CHAPTER 4

AN OVERVIEW OF THE ENANTIOSELECTIVE DETERMINATION OF ILLICIT DRUGS IN ENVIRONMENTAL SAMPLES

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ABSTRACT

In last decades, the appearance and consumption of illicit drugs has been growing gradually, which eventually has led to their occurrence in environmental samples. Some of the illicit drugs (namely amphetamines, cathinones, ketamine, among others) are in form of enantiomers, whose enantiodetermination is of crucial relevance to be able to distinguish not only ecotoxicological but also risk assessment effects. In view of this, development of analytical methods for the enantiodetermination of illicit drugs in environment is an evolving research field. Among various analytical techniques, chiral liquid chromatography (LC) coupled with mass spectrometry (MS) has been widely used due to its simplicity, wide applicability and high sensitivity to determine chiral illicit drugs in environmental samples. However, other separation techniques including gas chromatography (GC) and capillary electrophoresis has been also explored either using direct (chiral stationary phases) or indirect methods (chiral additives or derivatisation using usual stationary phases) in such conditions feasible to be coupled to MS.

This chapter covers the different aspects of chiral illicit drugs in environmental samples, with special emphasis on their determination. Thus, different sample treatment strategies for water and solid analysis are overviewed. Moreover, separation techniques including mainly LC using several chiral stationary phases and elution modes are summerised, but also GC enantioseparation using either direct and indirect methods are outlined. Finally, some applications for the enantiomeric fraction determination of chiral drugs in environmental matrices are also described.

Keywords: chiral illicit drugs; sample treatment; gas chromatography; liquid chromatography; chiral stationary phases; derivatisation; mobile phase elution modes; mass spectrometry.

1. INTRODUCTION

Illicit drugs are psychoactive or psychotropic substances that stimulate the central nervous system, altering a person's behavior and consciousness and potentially leading to dependence and addiction. Over the decades, the global consumption of illicit drugs has been steadily increasing, resulting in the emergence of new types of drugs, new trafficking methods or distribution routes, and the growth of on-line markets [1]. Due to these factors and the significant social impact of illicit drug consumption, various organizations have been established to monitor and control these substances. Examples include the National Institute on Drug Abuse (NIDA) in the United States and the recently rebranded European Union Drugs Agency (EUDA), formerly known as the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), in

Europe. These organizations aim to provide evidence-based information on drugs, addiction, and their consequences. Additionally, they collaborate with other organizations, such as the United Nations Office on Drugs and Crime (UNODC), which operates globally through an extensive network of field offices.

Therefore, as the drug consumption increases, the discharge of drugs and their metabolites into the environment also increases, and consequently the occurrence of these compounds in the environment has become of global concern, and illicit drugs constitute a group of emerging environmental contaminants. In this line, studies monitoring illicit drugs in environment have increased exponentially over the last decades [2–4].

An important characteristic of some illicit drugs is the presence of a chiral center in their structures, resulting in the formation of two enantiomers. Although these enantiomers have identical chemical structures, they can exhibit different pharmacological, toxicological, and pharmacokinetic effects in the human body. This variation arises because enantiomers may interact stereoselectively with chiral molecules such as proteins, enzymes, and amino acids within the organism. For chiral drugs, typically only one enantiomer (the eutomer) is pharmacologically active, while the other (the distomer) may be inactive or even harmful. For this reason, identifying the enantiomeric composition of a substance has become increasingly important in forensic and toxicological fields, as well as in environmental risk assessment, where it can affect the correct evaluation of a drug's ecotoxicity. Therefore, research into the enantiospecific determination, occurrence, fate, and toxicity of chiral drugs is important for achieving accurate ecological risk assessment.

The following sections overviews enantiomeric illicit drugs and their main metabolites and discuss their enantiomeric occurrence in the environment addressing the significance of enantiomeric determination of chiral drugs in the environment.

1.a CLASSIFICATION

Drugs can be classified in many different ways, including their uses, their effects, their therapeutic value or their chemical structures. Thus, one of the most interesting classifications is according to their medical uses, potential additive effects and health risks. Likewise, illicit drugs can be classified into different groups according to their potential effects on the nervous system as well as the risks associated with their abuse. In this section, drugs will be separated into two large groups, on the one hand, traditional drugs such as cannabis, cocaine, amphetamine or opioids, among others, and on the other hand, a group of novel psychoactive substances (NPS) that include synthetic cathinones, synthetic cannabinoids, ketamine analogues or synthetic opioids, among others. Table 4.1 summarises the main categories of traditional drugs and NPS present in the market considering the most common drugs in each category. In addition, only some categories (namely amphetamine-type substances, synthetic cathinones, ketamine analogues, phenethylamines, and synthetic opioids) are in form of pure enantiomers or racemic mixtures. This information is also highlighted in Table 4.1 and detailed in the text below.

Amphetamine-type substances are chemically synthesised in form of racemic mixtures of R and S enantiomers because obtaining a single enantiomer is more expensive. R and S enantiomers have different pharmacokinetic and pharmacological characteristics. Thus, S-enantiomer of amphetamines (i.e. amphetamine (AMP), methylamphetamine (MAMP) and 3,4-Methyl enedioxymethamphetamine (MDMA)) have more pronounced activity, so it is more easily absorbed and therefore, it is excreted at a lower rate than the R-isomers [5]. Moreover, the

enantiomers are used for different applications, as for instance, S-AMP or the prodrug lisdexamfetamine is used for attention deficit hyperactivity disorder (ADHD) treatment, while the R enantiomer is responsible for the euphoric, appetite suppression effects, among others [6]. In the case of MAMP, its R-enantiomer is used as a decongestant in some commercial devices while the S-enantiomer is related to stimulant effects [6].

Synthetic cathinones are similar to amphetamine as their structures are very similar, except for the ketone group in the cathinones. Synthetic cathinones can include a wide variety of synthetic substances, since different substituents can be placed in four different positions. They can be alkylated or halogenated in the different substituent positions to obtain different cathinones. For example, if the cathinone is methylated in positions R1 and R4, 4-methylmethcathinone (mephedrone) is obtained, whereas if a fluoride is in position R1 and a methyl in R4, this results in 4-fluoromethcathinone (flephedrone). Depending on the substituents, synthetic cathinones can be divided in four groups: N-alkyl compounds or those with an alkyl or halogen substituent in the aromatic ring; methylenedioxy-substituted substances with substituents in the aromatic ring; analogues of natural cathinone with an N-pyrrolidinyl substituent; and methylenedioxy and N-pyrrolidinyl substituent substances [7]. Similar to amphetamines, they have a chiral center and thus two enantiomers are present, which are important to identify because of their different metabolic pathways and pharmacokinetic and pharmacological properties in humans [7,8]. By a way of example, S-methcathinone showed higher stimulating effects in central nervous system than its R-analogue [9]. A similar conclusion was drawn for the isomers of 4-methylcathinone in rat studies [6]. It should be mentioned that the number of studies dealing with the enantioselectivity of cathinones has growing in last years, some of which are collected in a recent review [7].

Ketamine is available as a racemate (R,S), though pure enantiomeric forms such as S-ketamine (esketamine) have been approved. Some studies have shown detrimental side effects induced by racemic ketamine and S-ketamine, suggesting that R-ketamine might be safer for humans [10]. In fact, an early study conducted in humans showed that S-ketamine produced psychotic reactions, including depersonalization and hallucinations, whereas R-ketamine did not produce any psychotic symptoms [11].

Ephedrine is a substituted amphetamine, and only differs from MAMP by the presence of a hydroxyl group. Because of this, it is highly used to synthetize MAMP and classified as a table-I precursor since it is highly sought-after chemical precursor in the illicit manufacturing. As amphetamine-type substances, it has a chiral center, and the pair of (1*R*,2*S*) and (1*S*,2*R*) enantiomers are designated ephedrine, while the pair of enantiomers with the stereochemistry (1*R*,2*R*) and (1*S*,2*S*) are called pseudoephedrine. It has also been reported evidences of difference in pharmokinetics among enantiomers since the binding affinity to adrenergic receptors of ephedrine is 20-fold as compared to the enantiomeric form pseudoephedrine when studied in rats [12].

Fentanyl is a potent synthetic opioid used as analgesic, which is up to 40 and 100 times more potent than heroin and morphine, respectively, and has adverse effects similar to other opioids (drowsiness and euphoria). Different fentanyl derivatives (called non-pharmaceutical fentanyl or designer fentalyls), which have recently led to acute intoxications and lethal effects, have been synthesised by adding various substituents to enhance potency, and some of the resulting compounds may exist as enantiomers. For instance, the enantiomers of 3-methylfentanyl displayed different analgesic properties [13,14]. Nevertheless, due to the novelty of such substances, little is known about their pharmokinetics.

In view of the differences among enantiomers, it is important to monitor the different enantiomers to discriminate them.

1.b REPORTED OR POTENTIAL METABOLITES AND/OR TRANSFORMATION PRODUCTS

After human consumption, illicit drugs undergo absorption, distribution, metabolism, and excretion processes. Knowing the metabolic pathways of chiral illicit drugs and their main urinary excretion products (parent compounds or free and/or conjugate metabolites) is fundamental. However, up to date, limited information related to pharmacokinetics is available and therefore, more studies are required for understanding their metabolism.

Regarding the metabolisation of amphetamine-type substances in the human body, compounds such as norephedrine or hydroxyamphetamine from AMP, 3,4-methylenedioxyamphetamine and 4-hydroxy-3-methoxymethamphetamine from MDMA and p-hydroxymethamphetamine and AMP from MAMP can be found in some biological matrices such as urine [15]. Therefore, the presence of AMP may be due either to its consumption or to the metabolisation of MAMP. In any case, the excretion of amphetamine-type substances is mainly as unchanged form (excretion rates from 22.5% for MDMA to 43% for MAMP) and as various metabolic products [15].

Synthetic cathinones are mainly excreted (partly) unchanged via urine because of their poor metabolising capacity [16]. Transformation products of some synthetic cathinones are starting to be identified and found in the environment, as for instance, 18 transformation products were identified that corresponded to demethylenation, demethylation, hydroxylation, and reduction of the β-keto groups reactions from methylenedioxypyrovalerone, methylone, mephedrone [17].

Ephedrine was mostly excreted unchanged (40.9%) in human urine, and only a small amount of phenylpropanolamine (less than 2%) was identified as its metabolite [18]. Nevertheless, 4-hydroxyephedrine is the major metabolite of ephedrine in rat urine after administration of *Ephedra sinica Stapf* [19].

It has already been described that norketamine is the main chiral active metabolite of ketamine. S-norketamine has shown to have also anesthetic potential, although at lesser extent [20]. A more recent study also reported that the metabolism of ketamine to R-hydroxynorketamine (a minor metabolite) was essential for the antidepressant effect of ketamine in rodents [21].

Fentanyl is mainly metabolised to norfentanyl in liver and duodenal microsomes; however, other metabolites such as hydroxylated fentanyl should be also considered since fentanyl and its analogues has several paths for metabolic transformation. All of these metabolites as well as their metabolic pathways are revised in [14].

In any case, more biotransformation studies should be promoted, to select the most appropriate biomarkers for environmental analysis and also disclose differences in enantioselectivity if any.

1.c. ENVIRONMENTAL OCCURENCE

The enantiomeric determination of chiral illicit drugs is a valuable tool for different purposes: a) to understand the different route of synthesis of the drugs; b) to assess the origin of the drug residue, such as consumption and disposal of unused drugs; c) to understand the potency of the abused drug, due to the different effect of each enantiomer.

Chiral illicit drugs are marketed or abused in their racemate o enantiomerically pure forms and each enantiomer may exhibit different enantiomeric activity in the same biological environment [22] and different toxicity, as has already been discussed. Chiral drugs can undergo enantiospecific metabolism not only in the human body, but also during the wastewater treatment. Therefore, the enantiomeric fraction (EF) can vary from the intake to excretion and during the wastewater treatment, and even along the river due to the different biological degradation. The enantiomeric fraction is typically defined as the ratio between the concentration of S-(+)-enantiomer or first eluted enantiomer and the sum of the concentration of S-(+)-enantiomer and R-(-)-enantiomer or second eluted enantiomer [23].

The illicit drugs have been determined in several types of environmental samples. Wastewater is the type of sample most analysed because it enables to estimate the consumption of drugs, namely wastewater epidemiology, covered in chapter 14 of this book. Other samples, such as sediment, sludge, river and sea water, have been analysed for the enantiomerically determination of illicit drugs.

As regards amphetamines-type substances, AMP is mainly synthesised as racemate but after consumption S-AMP is metabolised faster than R-AMP, which means that excreted AMP is enriched with R-AMP and therefore higher R-AMP concentration is found in influent wastewater [23,24], although some studies found a higher concentration of S-AMP in the same type of water [25,26]. There are several reasons to justify these findings, among which the fact that the breakdown of S-MAMP will exclusively led to S-AMP, as S-MAMP is predominant in wastewater because S-MAMP is the dominant abused enantiomer [27] due to the stronger stimulant effects over R-enantiomer. In influent wastewater S-MAMP is the predominant [22] and R-MAMP presence can be attributed to consumption of racemic MAMP. During water treatment, the microbiological processes favor S-enantiomers and R-enantiomers are more recalcitrant [22].

In estuary water, only R-enantiomer of AMP was found in UK [28] while in river water from Portugal both AMP (EF of 0.5) were found but only the S-MAMP was detected [29]. In sediment of a small stream impacted by septic tank discharge AMP was only detected (2.1-5.4 ng/g) with an EF of 0.39-0.44, but no presence of AMP was found in sediments of the receiving river [30] while MAMP was not found in any sample.

As regards MDMA, concentrations between method detection limit (MDL) and method quantification limit (MQL) were found in effluent and river waters in Portugal [29] and MDMA metabolism is stereoselective favoring S-MDMA with subsequent enrichment of R-MDMA excretion leading to enrichment of the enantiomer in the environment [29], as conventional methods of clandestine manufacture produce MDMA racemate. R-MDMA was also only detected in influent from China (between 0.2 ng/L and 2.4 ng/L) [22] and in Portugal (<15 ng/L) and was the predominant enantiomer in influent waters from several European cities (0.32<EF<0.4) [27]. Values of 34.3 ng/L, 45.3 ng/L and 16.3 ng/L were found in influent, effluent and digested sludge of UK, with values of EF 0.7, 0.9 and 0.4 respectively [31].

Cathinones have also been enantiomerically determined and, for instance, buphedrone (BFD) and 3,4-dimethylmethacathinone (3,4-DMMC) were found in influent waters from Portugal [3] and in some samples only one enantiomer was determined while in others, both enantiomers, although in a previous study of the same group only one of each cathinone were determined

[29]. Mephedrone was found to be enriched with R-enantiomer in wastewater from European cities (EF= 0.57) [27]. In samples from Ebro River (Spain) methylone and methedrone were determined while flephedrone, mephedrone and butylone were not detected [32]. Both enantiomers of methylone were found in one river sample at 5.1 and 5.3 ng/L (EF=0.49) and those of methedrone were also found in one sample at 3.8 and 4.4 ng/L (EF=0.46) and concentrations in effluent wastewater samples of Tarragona region were below MQL.

Ephedrine, pseudoephedrine and norephedrine were also determined in influent and effluent wastewater from UK [31] but only norephedrine in digested sludge. However, EF were 0 for ephedrine and 1 and 0.2 for pseudoephedrine. Values for norephedrine were 0, 0.3 and 0.1 for norephedrine, respectively. Norephedrine was also found in different European cities with EF between 0.48 and 0.56 while ephedrine and pseudoephedrine provided EF of 1 [27].

2. SAMPLE EXTRACTION AND CLEAN-UP METHODS

Sensitive and selective analytical methods are essential for detecting the low concentrations of chiral drugs in the environment. A well-designed protocol for sampling and storage is critical to ensure sample representativeness and analyte stability. Additionally, an effective sample extraction procedure is crucial for isolating the target compounds and minimizing matrix interferences in complex environmental samples.

Chiral drugs have been primarily studied in aqueous environmental samples such as river and wastewater (influent and effluent) samples. However, other solid matrices including sediments, sludge or suspended particulate matter are also discussed, although at lesser extent.

2.a LIQUID SAMPLES

Grab sampling is usually performed for all environmental samples covered, although in the case of influent wastewater samples that aim to release with consumption or daily load data, they are usually collected by auto-samplers (take multiple individual samples proportionally to specific periods of time or volume) to generate “24h composite” sample per day.

Samples are collected in amber glass or plastic (polytetrafluoroethylene or polypropylene) bottles, transported and stored before analysis to preserve the stability of the drugs [33], although little is known about the enantiotransformation of these drugs. The most common procedure is to freeze samples at -20 °C after collection and maintain this temperature until analysis. Stability can be extended adding preservatives to the sample, like acidification with hydrochloric acid. It has been demonstrated that samples acidification at pH=2 improves the stability of drugs, including some cathinones, up to 14 days [34].

The samples are filtrated using 1.2 or 0.7 µm glass microfibers for wastewater samples or 0.45 µm membrane filters for surface water to eliminate solid particles and to avoid plugging of cartridges of solid-phase extraction (SPE). After this filtration, the sample is ready for extraction.

SPE is used in all the studies as extraction technique to preconcentrate the chiral illicit drugs from aqueous environmental samples. All the specific information regarding to extraction as well as the protocol used is detailed in Table 4.2.

Regarding the type of cartridge, as the chiral drugs covered are polar and in most case with basic character, all the studies selected either Oasis HLB or Oasis MCX. Oasis HLB is a polymeric based

sorbent with hydrophilic-lipophilic balance character that enables reversed-phase interactions including polar ones; whereas Oasis MCX is a mixed-mode cation-exchange sorbent, with Oasis HLB network functionalized with sulfonic acid groups that enables establishing reversed-phase and selective cation-exchange interactions with the basic compounds. In most of the studies, the sorbent was already established; however, Wang et al. [22] compared the extraction effectiveness through % recoveries of four cartridges: Envi-C18 (silica based modified with octadecyl groups), Oasis HLB and Poly-Sery HLB (two polymeric based with HLB features) and Oasis MCX for a group of chiral drugs. Figure 4.1 compares the recoveries obtained with these four sorbents for the studied chiral drugs. Enantiomers of ephedrine, MAMP and MDMA were not recovered with Envi-C18 sorbent, whereas Oasis HLB and Oasis MCX achieved the best recoveries for all compounds, with slightly better recoveries for Oasis HLB that was finally selected. The performance of Oasis MCX and Oasis WCX was also compared in the enantioselective determination of a group of cathinones [32], whose optimised protocols are in Table 4.2. Initial experiments in ultrapure water provided similar results with both sorbents; however, when moving to environmental samples with Oasis WCX sorbent, the peak retention times measured in river water were significantly decreased compared to those in ultrapure water, fact that was attributed to interference competition for the active sites of the sorbent; thus, the eluates from SPE should be diluted 10 times to recover the enantioseparation of the compounds. For this reason, Oasis WCX was ruled out and Oasis MCX, which did not experience such problems, was selected.

[Insert Figure 4.1 here]

Figure 4.1. Comparison of the recoveries of the studied chiral drugs in the four different SPE cartridges. Reprinted from [22] with permission of Elsevier.

Oasis HLB sorbent enables the use of generic protocol that is based on loading the sample (without pH adjustment or adjusted at pH 7) followed by the elution of the target compounds with 4-8 mL of methanol (MeOH) or acetonitrile (ACN). Despite these generic trends, some studies acidified the sample, probably to better preserve it [29,35]. And, as for the elution, only in one study [22] selected 5% NH_4OH in MeOH over pure MeOH, ACN and ethanol (EtOH) or basic (5% NH_4OH) ACN and EtOH due to the slightly better recoveries with the selected solvent, fact that the authors attributed to the better elution of the compounds in its neutral form. As for the washing step, this is not conducted or it is based on pure water [23,28] or acidic water [3,29], highlighting that a washing volume up to 50 mL of ultrapure water was optimised over 0 mL and 10 mL in the extraction of a group of drugs (including the enantiomers of AMP and MAMP) from sea water. The use of such high volume of water in the washing step eliminated sodium chloride from sea water, salt that was further eliminated by eluting the compounds with ACN (poor solubility of sodium chloride) to prevent the loss of enantioselectivity of two of the target drugs (acebutolol and sotalol) [28].

On the other hand, Oasis MCX needs a more specific protocol to turn on and off the chargeability of the basic compounds and promote the cation-exchange interaction between the sorbent (negative form) and the protonated basic compounds. To do so, the sample is adjusted at acidic pH [32,36], although in some studies this is not mentioned, thus, it is assumed that the samples were loaded without pH adjustment [24,29]. In any case, due to the pK_a (8-9) of such analytes, they might be at least partially protonated at the working pH. As for the elution, 2 to 7 mL of basic (0.5%-7% NH_4OH) MeOH was applied to neutralise the basic chiral drugs and in this way disrupt the cationic interactions with the sulfonic acid moieties of the sorbent. Estevez-Danta et al. [24] optimised the elution protocol by assaying three consecutive fractions of 3 mL of 5%

NH_4OH in MeOH, and they found that 94% of the studied analytes (AMP, MAMP and MDMA) eluted in the first fraction, therefore, the elution volume was fixed in 3 mL. One of the main advantages of Oasis MCX over Oasis HLB is the selective extraction of the basic compounds, achieved when an effective washing step based on organic solvent such as MeOH is applied. In this way, in order to exploit the selectivity, most of the studies that used Oasis MCX washed with 4 to 10 mL of pure MeOH. In some studies [36] the washing step was based on the combination of water followed by MeOH or the use of this organic solvent to elute the acidic target compounds [37] and so fractionate the elution between acidic and basic compounds.

The sample volume loaded into the cartridges depends on the complexity of the matrix as well as the amount of sorbent in the cartridge. Table 4.2 details these variables for each specific study. Thus, volumes ranging from 100 mL to 1 L of surface water are the most usual; whereas for the more complex samples as influent wastewater the volumes ranged from 25 mL to 100 mL.

As for the sorbent amount, the cartridges from 60 mg to 500 mg have been used. The higher the amount of sorbent, the higher the sorbent capacity and thus the volume to be percolated. Nonetheless, Oasis HLB and Oasis MCX in 60 mg format was able to percolate up to 100 mL of influent and effluent wastewater sample to extract a group of amphetamine-type substances [38] with recoveries > 70% for all compounds, except norephedrine (%R ca 55% and 21% in effluent and influent samples, respectively) which was attributed to high signal suppression. It should be mentioned that the authors also found that the extraction process was not enantioselective, and did not favor one enantiomer over the other [38].

A molecularly imprinted polymer (MIP) prepared using different enantiomers of ephedrine: (1R,2S)-(-)-ephedrine and (1S,2S)-(+)-ephedrine as template and at different proportions to end up with four different MIPs. Under the optimised SPE protocol (Table 4.2) to selectively extract a group of cathinones, it was disclosed that the MIPs did not show any stereoselectivity since the EF values in all the eluates was around 0.5 [39]. Figure 4.2 shows the chromatogram obtained when the extract by molecularly imprinted solid-phase extraction (MISPE) was analysed in liquid chromatography-high resolutions mass spectrometry (LC-HRMS) using a chiral cellobiohydrolase (CBH) column (the rest of chromatographic conditions are in Table 4.2).

[Insert Figure 4.2 here]

Figure 4.2. Enantioselective chromatograms of a group of cathinones extracted by MISPE followed by LC-HRMS with Chiral CBH column. See Table 4.2 for the rest of conditions. Reprinted from [39].

2.b SOLID SAMPLES

The studied solid samples (i.e. sludge, sediments and suspended particulate matter) are collected as grab samples, that are transported refrigerated to the laboratory, which were dried [3], freeze-dried [30,31] and sieved across 2 mm [30] for suspended particulate matter, sludge and sediments, respectively, to obtain homogeneous solid ready for extraction. If solid samples are not readily analysed, they are stored at -20 °C. It has been demonstrated that the stability of some drugs such as ketamine analogues in sludge or sediments stored at -20 °C is up to three months [20]; however, no information about its enantiotransformation is available so far. Some studies have only evaluated the stereoselective biodegradation of some chiral drugs including ketamine [20] and amphetamines [40,41] under usual conditions during sewage treatment, which resulted in similar enantioselective biodegradation in the case of ketamine and its main

metabolite (norketamine) [20], but strong enantioselectivity that favors the S-enantiomers in the case of amphetamine-type substances [40,41].

Table 4.2 details the extraction technique and their optimum conditions to extract chiral drugs from solid samples. Solid-liquid extraction was selected to extract chiral amphetamines and cathinones from suspended particular matter of wastewater samples [3]. During optimisation different extraction solvents (diethylether, MeOH and a mixture of MeOH/H_2O) were evaluated, and MeOH was selected since it provided similar recoveries (72-117%) than MeOH/H_2O mixture, but it uses low solvent volume (6 mL) and less extraction time (< 2h over 6h).

Microwave assisted extraction (MAE) was selected for the extraction of 18 chiral drugs that includes illicit drugs as amphetamines and ephedrines from sludge [31]. During the optimisation of the extraction different variables that include temperature, mass of the sample and extraction solvents were assayed with the optimum values detailed in Table 4.2. One interesting result was during the optimisation of the temperature, since it was found that better recoveries were achieved at lower temperature in the initial trials using silica as matrix; however, when moved to sludge matrix, the recoveries at low temperature (90 °C) were lower than at 120 °C, reason why this temperature was selected for extraction. In addition, the extract from MAE was cleaned-up using an SPE sorbent. During the sorbent selection (Oasis HLB, MCX and MAX), Oasis MAX proved to be the most effective sorbent when coupled to chiral LC column since good enantioseparation was achieved (loss of enantioseparation when injecting extracts from Oasis MCX) and no backpressure was observed (obtained when injecting extracts from Oasis HLB), although the recoveries were low.

Pressurized liquid extraction (PLE) was used to extract a group of 15 drugs that included anti-depressants and stimulants from river sediments [30], where the sediment mass from 5 g to 2 g was adapted from a previous method in order not to lose the enantioseparation in the subsequent chromatographic analysis. The other PLE parameters including extraction temperature and proportions of H_2O/MeOH as extraction solvent were also optimised to enhance the extraction recoveries. Figure 4.3 shows the results obtained from this optimisation, where it can be observed that the extraction parameter influence is the same for both enantiomers.

[Insert Figure 4.3 here]

Figure 4.3. Effect of the extraction temperature (A) and H_2O/MeOH proportion as extraction solvent (B) on the enantiomer recovery during SPE. See Table 4.2 for the rest of conditions. Reprinted from [30].

3. ENANTIOMERIC DETERMINATION

Different analytical techniques can be applied for the determination of chiral illicit drugs. Among them, liquid chromatography (LC) is the most commonly used but gas chromatography (GC), capillary electrophoresis (CE) and supercritical fluid chromatography (SFC) have also been applied.

Two different approaches can be used for the enantiomeric determination, which can be divided in indirect methods and direct methods. The indirect methods are based on derivatisation of the enantiomers with an enantiomerically reagent via a covalent bond, and further separation under

achiral conditions. On the other hand, the direct methods use a chiral selector present in the separation compartment, and in the case of chromatographic separation, the chiral selector can be an additive of the mobile phase or a component of the stationary phase. The direct methods are most preferred because of no need of previous derivatisation of the drugs. Both indirect and direct methods can be performed in LC; in GC, indirect method is the most common because only few chiral stationary phases are available and direct methods are the most used in SFC, CE and capillary electrochromatography (CEC).

Although the previous techniques have been used for the determination of chiral illicit drugs, in most applications to environmental samples, LC and, GC to a lesser extent, are the most used. Therefore, examples of these two techniques are more extensively described and a few examples of the application of SFC, CE and CEC will be given even most of them have not been applied to environmental samples so far.

3a. GAS CHROMATOGRAPHY

Although LC using chiral stationary phases are the most used technique to determine enantiomers of illicit drugs in environmental samples, some studies applied GC as an alternative to the expensive chiral separation phases (CSP). Due to the low volatility of illicit drugs and the low availability of temperature resistant CSP, the indirect method is the most used in GC [3,36]. The presence of amino and carboxylic groups in the illicit drugs enables an easy derivatisation with enantiomeric derivatising agents. For instance, for cathinones, *L*-N-(trifluoroacetyl)propyl chloride (L-TPC) and (1R)-(-)-menthylchloroformate (MCF) have been used with an achiral stationary phase [7] for the gas chromatography-mass spectrometry (GC-MS) determination with electron ionization and even some authors use negative chemical ionization in GC-NCI-MS with L-TPC derivatising agent [42] or in GC-NCI-MS/MS with MCF [43]. In both studies urine or urine and plasma are analysed. These examples demonstrate that cathinone can be determined by GC-MS but this technique has not been applied to environmental samples so far.

Although less studied, CSP has also been applied to the determination of illicit drugs. For instance, amphetamines have been enantiomeric separated using a γ-cyclodextrin CSP after derivatisation with trifluoroacetic anhydride [44] but, once more, it was not applied to environmental samples.

Focusing on environmental samples, some examples are included in Table 4.2. For instance, AMP, MAMP, fluoxetine, norfluoxetine. norketamine, sertraline, paroxetine together with some beta-blockers were determined in wastewater samples by GC-MS using an indirect method [35]. The authors used a common GC column 5% diphenyl/95% dimethyl polysiloxane, and optimised the chiral derivatisation reagent to allow the formation of diastereomers for the majority of the compounds, the highest sensitivity and lower retention time. The most suitable chiral reagent was (R)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl ((R)-MTPA-Cl) whose derivates were further derivatised with N-methyl-N-trimethylsilytrifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS). The derivatisation conditions were also carefully optimised. Of 12 analytes, the diastereomers of 10 of them were enantiomerically separated. After SPE procedure, described in Table 4.2, the method enables MDL between 0.03 ng/L and 26 ng/L with good accuracy and precision.

Another example of GC-MS application to environmental waters is the study of Verovsek et al [36] where they determine amphetamines by GC-MS/MS after derivatisation with (R)-MTPA-Cl. The authors achieved a good enantiomeric separation (Rs>1) of the AMP, MAMP and MDMA and after an SPE they could determine these drugs in some of the influent wastewater analysed. When they compare their results with those obtained by chiral LC-MS/MS, they found comparable methods except in terms of MQL that were higher in GC-MS/MS.

The same derivatisation agent was used for the determination of amphetamines and cathinones from influent and effluent wastewater, and river water [29], and influent wastewater and suspended particulate matter (SPM) [3], studying in the last paper the impact of sorption onto SPM in the underestimation of psychoactive drugs in the influent wastewater analysis, especially due to the possible enantioselective sorption. Figure 4.4 shows the GC-MS chromatogram of a spiked influent wastewater where the separation of enantiomers of 3 amphetamine-type substances and 4 synthetic cathinones, as well as the internal standard amphetamined-d3 is demonstrated [3]. In previous figure, despite the coelution of two compounds, the method was selective for quantification all analytes in both matrices applying selected ion storage mode. Applying the sample treatment detailed in Table 4.2 and previously discussed, MQL between 15 and 30 ng/L in liquid samples and between 0.30 and 0.60 ng/mg in SPM.

[Insert Figure 4.4 here]

Figure 4.4. Comparison of the GC-MS chromatograms of the standard solution, spiked and unspiked extract blank matrices of the liquid phase (A) and SPM (B) of a standard mixture at 150 ng/mL and 2 ng/mg, respectively, for the enantiomers of the amphetamines: AMP, MAMP, and MDMA; and synthetic cathinones: buphedrone (BPD), 3-methylmethcathinone (3-MMC), 3,4-dimethylmethcathinone (3,4-DMMC) and butylone (BTL). D1 and D2 correspond to the first and second eluted diastereomers, respectively. Reprinted from [3].

3b. LIQUID CHROMATOGRAPHY

Liquid chromatography is the most used technique for the enantiomeric separation of illicit drugs in environmental samples. As mentioned before, both direct and indirect methods can be used in LC although the direct method is the most preferred. Indirect methods have also been used which avoid the use of pricey chiral columns. For instance, to chirally separate 17 cathinones using a reversed-phase C_{18} column, 2% sulfated β-cyclodextrin as chiral selector was added to the mobile phase [45], but this method was not been applied in environmental samples.

Direct methods are the most used due to the commercially availability of different types of chiral separation phases and the versatility provided by the different elution modes, depending on the composition of the mobile phase (reversed-phase (RP), normal phase (NP), polar-organic phase (PO) and polar-ionic phase (PI)), although not all of them are compatible with all the columns.

In environmental analysis, the most used CSP are macrocyclic glycopeptides antibiotic-based and protein-based, followed by polysaccharide-based and Pickle type [46]. An interesting description of the different CSP is included in recent reviews [46,47].

Focusing on illicit drugs, the methods used for their enantiomeric determination in environmental samples are summarised in Table 4.2. As can be seen, most of the methods are based on chiral chromatography coupled to mass spectrometry-based detection. And, the most used columns are Chirobiotic V and Chiral CBH, while other columns such as Poroshell Chiral-V, Lux Cellulose, Lux AMP and Whelk-O1 are used in few examples.

Chirobiotic V is a type of macrocyclic glycopeptide antibiotic-based CSP which contains the antibiotic vancomycin as selector. It contains a variety of functional moieties which can interact with the enantiomers through a wide range of molecular interactions. This fact, together with the possibility of using different elution modes (RP, NP, PO an PI) make them suitable for the separation of a broad range of chiral analytes. However, when coupling to MS, RP and PI are the most common elution modes because NP is usually incompatible with MS detection and PO is more suitable for neutral analytes [46]. The Poroshell Chiral-V is also based on the same selector but is a superficially porous column, which enable a fast, efficient, high-resolution chiral separations.

The Chiral CBH is a protein-bonded CSP containing cellobiohydrolase (CBH) and it contains a large number of chiral centers and different binding sites, enabling different interactions to be stablished that contribute to the chiral separation. However, this type of column can only be used in RP mode and no more than 20% of organic modifier is required, which limits the sensitivity in MS detection. This column is more effective in the separation of basic drugs and pH, ion-strength and organic content must be carefully optimised.

The Lux-cellulose is a type of polysaccharide-based CSP that has been widely used but since the interactions are more effective in NP, its use has been limited when MS detection is used. However, by carefully optimising the mobile phase conditions, other elution modes such as RP, PO or PI can be applied [46]. But the applications to the determination of illicit drugs in environmental samples, where MS detection is required, are limited.

The Whelk-O1 CPS is the most used Prikle-type CSP and as it contains a strong π-acceptor aromatic group and a π-donor aromatic moiety, it can perform strong interactions with the enantiomers. It is extremely suitable for the enantioseparation of analytes with aromatic moiety and a hydrogen-bond acceptor group located close to the stereogenic center. They can be mainly used in NP but can also work in RP mode. They are more effective for the separation of acidic compounds and few examples are described for the illicit drugs because most of them contain basic groups in their chemical structure.

As can be seen in Table 4.2, the particle size of the columns is 5 μm in most cases, with the exception of the superficially porous Poroshell Chiral-V column of 2.7 μm [28] and the Lux AMP, of 3 μm.

Due to the complexity of environmental samples and in order to protect and enlarge the life of the costly columns, a guard column is generally used before the column, with the same CSP and of smaller dimensions [22,23].

As regards detection, mass spectrometry is the most used because of the low concentrations at which the illicit drugs are present in the environmental samples and the high confirmatory power required which limits the use of other detection techniques. The different mass

spectrometry-based detectors based on MS/MS or high-resolution mass spectrometry (HRMS), or the hybrid ones, are used as can be seen in Table 4.2. Triple quadrupole (QqQ) analyser is the most used due to the highest sensitivity and has been applied in several examples [22,24,46]. Higher selectivity can be achieved when using HRMS [32] and also the possibility of retrospective analysis of target and non-target because these analysers acquire in full scan mode. This is also important in the field of illicit drugs because of the continuous introducing of new drugs in the market which can be detected in the environmental samples. The hybrid high resolution mass spectrometry such as quadrupole- time of flight (Q-TOF) offers an increase of mass accuracy and a more reliable identification of the analytes in environmental complex samples [40,48].

Due to the polar characteristics of illicit drugs, electrospray ionization is the most used in the MS-based detection systems [46]. However, they are well known for the matrix effect present in the ionization process and in particular in electrospray ionization. This involves the use of a pretreatment of the sample in order to obtain selective methods which reduce the matrix effect, as has been previously described.

The selection of mobile phase is a key point in the chiral separation since several factors can affect the enantioseparation. It should be pointed out that, even using a very selective MS-based detector, complete separation of enantiomers has to be achieved because they both present the same MS spectrum. Therefore, optimisation of the mobile phase composition is quite tricky. Examples of CSP and mobile phase composition will be discussed below for different groups of chiral illicit drugs.

For the separation of amphetamines-type substances, Chirobiotic V [30,48] and Chiral CBH [22,38,49,50] are the most used. However, in recent years a specific column designed to enantiomerically separate amphetamines, Lux AMP, has been commercialized by Phenomenex but no information about the chiral selector is available [51] and few applications have been described in environmental analysis [24].

As regards CBH columns, the separation of amphetamines can be achieved in RP mode, using a mobile phase containing H_2O/isopropanol (IPA) (90/10) containing 1 mM of ammonium acetate [31,48], which is a mobile phase compatible with electrospray ionization in LC-MS. In this separation mode, pH of the mobile phase, organic modifier and aqueous buffer play an important role and must be carefully optimised. For instance, for the optimisation of the enantiomeric separation of a group of chiral substances [22], the authors selected three compounds, MAMP, norketamine (NK, metabolite of ketamine) and norephedrine (NE, metabolite of ephedrine) whose enantiomeric separations were more difficult. The addition of the organic modifier MeOH improved the separation of NK and MAMP enantiomers and the increase from 5% to 10% of methanol shortened the analysis time. The $\%NH_4Ac$ and pH were also adjusted and when the % increased the resolution decreased, for which 1% was the optimum, and pH was fixed at 6.9 since lower pH did not enable the separation of NE and MAMP. The flow-rate had to be fixed at 0.15 mL/min because higher flow-rate did not enable the separation of NE and NK ($Rs < 1.0$). Fig. 4.5 shows the separation of some illicit drugs with the method optimised using a Chiralpak CBH column [22].

[Insert Figure 4.5 here]

Figure 4.5. LC-MS/MS chromatogram of chiral illicit drugs spiked in water obtained with the optimised method shown in Table 4.2. Chiralpak CBH column (100 mm x 2 mm, 5µm) with -Chiral CBH guard column (10 mmx 2 mm); mobile phase: 1 mM NH_4OAc/ MeOH (90/10); flow-rate: 0.15 ml/min; column temperature: 30 ^oC; injection volume: 20 µL. Reprinted from [22] with permission of Elsevier.

In a study of the group of Kasprzyk-Horden [31], group that has significantly contributed to the chiral determination of drugs in environmental samples, for the chiral separation of a group of pharmaceuticals and illicit drugs in wastewater and sludge, the authors use a CBH column for 4 amphetamines, ephedrine and norephedrine while 15 pharmaceuticals are enantiomerically separated with a Chirobiotic V. In a further study of the same group [23] three chiral columns were evaluated for the separation of 56 drug biomarkers, among which stimulants (e.g. amphetamines, cathinones) and opioids (e.g. morphine, methadone, tramadol) were included. The authors tested a Chiralpak CBH, a Chirobiotic V and a Chirobiotic T (a macrocyclic glycopeptide-based CSP containing the antibiotic teicoplanin as selector) with their respective guard columns. The CBH column was 100 x 2.0 mm, while the Chirobiotic ones were 250 x 2.1 mm, all of them with 5 µm diameter particles. After optimisation of the mobile phase compositions, the authors concluded that CBH column provided better enantiomeric resolution than Chirobiotic V, in particular, for amphetamine-type substances. Chirobiotic T showed higher enantioselectivity for benzodiazepines only, a class of compounds that are not enantiomerically separated by CBH column. 18 pairs of enantiomers were satisfactory separated with the CBH column under optimum conditions.

Chirobiotic V and a Pirkle type Whelk-O 1 (based on 1-(3,5-dinitrobenzamido)-1,2,3,4,-tetrahydrophenanthrene) working in RP elution mode were also compared for the determination of a group of chiral drugs, including few illicit drugs such as ketamine [37]. The authors used the Whelk-O column for the separation of acidic drugs while Chirobiotic V for the basic drugs. However, the AMP and MAMP were not enantioseparated although they achieved the separation of 10 basic drugs. In other studies [28,30], a Poroshell Chiral-V working in polar ionic mode enables the enantiomeric separation of AMP, MAMP, among others drugs, but not of MDMA, and they were determined in sea water [28] and sediments [30]. Chromatographic conditions are detailed in Table 4.2.

Chiralpak CBH was also compared with a Lux-Cellulose 1 in RP and PO modes, respectively, for the determination of 4 cathinones, 3 β-blockers and one antiacid drug [52] although no application to real samples is included. Lux Cellulose-1 contains cellulose tris(3,-5-dimethyl carbamate) as chiral selector and although good separation is achieved with these columns in NP, the incompatibility with MS detection, limits the use of this mode. The optimised mobile phase for Chiralpak CBH consists of 5 mM ammonium acetate aqueous (pH=6.4)/MeOH (95/5, v/v) whereas for Lux-Cellulose 1, was ACN/IPA/HCOOH/diethylamine (DEA) (90/10/0.1/0.1). The authors observed that CBH column provided the enantioseparation of the 8 analytes, whereas with Lux-Cellulose, cathinones were not enantioseparated.

Lux-Cellulose columns were also applied to the determination of other NPS, such ketamine and it metabolite norketamine in sludge [29]. In this case, they used a Lux-Cellulose-4 (cellulose tris(4-chloro-3-methylphenylcarbamate) and after optimising the different parameters of the

separation, the best conditions were 20 mM ammonium acetate in water (with 0.1% of DEA and ACN (70/30) at 1 mL/min. With ethanol instead of ACN no enantioseparation was achieved and even the careful optimisation, the final analysis time was almost 40 min.

In another example, 40 out of 43 NPS were enantiomerically separated using a Lux-cellulose-2 in polar organic phase consisting on ACN/IPA/DEA/formic acid (100%) (95/5/0.1/0.1), although no application is described [53]. The enantioseparation of NPS is described in the review of Schmid et al. [47] although it is not applied to environmental samples but solid ones.

Different cellulose-based stationary phases were evaluated for the enantioseparation of new emerging NPS, fentanyls in polar-ionic conditions with UV detection [14]. The authors compare Lux-Cellulose-2 and Lux-Cellulose-4 and found different performance among the two columns even the slight differences in the selector. The authors optimised the conditions in LC-UV with a 250 x 4.6 mm, 5 µm column but they use a column with smaller dimensions (150 x 3 mm, 3 µm) when they move to LC-HRMS. Even this method has not been applied to environmental samples, it could be of interest for the coming social problem with fentanyl type drugs.

Another column, specifically designed for the enantiomeric separation of amphetamine-type substances is the Lux AMP. The previous Lux columns were based on cellulose or amylose linked to tris-phenylcarbamates, but for this new column no information of the selector is available. The advantage of this column is its wide range of working pH, from 1 to 11.5. After optimisation, a mobile phase of 5 mM ammonium bicarbonate adjusted to pH 11.3 with ammonia/ACN (70/30) enable 83 of the 95 NPS to be enantiomerically separated [51] within 40 minutes. An application of this column to the environmental samples is the study of Estévez-Danta [24] where a group 3 amphetamines (AMP, MAMP, MDMA) were determined in wastewater in order to obtain information of the origin of the occurrence of these drugs in wastewater. On the contrary to most the examples included in literature, that worked in isocratic mode, in this study the authors use a gradient of A) 50 mM of NH_4OH in ultrapure water and B) MeOH, at a flow rate of 0.4 mL/min shown in Table 4.2. The analytes eluted in less than 22 minutes. The authors stated that pH 11 is necessary to achieve the complete separation but it can be adjusted by NH_4OH or ammonium bicarbonate, although ammonia was selected due to the higher signal intensity and lower noise obtained in ESI-QqQ compared to ammonium bicarbonate. Fig 4.6 shows the effect of the mobile phase on the separation of the amphetamines.

[Insert Figure 4.6 here]

Figure 4.6. Chromatograms illustrating the separation of AMP, MAMP and MDMA with different eluent modifiers. Reprinted from [24].

As can be seen in Table 4.2, using the sample treatment described and the highly sensitive MS-based detectors, the MDL and MQL are at low ng/L for environmental waters, which enable de quantification of the enantiomers in complex samples with different purposes as has been explained in section 1.c.

3.c OTHER TECHNIQUES

Although LC is the technique most used for enantiomeric separation of illicit drugs, and to a lesser extend GC, other techniques such as CE and SFC are quite suitable for the determination of enantiomers but they have been hardly used in environmental analysis. In the study of Albals et al [54], SFC, CEC and three LC modes are compared for the chiral separation of cathinone and amphetamine-type substances and advantages and limitations of these techniques are discussed.

As regards SFC, in the last years a few applications have been reported for the determination of chiral illicit drugs and NPS mainly due to fact that modular LC systems can be upgraded to SFC instruments rather easily, and that many chiral stationary phases initially designed for LC can be used in SFC without further modification [47]. Several examples can be found in literature [47,54–57] but none of them have been applied to environmental samples. In these examples, different columns are used, such as teicoplanin and vancomycin-based superficially porous particles-packed columns [55] for the separation of a group of biologically active compounds, such as ketamines, cathinones, etc, or Chiral zwiterionic columns for the separation of cathinones [55].

As regards CE and CEC, several methods have been developed [47,54,58]. Chiral selectors can be added to the running buffer and chiral stationary phases can be used in CEC. The improved peak shape and the low electrolyte consumption makes CE quite used in enantiomeric determination although its lower sensitivity limits the use in environmental samples. The chiral selectors most frequently used are cyclodextrin and their derivates, which are added to the running buffer. They are quite used with UV detection since they are UV transparent but when using MS, since they are nonvolatile, other strategies have to be applied such as the countercurrent migration approach [59] which avoids the selector entering to the MS detector. Another option is the use capillary packed with chiral stationary phases mainly based on polysaccharides such as amylose and cellulose derivatives [7,47,54] in CEC. However, as in SFC applications, these techniques have not been applied to environmental samples.

4. CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Determination of enantiomeric composition of illicit drug is of crucial importance in different fields, which include environment to achieve accurate risk assessment.

Different analytical methods have been successfully developed and applied during the last years to achieve successful enantiodetermination of chiral illicit drugs in environmental samples. These methods include such separation techniques as gas chromatography, liquid chromatography or capillary electrophoresis using either direct and indirect methods coupled to mass spectrometry-based detectors. Nevertheless, liquid chromatography using chiral stationary phases is by far the technique of choice to determine chiral illicit drugs in environmental samples. In any case, the separation conditions should be adapted and optimised to be feasibly coupled to MS detectors.

In addition, to reach the low concentration levels of illicit drugs in environmental samples, the analytical methods should also include a preconcentration technique which is not different from the well-established to pretreat illicit drugs in environment.

Further studies are expected in future to deal with the enantiodetermination of novel illicit drugs that are continuously introduced.

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Enantioselective potential of teicoplanin- and vancomycin-based superficially porous particles-packed columns for supercritical fluid chromatography, J. Chromatogr. A. 1612 (2020) 460687. https://doi.org/10.1016/j.chroma.2019.460687.

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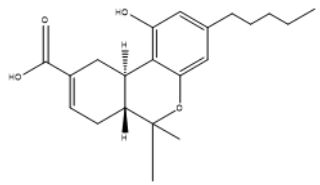
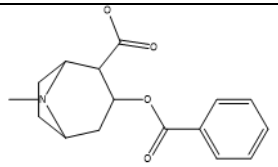
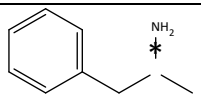
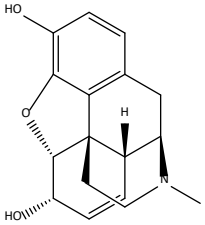
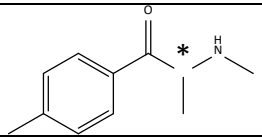
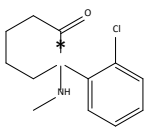
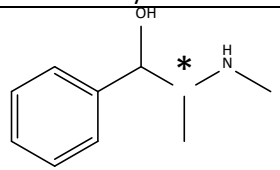
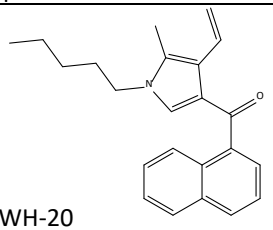
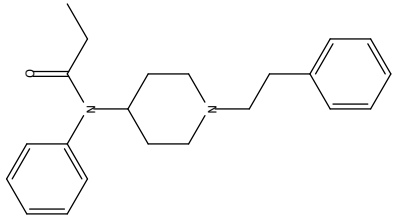
Table 4.1. Illicit drug categories, examples of structure and the common drugs included.

Type	Category	Structure	Common drugs
Traditional drugs	Cannabis	 Δ9-tetrahydrocannabinol (THC)	Δ9-tetrahydrocannabinol (THC) *Degradative products* cannabidiol (CBD) cannabinol (CBN) *Metabolites:* 11-hydroxy-THC (THC-OH), 11-nor-9-carboxy-Δ9-THC (THC-COOH)
Traditional drugs	Cocaine	 Cocaine	Cocaine *Metabolites:* benzoylecgonine (BE), ecgonine methyl ester (EME).
Traditional drugs	Amphetamine-type substances	 Amphetamine	**(R,S)-Amphetamine** **(R,S)-Methamphetamine** **(R,S)- 3,4-methylenedioxy-methamphetamine (MDMA or ecstasy)**
Traditional drugs	Opioids	 Morphine	Morphine Heroine Codeine
NPS	Synthetic cathinones		**(R, S)-Mephedrone** **(R, S)-Methedrone** **(R, S)-Methylone** **(R, S)-Butylone**

		Mephedrone	**(R, S)-α-PVP** **(R, S)-MDPV, etc**
NPS	Ketamine	 Ketamine hydrochloride	**(R, S)-Ketamine** *Metabolite:* **(R, S)-Norketamine**
NPS	Phenethylamines	 Ephedrine	**(R,S)-Ephedrine** 25c-NBOMe
NPS	Synthetic cannabinoids	 JWH-20	JWH-20 Spice K2
NPS	Synthetic opioids	 Fentanyl	Fentanyl *Metabolites:* **(R, S)-3-Methylfentanyl** **(R, S)-Hydroxyfentanyl** Methadone Oxycodone Buprenorphine Carfentanyl

Chiral center marked with "*". Bold denoted the chiral illicit drugs.

Table 4.2. Chiral methods employed in the determination of chiral illicit drugs in environmental matrices.

Compounds *(in bold illicit drugs)*	**Environmental matrix**	**Sample preparation**	**%Recoveries**	**Analytical methodology**	**Chromatographic conditions**	**MDL/MQL**	**Ref.**
Fully enantioseparated: **AMP, MAMP, MDMA**, **norketamine,** alprenolol, fluoxetine, norfluoxetine, metopropolol, propanolol, bisoprolol **No separated**: Paroxetine and sertraline	Influent and Effluent water	SPE: Oasis HLB (150 mg) L: 500 mL waste water at pH 2 W: 4 mL 2% formic acid in water Vaccum for 1 h E: 4 mL of 5% NH_4OH in ethanol Evapor. to dryness Reconst.: 0.25 mL MeOH	81-114	GC-IT	Derivatisation with R-MTPA-Cl Column:5%diphenyl and 95% dimethyl polysiloxane (30mx0.25 mmx0.25 µm) Mobile phase: He 1 ml/m	MDL: 0.03 ng/L-26.01 ng/L MQL: 0.15 ng/L-104.2 ng/L	[35]
Fully enantioseparated: **AMP, MAMP, MDMA**	Influent water	SPE: Oasis MCX (150 mg) L: 25 mL influent waste water at pH 2 W: 5 mL MilliQ-water+ 6 mL MeOH E: 4 mL 0.5% NH_4OH in MeOH Evapor. to dryness Reconst.: 2 mL 0.02% Et_3N 1-cholobutanol	81-99	GC-QqQ	Derivatisation with R-MTPA-Cl Column: 5% phenyl-methylpolysiloxane (30mx0.25 mmx0.25 µm) Mobile phase: He 1 ml/m	MDL: 400 ng/L- MQL: 160 ng/L	[36]
Fully enantioseparated: **AMP, MAMP, MDMA, norketamine, buphedrone, butylone, 3,4-dimethylmethcathinone (3,4-DMMC), 3-methylmetcathinone**	Influent water Effluent water River water	SPE: Oasis MCX (150 mg) L: 1 L estuarine or effluent water W: 4 mL 2% HCOOH in water E: 4 mL 0.5% NH_4OH in MeOH Filt.: 0.22 µm PTFE Evapor. to dryness Reconst.: 250 µL MeOH	19-98	GC-Q	Derivatisation with R-MTPA-Cl Column: 5%diphenyl and 95% dimethyl polysiloxane (30mx0.25 mmx0.25 µm) Mobile phase: He 1 ml/min	MDL: 14.2-89.5 ng/L MQL: 50.0-250 ng/L	[29]

Fully enantioseparated: **AMP, MAMP, MDMA, buphedrone, butylone, 3,4-dimethylmethcathinone (3,4-DMMC), 3-methylmetcathinone**	Influent water Suspended particulate matter	**Water** SPE: Oasis MCX (150 mg) L: 100 mL influent water W: 4 mL 2% HCOOH in water E: 4 mL 0.5% NH_4OH in EtOH Filt.: 0.22 µm PTFE Evapor. to dryness **SPM** 50 mg SPM 6 mL of MeOH stirred for 30 min Centrifugation 3000 g for 10 min Filt.: 0.22 µm PTFE Evaporation to dryness	Water 83-107 SPM 84-106	GC-Q	Derivatisation with R-MTPA-Cl Column: 5% diphenyl and 95% dimethyl polysiloxane (30mx0.25 mmx0.25 µm) Mobile phase: He 1 ml/m	**Water** MDL: 5.1-9.6 ng/L MQL: 15-30 ng/L **SPM** MDL: 0.10-0.18 ng/mg MQL: 0.30-0.60 ng/mg	[3]
Fully enantioseparated: **AMP, MAMP, MDA, MDMA,** atenolol, propranolol, metoprolol, fluoxetine, venlafaxine	River Effluent	SPE: Oasis HLB (no info) L: 250 mL river water, 100 mL effluent E: 4 mL MeOH Evapor. to dryness Reconst.: 0.5 mL MP Filt.: 0.2 µm PTFE		LC–(ESI)QTOF	1) Chiral CBH column (100×2 mm, 5 µm) H_2O/IPA (90/10, v/v) with 1 mM NH_4Ac (pH=7) 0.075 mL/min, 25°C 2) Chirobiotic V column (250×4.6 mm, 5 µm) MeOH containing 4 mM NH_4Ac and 0.005% FA 0.1 mL/min. 25°C	CBH column: River water: MDL: 2.1-10.7 ng/L MQL: 9.1-51.7 ng/L Chirobiotic V column: River water: MDL: 0.2-10.4 ng/L MQL: 0.3-39.0 ng/L Effluent:	[48]

						MDL: 0.6-22.8 ng/L MQL:1.3-85.7 ng/L	
Fully enantioseparated: **AMP, MAMP, MDMA, MDA,ephedrine/pseudoephedrine** *Partially enantioseparated:* **3,4-methylenedioxy-N-ethyl-amphetamine (MDEA), norephedrine**	Influent Effluent	SPE: Oasis HLB, 60 mg L: 100 mL wastewater, pH=7,5 E: 4 mL MeOH SPE: Oasis MCX, 60 mg L: 100 mL wastewater, pH=2 E: 4 mL MeOH + 2 mL 7% NH4OH in MeOH In both cases: Evapor. to dryness Reconst.: 0.5 mL MP Filt.: 0.2 µm PTFE	Influent 21-91 Effluent 54-90	LC-(ESI)QqQ	1) Chiral CBH (100×2 mm, 5 µm) H_2O/IPA (90/10, v/v) with 1 mM NH_4Ac 0.075 mL/min, 25°C	Influent: MDL: 0.55-3.3 ng/L MQL: 2.4-11.75 ng/L Effluent: MDL: 0.6-1.4 ng/L; MQL: 2.4-10.1 ng/L	[38]
1) *Fully enantioseparated:* **AMP, MAMP, MDMA, MDA,ephedrine/pseudoephedrine** *Partially enantioseparated:* **3,4-methylenedioxy-N-ethyl-amphetamine (MDEA), norephedrine** 2) *Fully enantioseparated:* alprenolol, propranolol, metoprolol, tramadol, salbutamol, sotalol, terbutaline, venlafaxine, fluoxetine, desmethylvenlafaxine, citalopram, desmethylcitalopram *Partially enantioseparated:*	Influent Effluent Sludge	1) **Sludge:** MAE: Sample: 1 g (d.w.) Solvent: 20 mL MeOH/H_2O (50:50, v/v), 120 °C 1200 W Exposure time: 30 min. SPE with Oasis MAX (60 mg) L: 20 mL MAE centrifuged supernatant + 65 mL H_2O, pH=7 E: 4 mL MeOH 2) Wastewater:	No data	LC-(ESI)QqQ	1) Chiral CBH (100×2 mm, 5 µm) with a 0.2 µm, 2.1 mm in-line column filter H_2O/IPA (90/10, v/v) with 1 mM NH_4Ac 0.075 mL/min, 25°C 2) Chirobiotic V column (250×2.1 mm, 5 µm) Guard column 20 x 1.0 mm, 5 µm MeOH containing 4 mM NH_4Ac and 0.005% FA	Influent: MDL: 0.03-28.74 ng/L MQL: 0.03-95.81 ng/L Effluent: MDL: 0.01-32.73 ng/L; MQL: 0.07-109.08 ng/L	[31]

norfluoxetine		SPE: Oasis HLB (60 mg) L: 50 mL wastewater, pH=7 E: 4 mL MeOH In both cases: Evapor. to dryness Reconst.: 0.5 mL MP Filt.: 0.2 µm PTFE					
Fully enantioseparated: **AMP, MAMP, mephedrone, MDA, MDMA, HMA**, norfluoxetine, venlafaxine, **pseudoephedrine,** zopiclone, **para-methoxyamphetamine** *Partially enantioseparated:* **MDEA, HMMA,** tramadol, fluoxetine, **ephedrine, norephedrine,** desmethylvenlafaxine	Influent	SPE: Oasis HLB (60 mg) L: 100 mL W: 3 mL H_2O E: 4 mL MeOH Evapor. to dryness Reconst.: 0.5 mL MP Filt.: 0.2 µm PTFE	No data	LC-(ESI)QqQ	Chiral CBH column (100×2.0 mm, 5 µm) Chiral CBH guard column 10 × 2.0 mm, 5 µm 1 mM NH_4Ac aqueous buffer (pH=6.4) /MeOH (85:15, v/v) 0.1 mL/min, 25°C	MDL: 0.1-61.2 ng/L MQL: 0.1-320.8 ng/L	[23]
Fully enantioseparated: **AMP, MAMP, MDMA, MDA ephedrine/pseudoephedrine** *Partially enantioseparated:* **3,4-methylenedioxy-N-ethyl-amphetamine (MDEA), norephedrine**	Influent	SPE: Oasis HLB, 60 mg L: 50 mL wastewater, no pH adjustment E: 6 mL MeOH Evapor. to dryness Reconst.: 0.5 mL MeOH/H_2O (25/75,v/v) Filt.: 0.2 µm PTFE	No data	LC-(ESI)QqQ	1) Chiral CBH (100×2 mm, 5 µm) H_2O/IPA (90/10, v/v) with 1 mM NH_4Ac 0.075 mL/min, 25°C	No data	[49]
Fully enantioseparated: **mephedrone, flephedrone, methedrone, methylone, butylone**	River Effluent	SPE: Oasis MCX (500 mg) L: 500 mL river water, 250 mL effluent at pH 3 W: 10 mL MeOH	84-102	LC-(HESI)Orbitrap	Chiral CBH (150x2 mm, 5 µm) Chiral CBH guard column (10 ×2 mm, 5 µm)	River: MDL: 0.3-1.2 ng/L MQL: 1.0 -2.9 ng/L	[32]

		E: 5 mL 5% NH_4OH in MeOH Evapor. to dryness Reconst.: 1 mL MP Filt.: 0.45 µm PTFE Oasis WCX (500 mg) L: 500 mL river water, 250 mL effluent at pH 7 W: 10 mL MeOH E: 5 mL 5% HCOOH in MeOH Evapor. to dryness Reconst.: 10 mL MP Filt.: 0.45 µm PTFE			1 mM NH_4Ac aqueous buffer /MeOH (98/2, v/v) 0.4 mL/min, 30°C	Effluent: MDL: 0.8-2.5 ng/L MQL: 2.3 - 6.0 ng/L	
Fully enantioseparated: **mephedrone, flephedrone, methedrone, methylone, butylone**	River	SPE: in house MIP (100 mg) L: 100 mL pH 7 W: 1 mL 0.02% CH_3COOH in MeOH E: 1 mL 5% NH_4OH in MeOH Evapor. to dryness Reconst.: 1 mL MP Filt.: 0.45 µm PTFE	68-83	LC-(HESI)Orbitrap	Chiral CBH (150x2 mm, 5 µm) Chiral CBH guard column (10 ×2 mm, 5 µm)1 mM NH_4Ac aqueous buffer /MeOH (98/2, v/v) 0.4 mL/min, 30°C	River: MDL: 0.3-0.8 ng/L MQL: 1.0 -2.0 ng/L	[39]
Fully enantioseparated: **AMP, MAMP,** salbutamol, propranolol, fluoxetine, venlafaxine, desmethylvenlafaxine, citalopram, sotalol, chlorpheniramine, acebutolol *Partially enantioseparated:* atenolol, metoprolol, bisoprolol	Sea water	SPE: Oasis HLB (200 mg) L: 50 mL W: 50 mL water E: 6 mL ACN Evapor. to dryness Reconst.: 0.25 mL MeOH	1-61	LC-(ESI)QqQ	Poreshell Chiral-V (150 x2.1 mm, 2.7 µm) 0.2 µm pre-filter 2 mM NH_4Ac in MeOH + 0.01% acetic acid 0.15 mL/min, 15°C	Sea water: MDL: 0.2-1.6 ng/L MQL: 0.7-6.2 ng/L	[28]

No separated: **MDMA**							
Fully enantioseparated: **AMP, MAMP, MDMA**, **MDA, ephedrine, norephedrine, norketamine,**	Waste water Surface water	SPE: Oasis HLB (60 mg) L: 400 mL surface water 200 mL waste water W: - E: 6 mL 5% NH_4OH in MeOH Evapor. to dryness Reconst.: 1 mL H_2O Filt.: 0.2 µm PTFE	Influent: 43-118 Effluent: 62-120 Surface water 36-116	LC-(ESI)QqQ	Chiral CBH (100x2 mm, 5 µm) Guard column Chiral CBH (5x2 mm, 5 µm) 1 mM NH_4Ac aqueous buffer /MeOH (90/10, v/v) 0.15 mL/min, 30^o	Influent: MDL: 0.04-3.2 ng/L MQL: 0.3-5.9 ng/L Effluent: MDL: 0.02-2.8 ng/L MQL: 0.25-5.0 ng/L Surface water MDL: 0.01-0.6 ng/L MQL: 0.06-1.1 ng/L	[22]
Fully enantioseparated: **AMP, MAMP, MDMA**	Influent Effluent	Oasis MCX 150 mg L: 100 mL influent water W: 4 mL MeOH E: 3 mL 5% NH_4OH in MeOH Evapor. to dryness Reconst.: 100 µm MeOH 0.22 PVDF filtre	82-116	LC-(ESI)QqQ	Lux AMP (150x3mm, 3 µm) Gradient elution: A) 50 mM NH_4OH B) MeOH 0.4 mL/min Gradient: 0 min, 60%B: 15 min, 60% B; 20 min, 95%; 25.1 min, 60%; 30 min, 60%.	MDL: 0.7-1.8 ng/L MQL: 2.4-5.5 ng/L	[24]
Fully enantioseparated: **AMP, MAMP,** atenolol, chlorpheniramine,	Sediments	PLE: 2 g sample, 1 g DE, H_2O:MeOH (1:1), 100^{o}C, 1500 psi, 2x5 min, 60% flush volume	22-93	LC-(ESI)QqQ	Poroshell Chiral-V (150 x2.1 mm, 2.7 µm) with a 0.2 µm pre-filter.	MDL: 0.03-0.91 ng/g MQL: 0.11-2.84 ng/g	[30]

citalopram, desmethylcitalopram, fluoxetine, propranolol, salbutamol,venlafaxine, desmethylvenlafaxine, bisoprolol, acebutolol, metoprolol and sotalol		Extract: ~ 22mL Clean-up: SPE SPE: Oasis HLB (60 mg) L: 250 mL diluted extract W: 4 mL 2% HCOOH in water E: 4 mL MeOH Evapor. to dryness Reconst.: 0.5 mL MP			2 mM NH_4Ac in MeOH + 0.01% acetic acid 0.15 mL/min, 15°C		

ACN, acetonitrile; AGP, α1-acid glycoprotein; AMP: amphetamine; CBH, cellobiohydrolase; DE, diatomeous earth; d.w., dried weight; E, elution; ESI, electrospray ionization; EtOH, ethanol; Evapor., evaporation; FA, formic acid; Filt., filtration; HMMA, 4-Hydroxy-3-methoxymethamphetamine; HSA, human serum albumin; IPA, isopropanol; L, loading; LC, liquid chromatography; MAE, microwave assisted extraction; MAMP: methamphetamine; MDA, methylenedioxyamphetamine; MDEA, 3,4-methylenedioxy-N-ethyl-amphetamine; MDL, method detection limit; MDMA, methylenedioxymethamphetamine; MeOH, Methanol; MIP, molecularly imprinted polymer; MP, mobile phase; MQL, method quantification limit; MTPA-Cl, α-methoxy-α-(trifluoromethyl)phenylacetyl; NH_4Ac, ammonium acetate; NH_4OH, ammonium hydroxide; NSAIDs, non-steroidal anti-inflammatory drugs; PTFE: polytetrafluoroethylene; PVDF: polyvinylidene difluoride; QqQ, triple quadrupole; QTOF, quadrupole-time of flight; Reconst., reconstitution; SPE, solid-phase extraction; THF, tetrahydrofuran; W, washing.

FIGURES LEGEND

Figure 4.1. Comparison of the recoveries of the studied chiral drugs in the four different SPE cartridges. Reprinted from [22] with permission of Elsevier.

Figure 4.2. Enantioselective chromatograms of a group of cathinones extracted by MISPE followed by LC-HRMS with Chiral CBH column. See Table 4.2 for the rest of conditions. Reprinted from [39].

Figure 4.3. Effect of the extraction temperature (A) and H_2O/MeOH proportion as extraction solvent (B) on the enantiomer recovery during SPE. See Table 4.2 for the rest of conditions. Reprinted from [30].

Figure 4.4. Comparison of the GC-MS chromatograms of the standard solution, spiked and unspiked extract blank matrices of the liquid phase (A) and SPM (B) of a standard mixture at 150 ng/mL and 2 ng/mg, respectively, for the enantiomers of the amphetamines: AMP, MAMP, and MDMA; and synthetic cathinones: buphedrone (BPD), 3-methylmethcathinone (3-MMC), 3,4-dimethylmethcathinone (3,4-DMMC) and butylone (BTL). D1 and D2 correspond to the first and second eluted diastereomers, respectively. Reprinted from [3].

Figure 4.5. LC-MS/MS chromatogram of chiral illicit drugs spiked in water obtained with the optimised method shown in Table 4.2. Chiralpak CBH column (100 mm x 2 mm, 5µm) with -Chiral CBH guard column (10 mmx 2 mm); mobile phase: 1 mM NH_4OAc/ MeOH (90/10); flow-rate: 0.15 ml/min; column temperature: 30 ^oC; injection volume: 20 µL. Reprinted from [22] with permission of Elsevier.

Figure 4.6. Chromatograms illustrating the separation of AMP, MAMP and MDMA with different eluent modifiers. Reprinted from [24].