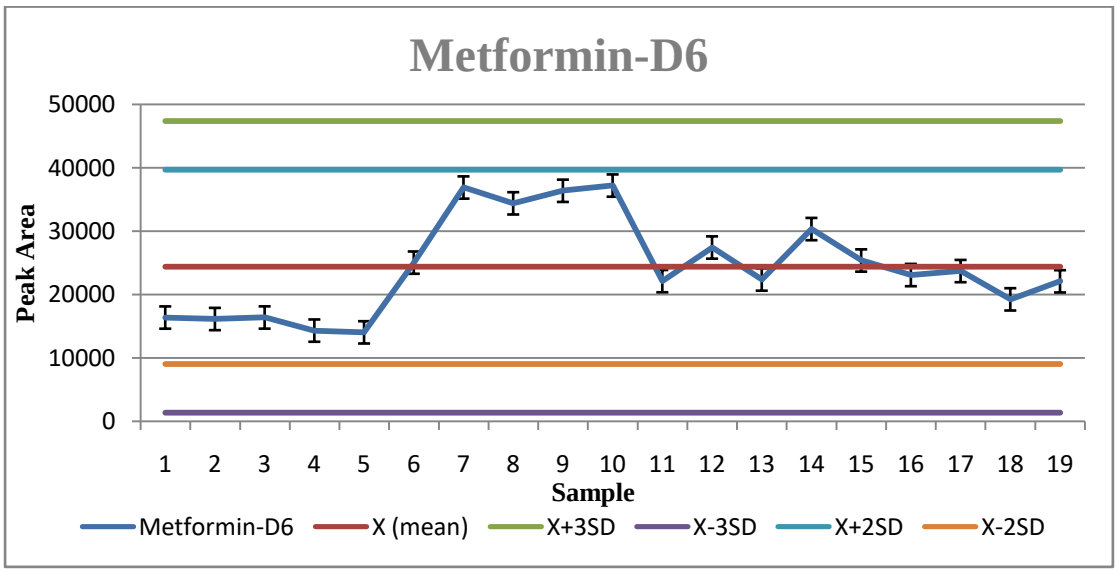

Codeine-D6
Peak Area
800000
700000
600000
500000
400000
300000
200000
100000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Codeine-D6
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD

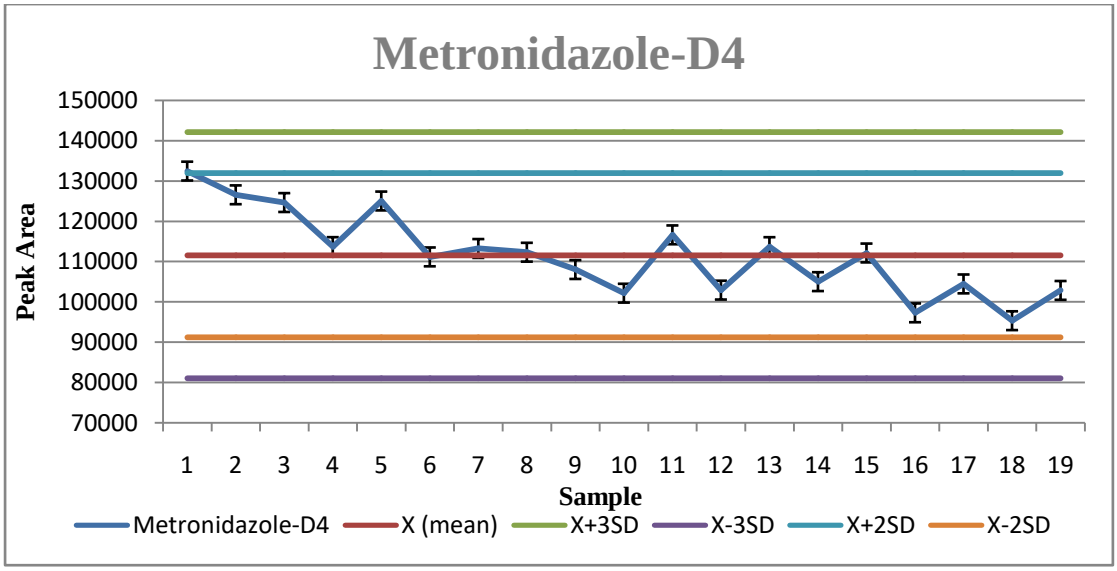

Diazepam-D5
Peak Area
700000
600000
500000
400000
300000
200000
100000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Diazepam-D5
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD

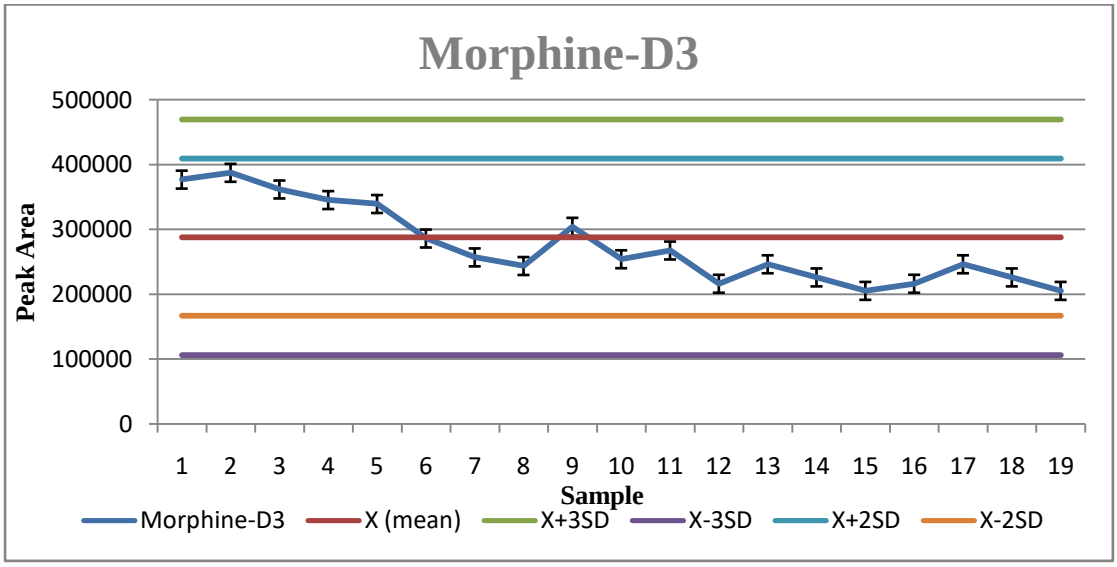

Diuron-D6
Peak Area
600000
500000
400000
300000
200000
100000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Diuron-D6
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD

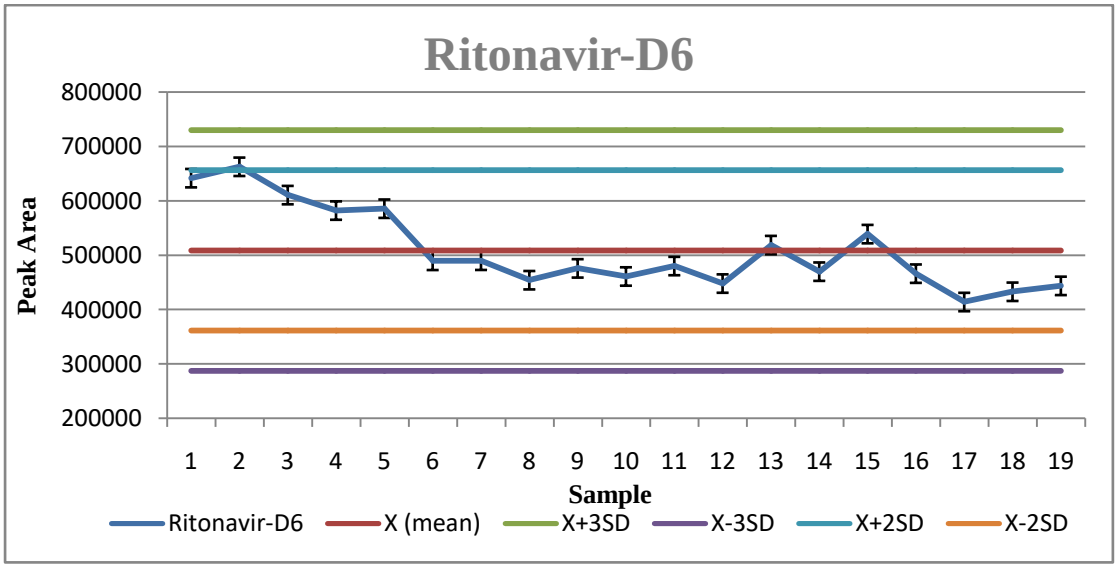

Ketamine-D4
1200000
1000000
800000
600000
400000
200000
0
Peak Area
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Ketamine-D4
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD

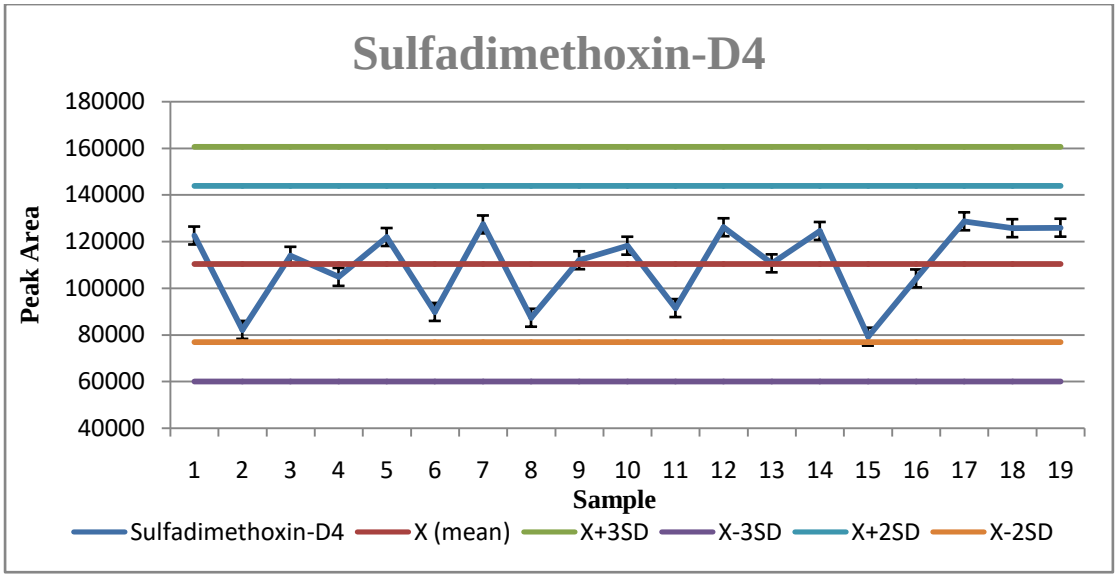

Lamotrigine-13C3,D3
400000
350000
300000
250000
200000
150000
100000
50000
0
Peak Area
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Lamotrigine-13C3 D3
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD


Metformin-D6
50000
40000
30000
20000
10000
0
Peak Area
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Metformin-D6
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD


Metronidazole-D4
Peak Area
150000
140000
130000
120000
110000
100000
90000
80000
70000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Metronidazole-D4
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD


Morphine-D3
Peak Area
500000
400000
300000
200000
100000
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Morphine-D3
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD


Ritonavir-D6
Peak Area
800000
700000
600000
500000
400000
300000
200000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Ritonavir-D6
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD


Sulfadimethoxin-D4
Peak Area
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160000
140000
120000
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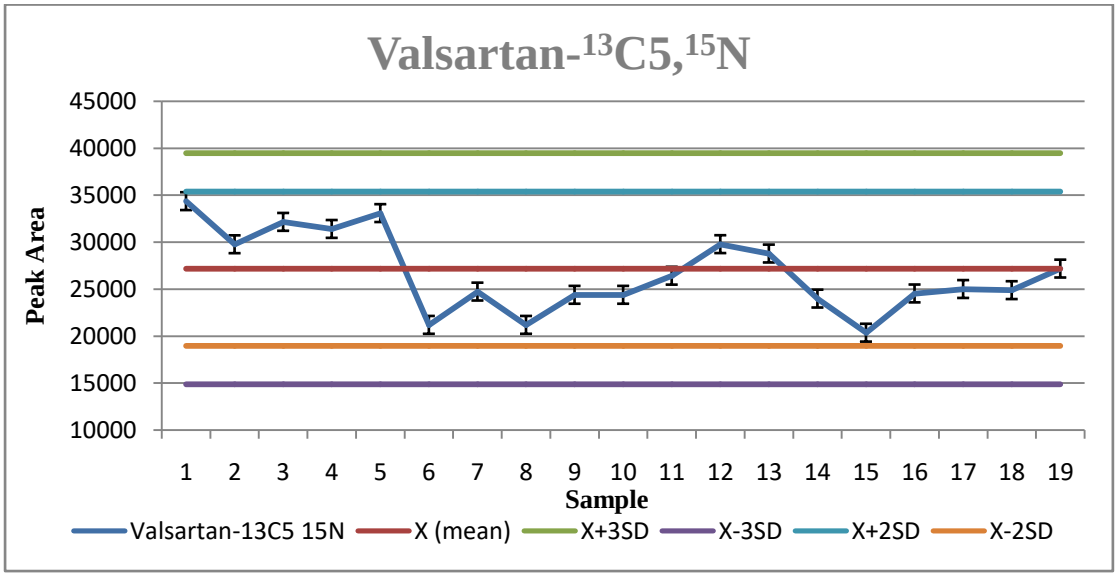
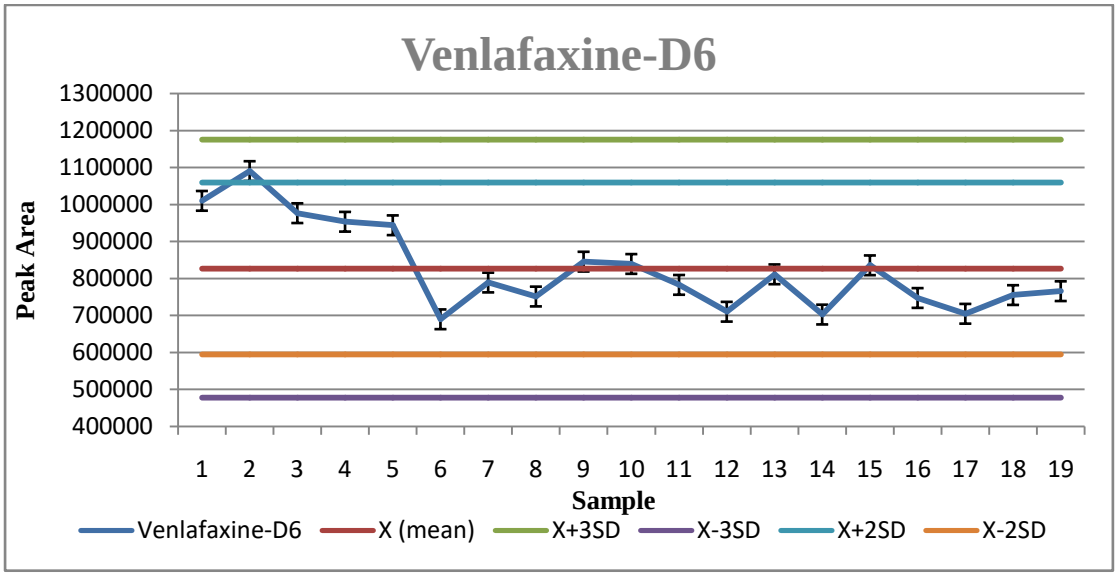
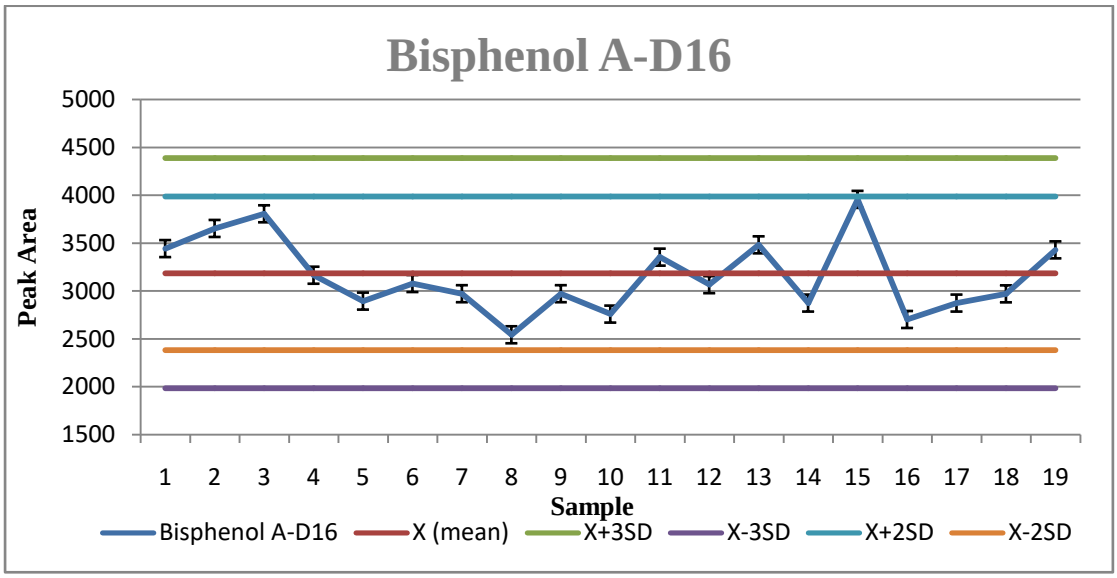
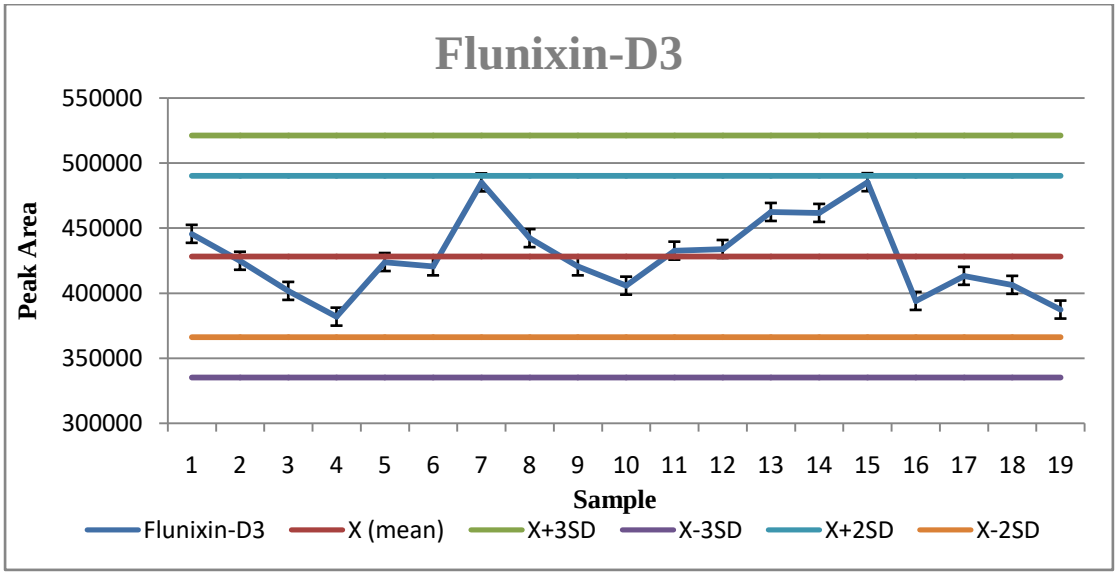
80000
60000
40000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Sulfadimethoxin-D4
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD

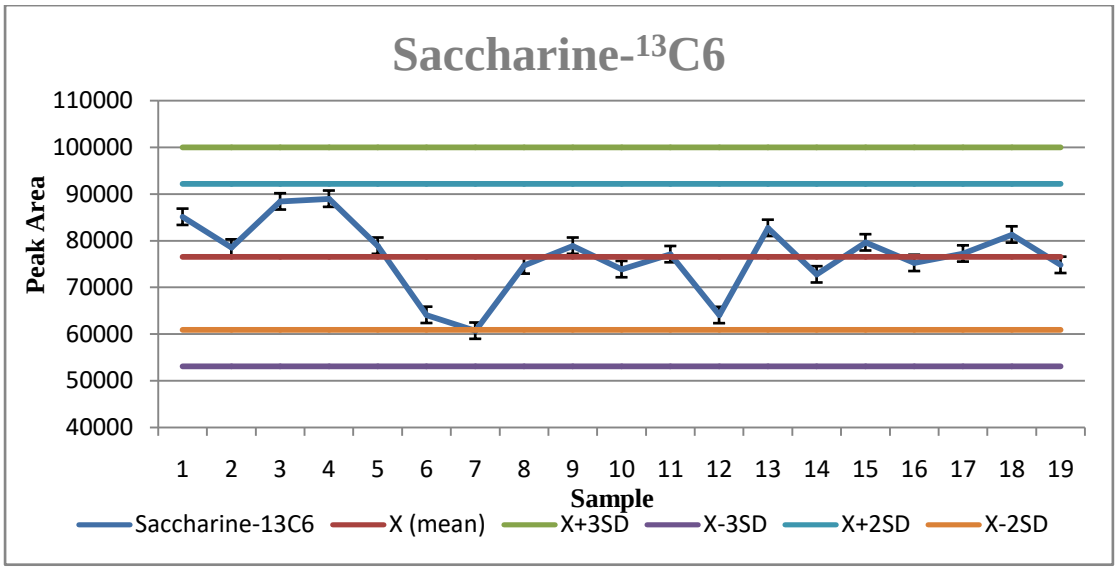

Valsartan-13C5,15N
Peak Area
45000
40000
35000
30000
25000
20000
15000
10000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Valsartan-13C5 15N
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD


Venlafaxine-D6
Peak Area
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sample
Venlafaxine-D6
X (mean)
X+3SD
X-3SD
X+2SD
X-2SD

QC Charts – Negative ESI







ANNEX III

Table 16: CRM spiked with following analytes, for quality control - No. of runs=3

N=3, CRM SPIKED 200 ng/g	Mean (peak area)	SD	% RSD
Acephate	899950	40004	4.4
Albendazole	368429	13064	3.5
Albendazole sulfone	181295	10223	5.6
Alprazolam	994013	7885	0.8
Ambroxol	19983	2040	10
Amisulpride	2827149	95307	3.4
Amitriptyline	2609066	100085	3.8
Amphetamine	1535778	68387	4.5
Amphetamine-Methyl	3333750	197159	5.9
Atenolol	1703320	110857	6.5
Atenolol acid (Metoprolol acid)	11505	1550	14
Atorvastatin	50786	3168	6.2
Atorvastatin-d5	49051	4167	8.5
Atrazine	528750	22790	4.3
Azithromycin	25815	4097	16
Benzotriazole (BTR)	544109	9666	1.8
Benzoylecgonine	1991897	99461	5.0
Bromazepam	69798	3039	4.4
Bromhexine	165587	8775	5.3
Buprenorphine (BN)	584988	45415	7.8
Caffeine	46362	8655	19
Cannabidiol	24863	2951	12
Carbamazepine	1233427	12773	1.0
Cathine	1058496	13300	1.3
Cefaclor	9750	626	6.4
Cefadroxil	6700	1982	30
Cefalexin	20022	1433	7.2
Cefazolin	7328	1734	24
Cefoperazone	2425	377	16
Cefquinome	3870	258	6.7
Cetirizine-D8	64955	3078	4.7
Chlorbufam	6520	1157	18
Chlordiazepoxide	246910	13604	5.5
Chlorpromazine	1536829	58569	3.8
Cimaterol	1425868	83968	5.9
Cimetidine	1877882	104310	5.6
Ciprofloxacin	55232	15417	30
Ciprofloxacin-D8	15697	2163	14
Citalopram	1049014	63089	6.0
Citalopram-D6	537052	37179	6.9
Clarithromycin	1328465	38277	2.9

Clenbuterol	708097	26319	3.7
Clobazam	638665	13348	2.1
Clonazepam	130539	2134	1.6
Clopidol	88907	10792	12
Clozapine	183727	8963	4.9
Clozapine-Nor	53682	1682	3.1
Cocaine	3624605	185544	5.1
Codeine	1085466	36481	3.4
Codeine-d6	604702	33113	5.5
Colchicine	769639	38866	5.0
Cortisol F	65748	3109	4.7
Cortisone E	35985	526	1.5
Coumaphos	521812	25760	4.9
Danofloxacin	131887	3237	2.5
Dapsone	297037	8202	2.8
Decoquinate	250236	14255	5.7
Dexamethasone	6746	1001	15
Diaveridine	2024298	109885	5.4
Diazepam	2205307	67775	3.1
Diazepam-Nor	167227	17649	11
Diazepam-d5	526526	21141	4.0
Diclofenac	21653	2523	12
DADMAC (C10:C10)	485551	29072	6.0
Difloxacin	75847	3013	4.0
Diltiazem	1102672	45108	4.1
Dimetridazole	174263	7454	4.3
Diuron-d6	465127	6853	1.5
Duloxetine	446454	22934	5.1
EDDP	1438450	85359	5.9
EME (Ecgonine methyl ester)	428149	19715	4.6
Emamectin B1a	557894	48566	8.7
Emamectin B1b	26907	7668	29
Enrofloxacin	41767	1762	4.2
Ephedrine-Nor	1057885	29516	2.8
Ephedrine-Pseudo	1209412	18943	1.6
Erythromycin	8560	1457	17
Ethopabate	570383	26761	4.7
Ethoprophos	1453270	40973	2.8
Fenbendazole	424134	26236	6.2
Fentanyl	637786	35670	5.6
Fentanyl-Nor	1092497	47945	4.4
Fluazuron	90758	7570	8.3
Flubendazole	194684	10499	5.4
Flumequine	1506757	26046	1.7
Flunitrazepam	308840	26432	8.6
Flunitrazepam- 7-Amino	432233	14234	3.3

Flunixin	1718929	27672	1.6
Fluoxetine	2411713	113778	4.7
Flurazepam	952585	70965	7.4
Gabapentin	158914	5645	3.6
Haloperidol	2224123	191175	8,6
Imipramine	2426915	128682	5.3
Ketamine	2466421	69228	2.8
Ketoprofen	104477	8798	8.4
LSD	1666086	94542	5.7
Lacosamide	1237585	55244	4.5
Lamotrigine-13C3 d3	311344	8897	2.9
Levamisol	1761877	100408	5.7
Levetiracetam	205626	9410	4.6
Lidocaine	2636583	30844	1.2
Lincomycin	270193	7962	2.9
Lorazepam	24276	3207	13
MAM (6-O-Monoacetylmorphine)	1365463	42939	3.1
MDA	727413	28674	3.9
MDMA	2129269	185162	8.7
Mabuterol	1219109	53147	4.4
Marbofloxacin	66629	5959	8.9
Mebendazole	215574	11491	5.3
Meclofenamic acid	6655	938	14
Medazepam	1465575	61800	4.2
Mefenamic acid	24926	1244	5.0
Meloxicam	201625	10643	5.3
Methacrifos	17145	2035	12
Methadone	2209589	110125	5.0
Methamidophos	2731	1993	73
Methomyl	269661	6512	2.4
Metronidazole	132609	6506	4.9
Metronidazole-D4	107430	1067	1.0
Midazolam	2566068	129242	5.0
Mirtazapine	73752	14012	19
Monocrotophos	1526532	54164	3.5
Morantel	1095965	65677	6.0
Morphine (MOR)	423251	18391	4.3
Moxidectin	37819	7157	19
Naproxen	29096	2778	9.5
Niflumic acid	493437	10237	2.1
Nigericin	26931	6054	23
Nitrazepam	70176	15719	22
Norfloxacin	68973	3737	5.4
Novobiocin	15416	812	5.3
Ofloxacin	80829	11616	15
Omethoate	2825819	59646	2.1

Oxamyl	222768	23084	10
Oxazepam	16851	1227	7.3
Oxcarbazepine	140558	7290	5.2
Oxfendazole	213882	10353	4.8
Oxolinic acid	244454	2795	1.1
Oxprenolol	2091260	136947	6.5
Paracetamol	247381	8386	3.4
Phenylbutazone	18688	2830	15
Phthalate-bis-(2-ethylhexyl) DEHP	3773425	171233	4.5
Pioglitazone	1683004	85857	5.1
Prazepam	1247901	22404	1.8
Prednisolone	82995	6896	8.3
Pregabalin	90071	1329	1.5
Primidone	116705	3517	3.0
Progesterone	296022	7587	2.6
Prometryn	3392781	113767	3.4
Propham	16421	5042	31
Propranolol	1546319	66311	4.3
Quetiapine	459503	35484	7.7
Ractopamine	1192145	41767	3.5
Ranitidine	28275	4263	15
Rifaximin	142521	12429	8.7
Risperidone	21447	1388	6.5
Ritonavir-D6	784707	42014	5.4
Robenidine	54680	1135	2.1
Ronidazole	124288	5940	4.8
Sarafloxacin	67390	5569	8.3
Simvastatin	59462	5046	8.5
Spinosad A (Spinosyn A)	1081323	55736	5.2
Sulfachloropyridazine	74558	1536	2.1
Sulfaclozine	30628	4228	14
Sulfadiazine	231726	4759	2.1
Sulfadimethoxin-d4	163276	1734	1.1
Sulfadimethoxine	235732	11253	4.8
Sulfadimidine (Sulfamethazine)	320169	5960	1.9
Sulfadimidine-d4	194243	8506	4.4
Sulfadoxine	479568	7695	1.6
Sulfamerazine	265277	11370	4.3
Sulfameter (sulfumetin)	120319	7512	6.2
Sulfamethizole	64348	2768	4.3
Sulfamethoxazole	167013	424	0.3
Sulfamethoxypyridazine	243524	3369	1.4
Sulfamonomethoxine	88833	4594	5.2
Sulfamoxole	167031	1261	0.8
Sulfapyridine	223778	5290	2.4
Sulfaquinoxaline	67090	2491	3.7

Sulfathiazole	100636	2834	2.8
Sulfisoxazole	160327	5645	3.5
Sulpiride	2195922	85605	3.9
THC	45014	3242	7.2
THC-COOH	43211	6501	15
Temazepam	639776	12960	2.0
Terbufos-sulfoxide	6395	1012	16
Terbutaline	326525	14096	4.3
Terbuthylazine	627299	21562	3.4
Ternidazole	145980	7572	5.2
Tetrazepam	6532	2563	39
Theophylline	42094	1883	4.5
Thiabendazole	1379164	43156	3.1
Tiagabine	974650	44877	4.6
Tiamulin	1698542	126416	7.4
Tilmicosin	244474	19278	7.9
Topiramate	5554	1569	28
Tramadol	2033225	109633	5.4
Triamterene	1300403	31637	2.4
Tributylamine	2694868	74172	2.8
Triclabendazole	98308	2908	3.0
Trimethoprim	2403732	88402	3.7
Tylosin	28705	7096	25
Valsartan	74231	4864	6.6
Valsartan-13C5 15N	49075	6218	13
Venlafaxine	2219371	168468	7.6
Venlafaxine-D6	1087856	60337	5.5
Zolpidem	4629640	401095	8.7
	Average %RSD		**7.2**

ANNEX IV

Table 17: Target screening quantitative results – Concentrantions in ng/g d.w.

	Analyte	Iponri 1	Iponri 2	Iponri 3	Iponri 4	Iponri 5	Alausa 1	Alausa 2	Alausa 3	Alausa 4	Alausa 5	Ijaiye 1	Ijaiye 2	Ijaiye 3	Ijaiye 4	Ijaiye 5	Ijaiye D2	Ijaiye D3	Ijaiye D4	Ijaiye D5
+	5-(2-Aminopropyl)indole (5-API)																384	454	365	167
+	Adenine	6287	6085	6479	7259	6593	8130	15565	19007	18839	12057	12100	16492	12878	25075	14852	18778	17993	15535	19349
+	Adenosine	1145	783	907	793	1032	1375	1381	2114	1458	1349	599	747	627	894	481	350	345	359	356
+	Albendazole	159	42	43	47	715	120	498	482	300	433	26	22	174			29	44	757	32
+	Allopurinol	489	358	337	370	358	479	647	506	559	514	415	514	348	424	383	332	236	335	306
+	Altretamine		30			1039			63	39	44	37	56				40	44		
+	Amiloride						21	33	33	34	34		8		10					
+	Aminobenzimidazole (2-)	22	19	20	16	22	42	150	125	119	66	26		20			24	20	17	20
+	Aminoglutethimide						89	61	41	59	57									
+	Aminoheptan (2-)							240	95	92	69									
+	Aminorex Isomer 1														83					
+	Amitriptyline	160	141	144	129	133	83	84	89	76	76	64	76	78	67	69	84	93	83	80
+	Amlodipine						83	73	97		101						100	96	75	
+	Amphetamine-P-Hydroxy-methyl								20									67		
+	Anatabine	63	147	123	226	104	348	479	80	151	142	139	73	81	169	166	105	104	47	107
+	Anilofos		<LOQ	5		7		14	13		9	99	257	254	371	217	93	82	78	78
+	Apophedrin (Phenylethanolamine)		214																	
+	Atazanavir						75													
+	Atrazine-desisopropyl	143	80	132	85	109	364	360	194	217	260									
+	Azinphos-ethyl	140	102	100	74	164		402	353	389	291	2874	8166	7202	13710	6267	2518	2245	2152	1898
+	Azithromycin	386	332	403	314	307	638	1157	921	927	890				150		201			
+	Baclofen											210	142	180	85	160	160	150	179	186
+	Befunolol	411	431	451	440	491	413	453	486	481	466	524	476	543	429	403	689	704	626	714
+	Benserazide	6133	4189	5275	5959	5889	6771	16218	14464	12744	16099	5047	7517	5574	8633	5387	5550	5142	5170	5515
+	Benzethonium											89		91	96	76	113	109	92	99
+	Benzododecinium	1798	1196	1379	1374	1241	70136	43366	41456	30998	46279	2366	6201	2154	135	676				
+	Benzylpiperazine						78													
+	Butoxycaine	<LOQ			10		63				81				2162	21				
+	Capsaicin		45									79	92	87			76	97	99	
+	Carbamazepine	107	61	91	77	95		36	43	45										
+	Carbendazim							19	20	16	10									
+	Chloroquine	4087	3743	3890	3937	4276	4895	4836	4699	4480	4463	4938	5421	5459	5876	4637	6665	7666	5776	7133
+	Chloroquine-Hydroxy	108	98	92	93	109				64	98	86	93	76	65	63	90	113	93	108
+	Chlorpheniramine	154	129	138	89	124	153	204	263	177	205	395	471	407	373	459	332	426	336	274

+	Chlorpromazine	35	43			39							53	49	77					
+	Chlorpyriphos						54	128	137	115	150									
+	Cimetidine	307	238	307	275	323	317	321	343	229	207	221	241	266	210	209	186	232	221	259
+	Ciprofloxacin	21768	21929	20704	32562	26099	59116	67558	82534	60625	52677	75772	112669	129030	152816	99693	243972	226440	160578	179644
+	Clopidogrel												209	144	318	119	236	217	196	173
+	Clopidogrel Carbon acid											245	102	155		170	408	344	316	260
+	Clotrimazole	473	417	455	302	448	192	230	197	195	177	614	627	647	698	487	713	747	530	617
+	Clozapine	160	113	126	75	118						35	50	49	35	22				
+	Crotamiton															111				
+	Cycloheximide						41	9	169	131	49									
+	Cyproheptadine	167	173	144	151	140	206	166	174	143	152	78	79	97	91	85	106	106	95	89
+	Cytarabin							3575	4196	3538	3354									
+	DNOC (4.6-dinitro-o-cresol)	75	73	87	53	76			62		76	104	109	79	89	87	132	105	134	128
+	Desloratadine	71	75	78	74	85	118	194	160	167	166	126	176	138	211	160	270	243	191	239
+	Diclofenac														723		266			286
+	DADMAC	47	37	32	29	47	3856	2791	3014	923	903	141	410	158	31	38	61	37	9	12
+	Diflufenzopyr		15	13	11													25	28	
+	Diosgenin	1088	1625	1621	1486	1661	2013	2674	4417	2116	1543	1230	1367	1080	1592	1260	1525			1269
+	Diphenhydramine	417	387	463	390	479	539	543	659	460	408	366	414	346	309	325	360	353	326	311
+	Diprophylline												634	627	769	675	685		581	
+	Diuron						72	143	134	110	74									
+	Domperidone												980	695	1758			824	554	551
+	E1 (1-3-5(10)-estratrien-3-ol-17-one estrone)						1771	1446					1612							
+	E1-4-OH (4-hydroxyestrone)							1474												
+	Efavirenz	341	357	265	264	271	468	581	467	454	395	2360	1673	2296	1485	2109	3147	3238	2531	2553
+	Ephedrine																	140		
+	Fenfluramine					28														
+	Flonicamid	130		83		112	86	88	134		64						91		85	
+	Fluometuron											108	96	87	57	67	64	79	65	62
+	Flurochloridone											618	815	624	1637	638	896	971	769	763
+	Formoterol											107	110	106	92	99	97	86	84	85
+	Galaxolide	52	50	66	53	70	189	156	154	91	112	104	95	68	77	57	49	71	29	61
+	Galaxolidone	1738	1646	1714	1741	1965	487	576	644	524	602	323	353	345	410	243	240	275	207	256
+	Haloperidol	140	131	155	117	145														
+	Harman	264	152	287	295	357	852	578	678	579	527									
+	Harmine	64						123												
+	Hydrocodone							16												
+	Ibuprofen	319	252	321	124	1006	429	435	526	346	361	225	259	275	260	360	228	230	163	181
+	Isoxsuprine											11	9	9	7	6				
+	Labetalol	29	47	21		28		117				2134	3090	2832	3515	2449	2497	2233	2180	2161
+	Lacosamide	58							54			81	120	110	152		110	99	85	67

+	Levetiracetam		133						817											
+	Lidocaine											25	26	28	18	24	28	32	25	23
+	Lidocaine-Nor											23	18	19		26	27	23	20	20
+	Lincomycin						84	150			130									
+	Lopinavir	243	210	228	243	263	105	131	105	75	61	225	255	234	344	213	206	221	191	196
+	Loratadine		108					89				1669	2946	2345	3719	1992	4718	4655	3673	3711
+	MDA												159	46	505	173	715	929	723	643
+	MDAI						412	819	806		695	213	174	138		121	244	183	221	217
+	MeOT (5-)							624												
+	MePPP					1066														
+	Mebendazole	125	38	71	74	84	210	617	820	444	393	112	73	370	76	57	88	93	93	78
+	Mebeverine								212	230		116								
+	Mefloquine	164	128	143	133	153	76	101	98	109	113	56	46	49	36	51	58	62	58	44
+	Melatonin								467	505	362									
+	Metformin												597		2959					
+	Methoprene	2396	1471	1264	902	1595	1390	3848	6777	4440	10735	1831	4102	2555	6990	1979	2452	3411	1748	2310
+	Metoclopramide			13		81						91	142	135	196	119	117	136	105	112
+	Miconazole	76	76	86	85	85	15	55	20		19	164	138	136	103	121	197	195	180	167
+	Morphine-Dihydro	38	29	41				58				135	301	225	336	173	288	275	162	119
+	Moxifloxacin							137	170	88	69		316	383	550		867	848	546	516
+	Nalbuphine						105													
+	Nalidixic acid											123	115	143	88	113	149	170	94	107
+	Nicotinamide	5697	3622	3664	3939	12899	8280	13386	12978	34162	14553	4019	4113	3849	4826	3552	3170	2931	3058	2304
+	Nicotine-Nor	46	87	50	83	41	89	29	40	22	18									
+	Nigericin																		399	3505
+	Nonylphenol di-ethoxylates	565	203	177	132	129	26716	27939	25452	17950	26270	564	1865	663	115	131	446	255	101	133
+	Nonylphenol mon-ethoxylates	1589	1905	1384	1836	1581						977	1193	1047	754	1022	891	1218	1172	1066
+	Norethisterone acetate	2008	1050	1388	1428	1560	3958	5981	7279	5885	6745		1378	984	1877	1313	1158	1619	702	1031
+	Norfloxacin	472	422	494	489	470	548	1963	2124	2064	1516	248	260	260	187	220	287	272	302	282
+	Obidoxime	424	698	415	2302	362										16054				
+	Octylphenol monoethoxylates							138	92		73				85					
+	Ofloxacin	15118	16468	14279	23071	17718	21289	24127	24306	18999	16957	93291	125626	153255	158113	120575	284004	275686	185680	210089
+	Orphenadrine	83	71	80	69	87	59	58	76	53	44	94	121	113	99	109	150	156	133	133
+	Oxitropium											149	256	292	442	270	371	288	221	106
+	Papaverine											95	86	98	82	100	107	121	111	111
+	Paracetamol	106	259	132		230	481			408	930	226	1911	216		968	366	1323	780	
+	Pentazocine	124	172	75	66	58		169				4067	2679	3527	1935	3635	4181	4276	3809	3779
+	Perindopril	262	1214	1016	1342	566		29		244	94									
+	Phenethylamine (2-)	148	83	102	146	146	121	478	134	187	118	164	197	148	360	142	111	173	87	114
+	Phentermine											208	190	179	181	211				
+	Pholedrine								21									69		

+	Phosphate-Triphenyl								23		22	53	225	186	394	164	132	157	105	108
+	Phosphate-triethyl	49					60					34	25							
+	Phthalate-Diethyl								1115		<LOQ				<LOQ					
+	Phthalate-bis-(2-ethylhexyl). DEHP		<LOQ	<LOQ	<LOQ	<LOQ	1167	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ								
+	Progesterone						244	155												
+	Proguanil	1055	998	922	861	956	770	917	856	859	841	2793	1414	2160	536	2065	2314	2332	2235	2093
+	Promethazine		229				774	702	442	393	453	8466	6864	6910	6375	5980	8400	7901	6674	6874
+	Propranolol	27	22	27	21	26		39		34	41	57	43	54	43	49	52	51	47	52
+	Protriptyline										13									
+	Pyrethrins: Cinerin I							5301			4188									
+	Pyrethrins: Jasmolin I	4000	1133	996			43633	46460	41437	27960	38545	3867	11705	4850			2909	1708		
+	Pyrimethamine	153	139	142	149	155	154	184	189	156	136	257	198	211	167	251	301	299	276	276
+	Quetiapine	12	9	13	14	12														
+	Quinidine							116	235	201	268						166	123		124
+	Quinine					64		112	230	194	267		298	236	552		166	116	57	122
+	Rabeprazole	12	13	12	12	10	11	22	20	24	20	42	48	48	47	40	70	62	59	59
+	Ritonavir											34	47		50	35	29			24
+	Salicylamide	69	50	55	46	70	124	101	77	36	87	89	188	90	194	72	100	215	166	124
+	Seneciphylline-N-oxide								515											
+	Sertraline	69	53	58	53	58				6302										
+	Sertraline-Nor									301										
+	Simvastatin						2957	1601	1090	1071	1538									
+	Sulfadimidine (Sulfamethazine)	283	266	275	292	280	1064	658	839	968	1100	2032	1233	1567	707	1476	1720	1533	1831	1627
+	Sulfadoxine									24		48	53	53	80		52	57	51	53
+	THC-COOH						5602	5450	6752	6142	4647									
+	Telmisartan	204	199	196	188	206	142	178	141	165	137	226	860	606	1545	459	436	439	305	388
+	Terbutryn						32	18	21	19	20									
+	Testosterone benzoate		858																	
+	Tralkoxydim peak 2						958	186				297								
+	Tramadol	16	29	14	16	16	38	81	90	64	69	91	46	68	23	67	194	191	181	143
+	Tramadol-O-Desmethyl	82	51	66	56	52	83	81	68	75	79	87	48	66	16	54	73	68	62	65
+	Tramadol-O-Desmethylnor						192	20	47		116									
+	Trapidil	132	128	75	52	95	302	507	849	499	429	216	181	239	335	349	252	405	212	204
+	Triclocarban	44	47	67	54	68	16	26	34	18	12	66	77	69	101	62	108	130	72	124
+	Trimethoprim												15		128	29				
+	Trinexapac-ethyl	139	145	143	158	169		277	46	35	19	3962	5383	4427	6000	3740	6686	8013	5414	6341
+	Triptyline-Nor		25	21	16	24														
+	Tyramine			421	626	507		3660				1363			1246					
+	Vigabatrin							3657												
-	3.5.6-Trichloro-2-pyridinol	68	31				463	716	845		857		33		25					
-	Androsterone-19-nor	828	715	814	800	1203	1018	1433	1249			783	961	1196	1714	1114	975	1080	682	981

-	Benzenesulfonate-4-hydroxy	28	26	27	32	34	34	33	42		36	37	24	36	20	21	53	49	24	30
-	Bisphenol A						3130	3775			561	1370	5127	1505						
-	Celecoxib	4	3	3		5														
-	Chloramphenicol											101	86	96	62	86	64	82	154	51
-	Chloroxuron (Chloroxifenidim)		116	59	87	74							89	140				64	60	
-	Corticosterone										8058									
-	Curcumin (E100)								1226		<LOQ									
-	Fluoxymesterone met.	6371	5408	6283	6549	7307	16589	7046	6716		5177	11429	4480	7409	7099	7868	9329	10849	6818	6762
-	Fuberidazole						34		30			27					18	<LOQ		
-	Gemfibrozil	429	349	445	428			572	729		577	560	488					292	286	
-	Meclofenamic Acid														501		245		162	
-	Mesterolone	3321	5553	6470	4713	7037	3976	10708	9649		2588	14276	19475	16939	33320	17843	20331	25333	11861	27386
-	Methenolone		1397				718		1449			670	848	536	950	674	751	1194	634	735
-	Nabumetone						1538	2140				740	2532	839						
-	Nonylphenol (4-NP)						11060	4213	3683		3941	240	625	143						
-	Nonylphenoxy-acetic acid (4-)	69		37			3631	2355	5010		2738	117	189	114	52	49	145	113	74	68
-	Norethandrolone met.	909	1360	2008	1354	1971	4376	6345	4690		958	3597	7284	4301	24417	7571	4105	5405	2243	4910
-	PFOS	4	4	<LOQ	<LOQ	4						<LOQ	<LOQ	<LOQ		1			<LOQ	<LOQ
-	Pyrimidinol	96	78	70	73	83	84	174	85		62									
-	Salicylic acid	50995	52596	51461	52302	57080	72369	43085	42983		32961	67257	46327	59696	28572	50142	50541	55298	45790	49351
-	Salicylic acid-5-Amino	357			196	238	300	388	211			274	295	380	314	296	343	327	281	280
-	THC					151	239	404	268			234	400	193	561	215	333	356	86	409
-	Topotecan																	396		
-	Triclosan	443	320	413	354	402	5317	4390	8148		12169	658	622	714	438	516	583	558	484	524
-	Ursodeoxycholic acid	631	431	481	545	689	1533	5476	3639		4059	235	734	341	1349	435	508	394	322	408

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