Fate of Drugs and their Metabolites in the Environment

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ABSTRACT

The individual use of these pharmacologically active substances which generate great but underappreciated levels of other toxicologically potent and associated bioactive metabolites through purposeful and inadvertent discharge to the environment via excreta and by illegal disposal has become a global issue. This work reviews aspects of drugs occurrence, metabolism, transport routes, stability, analysis and environmental distribution of these emerging contaminants and highlights current developments in investigating and monitoring their fate and potential effects in aquatic environments. Gas chromatography-mass spectrometry (GCMS) and high performance liquid chromatography are the preferred methods for trace drugs analysis in wastewaters as their measurements depend largely on successful application of a fast and reliable method for qualitative and quantitative determination. The application of this method to the actual influents, effluents, sludge and environmental sediments from sewage treatment works (STWs) allows the assessment of drugs content and the extent at which STW helps in the transport of these pollutants (via different media) into the environment. The capability is also outlined for furthering our understanding of fate and behaviour of drugs with particular reference to illicit drugs, abused pharmaceuticals and environmental processes in our quest to understand the overall issues of drugs and make available exposure data for the aquatic realm.

Introduction

Environmental occurrence of organic pollutants through interconnectedness of human actions and activities impacts the environment in many ways. The impacts arising from the potential of global warming, deforestation and deposition of drugs comprising myriads of chemical and therapeutic classes harbours risks to our daily lives. The high consumption of drugs have significant worldwide consequences and the comprehensive assessment to provide updates regarding the occurrence, metabolism, measurement as well as removal of these contaminants from STWs effluent-waters have therefore become important for humans and aquatic environment.

Occurrence of chemical substances in the aquatic environment

Heavy metals, solvents, dyes, pesticides etc. are some of the chemicals that enter the aquatic environment in several ways causing chemical pollution. Some are either from sewage treatment works (STWs) or are dumped directly from industrial effluents. Other sources include the use of herbicides and fertilizers in agriculture. Apart from phytoestrogens that come from plants; humans and animals also excrete natural hormones which are disrupting chemicals in the environment [1]. In effluents, bisphenol A (BPA), nonylphenol, nitrates found in fertilizers as well as animal excrements and industrial chemicals occur [2]. Figure 1, shows also the presence of polycyclic-aromatic hydro carbons (PAHs), heavy metals and phthalates are shown.
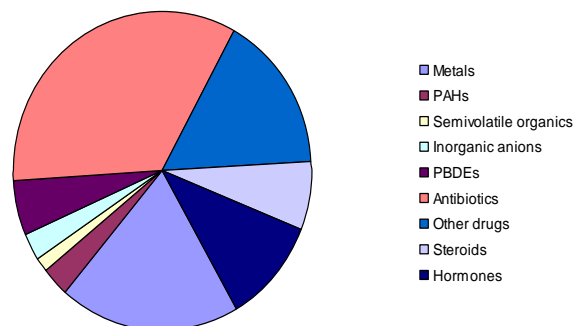

Figure 1: Chemical components in sewage sludge. Data from The U. S. Environmental Protection Agency. (Renewable Energy Venture, Austin, Texas) [4]

Other classes of endocrine disrupting compounds (EDCs), which includes multitudes of chemicals are considered in the studies of accumulation of potential toxic elements exposed to sheep grazed on grassland with repeated applications of sewage sludge and its exposure effects on sheep foetal testis development at different gestation periods [3, 4] have been reported. The chemicals used in the plastic industry includes phthalate esters and other major environmental pollutants [5] and Koppe *et al* [6] studied the metabolism of the parent phthalate and argued that a very active glucuronidated metabolite (monoester) was excreted, while in the digestive system their higher monomers has been detected [7].

Table 1 shows most of the reported data of pollutants and residual analytes in sewage samples showing the sources and the

analytes found. In foetus studies, high bioaccumulation of phthalates due to easy placental transfer [8] has been observed and the effects of high doses of phthalates on male's reproductive organs have been shown but in most organ systems, they are relatively non-toxic. A reduction in testosterone production in rats exposed to phthalates confirmed extensive studies of phthalates with the increased high levels of human exposure in human spermatozoa increased damage to DNA [9-14].

Activity effects of dibutyl phthalate (DBP) [62], di-ethylhexyl phthalate (DEHP) induced 'anti-androgenic' on humans testicular dysgenesis syndrome [63], multinucleate gonocytes in rats [64-66] and occurrence of oestrogens on breast cancer cells have been linked to phthalate exposures [67].

Polychlorinated biphenyls (PCBs) are (unreactive) organic compounds, which constitute a class of 209 congener groups. The commercial production of pulp bleaching, herbicides, metal smelting, by-products in combustion processes of incineration, chlor-alkali and coal-fired power stations or processes are main sources of PCBs stable compounds. Rudel *et al* [68] had listed their uses as electronic components, pesticides extenders, cutting oils, sealants, adhesives, stabilizing additives in flexible PVC coatings of electrical wiring, wood floor production, finishers, flame retardants, hydraulic fluids, paints, de-dusting agents coolants, insulating fluids for transformers, capacitors, and in carbonless copy paper. PCBs are stable, very resistant to oxidative degradation, only degrade anaerobically and readily persist longer [69]. On human health effects, anemia, thyroid gland injuries, impaired reproduction, stomach and liver injuries have all been reported [70, 71]. Exposures of PCBs can interfere with oestrogen levels of animals [72]. Impairments of immune system, lowering of testosterone levels in males, elevating the levels of progesterone in females and disruption of thyroid hormone function [73]. Bioaccumulation of PCBs induces oestrogenic effects in animal tissues [74]. Additional studies from Whyatt *et al* [75], Lilienthal [76] and Korach [77] have all further confirmed that the chlorinated congeners are more stable and persistent longer than less chlorinated compounds.

Pharmaceuticals in aqueous environment

Pharmaceutical substances are pollutants that are steadily increasing in wide variety in the aquatic environment apart from the traditional pollutants like polychlorinated biphenyls (PCBs), pesticides and polycyclic aromatic hydrocarbons (PAHs) in recent years [78-83]. Despite the rapid rise and continuous discharge of these chemicals of which some are carcinogenic, reproductive toxic and mutagenic in environmental matrices [84-87], studies have indicated that their removals have been found to be incomplete and inadequate attention on the fate and behaviour during the transport of many drugs after their intended use have increased the risks of possible environmental effects [88-92]. The active ingredients of medicinal products with a wide range of chemical structures are excreted as parent drugs with associated metabolites after metabolism by dosed user and these are further subjected to biotransformation in the sewage treatment processes producing more polar degradation products of which many complex modes of biochemical pathways are poorly understood. This has led environmental research's increasing attention to pharmaceuticals and their corresponding metabolites considering the production of large number of registered pharmaceuticals and those procured illegally for illicit use or without prescription [89]. Yet, large quantities of different chemical classes of new pharmaceuticals enter the already saturated marketplace and these are disposed

through agrochemicals runoff and the sewage systems to the aquatic environment

One of the major sources is excreta and urine containing the unmetabolized drug residues and its active metabolites being flushed down in the toilets, many unwanted and expired prescription drugs are deliberately disposed of via drains [95-97]). Also, Richardson & Bowron [93] reported that most of the drugs like antiseptics and lotions are assumed acceptable to be diluted to low levels in crude sewage when sluiced away. Numerous papers have reported the distribution of different chemicals belonging to different therapeutic classes such as antibiotics, anti-inflammatory drugs, lipid regulators, beta-blockers, β_2 –sympathomimetics, antiepileptics, antidepressants, antineoplastics, contraceptives, tranquilizers, diagnostic contrast media, preservatives and sunscreen agents in different media of the environment at the specific levels ranging from ngL^{-1} to µg L^{-1} [98-101]. Also reported at microgram levels in rivers were theophylline, erythromycin and tetracycline and some amounts of oestrogen from oral contraceptive in sewage systems excreted by human population [102]

In Switzerland, about 4 tonnes/year of fluoroquinolones (antibacterial drug) are sold and 14 tonnes/year in Italy [90, 103, 104], while 100 tons of annual drug prescription in Germany alone does not include several other pharmaceuticals that have been reported in aquatic samples in numerous papers ranging from ngL^{-1} to µg L^{-1} levels [87, 90, 104-106]. The recent analytical studies in UK further show that some pharmaceuticals are incompletely removed from sewage treatment works and surface waters such as lakes, rivers and seas have some detectable pharmaceuticals present [84, 105, 107-110].

Table 2 lists data of the main pharmaceuticals monitored in German STWs as well as German rivers and streams with 10,11-dihydro-10,11-dihydro-carbamazepine (DHH), a metabolite of antiepileptic carbamazepine having highest influent and effluent concentration of 4100 and 2600 ng L^{-1}.

Table 3, shows antiepileptic carbamazepine has highest concentration of 6300 ng L^{-1}, X-ray contrast media were between 11, 000-15, 000 ng L^{-1} [117]. About 31 pharmaceuticals and five metabolites were found in at least one sample of 40 German rivers. Out of 69 target compounds, only 10 were found in drinking water [119]. The survey of exposure effects and other environmental relevance is in the literature reviews [83, 120].

Commonly used illicit drugs and their metabolites in aqueous environment

The term "illicit drug or drug of abuse" is normally used to describe those drugs that are controlled under the Misuse of Drugs Act, 1971. The legislation regulates controlled drugs into classes depending on the harm they cause, and there are various offences including the unlawful possession of a controlled substance [121]. The emerging risks with the prevalence and trends in the illegal production and abuse of illicit drugs have prompted the establishment of many International Agencies [122, 123] to monitor and conduct the risk assessments of the social, economic and environmental impacts the menace are eliciting, particularly in the consumer countries. The common classes of illicit drugs are cocaine, amphetamine, opioid, lysergic acid diethylamide (LSD), hallucillogen and cannabinoid, and by the hidden activity of their users, it has helped its purported widespread and continual escalating use [83]. The idea of Daughton [101] to use a non – intrusive approach to approximate the level of illicit drugs consumption at community level which was later demonstrated by Zuccato *et al*

[124] determined the levels of cocaine in waters and related the quantity to the amount of drug consumed by a local population. The approach apparently provided information needed by environmental scientists and appropriate authorities involved in the fight against the drug menace. It has been argued that the sewage systems constitute one of the potential routes those drugs enter the environment; other highly dispersed sources include disposals by drug and manufacturing laboratories [125].

Human metabolism of environmentally relevant drugs.

In the human body, drugs are bio-transformed into one or more metabolites and after the loss of pharmacological activity the metabolites and unchanged parent drugs are eliminated from the body systemic circulation via urine or faeces. A number of parameters which include age, gender, ethnicity, patient and the time of administration have been associated to degree of metabolism. In Figure 9, the metabolism of drugs in the human body shows Phase I and Phase II reactions. The phase I comprises of oxidation reaction such as in aliphatic hydroxylation of ibuprofen and diclofenac, epoxidation of carbamazepine and ring oxidation of propranolol, while reductions, alkylations and dealkylations are other reactions.
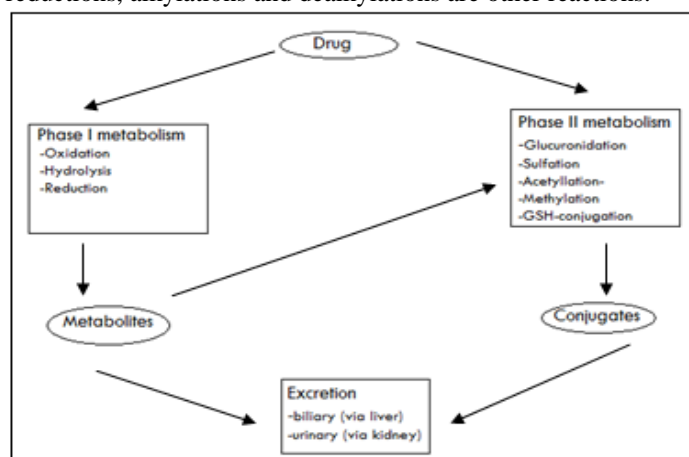

Figure 2: Simplified scheme of dug metabolism in the human body [92]

The conjugation reaction type occurs when polar molecules in Phase I transfer to the metabolites in Phase II such as the transfer of glucuronic acid to phenols, hydroxyls, caroxyls, thiols, amines and hydroxylamino groups. [92]. There is therefore interest in identifying the metabolites that may pass on to the sewage, and those that might stay longer in the STWs and enter the environment through untreated water effluents or sewage biosolids.

In the following sections, the metabolisms of the five major classes of illicit drugs found in various wastewaters are discussed.

Human metabolism of cocaine

Cocaine is extracted from the leaves of two species of coca: Erythroxylum coca and Erythroxylum novogranatense. The cocaine hydrochloride is normally formed after the alkaloids are precipitated with sodium carbonate and then dissolved in dilute HCl containing about 40% of cocaine, but when cocaine hydrochloride is extracted with ether in aqueous alkaline solution, it produces "free base" which contains 85-90% of pure cocaine [94,126]. The street cocaine used by addicts is often mixed or cut with a number of diluants [127], and these adulterants are sometimes the cause of poisoning. Cocaine is a powerful addictive stimulant drug with three common routes of administration: smoking, intravenously and intranasally (through the nose). Figure 10 shows only the compounds we determined in the results, however, cocaine is spontaneously

metabolized by the action of pseudo cholinesterase and hepatic esterase to give ecgonine methylester (EME) with the loss of benzoyl group [128-131]. A non-enzymatic hydrolysis at pH above 6 converts cocaine to benzoylecgonine (BZE) by demethylation as its main metabolite. BZE can be detected in the urine 48 hours after cocaine administration with a urinary excretion half-life of 6-8 hours [132-133]. The N-demethylation of cocaine leads to norcocaine (NC) (the most toxic metabolite) by P450 enzymes and then metabolized to N-hydroxynorcocaine by brain FAD –containing mono- oxygenases [134-135]. Norcocaine can further be hydrolysed to benzoylecgonine. Cocaine undergoes trans-esterification by enzymatic reaction in the liver in the presence of alcohol to form cocaethylene (CE); which has been reported to be more toxic than cocaine [136]. When cocaine is smoked, anhydroecgonine methylester (AEME) is produced and through enzymatic hydrolysis get converted to anhydroecgonine (AE) or ecgonidine [137]. The other metabolites of cocaine (ecgonidine, norecgonidine methylester, p- hydroxyl-benzoylecgonine, and m- hydroxyl-benzoylecgonine) found in human urine have minor metabolic-pathways that involve the aromatic meta- and para-hydroxylation of cocaine followed by partial hydrolysis to the corresponding HO-Be isomers [138]. About 1-9% of cocaine has been excreted unchanged in the urine with much higher proportion in acid urine; its metabolites are recovered in variable proportions which depend on the route of administration [139].
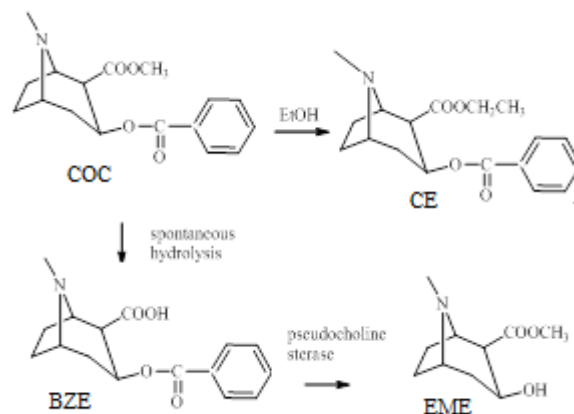

Figure 3: Degradation pathways of cocaine in the human body [140].

Human metabolism of amphetamine

Among the drugs classified as amphetamines are amphetamines (AM), methamphetamines (MA, "speed") and methylenedioxymethamphetamine (MDMA, "ecstasy or Adam"). They are usually taken orally but can be snorted, smoked or injected. They are addictive stimulant drugs that affect the central nervous systems among other risks of dependence and abuse. Other designer drugs are methylenedioxyethylamphetamine (MDE, "eve") and 3, 4-methylenedioxyamphetamine (MDA, "love pills") [141].

The major metabolic pathway involves deamination of cytochrome P450 to para- hydroxyl amphetamine and phenylacetone, this later compound is oxidised to benzoic acid and excreted as glucuronide or glycine (hippuric acid) conjugates. Smaller amounts of amphetamine are also converted to norepheridine by oxidation. Although most enzymes involved in amphetamine metabolism have not been clearly defined, CYP2D6 is known to be involved with the formation of 4-hydroxylamphetamine [142-143]

Table 1: Organic contaminants in sewage sludge [15 – 61].

Pollutants	Sources/usages	Analytes	Matrix	Ref
Organochlorine Pesticides and PCBs	Agricultural control of pests, transformer fluids, plasticisers PVCs and artificial Rubbers	g-HCH, Aldrin, Endrin, PCBs, Dieldrin	sewage	15, 16
Chlorophenols & chlorophenoxy acids	Herbicides	4-chlorophenol 2-chlorophenol, 2-chloro-6-MP MCPA; 2,4-D	Sewage	16-19
Organophosphorus Compounds	Pesticides	residues	Sewage	20, 21
Nitrosamines & Nitroaromatics	Control nematodes	Dimethylnitrosamine; NDMA; NDEA; NPYR; NMOR.	Sewage	21, 22
Mineral oils	Engine oils, paints	Paraffine, alkybenzene cycloparaffine	Sewage	23, 24
Alkylphenols	Detergents, surfactants	4-alkylphenol; polyetho- xylates; 4-nonylphenol (NP); Monoethoxylates (NP1EO); (NP2EO)	Sewage	25, 26
Lipids	Petroleum hydrocarbon	Phosphatidyl serine, Phosphatidyl ethanolamine, Phosphatidyl choline	Sewage	24, 27
Acrylamide Monomer	Coagulants	Polyacrylamide	Sewage	28
Phthalates esters	Plasticisers	bis-(2-ethylhexyl)phthalate	Sewage	29
Organotin compounds	Stabilisers in PVCs biocides, foams	Tributyltin oxide	Sewage	30-32
Surfactants & Related residues	Detergents	Linear alkylbenzene Sulphonates (LASs),	Sewage	33-38
	Paint removers		Sewage	39-46
Chlorobenzenes		chlorobenzenes		
Polychlorinated dibenzodioxins (PCDD)	Pulp bleaching	Congener group	Sewage	47-54
Polycyclic-aromatic hydro carbons (PAHs)	Pyrolysis of organic materials.	Naphthalenes	Sewage	55-61

Table 2: Occurrence of psycho-active drugs and beta blockers in STWs [116]

Substances	Influent			Effluent		
	LOQ [ng L^{-1}]	No of samples	Max [ng L^{-1}]	LOQ [ng L^{-1}]	No of samples	Max [ng L^{-1}]
Antiepileptics						
Carbamazepine	200	9	1000	100	9	1200
DH-CBZ	100	7	30	50	8	30
DHH	200	9	4100	10	9	2600
Primidone	200	9	420	10	9	250
Antidepressants						
Doxepin	200	9	100	10	9	190
Opioids						
Oxycodon	200	0	-	10	0	-
Dihydrocodeine	200	9	140	10	9	70
Codeine	200	9	160	10	9	30
Morphine	200	9	440	10	9	29
Methadone	100	9	130	5	9	120
Tramadol	200	6	470	10	6	370
Tranquilizers						
Diazepam	200	0	-	10	0	-
Nordiazepam	200	0	-	10	0	-
Oxazepam	200	6	190	10	6	180
Beta blockers						
Atenolol	100	9	910	5	9	370
Sotalol	100	9	1300	5	9	1200
Metoprolol	100	9	1200	5	9	1100
Propranolol	5	9	70	3	9	60
Bisoprolol	100	9	380	5	9	270
Celiprolol	100	9	160	5	9	160
Betaxolol	5	4	10	3	1	-

Note: DH-CBZ (10, 11-dihydrocarbamazepine

Table 3: Pharmaceuticals in German STW effluents, rivers and streams [81, 105, 116, 117]

Analyte	STWs			Rivers/streams	
	LOQ (ng L^{-1})	Number STWs	Maximum (ng L^{-1})	LOQ (ng L^{-1})	Maximum (ng L^{-1})
Lipid regulator					
Bezafibrate	250	49	4600	25	3100
Gemfibrozil	50	49	1500	10	510
Clofibric acid	50	49	1600	10	550
Fenofibric acid	50	49	1200	10	280
Antiphlogistics					
Diclofenac	50	49	2100	10	1200
Ibuprofen	50	49	3400	10	530
Indomethacin	50	49	600	10	200
Naproxen	50	10	520	10	390
Ketoprofen	50	49	380	10	120
Phenazon	100	30	410	20	950
Acetylsalicylic acid	100	49	1500	20	340
Salicylic acid	50	36	140	10	4100
Betablocker					
Metoprolol	25	29	2200	10	2200
Propranolol	25	29	290	10	590
Betaxolol	25	29	190	10	30
Bisoprolol	25	29	370	10	2900
β$_2$-Sympathomimetics					
Terbutalin	50	29	120	10	<LOQ
Salbutamol	50	29	170	10	35
Psychiatric drug					
Diazepam	30	20	40	30	<LOQ
Antiepileptic					
Carbamazepine	50	30	6300	30	1100
Antibiotics					
Clarithromycin	20	8	260	20	260
Roxithromycin	20	10	1000	20	560
Chloramphenicol	20	10	560	20	60
Sulfamethoxazol	20	10	2000	20	480
Trimethoprim	20	10	660	20	200
Dehydrato-erythromycin	20	10	6000	20	1700
X-ray contrast media					
Iopamidol	10	25	15000	10	2800
Iopromide	10	24	11000	10	910
Diatrizoate	10	25	8700	10	ca.100
Iomeprol	10	12	3800	10	890
Estrogens					
Estrone	1	38	70	0.5	1.6
17β-Estradiol	1	38	3	0.5	<LOQ
17β-Estradiol-17-valerate	4	38	<LOQ	2	<LOQ
17α-Ethinylestradiol	1	38	15	0.5	<LOQ
16α-Hydroxyestrone	1	15	5	0.5	<LOQ

Table 5: Sewage sludge disposal in England, 2008-2011 [196]

Mode of disposal	% Total disposed							
		2008/2009		2009/2010		2010/2011		Jan-Dec 2011	
	Tonnes		%	Tons	%	Tons	%	Tons	%
Land fill	13784		50	12490	47	11391	43	10135	39
Incineration with EfW*	3325		12	3610	14	3975	15	4577	18
Inicineration without EfW	6		0	6	0	5	0	4	0
Recycled/Composted/Reused	10082		37	10275	39	10588	4	10844	42
Other	198		1	255	1	356	1	404	2

*EfW = Energy from waste

Table 6: Illicit drug metabolites of human origin detected in the environment*

Compound	Human metabolites identified in biological fluids [150, 225-242]	Human metabolites identified in the aquatic environment [116158, 221, 243-251]
Amphetamine	Amphetamine (AM)	Detected
	3, 4-methylenedioxyamphetamine (MDA)	Detected
	Methylenedioxymethamphetamine (MDMA)	Detected
	Methylenedioxyethylamphetamin (MDEA)	Detected
	Methylbenzodioxolylbutanamine (MBDB)	Detected

	Metamphetamine (MA)	Detected
	p-hydroxy-metamphetamine (*p*-OHMA)	-
	p-OHMA-glucuronide (*p*-OHMA-Glu)	-
	p-OHMA-sulfate (*p*-OHMA-Sul)	-
Cocaine	Cocaine (Cocaine)	Detected
	Benzoylecgonine (BE)	Detected
	Ecgonine methyl ester (EME)	Detected
	Cocaethylene (CE)	Detected
	Norcocaie (Nor- COC)	Detected
	Ecgonidine	-
	nor-ecgonidine	-
	nor- ergonine methylester	-
	m-OH-benzoylegonine	-
	ecgonine	-
	ecgonidine methylester	-
Opiates	Heroin	Detected
	Morphine	Detected
	Nor-morphine	Detected
	6-monoacetylmorphine (6-ACM)	Detected
	Morphine -3- glucuronide (M3G)	Detected
	Methadone	Detected
	2-ethylene-1,5-dimethyl 1-3,3-diphenylpyrolidene (EDDP)	Detected
	Ethyl morphine	-
LSD	Lysergicdiethylamide (LSD)	Detected
	Hydroxyl Lysergicdiethylamide (OH-LSD)	Detected
	Nor - Lysergicdiethylamide (Nor-LSD)	Detected
	Iso - Lysergicdiethylamide (Iso-LSD)	Detected
	2-oxo-3-hydroxy-LSD (2-Oxo-3-OH-LSD)	-
Cannabinoids	Δ^9-tetrahydrocannabinol (THC)	Detected
	Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH)	Detected
	THC-COOH- glucuronide (THC-COO gluc.)	Detected
	Hydroxyl -THC- conjugate (OH-THC)	Detected

* No identified microbial degradates in the literature

Table 7: Assessment of the biodegradability of pharmaceutical chemicals [93, 111]

Compound	Test result
Amitriptyline	Non-biodegradable
Ampicillin	Biodegradable
Aspirin	Readily biodegradable
Caffeine	Readily biodegradable
Chlorhexidine	Non-biodegradable
Clofibrate	Non-biodegradable
Codeine phosphate	Non-biodegradable
Dextropropoxyphene	Non-biodegradable
Ephedrine	Readily biodegradable
Erythromycin	Non-biodegradable
Ibuprofen	Biodegradable
Menthol	Readily biodegradable
Meprobamate	Non-biodegradable
Methyldopa	Non-biodegradable
Metronidazole	Non-biodegradable
Naproxen	Non-biodegradable
Paracetamol	Readily biodegradable
Phenylpropanolamine	Readily biodegradable
Sulphamethoxazole	Non-biodegradable
Sulphasalazine	Non-biodegradable
Tetracycline	Non-biodegradable
Theobromine	Readily biodegradable
Theophylline	Readily biodegradable
Tolbutamide	Non-biodegradable

Table 8 Chromatographic (LC-MS/MS) methods for the determination of illicit drugs and human metabolites in water (2000-2011)

Analytes Ref. _____(LOD/LOQ [1])	Matrix	Sample Preparation Volume (mL)	Extraction	Method Recovery (%)	LC C'graphic Column	Mobile Phase	MS Detector (Interface)	Acq. Mode	Method (ng L^{-1})
3 Cocaine: (CO, BE, EME)	WW SW	100	SPE (Oasis, HLB, 500 mg)	73-96	Zorbax Extended C$_{18}$(2.1mm x 50mm x 3.5µm) HILIC: Rx-SIL (2.1 x 150mm,5µm)	250 µL/min. A:H$_2$O/AcN (92:2),10mM NH$_4$HCO$_2$ (pH 3). B: ACN.	ITMS (ESI$^+$)	MRM	2-4^{WW} 20^{SW} [219]
1Cocaine 1 Opoiod. 1Cannabiniod 3 ALC.	WW	-	SPE (Oasis, MCX, 200mg)	-	HPLC: XTerraMS C$_{18}$ (100mm x 2.1mm,3.5µm)	250 µL/min. A: 0.1% in H$_2$O. B: AcN	QqQ (ESI$^+$)	SRM	- [270]
5 Cocaine 4 ALCs 3 Opiods 1Cannabiniod	SW	250 mL	SPE (Oasis, MCX,60mg)	96-105 97 10 85-90 69-84	HPLC: XTerraMS C$_{18}$(100mm x 2.1mm,3.5µm)	250 µL/min. A: 0.1% in H$_2$O. B: AcN	QqQ (ESI$^+$)	SRM	0.02-0.05 0.01-0.35 0.02-0.28 0.14-0.36 [158]
2 Cocaine	SW WW	100 mL 500 mL	SPE (Oasis, HLB, 500 mg)	-	HILIC: Zorbax Rx-SIL (2.1 x 150mm, 5µm)	-	ITMS (ESI$^+$)	MRM	≤ 20 [158]
2 Cocaine	SW	500 mL	SPE (Oasis, MCX, 60mg)	90	HPLC: A Luna C$_{18}$ (50mm x 2mm i.d, 3 µm)	250 µL/min A: 0.1% in H$_2$O. B: AcN	-	MRM	- [124]

Analytes Method (LOD/LOQ) Acq. [1])	Matrix Ref.	Sample Preparation Volume (mL)	Extraction	Method Recovery (%)	LC Chromatographic Column	Mobile Phase	MS Detector (Interface)	Mode	(ng L^{-1})
3 Cocaine 1ALC 3 Opiods 1LSD	WW	500 mL	SPE (Strata-XC, 200mg)	50-65	HPLC: Phenomenex Onyx C$_{18}$ (200 x 3.0mm				36-120 [244]
5 ALCs 5 Cocaine 5 Opiods 1Cannabiniod	WW	50 mL	SPE (Oasis, MCX, 60mg)	50-105	HPLC: XTerraMS C$_{18}$(100mm x 2.1mm, 3.5µm)	250 µL/min A:CH$_3$COOH/ H$_2$O. B: AcN A$_2$: 0.05% TEA/ H$_2$O	QqQ (ESI$^+$)	MRM	300 pg/L^{wwinf} 1 ng/L$_{wweff}$ [248]
2 Cocaine	SW WW	100 mL 500 mL	SPE (Oasis, HLB, 500 mg)	-	Zorbax Rx-SIL (2.1 x 150mm, 5µm)	-	ITMS	MRM (ESI$^+$)	20 [271]
1 ALC	WW	250 mL	SPE (Oasis, HLB, 200 mg)	36-49	HPLC:Varian Pursuit XRs C$_{18}$ (100mm x 2.0mm, 3µm)	A:water/0.5% HCOOH B: 82% CH$_3$OH/ 18% AcN/0.5% HCOOH	-	Scan (CID)	0.25-5.0 [247]
5 ALCs 2 Cocaine. 1LSD 1Opiod	WW	100 mL	SPE (Oasis HLB, 200 mg)	70-110	UPLC:Acquity BEH C$_{18}$ (100mm x 2.1mm, 1.7 µm)	A: AcN/0.1% HCOOH. B: 30mM HCOOH/ NH$_4$HCO$_2$	QqQ (ESI$^+$)	-	5 - 850 [272]

Table 8 LC-MS/MS methods for the determination of illicit drugs and human metabolites in water (2000-2011)

Analytes Method (LOD/LOQ) Acq. (ng L⁻¹)	Matrix Ref.		Sample Preparation Volume (mL)	Extraction Recovery (%)	LC Method Column	Chromatographic Mobile Phase		MS Detector (Interface)		Mode	
1 ALC 2 Cocaine.	SW	100 mL	SPE (Oasis, MCX,60mg)	65-106	UPLC: Acquity BEH C$_{18}$ (1.7μm, 1 mm x 100mm)	A:94.5% H$_2$O. 5% MeOH, 5% CH3COOH (pH 2.8) B:99.5% MeOH + 0.5% Acetic	QqQ (ESI⁺)	MRM		0.3-50	[259]
8 Opiods 2Cannabiniod	SW WW	200 mL	SPE (Oasis, HLB, 200 mg)	40-70	UPLC: Acquity BEH C$_{18}$ (1.7μm, 1 x 100mm)	A: MeOH B: 5 mM NH$_4$HCO$_2$	QqQ (ESI⁺)	SRM		0.1-25	[258]
8 Opiods 2 Cannabiniod	SW WW	50 mL	SPE (Oasis, MCX,(150mg)	69-94%	UPLC: Acquity BEH C$_{18}$ (1.7μm, 2. 1 mm x50mm)	A: MeOH B: 5 mM NH$_4$HCO$_2$ + 1% formic acid	QqQ (ESI⁺)	SRM		-	[247]

Acq. Mode - Acquisition mode- SRM, Selected reaction monitoring; CID, Collision-induced dissociation.
Detector and Interface used – QqQ, Triple quadrupole; ITMS, Ion Trap mass spectrometry, ESI, Electrospray ionization.
MeOH – Methanol; TEA, Triethylamine; NH$_4$HCO$_2$ Ammonium acetate; AcN, Acetonitrile, H$_2$O, Water.
WW, Wastewater; SW, Surface water; WW^{inff}, Waste water influent; WW^{eff}, Waste water effluent.
RPLC, Reversed-phase liquid chromatography; UPLC, Ultra- performance liquid chromatography; HILIC, Hydrophilic interaction chromatography.

Table 9. Solid Phase Extraction (SPE) protocols in wastewater pre-treatment

		Protocols			
Types	Sorbent materials	Conditioning	Washing	Elution	Ref
Isolute, pH® (1000 mg/6 mL)	Silical treated with phenyl groups in which silanol group are end-capped.	2 mL of MeOH and 6 mL of milli-Q water, sample loading at (pH 6)	6mL of 5% MeOH in water, drying in in vacuum for 15 min	2 x 4 mL of 5% NH$_3$ in acetone	[219]
Oasis, MCX® (500 mg/6 mL)	Polymeric sorbent with strong cation –exchange sulfonic group located on surface of poly(Divinyl benzene-Co-N-vinyl py rrolidone) copolymer .	6 mL of MeOH , 3mL of milli-Q water and 3 mL of water at pH 2, sample load-ing at pH 2.	3 mLof milli-Q water at pH 2, dryingfor 15 min. under vacuum	6 mL of MeOH and 6 mL of 5% NH$_3$ in MeOH	[124, 159, 186, 259, 270]
Bond Elut Certify® (300mg/6 mL)	Lipophilic and strongly cationic properties	3 mL of MeOH and 3 ml of milli-Q water, sample Loading at pH 6.	2 mL of of milli-Q H$_2$O at at pH 2, and 3 mL of MeOH, drying for 15 min under vacuum	2 x 4 mL of 80: 20 DCM/isopropanol mixture with 2% NH$_3$	[225,226]
SCX® (500 mg/6 mL)	-	2 mL of MeOH, I mL of milli-Q water and 1 mL of 0.25 M phosphate buffer (pH 3), loading at pH 3	I mL of 0.25 M phosphate (pH 3), 0.5 mL of 0.1M acetic acid and 1 mL of MeOH, drying for 30 min	1.5 mL of 3% NH$_4$OH in 1.5 mL of MeOH	[258]
Phenomenex Strata-X™ (200 mg/6 mL)	-	2 x 6 mL of MeOH and 2 x 6 mL of H$_2$O, sample loading at pH 6.	50 mL of 10% MeOH in 100 mM formic acid + 500 µL of acetic acid, drying for 30	10 mL of 5% v/v NH$_4$OH in 1:1 acetone: ethyl acetate	[244]
Strata-XC™ (200mg/6mL)	-	-same-	-same-	-same-	[244]

		Protocols			
Types	Sorbent materials	Conditioning	Washing	Elution	Ref
Chrolut, ENV® (500 mg/6 mL)	Hyper-crosslinked polystyrene-divinyl benzene polymer based.	3 mL of MeOH and 3 mL of milli-Q water, sample loading.	air through the column for 1 hr.	5 mL of MeOH	[82]
Isolute, ENV® (500 mg/6 mL)	Hydrophobic sorbent with hydroxylated polystyrene divinyl benzene copolymer	2 mL of MeOH and 6 mL of milli-Q water, sample loading at pH 6	6 mL of 5% MeOH in water, drying under vacuum for 15 min.	2 x 4 mL of MeOH.	[219]
Chromabond, Easy (500 mg/6 mL)	Bifunctional polystyrene divinyl benzene copolymer	5 mL of hexane, 5 mL of ethyl acetate, 10 mL of MeOH and 1 mL of Milli- Q water.	5 mL of milli-Q water drying under vacuum for 15 min.	2 x 4 mL of MeOH	[219,271]
Oasis, HLB® (500 mg/6 mL)	Divinylbenzene/N-vinyl pyrrolidone) copolymer with hydrophilic/lipo philic properties	3 mL of MeOH and 3 mL of milli-Q water, sample loading at pH 6	3 mL of 5% MeOH in milli-Q water drying under vacuum for 15 min	2 x 4 mL of MeOH	[219,243]
Oasis, HLB® (500 mg/6 mL)	-same-	5 mL of hexane, 5 mL of ethyl acetate, 10 mL of MeOH and 1 mL of Milli- Q water	5 mL of milli-Q water drying under vacuum for 15 min.	2 x 4 mL of MeOH	[83,243]
Isolute ,C_{18} (EC)® (500 mg/6 mL)	Strongly apolar and lipo- philic based on octadecyl silica with end capping of free silanol group.	2 mL of MeOH and 6 mL of milli-Q water, sample loading at pH 6	6 mL of 5% MeOH in milli-Q water drying under vacuum for 15 min.	2 x 4 mL of 5% NH_3 in acetone	[219,272]
Oasis, Max (60 mg)	Strong anion-exchange mixed mode polymeric	2 mL of MeOH and 2 mL of 2% HCOOH (pH 2.1)	2 mL of 2% HCOOH/ H_2O, wrapped in aluminium	1 mL of MeOH and 2 mL of 5% NH_4OH in MeOH	[259]

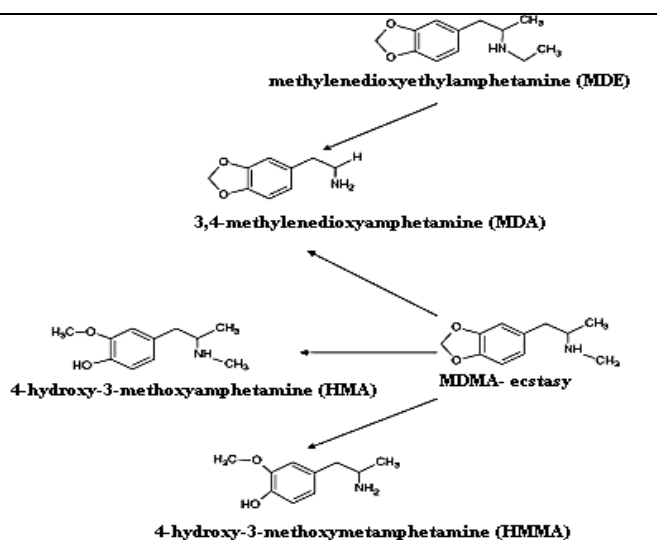|		Protocols			
Types	Sorbent materials	Conditioning	Washing	Elution	Ref
	sorbent built upon HLB copolymer (application: acids)		foil and stored in a freezer until eluted.		
Oasis, WCX (60 mg)	Weak cation-exchange mixed mode polymeric sorbent built upon HLB copolymer (application: strong bases).	2 mL of MeOH and 2 mL of 2% HCOOH (pH 2.1)	-same-	1 mL of MeOH and 2 mL of 5% NH_4OH in MeOH	[259]
Oasis, WAX (60 mg)	Weak anion- exchange mixed mode polymeric sorbent built upon HLB copolymer (application: strong acids).	-same-	-same-	-same-	[259]
Chromabond, C_{18} (200 mg).	Silical-based, endcapped sor-bent (non-polar compounds).	-same-	-same-	-same-	[259]
Isolute, HCX (200 mg)	Weak anion- exchange mixed mode (non-polar and basic analyte).	-same-	-same-	-same-	[259]

Table 4 Survey of illicit drugs and pharmaceuticals concentration in wastewaters.

Analytes	Matrix	Influent (ng L^{-1})	Effluent (ng L^{-1})	Surface Water (ng L^{-1})	Ref
Cocaine	5 STPs, Spain	225.0	47	10	[158]
	5 STPs, Belgium	22 -678	-	1.2 - 26	[219]
	37 STPs, Belgium	32 – 753	-	-	[158]
	3 Rivers, Italy	-	-	0.3 – 44	[186]
	5 STPs, Ireland, UK	489 ± 117	25 - 248 ± 20	0 – 33 ± 11	[244]
	Eastern Spain	370 – 1000.24	30 – 560	-	[258]
	30 STPs, Belgium	09 – 683	-	-	[245]
	2 STPs, Italy	218.4 – 421.4	0.9 – 10.7 ± 3.2	-	[159]
	4 STPs; River Po.	42 – 120	-	-	[124]
	42 STPs, NE Spain	04 – 4700	01 – 100	-	[272]
	Barcelona, Spain	2.40	-	-	[246]
Benzoylecgonine	5 STPs, Spain	2307.0	-	111	[158]
	5 STPs, Belgium	82 – 1 898	928	44 - 191	[219]
	37 STPs, Belgium	46 –2258	-	-	[186]
	3 Rivers, Italy	2.2 – 183	-	-	[186]
	5 STPs, Ireland UK	290 ± 11	22	-	[244]
	Eastern Spain	150 – 1000.5	22 ± 4 – 31 ± 18	-	[258]
	30 STPs, Belgium	37 – 1550	6.0 – 7.9	-	[245]
	2 STPs, Italy	547.4 -197.2	-	-	[159]
	4 STPs; River Po.	420 - 750	0.92 – 100.3 ± 28.6	-	[124]
	42 STPs, NE Spain	09 – 7500	-	-	[272]
	Barcelona, Spain	5.24	01 – 1500	-	[246]
	12 STPs, Germany	65± 5	77± 9	71	[276]

Analytes	Matrix	Influent (ng L^{-1})	Effluent (ng L^{-1})	SW (ng L^{-1})	Ref
Nor- BE	3 Rivers, Italy	-	-	0.2 – 8.4	[186]
	Eastern Spain	150 - 430	30 – 170	-	[258]
	2 STPs, Italy	18.8± 5.6 – 36.6 ± 7.8	<LOQ – 7.5 ± 2.9	-	[159]
Cocaethylene	2 STPs, Italy	5.9 ± 2.6 – 11.5 ± 5.1	0.2 ± 0.5	-	[159]
	Barcelona, Spain	77.5– 78.5±33.2	1.71– 4.2± 1.2	4.63	[246]
	3 Rivers, Italy	-	-	0.07 – 0.2	[186]
Nor-cocaine	3 Rivers, Italy	-	-	0.15 – 3.6	[186]
	Eastern Spain	0.15 – 0.43	0.03 - 0.17	-	[258]
	2 STPs, Italy	4.3 ± 0.9 – 13.7 ± 5.3	0.7 ± 0.5	-	[159]
Amphetamines	3 Rivers, Italy	-	-	<0.65	[186]
	Eastern Spain	1400	110 – 210	-	[258]
	2 STPs, Italy	5.4 – 14.7± 10.6	2.8	-	[159]
	42 STPs, NE Spain	03 - 6880	04 - 2100	-	[272]
	Barcelona, Spain	20.8 – 41.1± 9.1	0.45– 2.2 ± 0.1	2.84	[246]
	5 STPs, Spain	15	<1.0	<0.8	[158]
Metamphetamines	5 STPs, Nebraska USA	1.3 ± 0.1 – 1.4	35.0± 7.3	-	[160]
	3 Rivers, Italy	0.1 – 62.6 ± 13	-	<0.41 – 1.7	[186]
	Eastern Spain	-	<100 - 540	-	[258]
	2 STPs, Italy	<500	<1.11 – 3.5 ± 2	-	[159]
	42 STPs, NE Spain	3 - 277	3 - 90	-	[272]
	3 STPs, USA	15 ± 2 – 66 ± 14	0.8 – 1.3	-	[160]
	Barcelona, Spain	4.8 – 18.2 ± 5.8	2.1 – 6.3 ± 0.6	2.87	[246]
	Murray, USA	6.0 - 34	03 - 7	-	[247]
MDA	42 STPs, NE Spain	03 - 266	01 - 200	-	[272]
	3 Rivers, Italy	-	-	3 ± 0.3 – 4	[186]
	Eastern Spain	500 - 1400	41.0 – 68.0	-	[258]
	2 STPs, Italy	4.6 ± 7.3 – 8.7	0.9 ± 1.9 – 1.1± 1.5	-	[159]
	5 STPs, Spain	03 - 266	1 - 200	-	[158]

Analytes	Matrix	Influent (ng L^{-1})	Effluent (ng L^{-1})	SW (ng L^{-1})	Ref
MDMA	3 Rivers, Italy	-	-	1.1 – 4.0	[186]
	Eastern Spain	326 – 2700.5	100 – 210.2	-	[258]
	2 STPs, Italy	13.6 – 14.2	4.4 ± 3.7 – 5.1± 3	-	[159]
	5 STPs, Spain	91	67	3.5	[158]
	STP, Italy	2 - 598	2 - 267	-	[272]
	Barcelona, Spain	133– 135.13 ± 29.8	8.2– 14.8 ± 2.2	129	[246]
	Murray, USA	<1.0 – 10.0	-	-	[247]
	42 STPs, NE Spain	2 - 598	2 - 267	-	[272]
MDEA	5 STPs, Spain	27	<2.1	-	[158]
	2 STPs, Italy	4.19 – 1.5 ± 3.8	<1.64	-	[159]
	STP, Italy	6 - 114	12	-	[258]
	STP, Spain	<500	<100	-	[272]
	42 STPs, NE Spain	06 - 114	12	-	[272]
Opiates	Barcelona Spain	2.4	1.2	-	[246]
Heroin	STP, Italy	20.0	<20.0	<1.5	[247]
	5 STPs, NE Spain	25.9 – 96.7	20.9 – 81.1	-	[247]
Morphine	3 Rivers, Italy	-	-	3.5 - 38	[186]
	5 STPs, Ireland	874 ± 86	452	-	[244]
	2 STPs, Italy	83.3– 204.4	5.5 ± 11.1	1-2L	[159]
	Barcelona, Spain	68.1 – 162.9 ± 20.0	21.8 ± 3.0	3.25	[246]
	12 STPs, Germany	123 ± 6	9.0 ± 1.2	83	[246]
	STP, Italy	7.1 – 96.7	0.1 – 8.1.	4.8 – 6.3	[247]
	5 STPs, NE Spain	30.7	-	-	[247]
Nor-morphine	1 STP, Italy	<25	<2.5 – 3.7	<12..5	[247]
	3 Rivers, Italy	-	-	0.93	[186]
6 ACM	2 STPs, Italy	10.4± 4.8 – 11.8 ± 8.5	-	-	[244]
	Barcelona, Spain	8.4 – 12.8 ±3.1	2.5 – 3.6 ± 0.5	-	[246]

Analytes	Matrix	Influent (ng L^{-1})	Effluent (ng L^{-1})	SW (ng L^{-1})	Ref
	12 STPs, Germany	8.4 – 12.8 ±3.1	0.9 ±1.2	83	[246]
	STP, Italy	102 ± 14	<3.1	<0.9 – 3.4	[277]
					[247]
M3G	2 STPs, Italy	2.5 ± 7.1 – 18.1 ± 30	<0.48	-	[159]
Methadone	5 STPs, Spain	4.0 – 239	4.0 – 24.7	-	[247]
	3 Rivers, Italy	-	-	4.9 – 10.1	[186]
	2 STPs, Italy	11.6± 1.7 – 49.7 ± 9.6	9.1 ± 0.5 – 36.2 ± 2.8	-	[159]
	12 STPs, Germany	123 ± 6	9.0 ± 12	83	[277]
	STP, Italy	4 – 23.9	2 – 2.7	4.9 – 10.1	[247]
Codeine	5 STPs, NE Spain	18.1 – 119.7	3.1 - 397	-	[247]
	3 Rivers, Italy	-	-	1.0 - 51	[186]
	12 STPs, Germany	80 ± 5	7.7 ± 8	90	[277]
Nor-codeine	5 STPs, NE Spain	5 – 68.0	15.5 – 22.9	-	[247]
6 Acetyl codeine	3 Rivers, Italy	-	-	<0.31	[186]
EDDP	3 Rivers, Italy	-	-	9.9 – 18.0	[186]
	5 STPs, Ireland UK	9.0 – 206 ± 10	-	-	[244]
	2 ST1 STP, Italy	19.8 ± 3.1 – 91.3 ± 19.2	22.6± 0.6 – 72.1± 8.7	-	[159]
	STP, Italy	4.5 – 41.3	4.9 – 56.7	9.61 – 17.5	[247]
THC	5 STPs, NE Spain	11.3 – 31.5	-	-	[247]
	2 ST1 STP, Italy	62.7 ± 5 – 91.2 ± 24.7	<0.94 – 7.2 ± 3.7	-	[159]
	Barcelona, Spain	4.3– 21.03 ± 7.8	8.4 ± 3.8 – 11. 23	2.65	[246]
	STP, Italy	8.3 – 31.5	<8.3	<7.0 – 13.6	[247]
THC-COOH	3 Rivers, Italy	-	-	0.48 -3.7	[186]
	STP, Italy	12.5 – 96.2	12.5	16.4 – 34.1	[247]
	5 STPs, NE Spain	37.8 – 96.2	14.8 – 48.1	-	[247]
OH- THC	Barcelona, Spain	8.4 – 46.3	4.8 – 15.3	10.7	[246]

Figure 4: Main metabolites of 3, 4-methylenedioxymethamphetamine (MDMA) in urine [144].
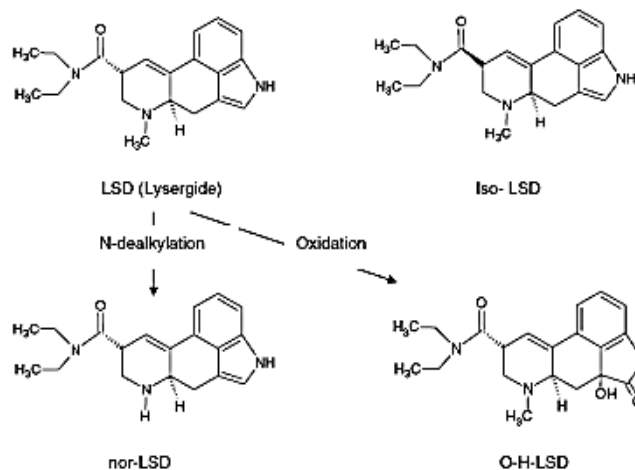
Human metabolism of opiates.

Opium comes from the opium poppy (papaver somniferum), a conjugated juice from the unriped capsule. It is an ingredient in morphine, codeine and theobaine. Several illegal drugs are produced from the opium poppy and the common ones are morphine and heroin, while 6-monoacetylmorphine/ and morphine are their related metabolites. The phenolic hydroxyl at position 3, the alcoholic hydroxyl at position 6 and the nitrogen atom plays important roles in morphine metabolism.

Figure 12 only show how heroin (diacetylmorphine) degradation pathways to produce main metabolites that we determined in the current work. But different morphine conjugates may arise from the actions of different enzymes, this emphasises the complexity of morphine metabolism [145]. Approximately 90% of an administered dose of morphine is excreted in the urine only about 10% is excreted as unchanged morphine. Morphine -3- glucuronide (M3G) is the major metabolite, while Morphine -6- glucuronide (M6G) is a minor one [146], and nor- morphine and nor-morphine-6-glucuronide have also been found in human urine and detected in wastewaters [Table 4]. Other minor metabolites like codeine (3-O-methylmorphine) and morphine- N- oxide have been identified in the urine of chronic users [147].

Figure 5: Degradation pathways of heroin and its main metabolites in living organisms. [150].
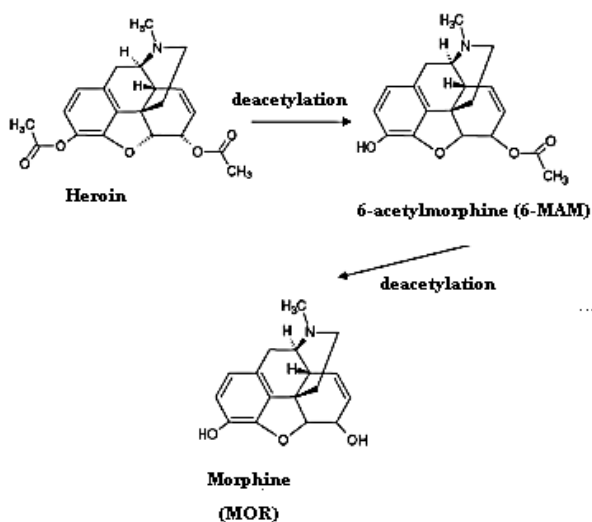
Human metabolism of Lysergic Acid Diethylamide (LSD)

Lysergic acid diethylamide is a compound derived from ergot alkaloids, a powerful hallucinogenic drug commonly sold as "acid" on the street as a drug of abuse. It is a non-addictive drug that comes in tablets or blotting paper, though liquid LSD is also available [148]. The drug is quickly metabolized in the body, where it is dispersed in the biological fluids in very low concentration and very small amount of the original dose is eliminated in the human urine [149]. In Figure 13, the following LSD metabolites have been identified in human biological fluids: 13-hydroxy-LSD, 14-hydroxy-LSD, N-demethyl LSD, 2-oxo-LSD, and 2-oxo-3-hydroxy-LSD [121-123]. The main metabolite of LSD is 2-oxo-3-hydroxy-LSD and 13- and 14-hydroxyl-LSD are excreted as glucuronide conjugates in urine [151]. In a review paper of Reuschel *et al* [152], evidences supporting a much higher concentration of 2-oxo-3-hydroxy-LSD in human urine of LSD users than the parent drug and 2-Oxo-LSD concentrations were reported. The iso-LSD and LSD exist as stereoisomers in illicit preparations and therefore iso-LSD is not a metabolite, it's frequently found in urine as a main contaminant of LSD [153]. Additional metabolites have also been identified in the laboratory animals but are yet to be found in human fluids [154]. The LSD compounds were however not studied in the current work.

Figure 6: Lysergic Acid Diethylamide and metabolites in human fluids [168]

Human metabolism of Cannabinoids

The cannabinoids, of which the most important one is tetrahydrocannabinol (THC) is the active chemical in *cannabis sativa L*, other active constituents are cannabidiol and cannabinol. Cannabis is commonly known as the source of the 'marijuana' drug and for centuries, this plant has been widely cultivated around the world for its fibres. The cannabinoids are non-polar compounds with low solubility in water but are soluble in fat, alcohol and many organic solvents, they are self-administered by smoking. The volatilized fractions are inhaled to give physiological effects. It is non-addictive and there are no withdrawal symptoms but one of the common side-effects of its use is making the user drowsy with reduced concentration and short term memory [155]. About 66 types of cannabinoids have been isolated from the cannabis plant but three of them have received most attention from researchers as a result of their natural prevalence. These are: phytocannabinoids (obtained from cannabis plant), synthetic cannabidiols (prepared from laboratory) and endogenous cannabinols (obtained from the body of humans and animals).

On ingestion, the cannabinoids are metabolized in the liver, especially by cytochrome P450 mixed-function oxidase, mainly CYP2C9. It is stored in the fat where Δ^9-THC is metabolized to 11-hydro-Δ^9-THC, which is metabolized to 9-carboxy-THC

[156], but the metabolism of THC is still not properly understood.

Figure 14 shows the structure of Δ^9-tetrahydrocannabinol (THC) and its metabolites in human urine [144]. The main metabolite is Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) and it is excreted as glucuronide-acid conjugate THC-COOH- glucuronide) [157], the metabolites can be detected in the body after weeks. It appears that the illegal status of the plant in most countries affected its systematic studying.
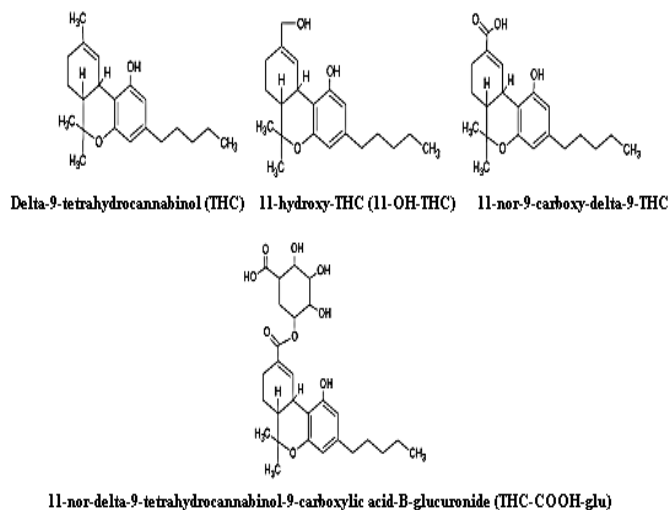

Delta-9-tetrahydrocannabinol (THC)   11-hydroxy-THC (11-OH-THC)   11-nor-9-carboxy-delta-9-THC

11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid-B-glucuronide (THC-COOH-glu)

Figure 7: Major metabolites of Δ9-tetrahydrocannabinol (THC) in urine [168].

Sewage Treatment Works as transport routes of pollutants.

Conventional sewage treatment works are the most significant routes through which the drugs enter the environment via untreated sewage and domestic sewage treatment systems. The ingested chemicals and associated metabolites are excreted via faeces and urine and passed onto sewage treatment systems. Discharges from manufacturers, commercial, domestic and run-off areas of unwanted and unused chemicals to the domestic sewage system are other major sources. Sewage sludge is the remaining residues after sewage treatment and the treated sewage sludge has several valuable properties which are agriculturally relevant; these include soil building potential giving it a strong hold, availability of nutrients and valuable trace elements essential to animals and plants, an efficient and sustainable alternative source to inorganic fertilisers and mineral fertilisers such as phosphate, and soil nutrient recovery through slow release of nitrogen.

Residues of pharmaceutical and illicit compounds have been found in surface waters in concentrations from ngL^{-1} to ugL^{-1} in many countries with the levels and distribution of these illegal compounds as found in wastewaters reported in Spain, Belgium, Italy, Germany, UK and USA [124, 158-167]. Apart from the active sludge processes, percolating filters, nitrification and de-nitrification facilities, investigations into the treatment technologies for the potential removal of drug residues and other organic compounds from the effluents of STWs have additionally identified ozonation [168-170] and membrane bioreactors (MBR) [171, 172] as biological means to provide improved potential in removing trace pollutants from the urban wastewaters. Microbial degradation has been suggested as the most important removal process in the sewage treatment works and with the continuing extensive studies on the metabolism and transformation of pharmaceuticals and other organics in humans and mammals, the microbial biodegradation pathways of some these chemicals, the persistence of their products and likely toxicity would largely be known [173]. Figure 16 below illustrates a typical interplay of complex physical, biochemical

and transformational routes of pollutants in STWs and each transport route depends on the nature of influents [173-179].
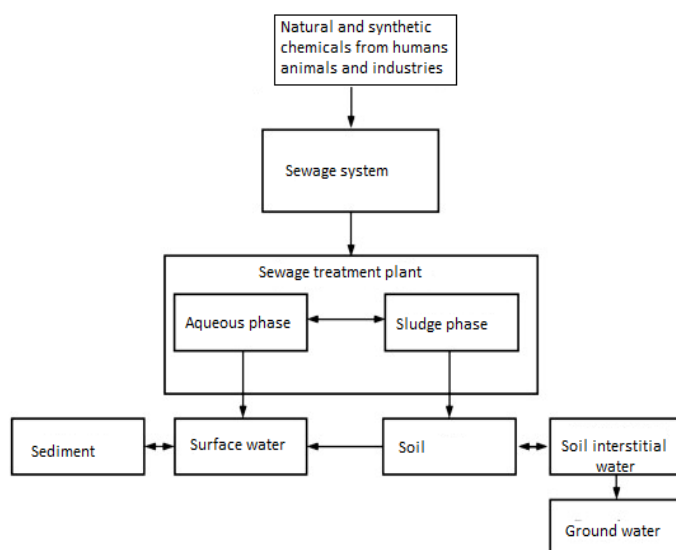

Figure 16: Organic contaminant fate and distribution in the environment [183]

The microbial degradability of the illicit drugs in the sewage as well as their degradation pathways has not been reported. However, small studies on selected pharmaceuticals with the identification of some microbial degrades suggest that similar processes are likely to affect the illicit drugs [176-178]. In 2009, the understanding of the STWs systems and the degradation processes involved were observed by Kasprzyk-Horden *et al* [179] on selected pharmaceuticals and illicit drugs (cocaine, benzoylecgonine and amphetamine) where the differences in the performance of activated sludge and trickling filter on a 5-month monitoring program was undertaken from two different STWs in South Wales, UK. However, the choice of sampling points was just to verify the removal efficiency of the two contrasting STWs and the work recorded over 85% removal efficiency of most drugs with STW utilising activated sludge compare to less than 70% reported for trickling filter.

However, for the first time, direct measurement of the illicit drug removal rates in laboratory (batch) studies would carried out to improve upon the understanding of the degradation rates of cocaine (COC); benzoylecgonine (BZE); heroin (HER); 6-monoacetylmorphine (6-MAM); morphine (MOR) and diazepam (Diaz) under different conditions to obtain removal rates. The capabilities of the current experimental batch data in generating removal rates of drugs would be applied in mass balance calculation to improve influent measurement.

Also, no publication to our knowledge has been found on the ecotoxicological impacts of chronic exposure of illicit drugs and their metabolites as the STWs procedures cannot effectively remove all the drugs or polar compounds due to their hydrophobic/lipophilic character [111]. Apart from volatilisation, hydrolysis (abiotic) and biodegradation (biological processes), physical-chemical adsorption of polar compounds onto the biosolids surfaces also occurs. The interaction of compounds with high adsorption coefficients in particular determines the extent of the removal. Natural solids like clay, sediment and micro-organisms and added solids (e.g. active carbon, coagulants) facilitate STWs removal processes [184]. Those adsorbed on solids and passed as sludge enter the environment when spread on agricultural lands as manure and the compounds continue in the ecosystems or are possibly leached into underground waters; while those with low adsorption coefficients are released as effluents into the receiving waters. The removal of organic compounds is often

incomplete in most municipal STWs, the sewage-sludge and effluent waters are therefore the primarily routes at which these chemicals enter the environment. Apart from the biodegradation, chemical degradation and sorption processes in typical STW details of which are not well understood because of the complex mixtures present are the other main removal processes during the wastewater treatment. The physicochemical properties of the contaminants ultimately determines their extent of persistence, toxicity and potential environmental effects after the sewage-sludge disposal to agricultural lands or effluents waters disposed of to seas.

The existing priority substance classifications by the European Communities Priority Substances Directive notwithstanding [185], the emerging priority contaminants groups like 'illicit drugs and their metabolites' have no safe-levels because of insufficient information on their biodegradability and persistence after their disposal to lands or receiving waters. Insufficient information, decisions and policy thrusts regarding the future practices of safe sewage-sludge disposal mean that complete removal of contaminants from STWs effluent-waters becomes difficult.

Existence of uncontrolled discharges of different types of compounds from humans and from veterinary treatment into the environment via STWs is shown in the anticipated exposure in Figures 17 and 18. Drugs for human treatment are primarily exposed to the environment from routes Fig 17 (F1 & F2) and enter different treatment fate processes at points F3 & F4 and terminate at F8 and F9.
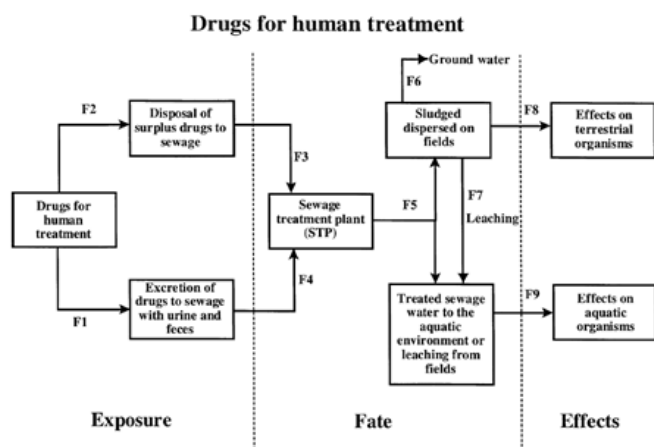
Figure 17. Anticipated exposure routes of drugs for human treatment in the environment [186]

The effects on terrestrial and aquatic organisms continue with drugs from veterinary treatment in Fig. 18 (F10-F13) in another complete process of bio-chemical reactions and mechanisms with anticipated toxicity impacts on the ecosystems not yet understood.

Studies in the literature have confirmed the enrichment of the sewage sludge partitioning of chemicals onto sludge solids or suspended in solution is due to their hydrophilicity/lipophilicity properties compared to influent sewage [187-189]. Understanding of the fate and behaviour of pollutants during sewage treatment will show the degradation possibility of compounds that are completely or partially degraded in aqueous and solid phases, sorbed to sludge solids or mineralised. In a study reported by Strachan *et al* [190], organic contaminants are located within the fraction of large organic wastes (biomass) which are repository of living and dead micro-organisms required for degradation processes.
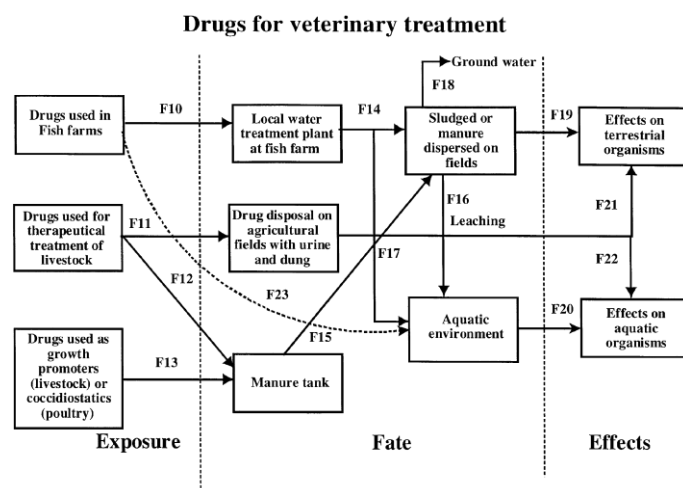

Figure 18. Anticipated exposure routes of drugs for veterinary treatment in the environment [186]

Microbial degradation in the aquatic environment

Investigations on the levels of removal of organic residues from a wastewater plant studies have shown toxicity correlation of wastewater effluents on aquatic organisms to determine the response levels with degree of contamination [191-193]. Biodegradability studies of organic priority pollutants and reduction in toxicity of these pollutants in wastewaters treatment processes have also been carried out [194-195]. Also evaluated were 22 priority pollutants belonging to the class of phthalates, pesticides, polycyclic aromatic hydrocarbons and phenols in an activated sludge pilot plant [111, 194]. About 80-99% removal efficiency was recorded from the parallel and spiked treatments of between 50-150 µg L^{-1} concentrations. The results indicated a degradation of phenols, enrichment of PAHs to about 64% from the mass balance calculations while pentachlorophenol was associated with the solid phases. Table 5 presents percent sewage sludge disposal in the last 3 years, and the subsequent transport of these residual organic pollutants in the sewage which enters the environment and becomes the issue of current concern.

Sewage is a complex association of wastes of human excreta containing mixtures of fats, sugars, lignin, protein, cellulose, humid materials, amino acids and fatty acids. Wang *et al* [197] studied the partitioning mechanism of the organic residues within the biomass and sorption onto the sludge surface as a two-stage process. Determination and prioritisation of typical sewage sludge can be a complex task because of many synthetic organic materials with various residues of diverse origins due to 1) interferences of co-contaminants in complex matrices, sample extraction and clean ups, sensitive techniques needed to determine low concentrations 2) the fate and behaviour of sludge-derived residues after disposals requires investigation to monitor persistence and environmental impacts and 3) bio-transformation arising from degradation of residues generates toxic by-products, but unavailability of some compounds, sorped onto sewage solids to bacteria for degradation can be significant as little is known about the final fate of these organics [198-202]. Difficult isolation of sludge samples arises also from non-uniformity of extraction procedures and variability in obtaining grab samples as a representative of all various genotoxins in the sludge matrix [203-205]. Different processes or techniques are often adopted for specific effluents depending on the origins of the contaminants. Generally factors often considered, though contaminants can be lost during treatments in a complex variety of ways are: 1) sorption/association with sewage solid surfaces 2) abiotic processes/hydrolysis involving chemical degradation 3) volatilisation and 4) biodegradation [111].

Humans are typically exposed to numerous organic and inorganic pollutants, as by-products from treatment of waste water from domestic, agricultural and industrial sources which constitutes sewage [206]. The presence of intestinal pathogenic bacteria and animal parasites in sewage sludge has been confirmed from several investigations [207, 208]. However, sewage sludge may contain relatively large amounts of heavy metals as well as organic pollutants such as phthalates, polychlorinated biphenyls (PCBs), alkyphenols, and organoclorine pesticides compared to normal environmental levels in soil, water, and air [2]. Increasing amounts of sewage sludge are used for land filling and agricultural land including pastures grazed by ruminants following the ban on ocean dumping of sludge [209]. The potential health risk imposed due to the presence of organic and inorganic compounds found in sewage sludge is of concern in humans [210, 211] if they are delivered at high enough doses to cause effects through the consumption of products derived from animals grazing on contaminated pastures [212]. Adverse effects which have been reported in humans include perturbation of male reproductive tract, certain male and female cancers, declined fertility, thyroid dysfunction and ill impacts on the central nervous system, gastroenteritis, damage to liver, kidneys and blood, hepatitis, occupational asthma, infection of skin or eyes and inflammation of the lung following sewage sludge exposure. Different groups of environmental chemicals with a variety of mechanisms and disrupting activities have been identified and discussed [213-218].

In the literature, degradation studies of pharmaceuticals have identified degradates of anti-inflammatory, analgesics and blood-lipid regulators. In batch studies of acetylsalicylic acid with suspended activated sludge, the decrease of about 70-99% in concentration after 6, 24 and 72 h was observed but no metabolites were detected using GCMS [89]. The degradation studies of anti-inflammatory and blood-lipid regulators such as bezafibrate, diclofenac, naproxen and ketoprofen in activated sludge were carried out, but only ketopofen biotransformed into [3-(hydroxyl-carboxy-methyl) hydratropic acid and [3-(keto-carboxyl- methyl) hydratropic acid [89]. Biodegradation of trimethoprim showed resistance to degradation in a reactor filled with activated sludge, but its degradation in a nitrification process was completed in 3 days. In a similar study, Ternes et al [89] investigated degradability of estrogens in aerobic batch reactors at two different concentrations using GCMS. The 17β-estradiol was oxidised to estrone without any detectable degradates. Also, 16α-hydoxy-estrone was similarly degraded without degradation products. In a subsequent work, the biodegradation studies of trimethoprim, anti-tumorals cisplatin, cyclophosphamide, cytarabine, X-ray contrast agents, iopromide and diatrizoate has been carried out but not all the details of metabolites identification were reported [89].

Concerning the degradability of illicit drugs, apart from sample degradation, biodegradation is a natural process that has been reported by the stability experiment conducted by Georghe *et al* [219] and which observed that the concentration of cocaine and ecgonine methylester changed in surface water by 40 and 95% after 5 and 24 h test period respectively. However, benzoylecgonine level was constant or increased in the study. Photodegradation is another abiotic process involving complex reactions and pathways that could affect the aquatic fate of compounds, particularly when degrades are resistance to hydrolytic processes [220]. Four relatively new metabolites of cocaine: ecgonidine, norecgonidine methylester, *p*- hydroxyl-benzoylecgonine, and *m*- hydroxyl-benzoylecgonine [221], and two conjugates of metamphetamines: *p*-hydroxy-metamphetamine (*p*-OHMA-sulfate), (*p*-OHMA-Sul) and (*p*-

OHMA) (*p*-OHMA-glucuronide) [220] have been identified in human urine.

The identification of this phase-II degradates and other metabolites in urine indicate likelihood of their presence in wastewater samples, unless they are further degraded in the sewage treatment works.

In a study, Pizzolato *et al* [150] observed the 40-80% degradation of cocaine and its metabolites in river waters under sunlight and pseudo-sunlight after 11 days of exposures as compared to HPLC grade water. Degradation was about 80% faster in river water as cocaine degraded to benzoylecgonine confirming the effects of both biodegradation and photodegradation.

Identification of microbial metabolites of ibuprofen has been found to be identical with the compound human metabolites [222-223]. During wastewater treatment, apart from the sorption behaviour of potential organic contaminants to the sludge solids, the removal of organic residues and associated metabolites are through microbial degradation as earlier reported as part of the removal mechanism of some pharmaceuticals and endocrine disrupting compounds (EDCs) in the sludge [80-83]. Hydrolysis (abiotic process) is the most important mechanism in the chemical degradation pathways through which compounds are removed [234]. The enrichment of the sewage sludge partitioning of chemicals onto sludge solids or suspended in solution is due to their hydrophilicity/lipophilicity properties compared to influent sewage [187-189].

Table 6 summarises the drugs and their metabolites identified from both human biological fluids and aquatic environments.

Appreciation of the degradation possibility of compounds whether they be completely or partially degraded in aqueous and solid phases, sorbed to sludge solids or mineralised is an important step in understanding the fate and behaviour of pollutants during sewage treatment. Within the large organic wastes in sewage is biomass of living and dead micro-organisms required for degradation processes within which some fractions of organic contaminants could be found. Sequential biological processes in alternating oxidative and reductive conditions for recalcitrant organic compounds plays a major role in removal mechanism [111, 190, 252].

In the degradation studies of alkylphenol polyethoxylate (APEO) surfactants, the recalcitrant and estrogenically active alkylphenols (APs) were produced from commercial NPEO using synthetic activated sludge in batch tests. The levels and distribution of the short chain compounds after NPEOs degradation confirmed in many ways these routes by which pollutants are discharged to the aquatic environment due to incomplete removal from treatment processes [253]. In activated sludge, viable and diverse bacterial population is maintained when the biological sludge is re-cycled from settling tank back to the aeration tank to produce high quality effluent, reduced biomass, maximised conversion of substrate and less production of waste sludge The oxidation of organic matter in an biological aerobic process generates carbon dioxide and water with the new but reduced biomass and dissolved residual organic matter in the effluent [224]. In related studies, Richardson and Bowron [93] assessed the biodegradability of some specific chemicals as presented in Table 7, but yet to be investigated are the degradation processes as well as the extent of transformations in producing different chemical metabolites [111]. Pathways of microbial degradation of selected acidic pharmaceuticals and their occurrence in municipal wastewater treated by a membrane bioreactor have been reported [254]. To further understand the behaviour of compounds in sewage plants, studies of metabolites from the biodegradation of pharmaceutical residual

of ibuprofen in biofilm reactor also confirmed the effects of biodegradations [222]

Elimination of selected acidic pharmaceuticals from municipal wastewater using activated sludge systems and membrane bioreactors [255], modelling versus measurement experiment of effluent from hospitals and private households to the total loads of diclofenac and carbamazepine in municipal sewage effluent [256] and identification of microbial degradation of trimethoprim in nitrifying activated sludge batch studies have been reported in the literature.

Stability of drugs and metabolites

The stability of drugs and their metabolites in the aqueous environment depends on some conditions of temperature and pH to minimise degradation of analytes. Studies recommended the acidification of samples to pH 2 and - 20° C for storage in a stability study of cocaine and its metabolites (e.g. benzoylecgonine and ecgoninemethylester) where a pond free of drugs was spiked with different concentration of cocaine and benzoylecgonine at modified pH values of 2 and 6 and temperatures (-20°C, +4°C and +20°C) for 5day stability tests. The 22% degradation of cocaine after 3 days and 35% after 5 days at pH 6 and +4°C were observed. Also, ca.75% degradation was observed at +20°C at pH > 6 for 1 day [158]. Using different preservation conditions, some decreases in the concentration of cocaine (36%), cocaethylene (13%), nor-cocaethylene (15%) and M3G (96%) and changes led to corresponding changes in the levels of metabolites (BZE, nor-BZE and MOR) respectively with the optimal conditions for storage similar to that observed for Cocaine, BZE and EME [219]. Similar works have also shown preserved samples at -20°C with addition of HCl (pH 2) stopping bacterial action. Cocaine stability in wastewater at 4° C for 48 hours was investigated but no changes were observed. Storage experiments with methanolic extracts for 7 days at different temperatures observed degradation of up to 15% with extracts stored at +4°C but no changes with those stored at -20°C [244, 159, 257]. Stability of other drugs of abuse like heroin, amphetamines-like substances and lysergic acid and their metabolites were not found in the literature.

Analytical Methodologies

In recent years, important advances in the development of chromatographic and mass spectrometric methods have been made, particularly in the detection and quantitative measurement of illicit drugs and their metabolites in various biological and aquatic matrices. The techniques based on liquid chromatography- mass spectrometry (LC-MS) or liquid chromatography tandem mass spectrometry (LC-MS^2) and gas chromatography-mass spectrometry (GC-MS) or gas chromatography tandem mass spectrometry (GC-MS^2) are very popular primarily because of their ability to detect and measure chemical substances at very low concentration. In addition to analytical methods for tracing pharmaceuticals residues in water and wastewaters that have been extensively used [93, 97-101], other analytical procedures for quick screening of drugs residue in aqueous environments including several inexpensive immunochemical approaches, as an alternative method to the chromatographic techniques for the efficient analysis of pharmaceuticals have also been published [96].

Chromatographic techniques

Table 8 shows the survey of chromatographic techniques from peer-reviewed literature in the determination of illicit drugs and human metabolites in waters. The review covers the extraction volumes, mobile phases, detectors (interfaces) and acquisition modes used by different scientists to provide sensitivity and selectivity. Also included are limits of quantifications depending on matrices for quantification and

confirmation of drugs. The HPLC separation procedures rely on the principles of reversed-phase columns with different solvent gradients depending on applications [247-249]. Recently, variations over conventional LC-MS method have appeared in the literature eg. Ultra-performance liquid chromatography (UPLC-ESI-MS/MS), the ultra-fast UPLC-MS^2 is unique for its short columns packed with small particles sizes and stability at different pH range [135, 266 -268]. With the development of this relatively new technology, a shorter analysis time as well as gain in separation efficiency, resolution and sensitivity has been reported. To minimised the effects of ion suppression on the analytical signal, a relatively new HILIC; Hydrophilic interaction chromatography technique was also carried out in some experiments. Analytes were better retained on HILIC column, unaffected by ion suppression and a reduction in analytical signal was minimised [158, 219].

The use of MS/MS with triple quadrupole (QqQ) analyzers with electrospray ionization (ESI$^+$) were mostly used in selected reaction monitoring mode (SRM) to minimize the matrix interferences. The choices of ionization in ESI positive-ion mode were to have achieved ionizations and simultaneous determinations of analytes.

The HPLC-MS methods are also used in the analysis of illicit substances in the literature [208,214]. Both HPLC and GC-MS have been applied in the determination of pharmaceuticals in different matrices of biological fluids [262-265] especially urine [266-269], oral fluid [225, 226], and blood [227] samples. The advantage of HPLC-MS in the determination of the main illicit drug classes including cocaine, amphetamines, opiates and synthetic opiods, cannabinoids and their metabolites is due to its no hydrolysis, no derivatization, one- step extraction and with the introduction of atmospheric pressure ionisation (API) interfaces, the technique has been popular [269]. HPLC is a good and popular technique for highly polar, high molecular weight and thermolabile compounds. Its reproducibility, sensitivity and overall lower costs have therefore made it a convenient method. The use of GCMS is very rapid, faster and highly specific with in-built NIST library softwares for compound identification and elimination of matrix effects.

Principle of the choice of method (SPE-GC-MS): The trace analysis in wastewater can be captured as simple liquid chromatographic process where the SPE sorbent acts as the stationary phase and water constitutes the mobile phase during the extraction. During the percolation step, analytes that are trapped and cannot elute constitute the sample matrix. The enrichment of analytes from a large volume of aqueous sample on sorbent depends on how strongly the analytes are retained while allowing low retention during elution with organic solvents.

The method of coupling of SPE to GC-MS can be directly integrated as an online analytical system or off-line where subsequent chromatographic analysis is completely separated from the sample treatment. As long as the compounds are sufficiently thermally stable and volatile enough, gas chromatography (GC) allows a broad variety of samples to be analysed. As for all other chromatographic techniques, a mobile (carrier gas e.g. helium, argon, nitrogen, etc.) and a stationary phase (packed column or solid support coated with the liquid stationary phase of high boiling polymer e.g capillary columns of a small-diameter tube like 0.25 mm film in a 0.32 mm tube) are required . Different compounds are separated due to the interaction of the compound with the stationary phase ("like-dissolves-like"-rule). The stronger the interaction is, the longer the compound remains attached to the stationary phase, and the more time it takes to go through the column (longer retention time). GC-MS is a good combination of coupled analytical

systems as GC separates the compounds then MS identifies them based on their fragmentation pattern.

Solid Phase Extraction (pre-concentration)

Table 9 shows the multi-step extraction procedures of different protocols which have been reported in the peer-reviewed literature, to eliminate the influence of matrices [124,159]. Apart from matrix effect, improved recovery, stability under pH and ability of delivering clean extracts have resulted into various tests of several SPE adsorbent to determine suitable parameters relevant to a particular application need. Several SPE methods and adsorbents have been developed and used in conjunction with LC-MS^2 or GC-MS^2 in the determination of illicit drugs and their metabolites in aquatic media at very low concentrations (ng L^{-1} levels). Recently, Oasis MCX$^®$ (500mg/6mL) adsorbent, a polymeric sorbent with mixed–reversed/strong cation-exchange sulfonic acid group located on the surface of a (divinylbenzene-co-N-vinyl pyrrolidone) has been used [259, 270] to extract drug analytes from aqueous samples. After the samples were adjusted to pH 2 with 37% HCl or 0.01NHCl, the cartridge was pre-conditioned with 6ml of MeOH, 3mL of milli-Q water and 3mL water at pH 2. Samples were loaded into the cartridges at flow rate between 5-20mL min^{-1}, vacuum-dried for 5min and eluted with 6mL of MeOH and 6ml of 5% NH$_3$ in MeOH. The cartridges were found to be stable perhaps because of its two phases that were assumed could retain all compounds investigated. In related development Wylie *et al* [225] and Miltona *et al* [266] have used Bond Elut Certify$^®$, a lipophilic and strongly cationic-adsorbent with similar conditioning and washing steps as used with Oasis MCX$^®$ adsorbent, the only difference was 2 x 4 mL of 80:20 DCM/isopropanol mixtures with 2% NH$_3$ in elution step. Traditional SPE materials such as the modified silica's e.g. C$_8$, (octyl), C$_{18}$ (octadecyl) or CN (cyanopropyl) materials have low pH range, poor selectivity and residual silanol group which often leads to low recoveries in aqueous sample [82,90].

Bones et al [94] investigated the use of three sorbents: Phenomenex Strata- X™, Strata- XC™ and Strata- XCW™, all in 200mg sorbent mass pre-packed in to 6mL cartridges, but Strata- XC™ provided the highest analyte recovery. In other experiments, the Oasis HLB$^®$ (500mg/6 mL) adsorbent [278], MCX$^®$ (500mg/6mL) [159], Isolute ENV+$^®$ (500mg/6 mL) and Isolute PH$^®$ (1000mg/6 mL) adsorbents [245], and Bond Elut Certify$^®$ adsorbent [225] were compared with other adsorbents by Gheorge *et al* [219] in the extraction of cocaine and its metabolites in waste and surface water and the authors recommended the use of Oasis HLB$^®$ (500mg/6 mL, protocol 1) as most suitable adsorbent for organic compounds because of its lower solvent usage, time, stability to pH range and over 75% recovery for most analytes in aquatic medium.

Results and conclusions of survey of drugs in wastewaters

Generally, the illicit drug detection has been limited to the continuous screening of individual's biological fluids (urine, blood, oral-fluids and sweat), population survey with crime, drug production data, drug seizures and medical records [273,274]. The official estimates of the community consumption of illicit drugs from these exercises can be very unreliable because of the hidden nature and network of manufacture, importation, supply and usage without authorisation. Globally, United Nation Office of Drugs and Crime, (UNODC) estimates that between 149 and 272 million people, or, 3.3% to 6.1% of the population aged 15-64 used illicit substances at least once in the previous year [275]. Drugs are used in many ways and in many combinations by prescription for medical purposes, some illicit drug users often utilise therapeutic pharmaceuticals to supplement their illicit drug use by diverting common

pharmaceuticals for illicit personal use and this illegal practice have affected societies in a myriad of ways. However, with the continuing pattern of escalation in use of illicit drugs and the discharge of their bioactive metabolites to sewage systems, and the present mode of sewage disposal (e.g. to grassland, landfills, incineration, horticulture, land reclamation) as complex mixtures so the processes involved in drugs removal at various STWs are not fully understood. Table 4 therefore summarises and compares the levels and distribution of the drugs from different STWs as reported in the literature in the last ten years. Also in Table 4, it was observed that the relative concentrations of drugs influents were higher compare to the effluents indicating the degree of removals. For example in 5 STWs in Spain, cocaine and benzoylecgonine in the influents were 225 and 2307 ng L^{-1} compare to only effluent cocaine concentrations of 47 ng L^{-1}. The relative concentration of benzoylecgonine for example is about 10 times higher than the parent drug.

The removal of organic compounds is often incomplete in most municipal WWTPs; the sewage-sludge and effluent waters are therefore the primarily routes at which these chemicals enter the environment. Apart from the biodegradation, chemical degradation and sorption processes in typical WWTP of which details are not well understood because of the complex mixtures present during the wastewater treatment. The physicochemical properties of the contaminants ultimately determines their extent of persistence, toxicity and potential environmental effects after the sewage-sludge disposal to agricultural lands or effluents waters disposed of to seas. The existing priority substance classifications by the European Commission and U.S. Environmental Protection Agency notwithstanding [185]; the emerging priority contaminants groups like 'illicit drugs and their metabolites' have no safe-levels because of insufficient information on their biodegradability and persistence after their disposal to lands or receiving waters. As a result of insufficient information, decisions and policy thrusts regarding the future practices of safe sewage-sludge.

The illicit drugs and their metabolites are mainly from faeces and urination, the pattern of lavatory use fluctuates between individuals, certain periods of work and the population of residents in a particular environment, the load pattern of illicit substances would likely fluctuate in similar way [144]. Since many active researches have been on detection of illicit drugs and related products, studies on their fate and behaviour are therefore most warranted.

We have therefore critically reviewed the current development on the occurrence, metabolism, treatment processes, measurement before and after discharges from STWs to the environment with the current analytical methodologies that meet particular application needs such as fate of drugs monitoring.

With the developments on different aspects of drug's transformations in the environment recently published in the chemical literature which includes occurrence and fate, treatability by conventional and non-conventional processes, and several miscellaneous others [276-293]. The pharmaceutical and illicit markets will continue to grow to provide numerous commercial and therapeutic purposes. Today, large numbers of new drugs are introduced into the market and the number of patents granted keeps increasing accordingly. As these products reach the market with already large number of pharmaceutical companies involve in their development continue to grow. Information in the literature on the distribution levels, laboratory studies of fate and behaviour of some classes of illicit and pharmaceuticals drugs and their environmental assessment are rapidly changing. It is not very clear if current chemical pollution approaches in terms of effective monitoring and control of chemical discharges into the aquatic environment

would achieve desired effect and environmental scientist will have to be ready for new challenges ahead.

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