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Analysis of psychoactive substances in surface waters

Ivan Marcelino Langa

Dissertation for the Degree of Master's in Forensic Sciences and Laboratory Techniques of the University Institute of Health Sciences (IUCS, CESPU)

Gandra, January 2021

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Analysis of psychoactive substances in surface waters

Under guidance of Professor Doctor Cláudia Ribeiro and Professor Doctor Maria Elizabeth Tiritan

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IVAN MARCELINO LANGA, estudante do MESTRADO EM CIÊNCIAS E TÉCNICAS LABORATORIAIS FORENSES do Instituto Universitário de Ciências da Saúde, declaro ter atuado com absoluta integridade na elaboração desta Dissertação.

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The results presented in this dissertation are part of the following manuscript and scientific communications:

Articles in international peer-reviewed journals

1. I.M. Langa, R. Gonçalves, M.E. Tiritan and C. Ribeiro. Wastewater analysis of psychoactive drugs: non-enantioselective *vs* enantioselective methods for estimative of consumption. 2021, Submitted to Forensic Science International (manuscript number FSI-S-21-00108).

Poster communication

1. I.M. Langa, M.E. Tiritan, D.D. Silva, C. Ribeiro. Monitorização de Drogas quirais e psicoativas em águas de superfície. XI Jornadas de Ciências and IV Congresso APCF, 11-12 April 2019, Porto, Portugal. (Abstract and poster communication in annex 1)
2. I.M. Langa, C. Ribeiro, M.E. Tiritan. Development and validation of an indirect GC-MS method for the quantification of psychoactive substances in surface waters. 10º Encontro da Divisão de Química Analítica da SPQ, ANALITICA 2020, 26-28 October, Lisboa, Portugal (Abstract and poster communication in annex 2)

RESUMO

As mudanças na lei para manter o tráfico e o consumo de drogas sob controle contribuem para o crescimento do mercado ilícito de substâncias psicoativas (SPA) e para a síntese e introdução de novas substâncias psicoativas (NSP). Por outro lado, muitas SPA são quirais e estão disponíveis como racemato ou enantiomericamente puras e a determinação da fração enantiomérica (FE) é essencial para uma análise abrangente do consumo de drogas e avaliação do risco ambiental.

Essas substâncias chegam ao meio ambiente de diferentes formas, como descarga direta pelas indústrias, descargas ilegais e, principalmente, produtos de excreção humana (de compostos originais e / ou seus metabolitos) por meio de efluentes das estações de tratamento de águas residuais (ETAR)

A ocorrência de SPA em águas superficiais pode fornecer informações sobre o seu consumo numa região específica e a FE pode fornecer informações valiosas sobre as vias sintéticas e a distinção entre descarga direta ou consumo. Portanto, o objetivo deste trabalho foi desenvolver e validar um método indireto por cromatografia gasosa-acoplada à espectrometria de massa (GC-MS) com base na formação de diastereoisómeros usando (*R*)-(-)-α-metoxi-α- (trifluorometil) fenilacetil cloreto ((*R*) -MTPA-Cl) como reagente de derivatização quiral, para a quantificação enantiomérica de oito SPA incluindo três substâncias relacionada com as anfetaminas, nomeadamente anfetamina (AMP), metanfetamina (MAMP), 3,4-metilenodioximetanfetamina (MDMA), o metabolito norketamina (NK) e quatro catinonas sintéticas, bufedrona (BPD), butilona (BTL), 3,4-dimetilmetcatinona (3,4-DMMC) e 3-metilmetcatinona (3-MMC).

Além disso, duas piperazinas ilícitas (PP), nomeadamente 1-benzilpiperazina (1-BP) e 1- (4-metoxifenil)-piperazina (1,4-MPP), foram incluídas. As PP também foram derivatizadas com (*R*)-MTPA-Cl.

As condições otimizadas permitiram a quantificação das SPA alvo (um total de 16 diastereoisómeros e dois derivados de PP) em menos de 24,0 min. O método foi validado de acordo com a International Conference on Harmonization (ICH) em termos de seletividade, linearidade, limite de deteção (LD), limite de quantificação (LQ), exatidão, intra

e inter-precisão e recuperação. O método mostrou-se seletivo e os coeficientes de correlação foram superiores a 0,98. O LD variou de 17 a 100 ng L^{-1} e o LQ variou entre 50 e 300 ng L^{-1}. O método demonstrou ser exato (82,4 a 116,9%), preciso (até 8,5%) e as recuperações variaram de 25 a 105,5%. O método foi utilizado para avaliar a ocorrência, distribuição espacial e FE das SPA alvo em águas superficiais portuguesas na região do Grande Porto e efluentes de duas ETARs. Para tal, 1 L de amostras de água estuarina superficial foram recolhidas em cinco pontos de amostragem ao longo do estuário do rio Douro. Além disso, foram recolhidas amostras de efluentes de duas ETARs que descarregam as suas águas residuais tratadas nos afluentes do rio Douro.

Dos 18 compostos incluídos neste trabalho, 5 foram detetados em águas estuarinas e 6 em ETARs. Ambos os enantiómeros de AMP e MDMA e apenas um enantiómero de MAMP e BPD foram encontrados em águas estuarinas, mas abaixo do LQ. As amostras de efluentes mostraram apenas um enantiómero de AMP, BPD, 3,4-DMMC, mas ambos os enantiómeros de MDMA (presentes embora em concentrações abaixo do LQ). MAMP também foi detetado em ambas as ETARs no intervalo de <LOQ - 57,30 ng L^{-1} com FE ≅1. Estes resultados mostraram a ocorrência de AMPs e pela primeira vez a presença de catinonas sintéticas ilícitas no estuário e efluentes do rio Douro. A ocorrência de MAMP em águas superficiais e amostras de efluentes sugere consumo, em vez de descarga direta. A ocorrência de catinonas sintéticas, nomeadamente BPD, foi detetada e documentada pela primeira vez nas águas superficiais portuguesas. A análise de amostras de efluentes também detetou pela primeira vez a ocorrência de catinonas sintéticas (BPD e 3,4-DMMC) e FE sugere processos enantiosseletivos. Mais estudos são necessários considerando a ordem de eluição dos diastereoisómeros para uma análise abrangente dos dados. Embora os dados deste estudo tenham sido obtidos em um período de amostragem, os resultados mostram o potencial do método para monitorizar as SPA alvo.

Palavras-chave: catinonas sintéticas; anfetamina; água estuarina; substâncias psicoativas; enantiosseletivo; cromatografia gasosa.

ABSTRACT

The changes in the law to keep drug trafficking and consumption under control have increased illicit market of psychoactive substances (PAS) and the synthesis and introduction of new psychoactive substances (NPS). On the other hand, many PAS are chiral and available either as racemate or enantiomerically pure and determination of the enantiomeric fraction is essential for a comprehensive analysis of drug consumption and evaluation of environmental risk.

These substances reach the environment through different ways such as direct disposal by industry, illegal discharges and manly as humans excretion products (of parent compounds and/or metabolites) through the effluents of wastewater treatment plants (WWTP).

Occurrence of PAS in surface waters can give insights about their consumption in a specific region and EF can provide valuable information about synthetic pathways and distinction between direct disposal or consumption. Therefore, the aim of this work was to develop and validate an indirect method by gas chromatography-mass spectrometry (GC-MS) based on the formation of diastereomers using (*R*)-(-)-α-methoxy-α- (trifluoromethyl) phenylacetyl chloride ((*R*)-MTPA-Cl) as chiral derivatization reagent, for enantiomeric quantification of eight PAS including three amphetamine like substances namely amphetamine (AMP), methamphetamine (MAMP), 3,4-methylenedioxymethamphetamine (MDMA), the metabolite norketamine (NK), and four synthetic cathinones, buphedrone (BPD), butylone (BTL), 3,4-dimethylmethcathinone (3,4-DMMC) and 3-methylmethcathinone (3-MMC).

Also, two illicit piperazines (PP) namely 1-benzylpiperazine (1-BP) and 1-(4-metoxyphenyl)-piperazine (1,4-MPP) were included. PP were also derivatized with (*R*)-MTPA-Cl.

The optimized conditions allowed the quantification of the target PAS (a total of 16 diastereomers and two PP derivatives) in less than 24.0 min. The method was validated according to the International Conference on Harmonization (ICH) in terms of selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, intra and inter-precision and recovery. The method showed to be selective and correlation coefficients were higher than 0.98. The LOD ranged from 17 to 100 ng L^{-1} and LOQ varied between 50 and 300 ng L^{-1}. The method demonstrated to be accurate (82.4 to 116.9%), precise (up to 8.5%) and recoveries ranged from 25 to 105.5%. The method was used to evaluate the occurrence,

spatial distribution, and the EF of the target PAS in Portuguese surface waters in the Great Porto region and effluents from two WWTPs. For that, 1 L of surface estuarine water samples were collected at five sampling points along the Douro river estuary. Further, effluents samples were collected from two WWTPs that discharge their treated wastewaters for Douro river tributaries.

Of the 18 compounds included in this work, 5 were detected in estuarine water and 6 in WWTPs. Both enantiomers of AMP, and only one enantiomer of MAMP, MDMA and BPD were found in estuarine waters, but below LOQ. Effluents samples showed only one enantiomer of AMP, BPD, 3,4-DMMC, but both enantiomers of MDMA (present though at concentrations below the LOQ). MAMP was also detected in both WWTPs in range of <LOQ - 57.30 ng L^{-1} with enantiomeric fraction (EF) ≅1. These results showed the occurrence of AMPs and for the first time the presence of illicit synthetic cathinones in Douro river estuary and effluents. Occurrence of MAMP in both surface waters and effluent samples suggests consumption rather than direct disposal. The occurrence of synthetic cathinones, namely BPD was found and reported for the first time in Portuguese surface waters. Analysis of effluent samples also detected for the first time the occurrence of synthetic cathinones (BPD and 3,4-DMMC) and EF suggests enantioselective processes. Further studies are needed considering the elution order of the diastereomers for a comprehensive analysis of the data. Though data from this study was obtained from one sampling period, results show the potential of the method to monitor the target PAS.

Keywords: synthetic cathinones; amphetamine; estuarine water; psychoactive drugs; enantioselective; gas chromatography.

GENERAL INDEX

LIST OF ABBREVIATIONS, SYMBOLS AND ACRONYMS

1-BP: 1-benzylpiperazine

1-PHP: 1-phenylpiperazine

1,4-MPP: 1-(4-metoxyphenyl)-piperazine

2,3-MDBZP: *N*-[2,3-(methylenedioxy)-benzyl-piperazine

3-MMC: 3-methylmethcathinone

3,4-DMMC: 3,4-dimethylmethcathinone

3,4-MDBZP: *N*-3,4-(methylenedioxy)-benzyl-piperazine

4-FMC: 4-fluoromethcathinone

4-MEC: 4-methylethcathinone

4-MMC: 4-methylmethcathinone

5-HT: Serotonin

α-PVP: α-pyrrolidinovalerophenone

ACN: Acetonitrile

ADHD: Attention deficit hyperactivity disorder

AMP: Amphetamine

$AMPd_3$: Deuterated amphetamine

AMPs: Amphetamines

BPD: Buphedrone

BTL: Butylone

BZE: Benzoylecgonine

CE: capillary electrophoresis

CG: Carrier gas

CHN: China

CNN: Cannabis

COC: Cocaine

CV: coefficient of variation

DA: Dopamine

DCAT: *N,N*-dimethylcathinone

DTR: drug target residue

DU: Drug use

D1: First Eluted Diastereomer

D2: Second Eluted Diastereomer

EF: enantiomeric fraction

EMCDDA: European Monitoring Centre for Drugs and Drug Addiction

EPD: Ephedrone

ERA: Environmental risk assessment

ESP: Spain

ETC: Ethcathinone

ETL: Ethylone

EtOH: ethanol

FS: Full Scan

GC: Gas chromatography

GC-MS: Gas chromatography coupled to mass spectrometry

LC-MS: Liquid chromatography coupled to mass spectrometry

ICH: International Conference on Harmonization

IUPAC: International Union of Pure and Applied Chemistry

LC: Liquid chromatography

LOD: Limit of detection

LOQ: Limit of quantification

MAMP: Methamphetamine

MDA: 3,4-methylenedioxyamphetamine

MDEA: 3,4-methylenedioxyethylamphetamine

MDMA: 3,4-methylenedioxy-*N*-methylamphetamine

MDPBP: 3,4-methylenedioxy-α-pyrrolidinobutiophenone

MDPPP: 3,4-methylenedioxy-α-pyrrolidinopropiophenone

MDPV: 3,4-methylenedioxypyrovalerone

MeOH: Methanol

METC: Methcathinone

MEPH: Mephedrone

MPH: methylphenidate

MPHP: 4-methyl-α-pyrrolidinohexanophenone

MPVP: 4-methyl-α-pyrrolidinovalerophenone

MS: Mass Spectrometry

MS/MS: Tandem Mass Spectrometry

MTL: Methylone

MW: Molecular weight

NA: Noradrenaline

NK: Norketamine

NMR: Nuclear magnetic resonance spectroscopy

NPS: New psychoactive substances

PAS: Psychoactive substances

PP: Piperazines

PRT: Portugal

PTD: Pentedrone

PTL: Pentylone

PVL: Pyrovalerone

(*R*)-MTPA-Cl: (*R*)-(-)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride

RSD: Relative standard deviation

S: Slop of the calibration curve

SD: standard deviation

SFC: Supercritical fluid chromatography

SIS: Selected ion storage

SPE: Solid phase extraction

TEA: Triethylamine

TFMPP: 1-(3-Trifluoromethylphenyl)-piperazine

THC: Tetrahydrocannabinol

U.K.: United Kingdom

UPLC: Ultra-Performance Liquid Chromatography

USA: United States of America

WBE: Wastewater Based Epidemiology

WWTP: Wastewater Treatment Plant

ZAF: South Africa

FIGURES INDEX

TABLES INDEX

1. INTRODUCTION

1.1. Psychoactive substances

The misused of psychoactive substances (PAS) have been reported all over the world with consequent negative social, economic, and public health problems. According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), the number of new psychoactive substances (NPS) increased since 2012 and reached a peak (close to 100 NPS) in 2014-2015 (EMCDDA, 2015; 2016; 2017; 2018; 2019; 2020). Since then, a reduction on the report of NPS has been observed possible due to the introduction of the new general prohibitions and regulation/legislation on generic and analogous substances (EMCDDA, 2019; 2020). Nevertheless, the number of the NPS remains worrisome due to the synthesis of new substances such as synthetic amphetamines and cathinones (considered second major group of NPS) to escape from those already regulated (EMCDDA, 2017; 2018). Most of these new drugs are produced by chemical and pharmaceutical companies in Asian countries and sold worldwide mainly in European market (EMCDDA, 2019), although Europe is also a production region, favouring its market and access by the consumers.

According to the EMCDDA, cannabis (CNN), cocaine (COC), 3,4-methylenedioxy-*N*-methylamphetamine (MDMA or 'Ecstasy') and amphetamines (AMPs) seem to be the most used by adults aged from 15 to 64 years old.

CNN products account for the largest proportion of the illicit drug market with the percent of drug use (DU) ranging from 5.7% to 7.6% in the last 6 years followed by COC (1-1.3), MDMA (0.6%-0.8%) and AMPs with no changes in the DU during the 6 years (0.5%) (**Table 1**).

Concerning the NPS, consumption data are available only for young adults (15-34 years old) with DU up to 3.0% (**Table 1**).

Regarding to the purity, differences have been observed for the resin and the herb of CNN with resin available with more potency (considering the % of Tetrahydrocannabinol, THC) than the herbs (**Table 1**). AMPs and MAMP are also available with different purities and wide ranges, for example MAMP ranges from 7 to 100 and AMP from 1 to 64 (**Table 1**). Regarding MDMA, the mg per tablet has been reported ranging from 18 - to 190 mg/tablet also showing a wide difference in the quantity of substance among samples.

Table 1- Data of percent drug use (DU%) and the respective potency or purity by adults (15-65 years old) across Europe.

Year	CNN		COC		AMPs		MDMA		NPS	Ref.
	DU (%)	Potency (%THC)	DU (%)	Purity (%)	DU (%)	Purity (%)	DU (%)	(mg/tablet)	DU (%)	
2015	5.7	3 - 22/ 2 - 13	1.0	20 - 75	0.5	5 - 47/ 7 - 89	0.6	34 - 144	-	(EMCDDA, 2015)
2016	6.6	7 - 29/ 3 - 15	1.1	26 - 64	0.5	1 - 49/ 9 - 73	0.8	18 - 131	3.0[(1)]	(EMCDDA, 2016)
2017	7.0	4 - 28/ 3 - 22	1	15 - 78	0.5	7 - 50/ 16 - 79	0.8	35 - 128	3.0[(2)]	(EMCDDA, 2017)
2018	7.2	7 - 27/ 2 - 18	1.1	40 - 84	0.5	14 -61/ 22 - 73	0.8	41 - 168	3.0[(2)]	(EMCDDA, 2018)
2019	7.4	9 - 25/ 3 - 15	1.2	27 - 88	0.5	13 - 50/ 12 - 90	0.8	36 - 190	–	(EMCDDA, 2019)
2020	7.6	9 - 31/ 4 - 15	1.3	23 - 87	0.6	15 - 64/ 21 - 100	0.8	39 - 188	1.9[(3)]	(EMCDDA, 2020)

AMPs: amphetamines; COC: cocaine; CNN: Cannabis; MDMA: 3,4-methylenedioxymethamphetamine; NPS: New psychoactive substances; [(1)]: Young adults (aged 15-24); [(2)]: 15- to 16-year-old school students; [(3)]: Young adults (aged 15-34) .

The most recent data about DU is shown in **Figure 1** with the trend of PAS (CNN, COC, AMPs and MDMA) use by the European population aged 15 to 64 years from 2015 to 2020.

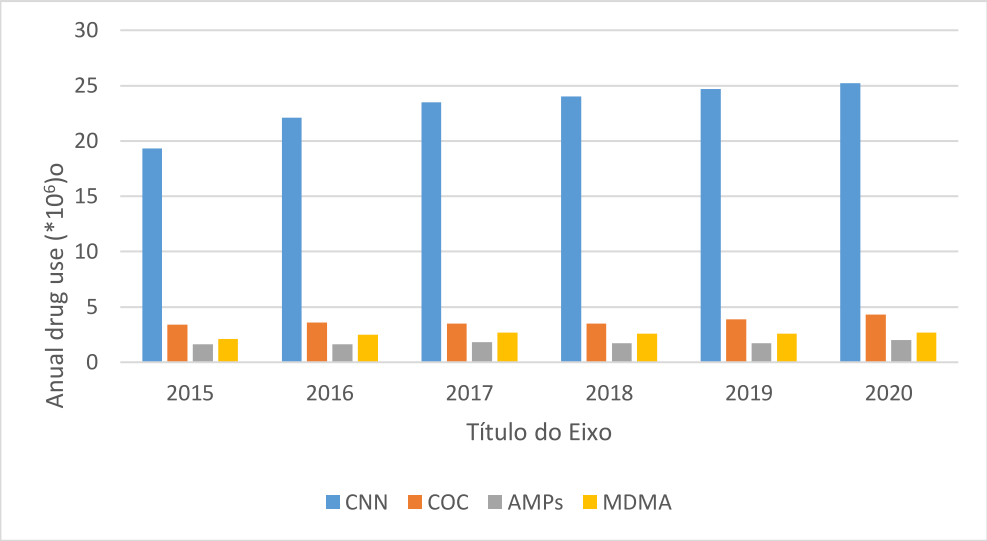


Figure 1 - Annual drug use of cannabis (CNN) cocaine (COC) amphetamines (AMPs) and MDMA by the European population aged 15 to 64 years.

Measure drug consumption is difficult but essential for health-care professionals, risk assessment and policymakers. Different sources of information have been used for a comprehensive analysis of drug consumption.

1.2. Epidemiological studies and sewage epidemiology

Estimative of consumption of PAS by traditional drug monitoring methods based on indirect data such as general statistics, hospital emergencies, syringe residues analysis, population surveys (web), consumer interview have been useful providing complementary data but on the other hand they are time consuming, expensive, may be inaccurate and not representative (Daughton, 2001; Gonçalves et al., 2019a; Löve et al., 2018; Zuccato et al., 2005). Based on the principle that all human excreted substances end up in the sewage system, Daughton in 2001, proposed an approach consisting in the measurement of an unchanged drug or its human metabolites in raw wastewater to estimative drug consumption. This tool recently referred as *Wastewater based epidemiology* (WBE) is nowadays used worldwide to estimate drug consumption at a community level (Castiglioni et al., 2013; Daughton, 2001; van Nuijs et al., 2018). Moreover, it may provide the snapshot about the licit and illicit drug use of a large population (EMCDDA, 2019; Zuccato et al., 2005), as well as may quickly reveal short and long-term trends in the scale of drug use.

WBE consists on the quantification of trace residual unchanged drugs or their metabolites in wastewater influent (raw wastewaters) followed by the back-calculation (Subedi, 2018). Estimative of consumption can be determined using the following equation (Xu et al., 2017):

$$Drug\ loads(mg/d/1000\ inh.) = \frac{Conc.of\ DTR\ \left(\frac{ng}{L}\right) \times IF\left(\frac{L}{d}\right)}{\frac{PS}{1000}} \times \left(\frac{1}{10^6}\right)\left(\frac{mg}{ng}\right)$$

The concentrations (ng/L) of the drug target residue (DTR) are analysed in daily samples of influent wastewater. The concentrations are multiplied by the daily average of the influent flow (IF) (L/day) to achieve daily mass loads (g/day). The mass loads are then normalized with respect to the population served (PS) and normalized to 1000 inhabitants to give mg/day/1000 people for comparable results. When excretion rates and drug stability of sewage is available, consumption can be determined using the following equation:

$$\text{Drug consumption } \left(\frac{\text{mg}}{1000\ \text{inh} \cdot \text{d}}\right)$$

$$= \text{Load}_{\text{DTR}}\left(\frac{\text{mg}}{1000\ \text{inh} \cdot \text{d}}\right) \times \frac{1}{\text{Stability}_{\text{DTR}}} \times \frac{1}{\text{Excretion}_{\text{DTR}}} \times \frac{\text{MW}_{\text{Drug}}}{\text{MW}_{\text{DTR}}}$$

where $\text{Excretion}_{\text{DTR}}$ is the excretion rate of the DTR; $\text{Stability}_{\text{DTR}}$ is the ratio of DTR concentration after in-sewer transformation and adsorption to initial concentration; MW_{Drug} is the molecular weight of the drug of interest and the MW_{DTR} is the molecular weight of the DTR.

With these equations and the proper data, it is possible to estimate the drug consumption for a specific population. These parameters are also important for the standardization of data between studies, being therefore possible to correlate patterns of consumption between cities, countries or even continents (Archer et al., 2018; Castrignanò et al., 2018; Löve et al., 2018; Thomas et al., 2012). On the other hand, many PAS are chiral and available either as racemate or as single enantiomers. Regarding chiral drugs, the determination of

the enantiomeric fraction (EF) is essential for a comprehensive analysis of drug use. (Emke et al., 2014; Ribeiro et al., 2018a; Rice et al., 2020; Sanganyado et al., 2020).

Actually, depending on the manufacturing procedure, licit and illicit drugs can be market either as racemates or single enantiomers, and human metabolism or transformation products can lead to a racemization or enantiomeric enrichment (Archer et al., 2018; Gonçalves et al., 2019b; Sanganyado et al., 2020). Thus, EF of chiral drugs is important in WBE as it can provide distinction between direct disposal and consumption, manufacturing procedure in addition to estimative of drug consumption by a specific population (Archer et al., 2018; Castrignano et al., 2018; Gonçalves et al., 2019b).

Different chromatographic analytical methods using either non-enantioselective and enantioselective methods have been reported for the quantification of PAS and estimative of consumption and were recently revised (Langa et al 2021, submitted).

1.3. Chiral psychoactive substances

Chiral (from the Greek *Kheir* "hand") refers to three dimensional molecules with one or more stereogenic centers or structural asymmetry, giving two non-superimposable specular image (Ribeiro et al., 2018b; Tiritan et al., 2016). The asymmetry can be inferred by a stereogenic center, usually the carbon atom linked to 4 different groups or atoms (**Figure 3**) (Ribeiro et al., 2014).

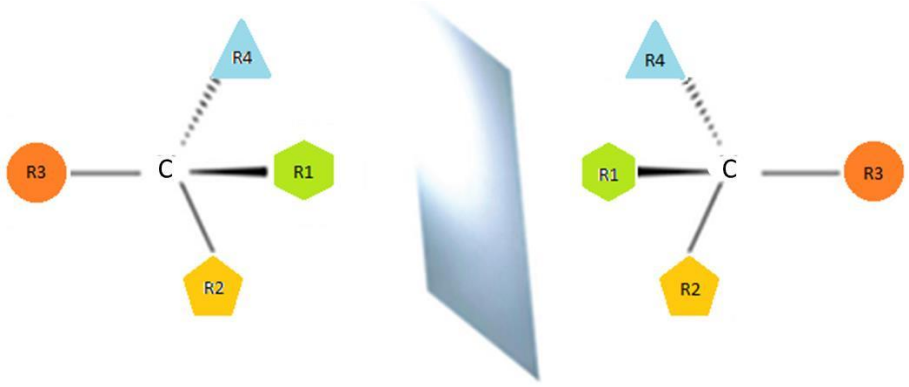


Figure 2 - Schematic representation of the stereogenic center (C) with 4 different substituents (R1, R2, R3 and R4). Adapted from (Tiritan et al., 2016).

Enantiomers have similar physico-chemical and spectrometric properties in an achiral environment, except for the rotation of polarized light that can be used for enantiomeric differentiation. For the right rotation (clockwise), the enantiomer is called dextrorotatory (+) and for the left rotation (counter-clockwise) the enantiomer is called levorotatory (-). Enantiomers, when stereogenic center is present, are structural identified through the configuration of the stereogenic center (spatial orientation of the substituents). The enantiomer can be (*R*), from the Latin *rectus* that means right or (*S*) from the Latin *sinister* that means left (**Figure 3**).

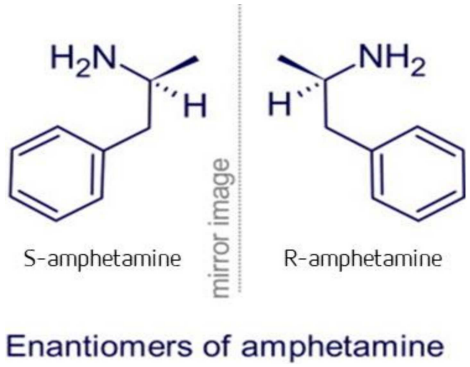


Figure 3 - Representative example of a pair of enantiomers (*R* and *S*).

Despite the similar physico-chemical properties, enantiomers may have different (pharmacokinetic and pharmacodynamic) including toxicity due to the enantioselective interaction with the enzymes, receptors and other chiral molecules present in living organisms (**Figure 4**) (Nallamuthu, 2018; Ribeiro et al., 2014; Tiritan et al., 2016).

Pharmacological/biological activity can be associated to only one of the enantiomers, the other may be inactive, present toxicity or a completely different activity (Nallamuthu, 2018; Tao and Zeng, 2002).

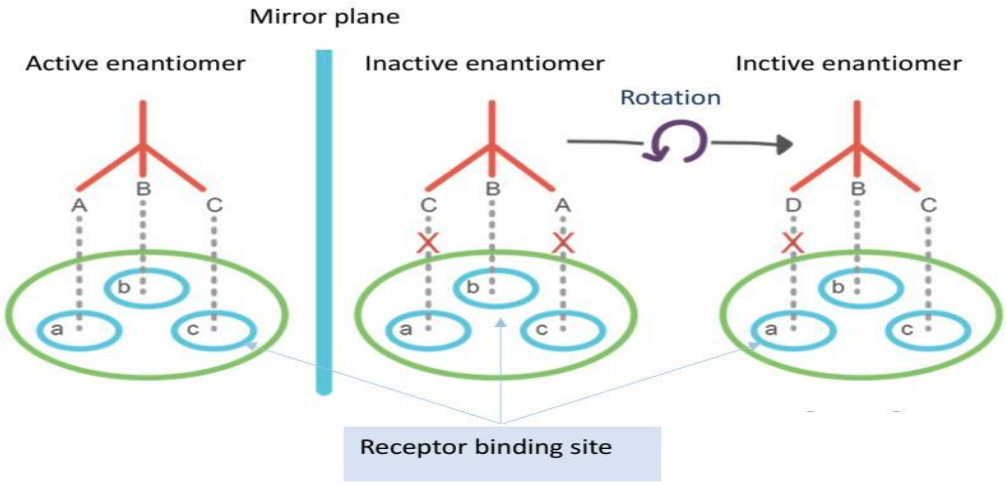


Figure 4 – Schematic representation of the 3 points proposed by Easson-Stedman representing the interaction between both enantiomers and the receptor binding site. Adapted from (Nguyen et al., 2006)

1.4. Amphetamine and amphetamine type substances

Amphetamine (AMP) and amphetamine type substances are chiral psychostimulant drugs structurally related with phenylethylamine (**Figure 5**). These substances have great potential for abuse and dependence (Newmeyer et al., 2014) though they are also used in clinical practice for treating attention deficit, hyperactivity disorder, narcolepsy and in some countries for the treatment of obesity (Capela J.P. and Carvalho, 2015; Heal et al., 2013).

These compounds were first synthesized in 1887 by the Romanian chemist Edeleanu at the University of Berlin and first marketed in 1932 for medicinal use. Amphetamine type substances were widely used in the world war II to promote the military's alertness. Moreover, they are used as medicines for diseases such as Selegiline (*L*-deprenyl, (*R*)-(−)-*N*-2-propynylmethamphetamine) used for treatment of Parkinson, Benzedrine® (racemate α-methylphenethylamine) used as treatment of narcolepsy, mild depression and post-encephalitic Parkinsonism (Heal et al., 2013). Also, dexamphetamine and methylphenidate (MPH) are used as first-line pharmacological treatments for attention deficit hyperactivity disorder (ADHD) (Thapar and Cooper, 2016). However, their notoriety worldwide is due to the recreational consumption of their synthetic derivatives and use by healthy individuals to improve performance at work or school or as doping in sports (Carvalho, 2003; Ribeiro et al., 2018b).

Among the most relevant compounds are MDMA, AMP, methamphetamine (MAMP) and MPH (Capela J.P. and Carvalho, 2015; Ribeiro et al., 2018b).

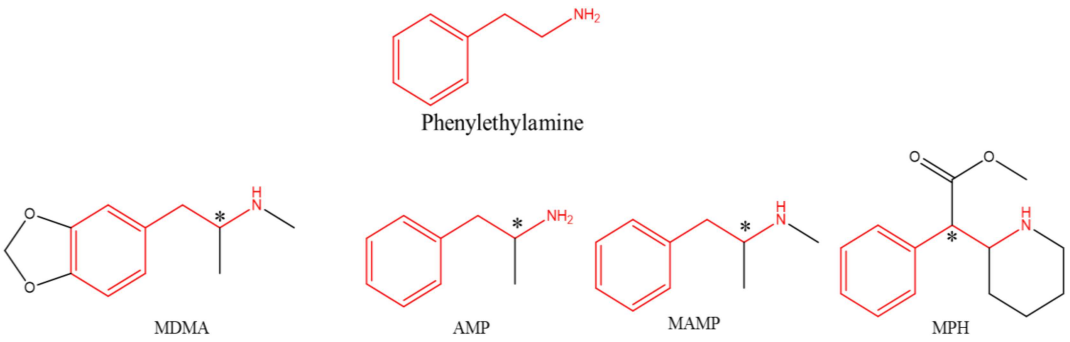


Figure 5 - Chemical structures of the amphetamine's precursor (phenylethylamine) and the main derivatives (MDMA, AMP, MAMP and MPH). * stereogenic center.

These compounds are used in conventional medicine either in their enantiomerically pure form or racemate. However, these substances can be produced illegally by synthesis in clandestine laboratories, usually available as racemate (Ribeiro et al., 2014; Ribeiro et al., 2018b). Nevertheless, their enantiomers may show different biological activities or potencies. For instance, the enantiomer (*S*)-MAMP has greater potency than (*R*)-MAMP, i.e., approximately 2-fold more potent in inhibiting vesicular uptake and approximately 3-fold more potent in evoking vesicular release. Illicit market distribution can occur as racemate and as the pure (*S*)-MAMP (Bardo et al., 2019; Partilla et al., 2006).

Every year, new amphetamine derivatives are identified on the illicit market and therefore not controlled in many countries. These NPS are proclaimed by traffickers as being legal highs and wrongly perceived by consumers as safer (Capela J.P. and Carvalho, 2015) leading to the continued growth of the drug consumption.

After consumption, these drugs can undergo stereoselective metabolism and thus residues of unchanged drugs and their metabolites are excreted into the sewage system in different EF (Gao et al., 2018; Gonçalves et al., 2019b; van Nuijs et al., 2011). Therefore, the occurrence of these substances in the raw wastewaters has been an important issue as it can give information about consumption by a specific community. In addition, the enantiomeric profile of these drugs has been used to differentiate between consumption or direct

disposal of drugs and the synthetic pathways and even possible discharges of clandestine laboratories. For instance, in recent study done by our research group, the EF values of chiral pharmaceuticals and illicit drugs were used to determine the consumption rates and sources of the drugs in a specific population (Gonçalves et al., 2019b). Only (*S*)-MAMP was found (EF ~1). This result was in accordance with most common synthesis process that results in the enantioselective synthesis of *(S)*-MAMP used as an illicit drug in Portugal. Also, only (*R*)-MDMA was found (EF ~1) suggesting that MDMA in wastewater was from consumption rather than direct disposal.

Further, these compounds are considered environmental contaminants due to their continuous disposal into the environment and potential adverse effects on non-target exposed organisms. For example, cyto-genotoxicity as well as a significant increase of the levels of carbonylated proteins and slight variation in lipid peroxidation was observed in zebra mussel (*Dreissena polymorpha*) after exposure of a mixture of illicit drugs including amphetamines (Parolini et al., 2016; Parolini et al., 2015).

Their environmental occurrence can present EF values from 0 to 1 as well their ecotoxicological effects are also expected to be enantioselective. However, there are only few studies regarding enantioselective environmental occurrence and the ecotoxicological effects of chiral PAS.

Chiral analysis is of highly importance in environmental studies as a tool to provide information for a further accurate Environmental risk assessment (ERA) and new proposals for environmental protection measures.

1.5. Cathinone and Synthetic Cathinones

Cathinone, also known as β-keto-amphetamine is a naturally occurring β-ketone, structurally related to amphetamine, found in leaves of the *Catha edulis* plant (**Figure 6**). This plant grows in east Africa and in southern Arabia where is known because of its therapeutic and recreational properties.

Synthetic cathinones are the latest version of sympathomimetic compounds chemically related to natural cathinone, that recently emerged as abused drugs due to their

stimulating, euphoric, and empathogenic properties (Banks et al., 2014; Majchrzak et al., 2018).

They are commonly sold in the form of odorless powder and fine crystals via "darknet" or smart shops as "bath salt", "Plant feeders" or "Plant food", associated with glamorous names such as Meow Meow, Bliss, Energy-1, Hurricane Charlie, White rush, Bloom, Blue magic, Cloud 9, Cloud 10, Mind Candy, Rocket Fuel and Sextasy (Karila et al., 2015), described as "not for human consumption or not tasted for hazards or toxicity" (Leffler et al., 2014; Levitas et al., 2018; Weiß et al., 2015).

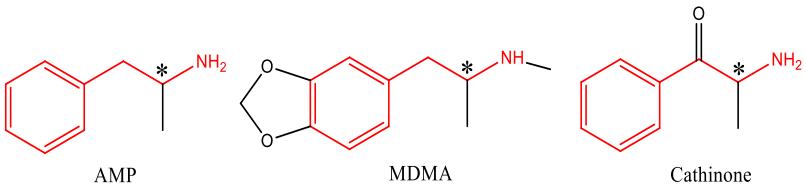


Figure 6 - Chemical structures of the amphetamine, MDMA and cathinone with the similar pharmacophore highlight in red. * stereogenic center.

This group of sympathomimetic compounds is mainly obtained by minor modification at the alkyl chain or at the aromatic ring being mainly classified as phenylalkylamine and can be divided into 4 groups according to whether they are *N*-alkylated derivatives, 3,4-methylenedioxy-*N*-alkylated derivatives, *N*-pyrrolidinyl derivatives and 3,4-methylenedioxy-*N*-pyrrolidinyl derivatives (Majchrzak et al., 2018). **Table 2** shows the chemical structures of the most common synthetic cathinone derivatives for each group.

Table 2 - Chemical structures, IUPAC name and usual name of the most common synthetic cathinones for each group.

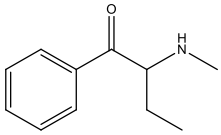
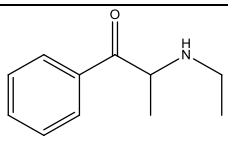
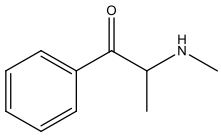
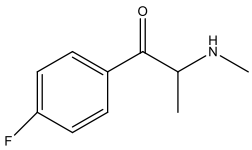
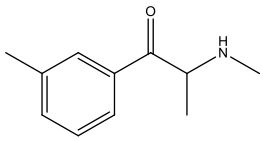
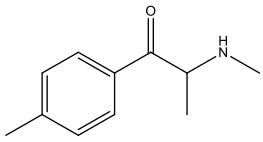
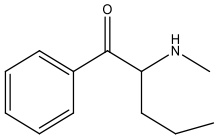
Precursor	Chemical structure	Chemical name (IUPAC)	Usual name
N-alkylated derivatives		[2-(N-Methylamino)-butan-1-onyl]-benzene	BPD
		[2-(*N*-Ethylamino)-propan-1- onyl]-benzene	ETC
		[2-(*N*-Methylamino)-propan-1-onyl]-benzene	METC, EPD
		1-[2-(*N*-Methylamino)-propan-1- onyl]-4-fluorobenzene	4-FMC, Flephedrone
		1-[2-(*N*-Methylamino)-propan-1- onyl]-3-methylbenzene	3-MMC
		1-[2-(*N*-Methylamino)-propan-1- onyl]-4-methylbenzene	MEPH, 4-MMC
		[2-(*N*-Methylamino)-pentan-1- onyl]-benzene	PTD

Table 2 – *Continued.*

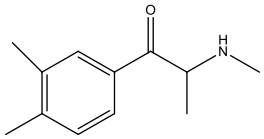
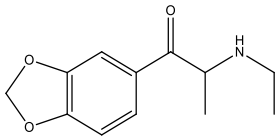
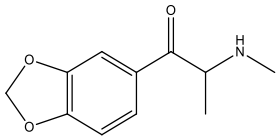
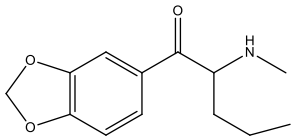
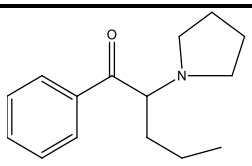
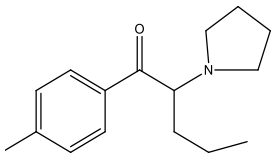
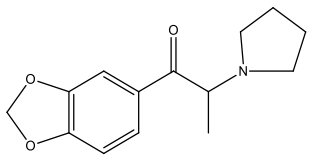
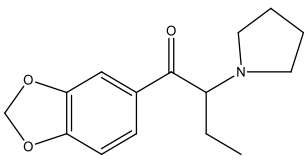
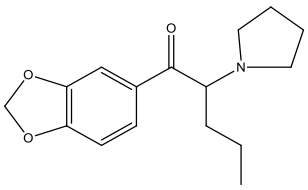
		1-[2-(*N*-Methylamino)-propan-1- onyl]-3,4-dimethylbenzene	3,4-DMMC
3,4-Methylenedioxy-*N*-alkylated derivatives		1-[2-(*N*-Ethylamino)-propan-1-onyl]-(3,4-methylenedioxy)-benzene	ETL
		1-[2-(*N*-Methylamino)-propan-1-onyl]-(3,4-methylenedioxy)-benzene	MTL
		1-[2-(*N*-Methylamino)-pentan-1-onyl]-(3,4-methylenedioxy)-benzene	PTL
N-pyrrolidinyl derivatives		1-[2-(Pyrrolidin-1-yl)-pentan-1-onyl]-benzene	α-PVP
		1-[2-(Pyrrolidin-1-yl)-pentan-1-onyl]-4-methylbenzene	PVL, MPVP

Table 2 – *Continued.*

3,4-methylenedioxy-*N*-pyrrolidinyl derivatives		1-[2-(Pyrrolidin-1-yl)-propan-1-onyl]-3,4-methylenedioxybenzene	MDPPP
		1-[2-(Pyrrolidin-1-yl)-pentan-1-onyl]-3,4-methylenedioxybenzene	MDPV
		1-[2-(Pyrrolidin-1-yl)-butan-1-onyl]-3,4-methylenedioxybenzene	MDPBP

BPD: Buphedrone; BTL: Butylone; 3,4-DMMC: 3,4-dimethylmethcathinone; EPD: Ephedrone; ETC: Ethcathinone; ETL: Ethylone; 4-FMC: 4-fluoromethcathinone (flephedrone); MDPBP: 3,4-methylenedioxy-α-pyrrolidinobutiophenone; MDPPP: 3,4-methylenedioxy-α-pyrrolidinopropiophenone; MDPV: 3,4-methylenedioxypyrovalerone; METC: Methcathinone; MEPH: Mephedrone; MPHP: 4-methyl-α-pyrrolidinohexanophenone; MPVP: 4-methyl-α-pyrrolidinovalerophenone; 4-MMC: 4-methylmethcathinone; MTL: Methylone; PTD: Pentedrone; PTL: Pentylone; PVL: Pyrovalerone; 3-MMC: 3-methylmethcathinone; α-PVP: α-pyrrolidinovalerophenone.

With medicinal purposes, the first synthetic cathinones derivatives were synthetized in the early twentieth century. Their illegal use began around the year 2000, however first notified to the EU Early Warning System was only in 2008 with 99 associated deaths and 107 non-fatal intoxication in 29 countries (EMCDDA, 2014; Majchrzak et al., 2018).

The occurrence of synthetic cathinones in effluents and surface waters has been reported. For example, the occurrence of 3,4-methylenedioxypyrovalerone (MDPV) was reported ranging from 0.5 ± 0.7 ng L^{-1} to 1.6 ± 0.5 ng L^{-1} in 13 effluent samples from Chinese WWTPs (Gao et al., 2017). The occurrence of 8 cathinones in river water including flephedrone (4-

FMC), methylone (MTL), methedrone, butylone (BTL), mephedrone (MEPH), 3,4-dimethylmethcathinone (3,4-DMMC), α-pyrrolidinovalerophenone (α-PVP) and 3,4-methylenedioxypyrovalerone (MDPV) was also reported (Fontanals et al., 2017).

Nevertheless, enantioselectivity studies of synthetic cathinones in surface waters are scarce. Recently, the occurrence of both enantiomers of MTL (5.1 and 5.3 ng L^{-1}) and methedrone (3.8 and 4.4 ngL^{-1}) in river water sample was reported (Fu et al., 2020). Ecotoxicity studies are also scarce although some studies are already reported. For examples, changes in the behaviour were demonstrated in zebrafish larvae exposed to Pyrovalerone (PVL) (100 μM) as well as decrease in total distance moved duo to the modulation of dopamine system (Souders et al., 2019).

1.6. Other psychoactive drugs: Piperazine

Piperazines (PP) are considered NPS with stimulant effects like those of amphetamines although with lower potency. Their mechanism of action includes the stimulation of the release and inhibition of the reuptake of dopamine (DA), noradrenaline (NA) and 5-HT (Baumann et al., 2004; Elliott and Smith, 2008).

The piperazine derivatives are totally synthetic and are derived from two main structural groups: 1-benzylpiperazine (1-BP) and 1-phenylpiperazine (1-PHP).

As well as the other NPS early described, PP are obtained by minor modification (**Figure 7** and **Figure 8**) (Elliott, 2011)

Although the first report of PP consumption in United States of America (USA) is from 1990, only later, by the year 2000, piperazines became available in illegal market in Europe and other countries. PP are usually sold as powder, tablets and less frequently as capsules and the principal consumption mode is oral and although intravenous and smoking are also reported (de Boer et al., 2001; Elliott, 2011).

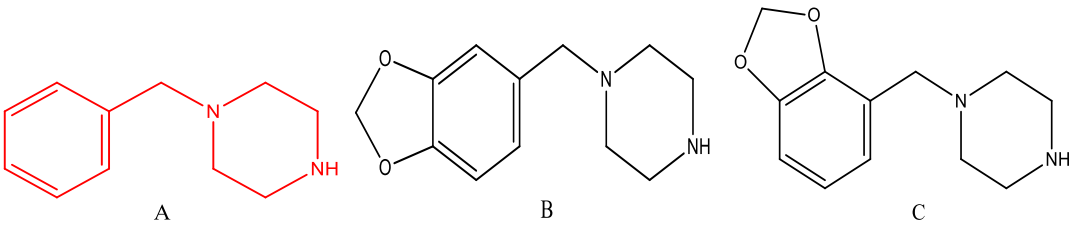


Figure 7 - Chemical structures of 1-benzylpiperazine (1-BP) (A) and the respective derivatives N-3,4-(methylenedioxy)-benzyl-piperazine (3,4-MDBZP) (B) and N-[2,3-(methylenedioxy)-benzyl-piperazine (2,3-MDBZP) (C).

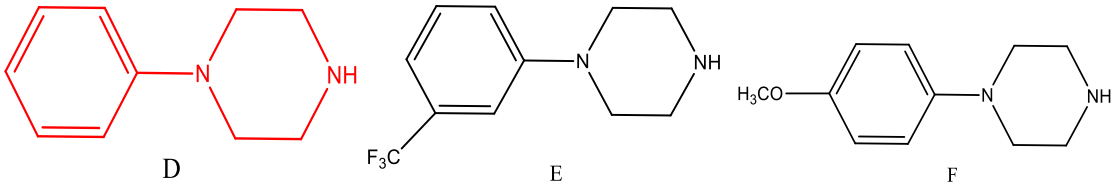


Figure 8 - Chemical structures of 1-phenylpiperazine (D) and the respective derivatives 1-(3-Trifluoromethylphenyl)-piperazine (TFMPP) (E) and 1-(4-metoxyphenyl)-piperazine (1,4-MPP) (F).

Regarding toxic effects of piperazines, although piperazines are often reported in co-consumption with other drugs, mostly MDMA and other amphetamine like substance, the adverse effects including agitation, tachycardia and seizures from single consumption of 1-BP (Gee et al., 2005) and death involving detention of 1-BP (Röggla and Moser, 2007), 1-BP and 1-(3-Trifluoromethylphenyl)-piperazine (TFMPP) was also reported (Elliott and Smith, 2008).

To the best of our knowledge no ecotoxicity studies are described although their occurrence in the surface waters have been reported (Baker and Kasprzyk-Hordern, 2013).

1.7. Enantioselective chromatographic methods for quantification psychoactive substances in environmental samples

After consumption, PAS are excreted with urine and faeces as unchanged form or metabolized and discarded into Wastewater Treatment Plant (WWTP) or directly to the aquatic environment. The conventional WWTP are not designed to completely eliminate this

type of residues leading to the contamination of the aquatic ecosystems (Bagnall et al., 2012; Baker and Kasprzyk-Hordern, 2013) (**Figure 9**).

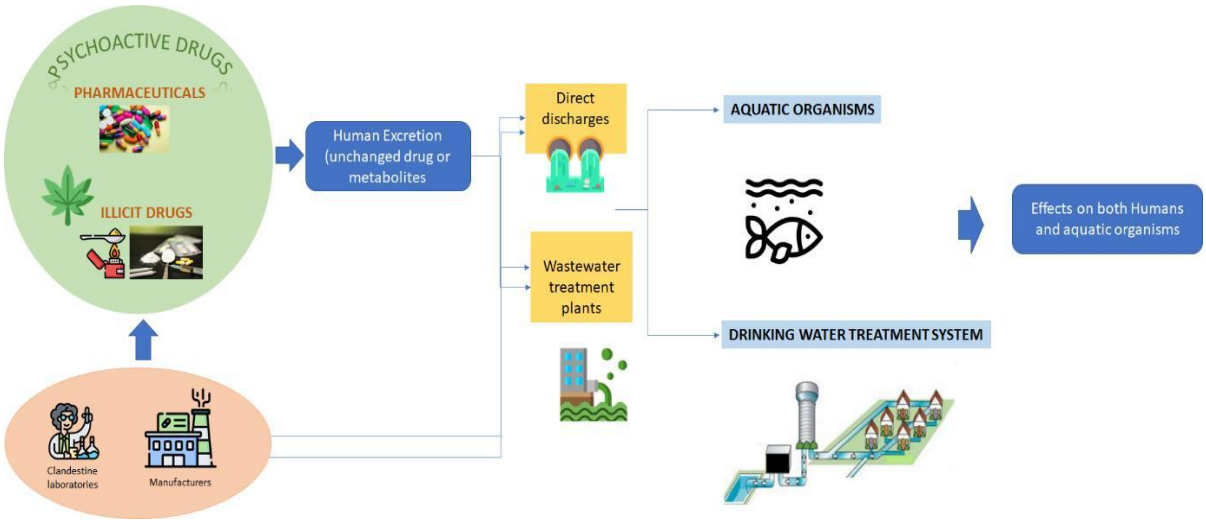


Figure 9 - Schematic representation of the pharmaceuticals and illicit drugs routes into the environment.

Various studies have been reporting the occurrence of PAS in streams, river, lakes and other aquatic ecosystems (Coelho et al., 2019; Evans et al., 2017; Evans et al., 2016; Fekadu et al., 2019; Li et al., 2020). Consequently, these biological active substances may cause harmful effects to non-target organisms leading to acute and chronic toxicity effects (Binelli et al., 2012; Nilsen et al., 2019; Pérez-Pereira et al., 2020; Wang et al., 2020).

Although they are usually reported at low concentration (ng L^{-1} to µg L^{-1}), their continued discharge and the combined exposure of multiple substances may cause adverse effects on non-target (Pal et al., 2013; Parolini et al., 2015; Wang et al., 2020). Furthermore, studies demonstrated that exposer to PAS at environmental concentrations can interact with genetic factors causing altered neurological gene sets expression associated with human neurological disorder including Idiopathic Autism, Alzheimer's disease and Schizophrenia, (Kaushik and Thomas, 2019).

Chromatographic methodologies including liquid chromatography (LC), gas chromatography (GC), capillary electrophoresis (CE) and supercritical fluid chromatography (SFC) are the tools for quantification of enantiomers of different classes of chemicals (Ribeiro et al., 2020a; Ribeiro et al., 2020b; Ribeiro et al., 2018b). The enantioseparation

can be done by direct method using chiral columns or chiral mobile phase additives resulting in formation of transient diastereomeric complex with analytes (Ribeiro et al., 2018a; Yu et al., 2013). The most used chiral columns are those derived from macrocyclic antibiotics, polysaccharides and Pirkle's type (Ribeiro et al., 2016a).

Enantiomeric separation can also be achieved by indirect method through formation of diastereomers by reaction with an enantiomerically pure reagent (Gonçalves et al., 2019a). The most widely used chiral derivatives are (*R*)-(+)-α-methylbenzylisocyanate, (*R*)-(-)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (*R*-MTPA-Cl) and (*R*)-(-)-3-methyl-2-butylisocyanate (Ribeiro et al., 2020b).

Regarding enantioselective methods for PAS analysis, LC and GC are among the most used (Ribeiro et al., 2020b; Ribeiro et al., 2018b) for aquatic matrices as wastewaters and surface waters (Coelho et al., 2019; Gonçalves et al., 2019a).

Enantioselective analysis of PAS in effluents demonstrated different EF values corroborating that effluents are a major source of these pollutants in environmental matrices and that biodegradation during wastewater treatment can affect the EF of PAS (Maia et al., 2016; Maia et al., 2018; Ribeiro et al., 2014). These results are of high importance for forensic analysis, risk assessment and for an accurate evaluation of the impact of these substances on aquatic systems. **Table 3** presents some examples of the application of chromatographic methods for monitorization of the occurrence of PAS in surface waters. LC methods are among the most used though both GC and LC methods have been reported. Concentrations reported on **Table 3** refers to surface waters while the EF values refer to effluent's samples.

Table 3 - Chromatographic methods for determination of PAS in environmental samples and effluent wastewater.

PAS	Local	Method	Concentration[a] (ng L^{-1})	EF[b]	Ref.
				n.d	
COC			0.1-6.3	nd	
BZE			0.2-72.4	-	
AMP		**UPLC-MS/MS**	0.9-4.3	-	
MAMP	U.K.	BEH C18 column (150 x 1 mm, 1.7 µm particle size), Chiral-CBH column (100 × 2 mm, 5 µm particle size)	0.1-0.4	-	(Baker and Kasprzyk-Hordern, 2013)
1-BP	(River water/EWW)		1.0-59.4	-	
TFMPP			0.1-6.1	0.78	
MDMA			0.5-24.8	-	
MDA			1.6-1.9	-	
NK			0.6-12.0		
MDMA	U.K. (EWW)	**UPLC-MS/MS** Chiral-CBH column, (100 mm × 2 mm, 5 µm) and Chiral-CBH (10 mm × 2.0 mm) guard column; MP: H_2O: IPA (90:10 v/v), 1 mM NH_4OAc, pH 5.0.	-	0.71	(Kasprzyk-Hordern et al., 2010)
MDMA	UK (River water/EWW)	**HPLC -MS/MS** CBH column (100 x 2 mm, 5 µm particle size); MP: H_2O: IPA (90:10 v/v), 1 mM NH_4OAc.	60	0.13	(Evans et al., 2017)
MDA			>5	1.0	

		UPLC-MS/MS			
AMP MAMP MDMA MDA	(U.K. River water/EWW)	CBH column (100×2 mm, 5 µm particle size); MP: H_2O: IPA I (90:10 v/v), 1 mM NH_4OAc	- - <70 <35	0.4 <0.5 <0.3 1.0	(Evans et al., 2016)
		LC-MS			
MTL Methedrone	ESP (River water/EWW)	Chiralpak CBH (150 × 2 mm, 5 µm) with a Chiralpak CBH guard column (10 × 2 mm, 5 µm particle size) MP: 1 mM NH_4OAc (aq): MeOH (98/2, v/v)	E1=5.1; E2=5.3 E1=3.8; E2=4.4	0.49 0.46	(Fu et al., 2020)
		UHPLC-MS/MS			
COC BZE AMP MAMP	PRT (River water)	Chirobiotic TMV CSP (150 mm × 2.1 mm i.d., 5 µm) MP: EtOH/10 mM NH_4OAc (aq) (92.5/7.5, v/v) pH: 6.8 Whelk-O®1 CSP (250 mm x 4.6 mm i.d., 5 µm particle size) MP: MeOH(aq) (60/40, v/v), 0.1% HCOOH	<12 <13 <14 <10	- - - -	(Coelho et al., 2019)
4-FMC	ESP	**LC-MS**	<LOQ	-	(Evans et al., 2015;

MTL	River water	Acquity UPLC HSS T3 (100 × 2.1 mm, 1.8 μm particle size); MP: 0.1% HCOOH (aq): 0.1% HCOOH, ACN	1.8-4.7	-	Fontanals et al., 2017)
Methedrone			<1.8	-	
BTL			<LOQ	-	
MEPH			<LOQ	-	
3,4-DMMC			<LOQ	-	
α-PVP			<LOQ	-	
MDPV			1.4-1.6	-	
		LC-MS			
AMP	CHN (Lakes and river water)	Gemini C18 column (100 mm × 2 mm, 3 μm particle size) MP: 30 mM ammonium formate (aq) with	<LOD	-	(Fu et al., 2020; Li et al., 2016)
COC			<LOD	-	
BZE			<LOD	-	
MDMA			<LOD	-	
MDA			<LOD-2.7	-	
NK			<LOD-5.6	-	
		GC-MS			
AMP	PRT (EWW)	CDR: (R)-(-)-MTPA-Cl IV: 1 μL, splitless FR: 1 mL/min IT: 280°C; TLT: 280°C; IST: 230°C; DT: 150°C; TP: 140°C for 0.5 min, up to 215°C at 11°C/min, up to 283°C at 29°C/min (held for 6 min), up to 300°C at 29°C/min (held for 7.7 min)		0.53	(Gonçalves et al., 2019a)
MAMP				≈ 1	
MDMA				≈ 1	
		LC–QTOF-MS			
AMP	U.K.	Chirobiotic V column, 250 ×	*S,R* = <LOQ	-	(Bagnall et al., 2012)
MAMP			*S,R* = <LOQ	-	

MDA	River water (July, 2011)	4.6 mm, I.D. 5 µm particle size) and (20 × 4.0 mm, I.D. 5 µm particle size) guard column MP: MeOH, 4 mM ammonium acetate and 0.005% HCOOH	E1,E2 = <LOQ	-
MDMA			E1,E2 = <LOQ	-
		LC–QTOF-MS		
	U.K. River water (October, 2011)	Chiral-CBH column (100 × 2 mm, I.D. 5 µm particle size) and Chiral-CBH (10 × 2.0 mm, I.D. 5 µm particle size) guard column MP: 90% H_2O, 10% IPAand1 mM NH_4OAc	*S,R* = <LOQ	-
			S,R = <LOQ	-
			E1,E2 = <LOQ	-
			E1, E2 = <LOQ	-

([a]). EF obtained in effluent; AMP: Amphetamine; BZE: Benzoylecgonine; 1-BP: benzylpiperazine; CG: Carrier gas; CHN: China; CNN: Cannabis; COC: Cocaine; ESP: Spain; GC-MS: Gas chromatography coupled to mass spectrometer; IPA: isopropanol; LC-MS: liquid chromatography coupled to mass spectrometer; MAMP: Methamphetamine; MDA: 3,4-methylenedioxyamphetamine; MDEA: 3,4-methylenedioxy-N-ethylamphetamine MDMA: 3,4-methylenedioxymethamphetamine; MEPH: Mephedrone; MS/MS: Tandem Mass Spectrometry; NK: Norketamine; PRT: Portugal; TFMPP: 1-(3-trifuoromethylphenyl)-piperazine; U.K.: United Kingdom; UPLC: Ultra-Performance Liquid Chromatography; ZAF: South Africa

2. OBJECTIVES

The main purpose of this study was to develop an enantioselective method by gas chromatography coupled to mass spectrometry (GC-MS) based on the formation of diastereomers for quantification of several classes of PAS in surface waters.

The specific aims proposed for this dissertation was:

- To establish the derivatization procedure for diastereomer formation, based in previous established study for other classes of PAS, for the target compounds: AMP and amphetamine type substances (MAMP, MDMA), synthetic cathinones (BTL, 3,4-DMMC, 3-MMC and BPD), and norketamine (NK), as well as derivatization of PP (1-BP and 1,4-MPP) using the enantiomerically pure reagent (*R*)-MTPA-Cl.
- To optimize a solid phase extraction (SPE) procedure and pre-concentration for the selected PAS in surface waters.
- To optimize and validate an indirect GC-MS for chromatographic separation and quantification of the targets PAS in surface waters.
- To apply the validated method to investigate the occurrence and spatial distribution of the selected PAS in Portuguese surface waters in the Greater Porto region and effluent samples from two WWTPs with different treatment technologies.

3. MATERIAL AND METHODS

3.1. Chemicals and materials

AMP and MAMP standards were acquired by Lipomed (Arlesheim, Switzerland). NK was purchased from Sigma Aldrich (Steinhein, Germany). MDMA, BPD, 3-MMC, BTL, 3,4-DMMC, 1-BP and 1,4-MPP standards were kindly provide by the Toxicology Laboratory of the Faculty of Pharmacy of the University of Porto. The internal standard (IS), D,L-AMP-D_3 was purchased from Lipomed (Arlesheim, Switzerland). The chiral reagent (*R*)-(-)-α-methoxy-α- (trifluoromethyl) phenylacetyl chloride (*R*-MTPA-Cl) (Ref. 65363; 100 mg), triethylamine (TEA) (Ref. T0886), ammonium hydroxide 25% (Ref. 30501), sodium hydroxide and sulfuric acid (Ref. 30743) were purchased by Sigma-Aldrich (Steinheim, Germany). Individual stock solutions of standards were prepared in methanol (MeOH) at concentration of 1 mg mL^{-1} and stored at -20 °C in amber vials. Work solutions were prepared freshly by dilution of stock solutions in MeOH. Stock solutions of IS were prepared at 200 µg mL^{-1}. The chiral reagent solution was obtained by dilution of 5 µL of (*R*)- MTPA-Cl in 95 µL of anhydrous acetonitrile and stored in amber vials at –20 °C.

All solvents used were of HPLC grade. Acetonitrile (ACN) (Ref. 83676.230), *n*-Hexane (Ref. 24575.320), methanol (MeOH, Ref. M / 4058/17) and ethanol (EtOH, Ref. E / 0665DF / 17) were purchased by VWR Prolab Chemical (Pennsylvania, USA). Anhydrous acetonitrile (Ref. 83676230) and anhydrous ethyl acetate (Ref.116668 333) were purchased by Merck, (Darmstadt, Germany). Formic acid 98—100% was purchased from Merck (Espoo, Finland). Ultra-pure water was supplied by a SG Water System (Ultra Clear UV model). Glass microfibers filter with 0.7 µm pore size was purchased from VWR (Leuven, Belgium). 2 mL syringes were purchased from BD Emerald (Madrid, Spain). Syringe filters with 0.22 µm pore size were purchased from Teknokroma (Barcelona, Spain). Oasis MCX 150 mg (6 cc) SPE cartridges was purchased from Waters (Dublin, Ireland).

3.2. Equipments

3.2.1. Chromatographic system

Chromatographic analysis was performed using a Varian CP-3800 gas chromatograph equipped with ion-trap Varian Saturn 2200 mass detector with electron impact (EI) ionization chamber, an autosampler (Varian CP-8400) and an electronically controlled split/splitless injection port. Chromatographic separation was achieved using a Zebron (5% phenyl, 95% dimethylpolysiloxane) capillary column (30 m × 0.25 mm I.D. × 0.25 µm film thickness, from Phenomenex, USA). High-purity helium (99,999%) was used as carrier gas.

3.2.2. Other equipment

A centrifugal vacuum evaporator (CentriVap Concentrator) with a cold trap was purchased from Labconco, (Kansas City, MO, USA) was used to evaporate sample´s extracts. Visiprep™ SPE Vacuum Manifold purchased from Supelco was used for SPE procedure. For determination of physico-chemical parameters of estuarine water samples: pH, total dissolved solids (TDS) and electrical conductivity (EC) were measured using a multi-parameter analyser Consort C863 (Turnhout, Belgium).

3.3. Derivatization procedure for diastereomer formation

For diastereomer formation the derivatization procedure described by Gonçalves et al. was adapted and used for the formation of the diastereomers of 8 chiral PAS (4 synthetic cathinones, 3 amphetamine like substances and NK) using (*R*) - MTPA-Cl) as chiral derivatization reagent. Further, two PP derivatives were also obtained (**Figure 10**) (Gonçalves et al., 2019a).

For the derivatization of the target compounds, 200 µL of standard mixtures or aliquots of SPE extracts were transferred into a vial and evaporated to dryness in a speedvac programmed at room temperature.

Then, 200 µL of ultra-pure water and 200 µL of NaOH (1M) were added to the residue and vortexed for 30 seconds. After that, 1500 µL of 0.02% TEA in *n*-Hexane was added and the

solution was vortexed for 10 minutes for compounds extraction and then centrifuged at 13000 rpm for 10 minutes for the phase's separation.

Then, the organic phase was transferred to a new vial and 10 µL of 0.5% of chiral reagent ((*R*)- MTPA-Cl) in anhydrous ACN was added followed by 2 hours of heating at 80 ° C. After that, the samples were cooled to room temperature and 100 µL of ethanol was added. The solution was heated in the oven at 70 ° C for 15 minutes to stop the derivatization reaction.

Finally, the samples were cooled and evaporated to dryness in the speedvac and the residue were reconstituted in 200 µL of anhydrous ACN and analysed by GC-MS (1 µL).

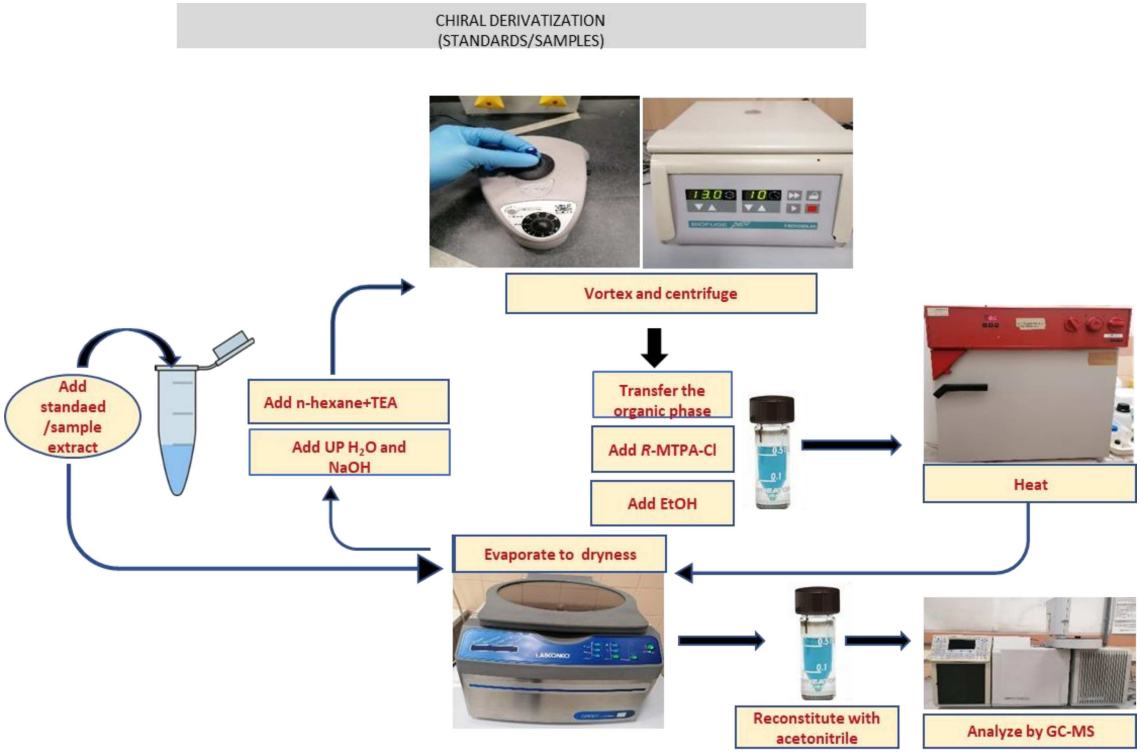


Figure 10 - Schematic representation of the derivatization procedure.

3.4. Chromatographic condition of the GC-MS

Different conditions were attempted to optimize separation of the diastereomers and PP derivatives using standard mixtures. Optimized chromatographic conditions were achieved using a Zebron (5% phenyl, 95% dimethylpolysiloxane) capillary column, 30 m x 0.25 mm I.D., 0.25 µm film thicknesses (Phenomenex, USA) at a constant flow rate of 1.0 mL min^{-1}.

The injection port temperature was programmed at 280 °C and 1 µL of sample was injected in splitless mode. The oven temperatures were programmed as followed: an initial temperature of 140°C and held for 50 s, followed by a ramp to 215 °C at 11 °C min^{-1} and held for 5 min, a ramp to 285 °C at 10°C min^{-1} and maintained for 20 min, with a total run time of 24.32 min. The MS operating conditions were EI mode with electron energy of 70 eV, operated in both full scan (FS) mode from *m/z* 40 to 650 (total ion count, TIC) and selection ion storage (SIS) according totheir *m/z* fragments obtained from the mass spectrum of each target compound.

3.5. Sample collection

For method validation, spring water samples from the source of the Leça river were collected to be used as blank matrix (**Figure 11**). Upon arrival to the laboratory, all samples were immediately vacuum filtered through a 0.7 µm glass fiber filter, acidified to pH ≈ 3 with H_2SO_4 (95–97%) and stored into amber glass bottles at 4 °C in the dark.

Figure 11 - Location of the sampling site of spring water used as blank matrix.

For method application, 1 L estuarine water samples from Douro river were collected at 5 sampling points (**S1** to **S5**) from the river outlet, near the Atlantic Ocean, to the Crestuma-Lever dam (**Figure 12**) in summer (August 2020). Sampling stations **S1** (mouth of the river Douro), **S3** (Freixo) and **S5** (mouth of the river Sousa) are located on the north bank of the

river at the Porto city margin, whereas **S2** (mouth of the river Douro) and **S4** (Crestuma-Lever dam) are located at the opposite side, bordering the other highly industrialized and densely inhabited region, the Vila Nova de Gaia city. Temperature was measured *in situ* at each sampling collection. Other physico-chemical parameters: pH, EC and TDS were measured upon arrived at the laboratory. During the transport, samples were kept refrigerated (± 4 °C) in the dark. In the laboratory samples were immediately vacuum filtered through 0.7 µm glass fibre filters to remove suspended particles, acidified to pH 3 with sulphuric acid (conc.) and were maintained at ± 4 °C in the dark for a maximum period of 12 h until SPE procedure.

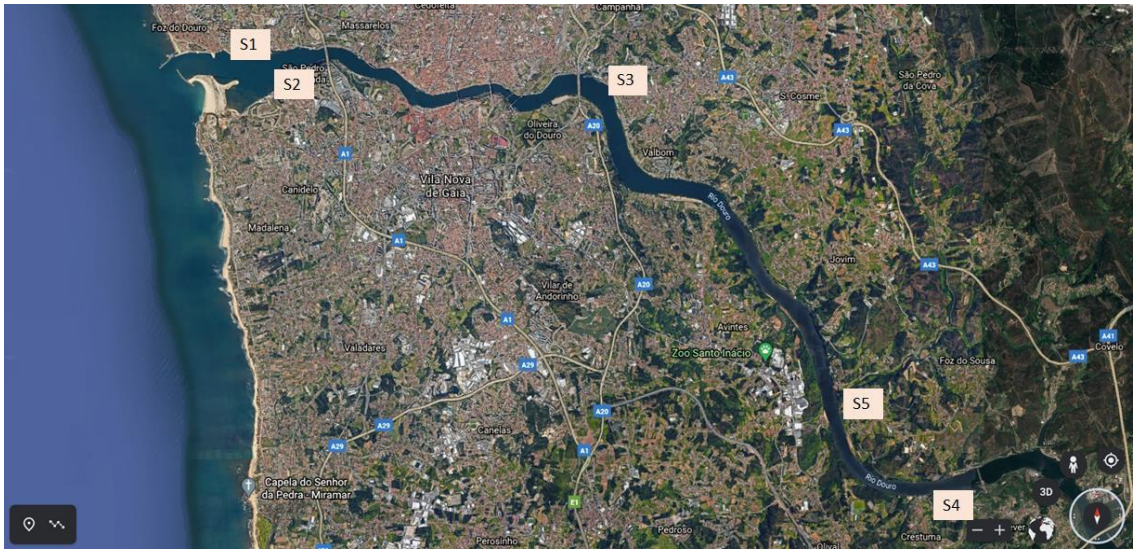


Figure 12 - Location of the five sampling points of Douro river estuary.

Also, 24 h composite samples of two WWTPs (WWTPA and WWTPB) with different treatment processes were collected using amber bottles in summer (July) of 2020. Wastewater treatment of WWTPA consists of a biological treatment with conventional activated sludge system operating under aeration regime. Effluents are discharge into a tributary of Douro River. WWTPB receives mainly urban wastewater and performs both secondary biological treatment with activated sludge system and tertiary treatment by UV light. Effluents are also discharge into a tributary of Douro River.

Enantiomeric fraction (EF) was used to express the relative concentration of diastereomer. The following equation was used for calculation of EF (Kasprzyk-Hordern and Baker, 2012):

$$EF = \frac{[D1]}{([D1] + [D2])}$$

Where D1 refers to the first eluted diastereomer and D2 to the second eluted diastereomer.

3.6. Solid-Phase extraction (SPE) procedure

SPE procedure was adapted from that described by Coelho et al as shown in **Figure 13** (Coelho et al., 2019). Briefly, for method optimization and validation 1 L of pre-filtered and acidified spring water were spiked with 250 µL of mixtures of the standards of the target compounds and the IS at 500 ng mL^{-1} final concentration. For method application, 1 L of estuarine water samples or effluent samples were spiked with the IS at the final concentration of 500 ng mL^{-1}.

The SPE was performed using a VisiprepTM SPE Vacuum Manifold and OASIS® MCX cartridges (150 mg, 6cc), without cartridge conditioning. Samples were directly loaded into the cartridges at a flow rate of 5 mL min^{-1}. Then, cartridges were washed with 4 mL of 2% formic acid in H_2O.

After washing, cartridges were dried under vacuum for 1 h. The elution was performed with 4 mL of 5% NH_4OH in EtOH. The eluates were then filtered, with a 0.22 µm syringe filter after syringes being rinsed with 1 mL of 5% NH_4OH in EtOH.

After filtration, the syringe filters were then washed with 1 mL of EtOH to ensure maximum recovery of the analytes. Eluates were evaporated to dryness using a centrifugal vacuum evaporator and then reconstituted in 250 µL of MeOH. After SPE procedure the extracts were derivatized according to the procedure described in **section 3.3**.

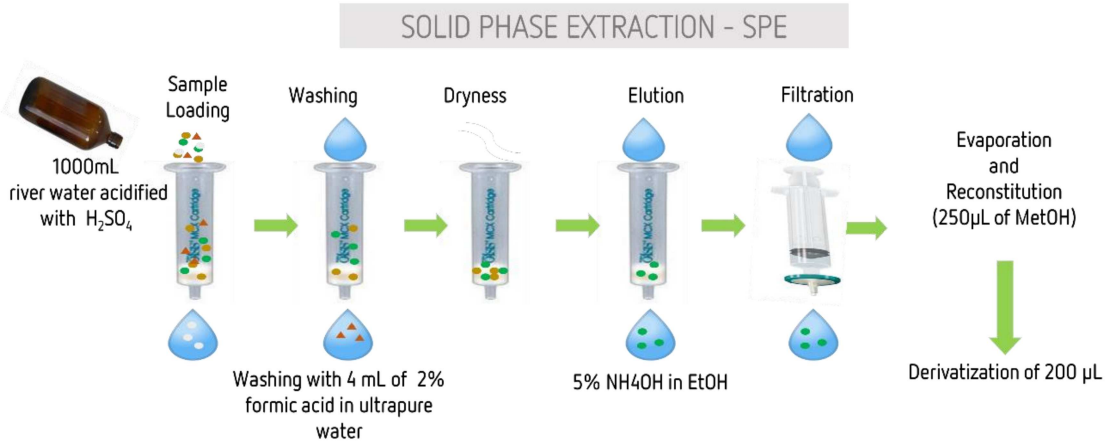


Figure 13 - Schematic illustration of the SPE procedure based on Coelho et al. 2019.

4. METHOD PARAMETERS AND VALIDATION

The method was validated according to the International Conference on Harmonization (ICH) considering the following parameters: selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and recovery (ICH, 1994).

Selectivity

Selectivity was verified by comparing the chromatograms in both FS and SIS and MS spectra of the solvent standard mixtures, spiked and non-spiked extracted samples from spring water samples (used as blank matrix) from the source of Leça River.

Linearity

Linearity was studied using matrix-matched calibration by spiking 1 L of blank matrices, i.e., spring waters from source of Leça River, at five or six calibration standards mixtures, each one in triplicate containing the IS. The IS, $AMPd_3$, was used as internal standard for amphetamine-like substances, synthetic cathinones and NK. Equations were obtained after least-squares linear regression of the ratio analyte / IS. For PP derivatives no IS was used. The calibration curve linearity was evaluated by its correlation coefficient (r^2). **Table 4** shows the range of concentration of each PAS for the construction of the calibration curves.

Table 4 - Range of method concentrations (ng L-1) of the calibration curve.

PAS	Calibration curve concentration (ng L^{-1})					
AMP	50	100	125	150	250	300
MAMP	50	100	125	150	300	
BPD	125	175	200	225	375	425
3-MMC	250	375	425	500	575	
3,4-DMMC	250	300	375	500	625	
1-BP	250	300	325	500	625	
MDMA	75	125	175	225	300	375
NK	75	100	125	150	200	250
BTL	75	125	175	250	300	375
1,4-MPP	75	100	150	175	250	

Limit of Detection and limit of quantification

The LOD and LOQ were determined based on the standard deviation of the response and the slop. The following equations were used to calculate LOD and LOQ (ICH, 1994; Shabir, 2003)

$LOQ = 10 \times (s / S)$, $LOD = 3.3 \times (s/S)$

Where s= standard deviation of the response

S= Slop of the calibration curve

Accuracy, precision and recovery

For accuracy, intra and inter-precision and recovery assays three quality controls (QCs) standard solutions covering the dynamic linear range (low, medium and high) and added to blank water samples, each one in triplicate and analysed (**Table 5**).

Accuracy was determined as the percentage of agreement between the method results and the nominal amount of added compound using the following equation.

$$Accuracy\ (\%) = (Real\ Conc./Nominal\ Conc.) \times 100\%$$

Precision was expressed by the relative standard deviation (% RSD) of the replicate measurements. Recovery was calculated by the ratio (peak area of analyte / peak area of IS) obtained after SPE procedure of blank water samples previous spiked with the standard mixtures and ratio (peak area of analyte / peak area of IS) of post-extracted blank water samples spiked at the same concentration of the standard mixtures.

Table 5 - Method quality control (QC) concentrations (ng L-1).

	Quality control concentration (ng L^{-1})									
	AMP	MAMP	BPD	3-MMC	3,4-DMMC	MDMA	NK	BTL	1,4-MPP	1-BP
QC1	100	75	150	300	300	125	100	100	87.5	300
QC2	137.5	112.5	212.5	350	350	175	175	175	125	350
QC3	225	175	300	500	575	325	300	300	200	575

5. RESULTS AND DISCUSSION

5.1. Derivatization method for diastereomer formation and piperazine derivatives

In this work the enantiopure derivatization reagent (*R*)-MTPA-Cl was used for the formation of the diastereomers of amphetamine type substances, synthetic cathinones and NK. A previous study done by our research group also used (*R*)-MTPA-Cl for the formation of diastereomer of PAS including amphetamines and NK (Gonçalves et al., 2019a). Nevertheless, this procedure was never reported for the formation of diastereomers of synthetic cathinones. Therefore, the derivatization procedure was optimized for the reaction with the synthetic cathinones allowing the formation of the diastereomers that were confirmed by the respective mass spectrum.

In this reaction, enantiomers of amphetamines type substances, synthetic cathinones and NK are converted into diastereomers by formation of amides by *N*-acylation. Further, it was possible to observe the formation of PP derivatives as a result of the reaction of the reagent with PP that was confirmed by the mass spectrum of both PP. The formation of these derivatives enhanced not only sensitivity of the method but also allowed the use of other *m/z* fragments to confirm the occurrence of these PP in complex matrices.

The scheme of reaction of (*R*)-MTPA-Cl with the enantiomers of amphetamines (**Figure 14**, synthetic cathinones (**Figure 15**) and NK (**Figure 16**) as well as reaction with piperazines (**Figure 17**): The products of reaction with (*R*)-MTPA-Cl of all target compounds are presented in **Table 6**.

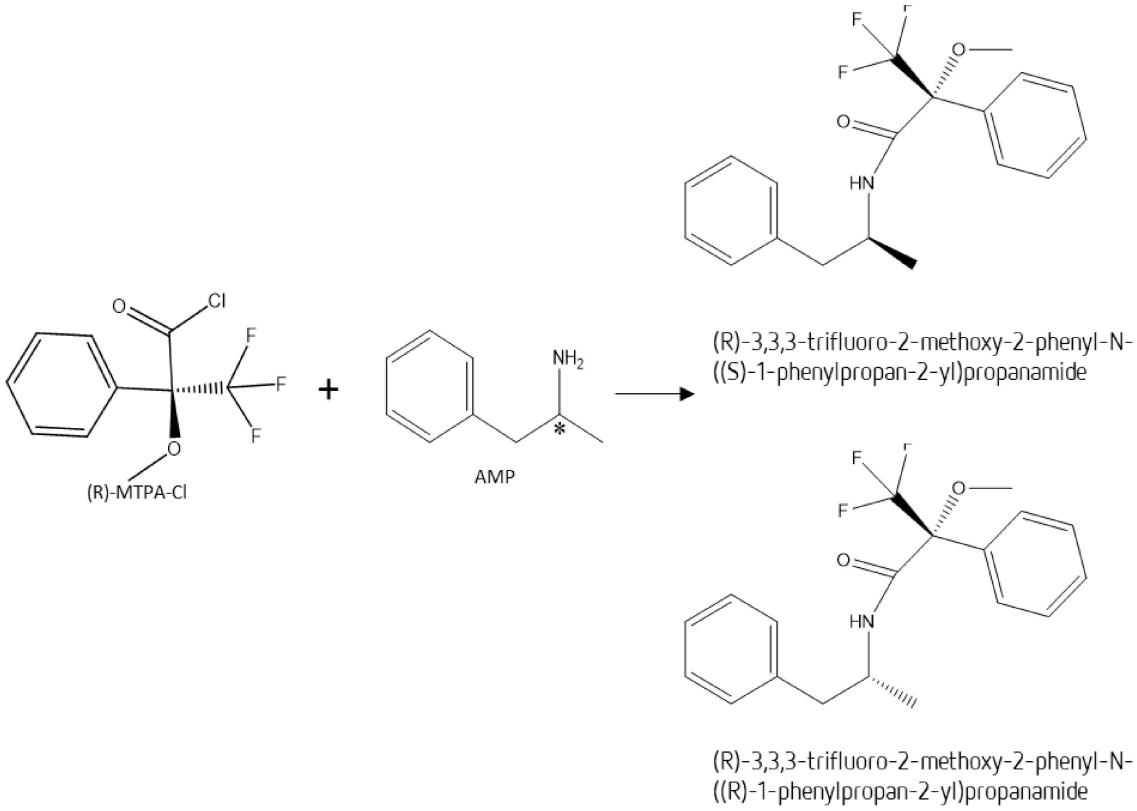


Figure 14- Reaction of (*R*)-MTPA-Cl with the enantiomers of AMP for diastereomer formation.

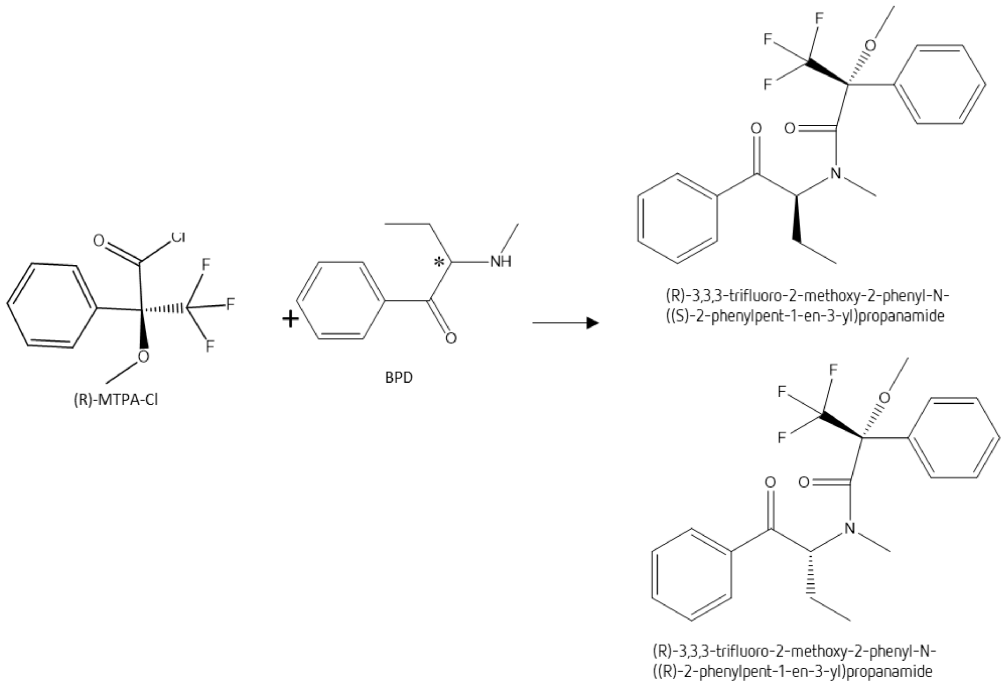


Figure 15 - Reaction of (*R*)-MTPA-Cl with the enantiomers of BPD for diastereomer formation.

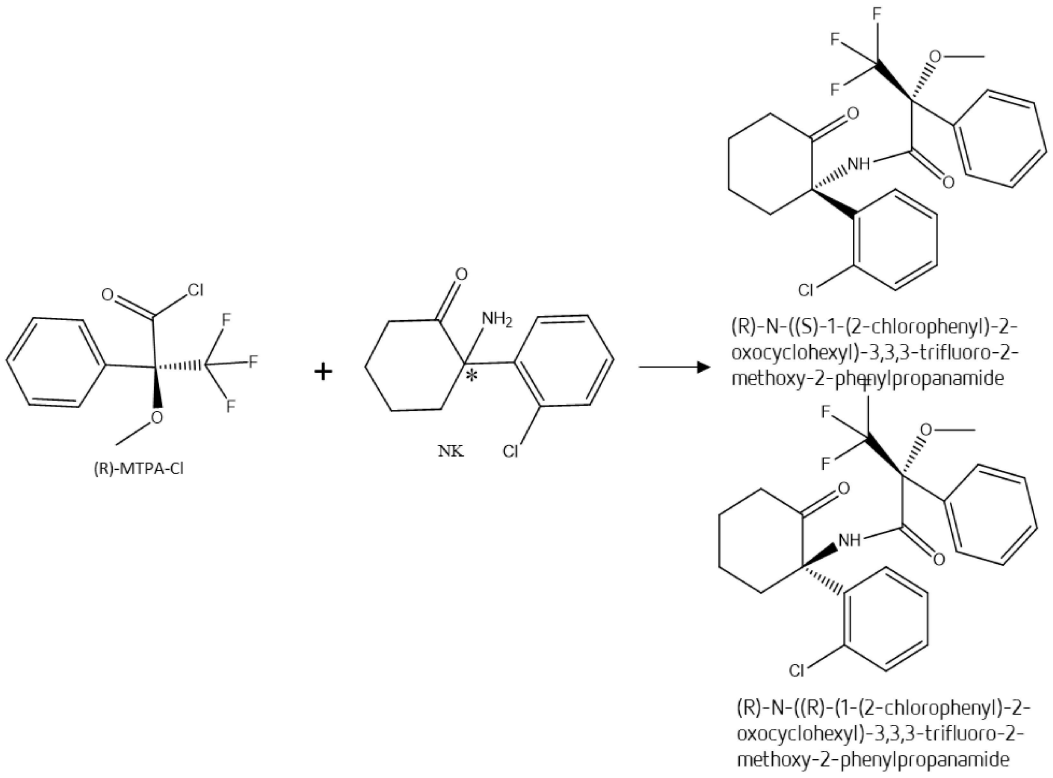


Figure 16 - Reaction of (*R*)-MTPA-Cl with enantiomers of NK for diastereomer formation.

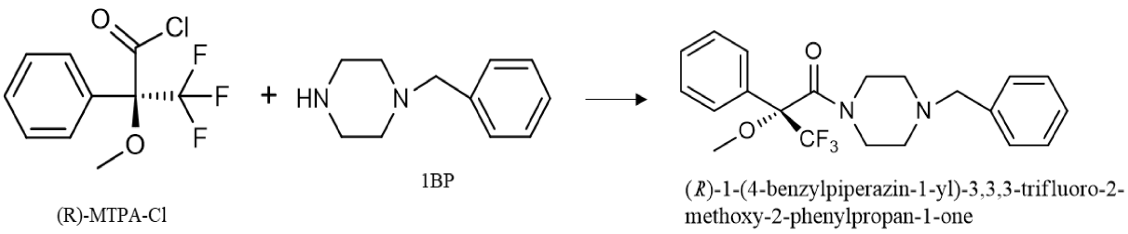


Figure 17 - Reaction of (*R*)-MTPA-Cl with 1-BP for the formation of the 1-BP derivative.

Table 6 - Products of the reaction of the enantiopure derivatization reagent (R)-MTPA-Cl with AMP and amphetamines type substances, synthetic cathinones, NK and piperazines.

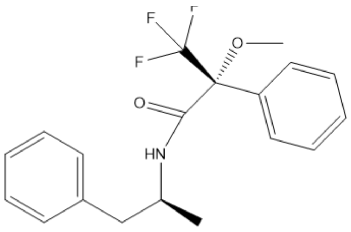
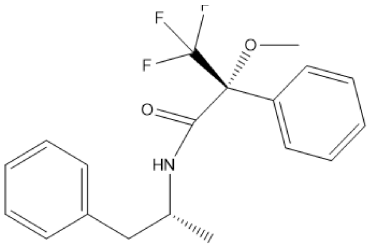
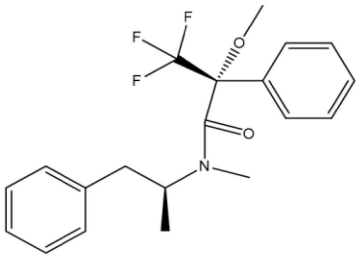
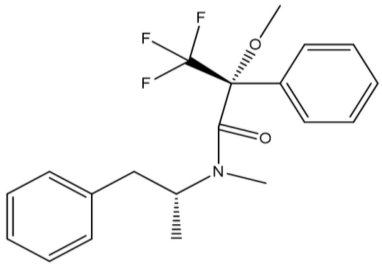
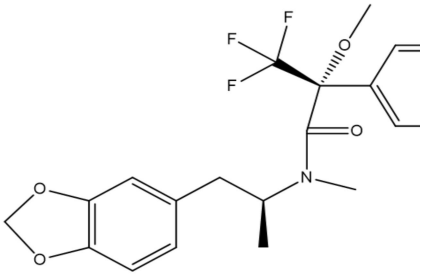
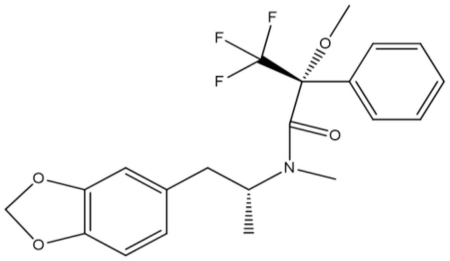
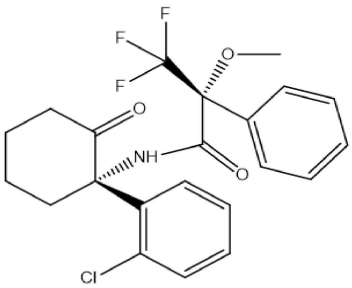
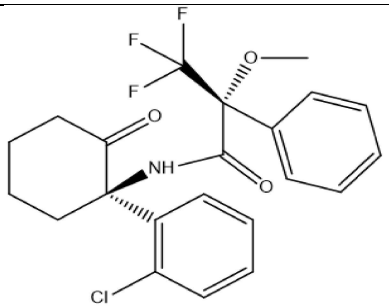
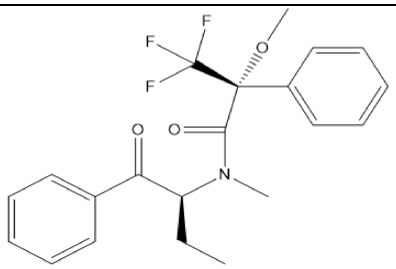
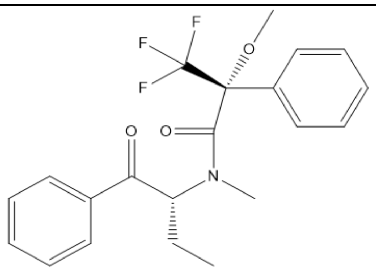
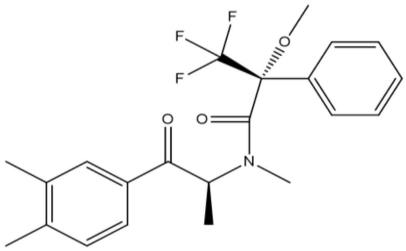
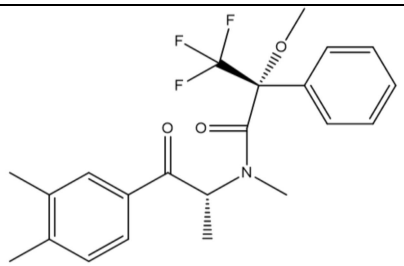
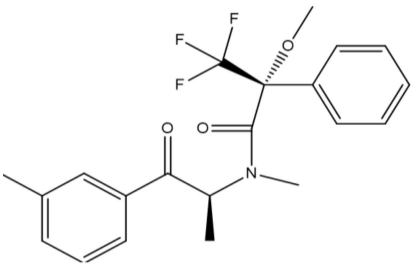
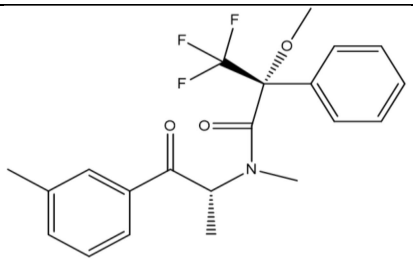
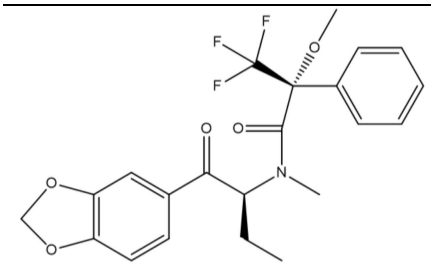
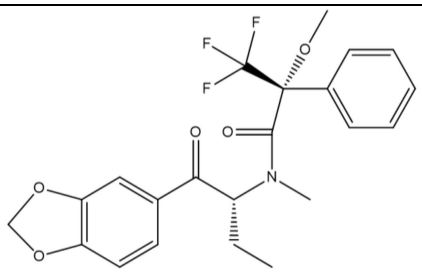
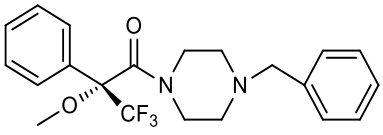
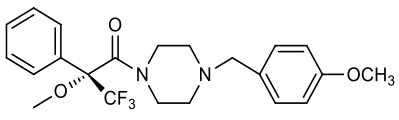
	 (R)-3,3,3-trifluoro-2-methoxy-2-phenyl-N-((S)-1-phenylpropan-2-yl)propanamide	 (R)-3,3,3-trifluoro-2-methoxy-2-phenyl-N-((R)-1-phenylpropan-2-yl)propanamide
AMPs diastereomers	 (R)-3,3,3-trifluoro-2-methoxy-N-methyl-2-phenyl-N-((S)-1-phenylpropan-2-yl)propanamide	 (R)-3,3,3-trifluoro-2-methoxy-N-methyl-2-phenyl-N-((R)-1-phenylpropan-2-yl)propanamide
	 (R)-N-((S)-1-(benzo[d][1,3]dioxol-5-yl)propan 3,3,3-trifluoro-2-methoxy-N-methyl-2-phenylpropanamide	 (R)-N-((R)-1-(benzo[d][1,3]dioxol-5-yl)propan-2-yl)-3,3,3-trifluoro-2-methoxy-N-methyl-2-phenylpropanamide
Norketamine diastereomers	 (R)-N-((S)-1-(2-chlorophenyl)-2-oxocyclohexyl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide	 (2R)-N-(1-(2-chlorophenyl)-2-oxocyclohexyl)-3,3,3-trifluoro-2-methoxy-2-phenylpropanamide

Table 6 – *Continued.*

Cathinones diastereomers	 (R)-3,3,3-trifluoro-2-methoxy-2-phenyl-N-((S)-2-phenylpent-1-en-3-yl)propanamide	 (R)-3,3,3-trifluoro-2-methoxy-2-phenyl-N-((R)-2-phenylpent-1-en-3-yl)propanamide
	 (R)-N-((S)-1-(3,4-dimethylphenyl)-1-oxopropan-2-yl)-3,3,3-trifluoro-2-methoxy-N-methyl-2-phenylpropanamide	 (R)-N-((R)-1-(3,4-dimethylphenyl)-1-oxopropan-2-yl)-3,3,3-trifluoro-2-methoxy-N-methyl-2-phenylpropanamide
	 (R)-3,3,3-trifluoro-2-methoxy-N-methyl-N-((S)-1-oxo-1-(m-tolyl)propan-2-yl)-2-phenylpropanamid	 (R)-3,3,3-trifluoro-2-methoxy-N-methyl-N-((R)-1-oxo-1-(m-tolyl)propan-2-yl)-2-phenylpropanamid
	 (R)-N-((S)-1-(benzo[d][1,3]dioxol-5-yl)-1-oxobutan-2-yl)-3,3,3-trifluoro-2-methoxy-N-methyl-2-phenylpropanamide	 (R)-N-((R)-1-(benzo[d][1,3]dioxol-5-yl)-1-oxobutan-2-yl)-3,3,3-trifluoro-2-methoxy-N-methyl-2-phenylpropanamide
Piperazine Derivatives	 (*R*)-1-(4-benzylpiperazin-1-yl)-3,3,3-trifluoro-2-methoxy-2-phenylpropan-1-one	 (*R*)-3,3,3-trifluoro-2-methoxy-1-(4-(4-methoxybenzyl)piperazin-1-yl)-2-phenylpropan-1-one

5.2. Optimization of chromatographic separation of the diastereomers and piperazine derivatives

Different conditions were attempted to optimize separation of the diastereomers and PP derivatives using standard mixtures. For that, different ramps of temperature, electron impact ionization voltages and flow of the carrier gas were tested.

After various attempts the optimized conditions (already described in section 3.5) allowed the separation of the diastereomers of all amphetamines type substances (AMP, MAMP, MDMA), NK and PP derivatives (1-BP and 1,4-MPP).

Considering synthetic cathinones, separation of diastereomers were achieved for BTL and 3,4-DMMC. Also, diastereomer resolution was possible for both BPD and 3-MMC, nevertheless, considering the standard mixture, coelution of the second BPD diastereomer (D2) and first 3-MMC diastereomer (D1) was observed. Various attempts were made for a better resolution but without success. However, identification and quantification of both compounds were possible due to some differences in the respective mass spectra.

The **figure 18** shows the chromatogram of a standard mixture of all the target with the separation of AMP, MAMP, MDMA, NK, BPD, 3MMC, 3,4-DMMC and BTL diastereomers as well as the derivatized piperazines (1-BP and 1,4-MPP) at 1 µg mL^{-1}.

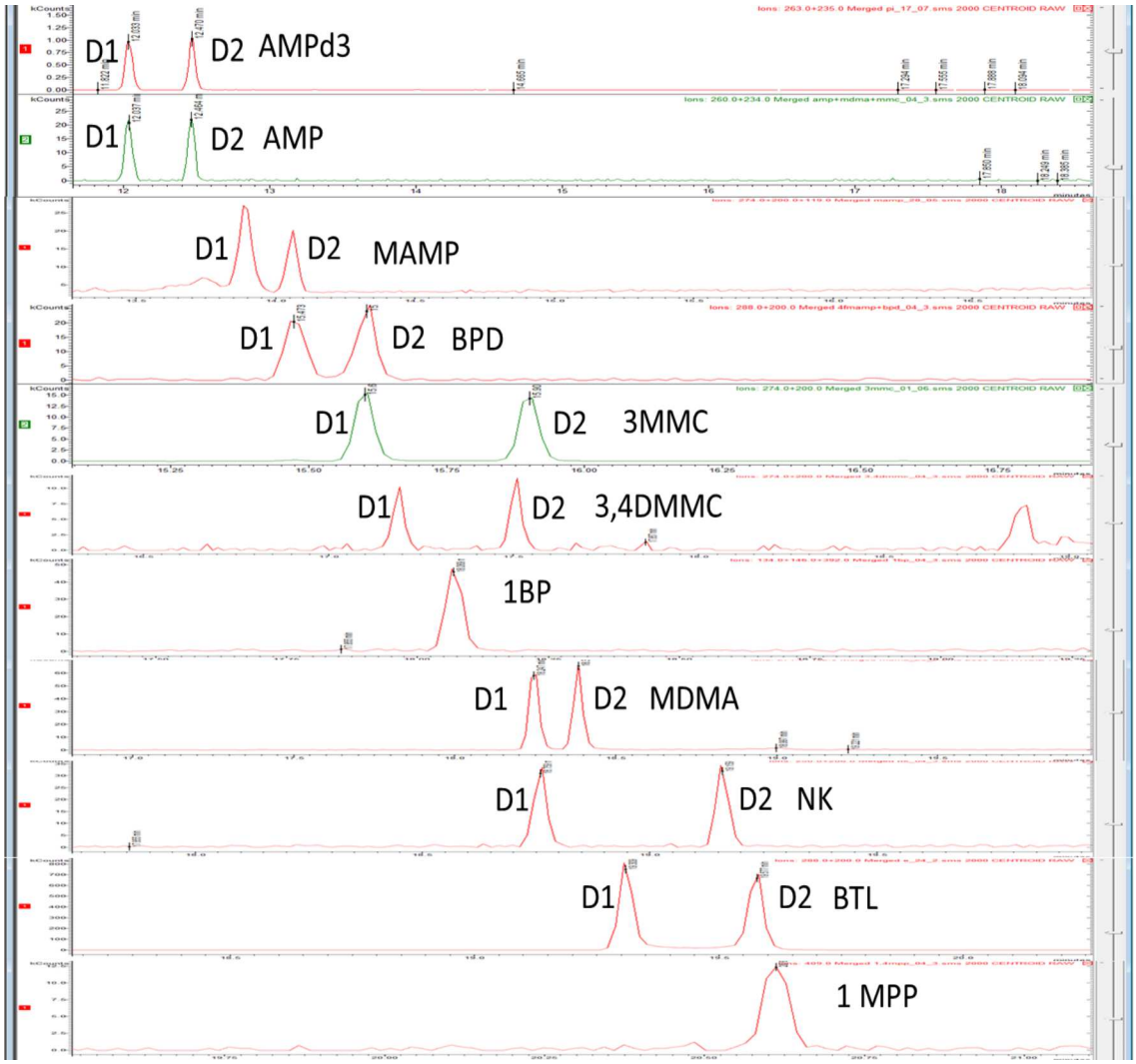


Figure 18 - Chromatograms of a standard mixture at 1 µg mL^{-1}, showing the separation of the diastereomers of the amphetamines: amphetamine (AMP), methamphetamine (MAMP), 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) and norketamine (NK); synthetic cathinones: buphedrone (BPD), 3-methylmethcathinone (3-MMC), 3,4-dimethylmethcathinone (3,4-DMMC) and butylone (BTL) as well as Piperazines derivatives: 1-benzylpiperazine (1-BP) and 1-(4-metoxyphenyl) piperazine (1,4-MPP). D1 and D2 corresponds to the first and second eluted diastereomer, respectively.

5.3. Mass spectra of the target compounds diastereomers and piperazine derivatives

For identification of the compounds, individual standards at concentration of 1 µg mL^{-1} were derivatized according to the procedure already described in section 3.4.

Acquisition of the MS spectra of each diastereomer and PP derivatives were performed in FS mode. **Table 7** shows the characteristic fragmentation ions (*m/z*) for identification and quantification ions (QI) as well as the retention time of each diastereomer: D1 corresponds

to the first eluted diastereomer and D2 to the second eluted diastereomer. **Figures 19 to 28** show the chromatogram and MS spectra with the fragmentation pattern of each target PAS.

Table 7 - Characteristic fragmentation ions (m/z), quantification ions (m/z) and retention time of the diastereomers of the target compounds.

Compound	*m/z*	QI	RT (minutes)	
			D1	D2
$AMPd_3$	91, 92 ,119, 165, 189; 235; 263	235; 263	12.02	12.45
AMP	91; 119; 162; 189; 234; 260	162; 234; 260	12.04	12.47
MAMP	91; 119; 148; 176; 189; 200; 274	200; 274	13.88	14.05
BPD	91; 105; 119; 189; 200; 288	288	15.47	15.60
3-MMC	91; 119; 189; 200; 274	274	15.60	15.89
3,4-DMMC	91; 105; 119; 133; 189; 200; 274	200; 274	17.18	17.50
1-BP	91; 175; 189; 392	134; 146; 392	18.06	
MDMA	91; 119; 135; 162; 189; 200; 274	162; 274	18.25	18.39
NK	91; 189; 207; 250; 404	206; 250	18.75	19.16
BTL	91; 119; 149; 189; 200; 207; 288	200; 288	19.32	19.58
1,4-MPP	91; 149; 189; 393; 408	408	20.61	

For the IS $AMPd_3$, the fragments *m/z* 235 and 263 were the most abundant and were used for both identification and quantification.

The fragment 189 (*m/z)* was observed in the MS spectra of all diastereomers (**Table 7** and **Figures 19** to **28**). This fragment is characteristic of the reagent (*R*)-MTPA-Cl (Gonçalves et al., 2019a; Jenske and Vetter, 2007; Paul et al., 2004) and therefore was not used for quantification.

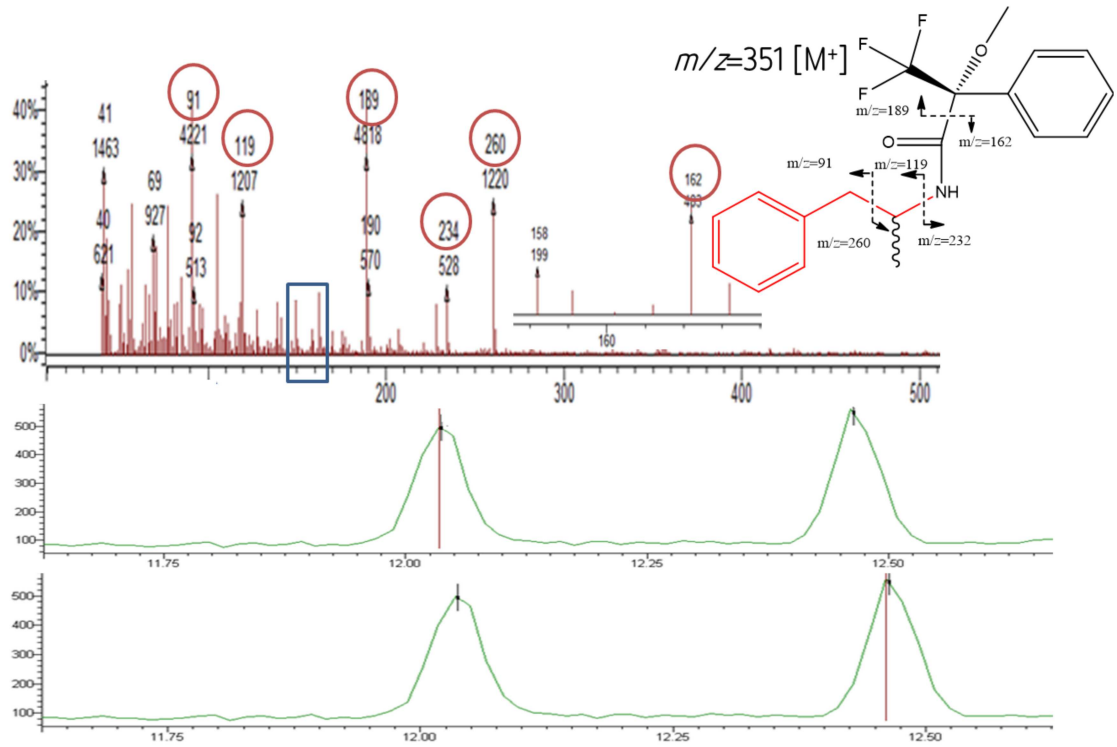


Figure 19 - Chromatograms showing the AMP diastereomers, D1 and D2 respectively, mass spectrum and the possible fragmentation pattern.

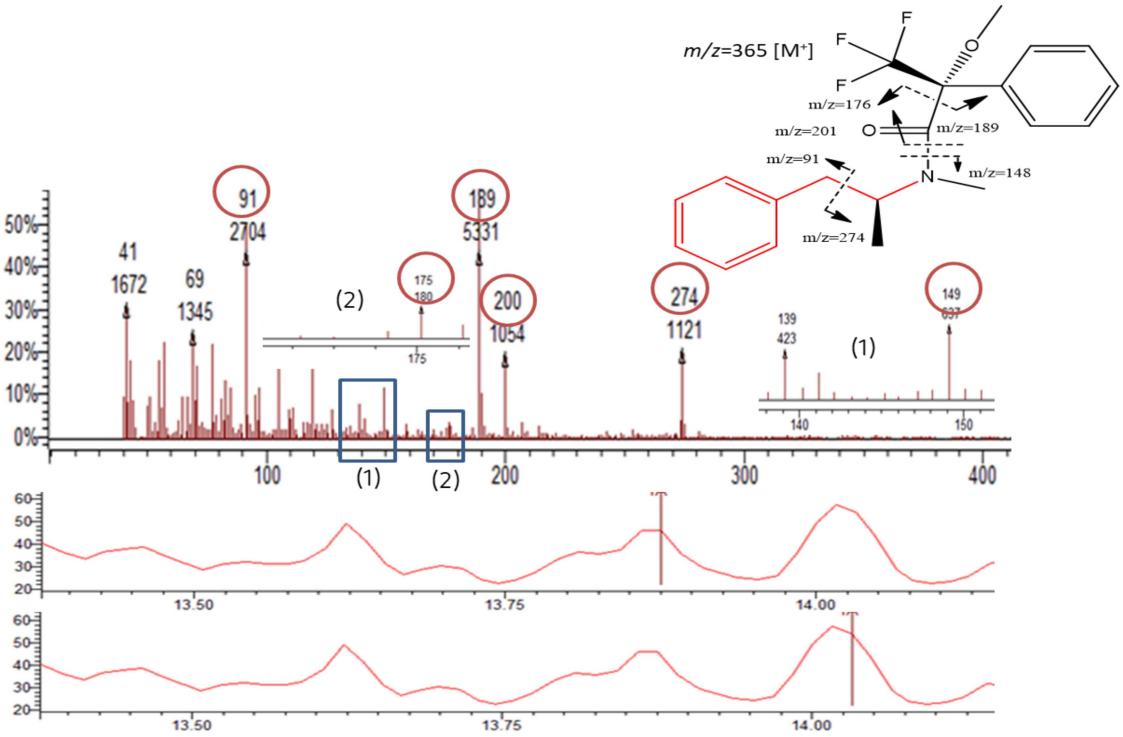


Figure 20 - Chromatograms showing the MAMP diastereomers, D1 and D2 respectively, mass spectrum and the possible fragmentation pattern.

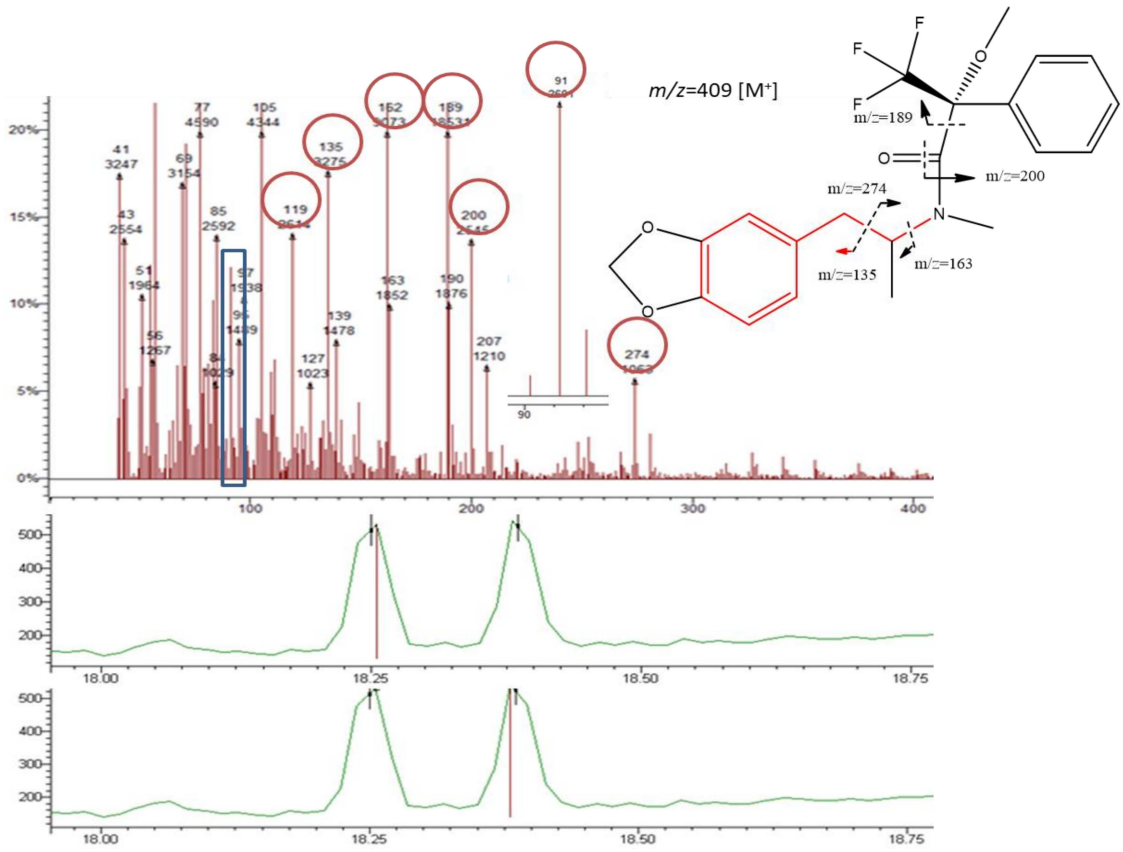


Figure 21 - Chromatograms showing the MDMA diastereomers, D1 and D2 respectively, mass spectrum and the possible fragmentation pattern.

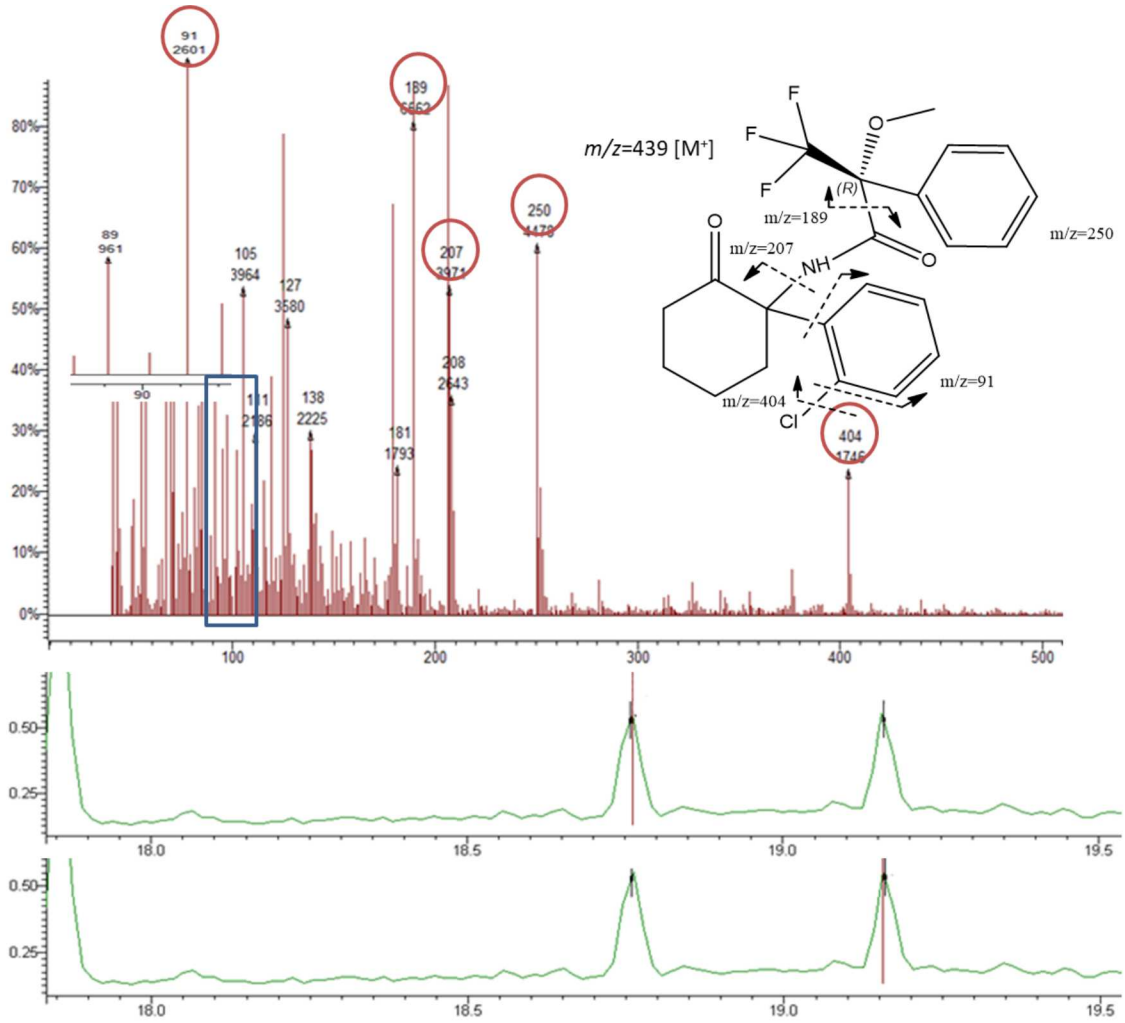


Figure 22 - Chromatograms showing the NK diastereomers, D1 and D2 respectively, mass spectrum and the possible fragmentation pattern.

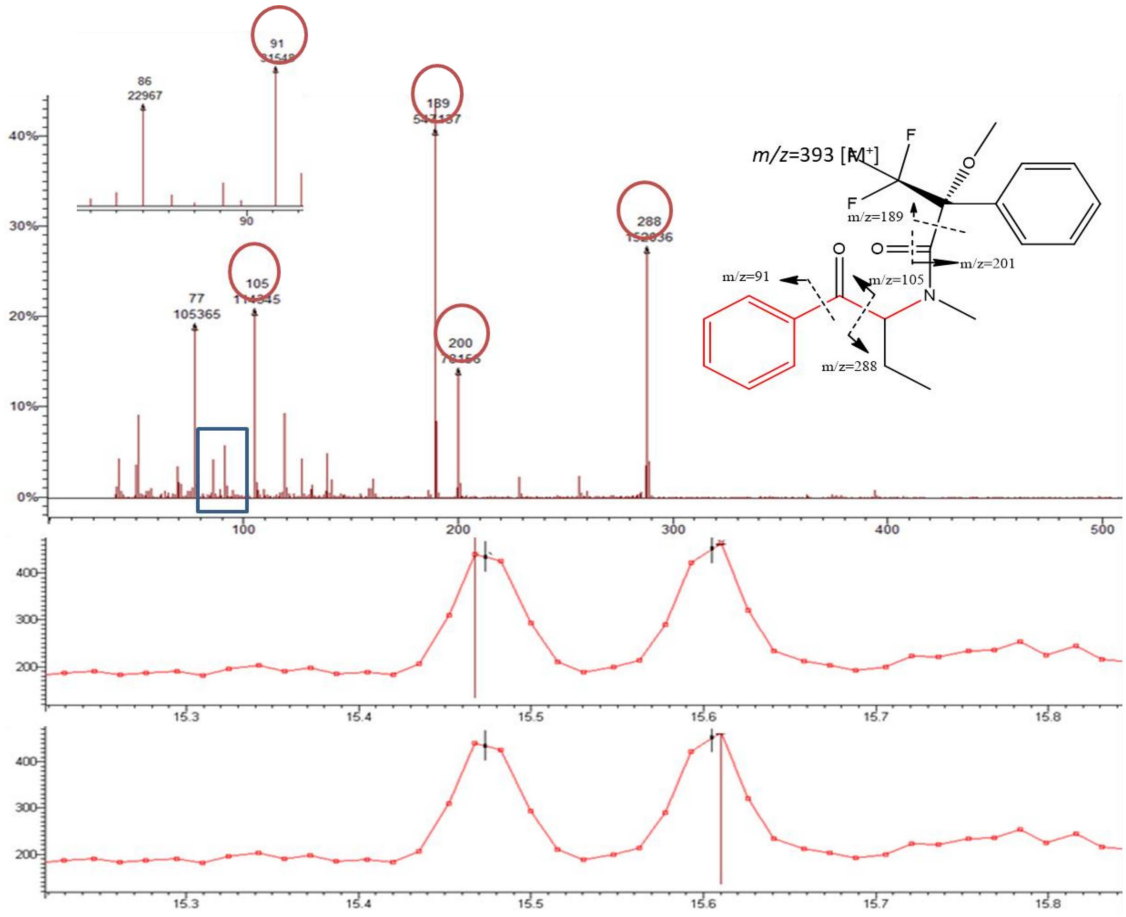


Figure 23 - Chromatograms showing the BPD diastereomers, D1 and D2 respectively, mass spectrum and the possible fragmentation pattern.

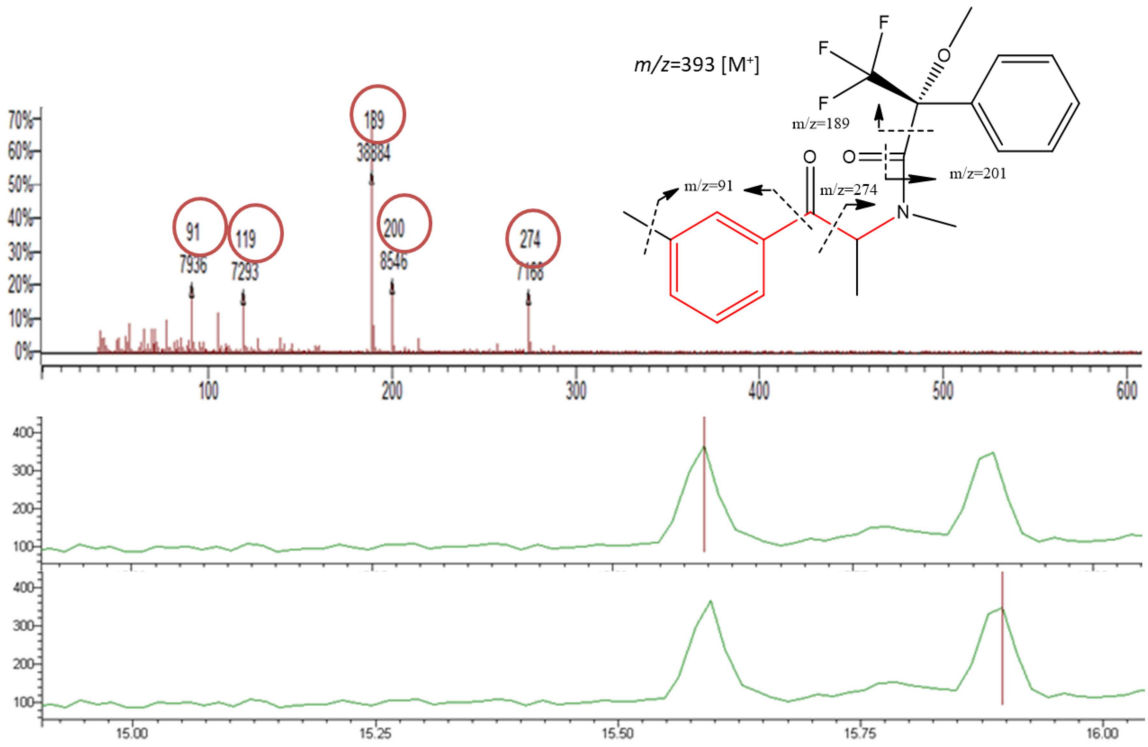


Figure 24 - Chromatograms showing the 3-MMC diastereomers, D1 and D2 respectively, mass spectrum and the possible fragmentation pattern.

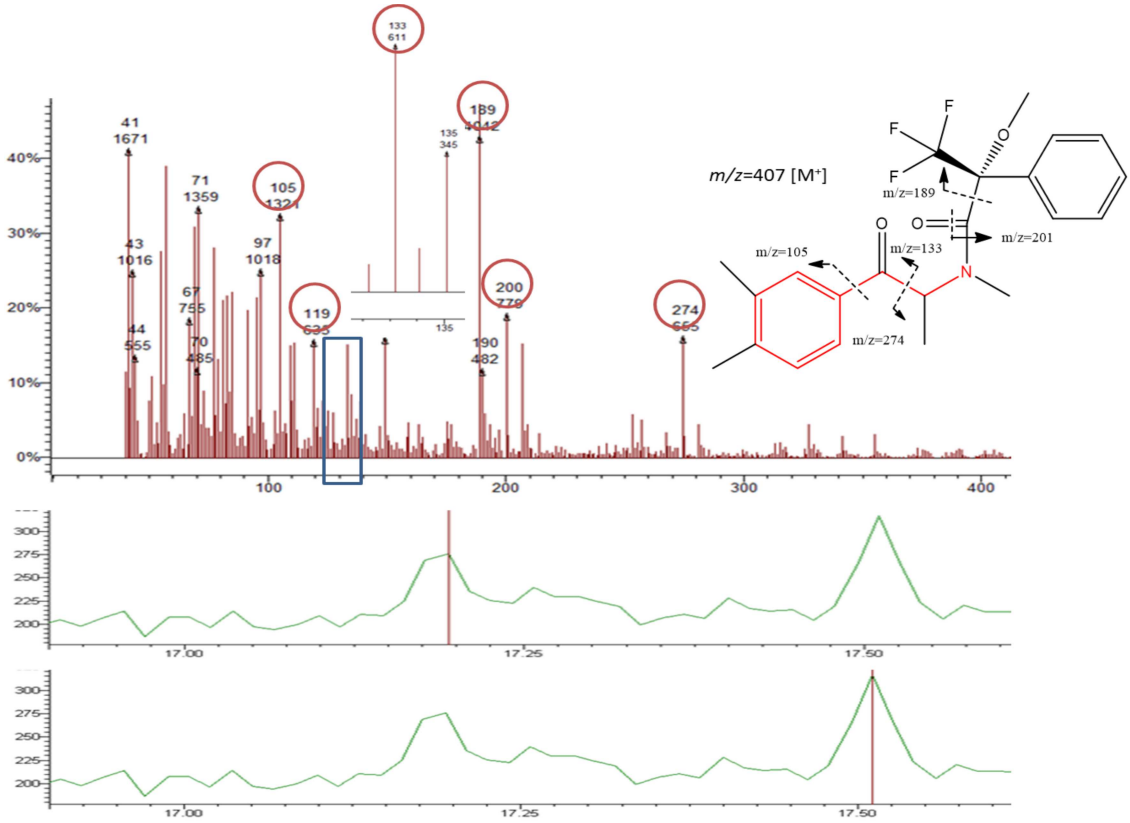


Figure 25 - Chromatograms showing the 3,4-DMMC diastereomers, D1 and D2 respectively, mass spectrum and the possible fragmentation pattern.

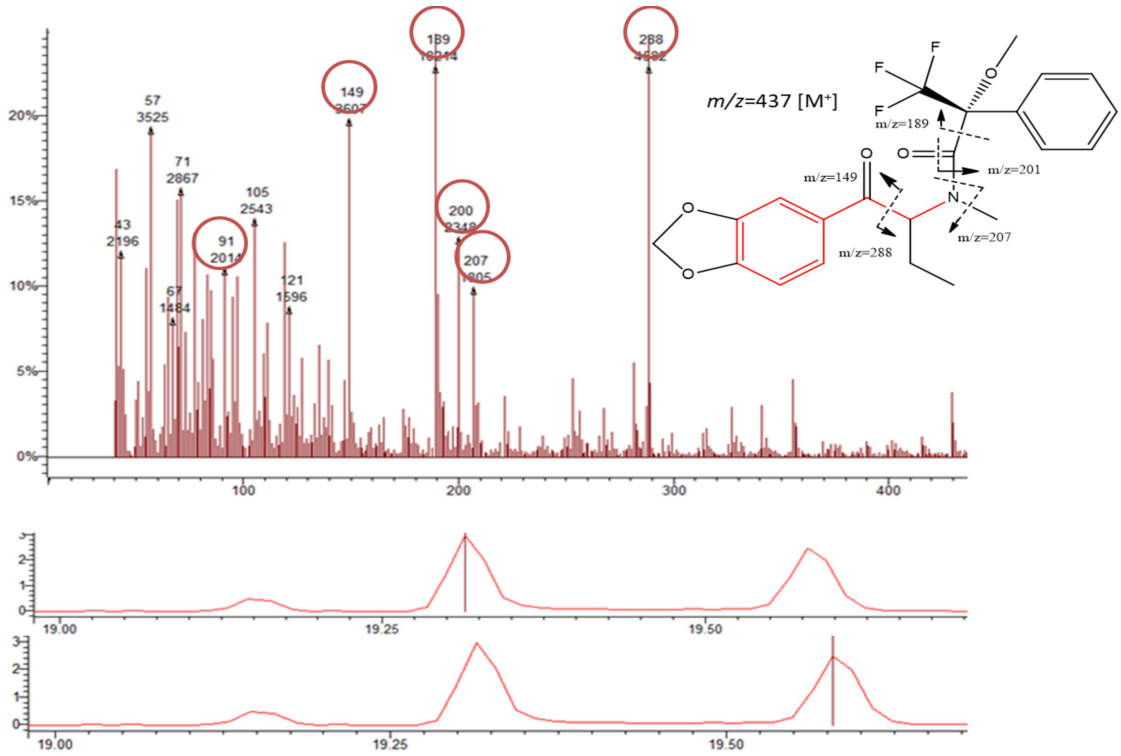


Figure 26 - Chromatograms showing the BTL diastereomers, D1 and D2 respectively, mass spectrum and the possible fragmentation pattern.

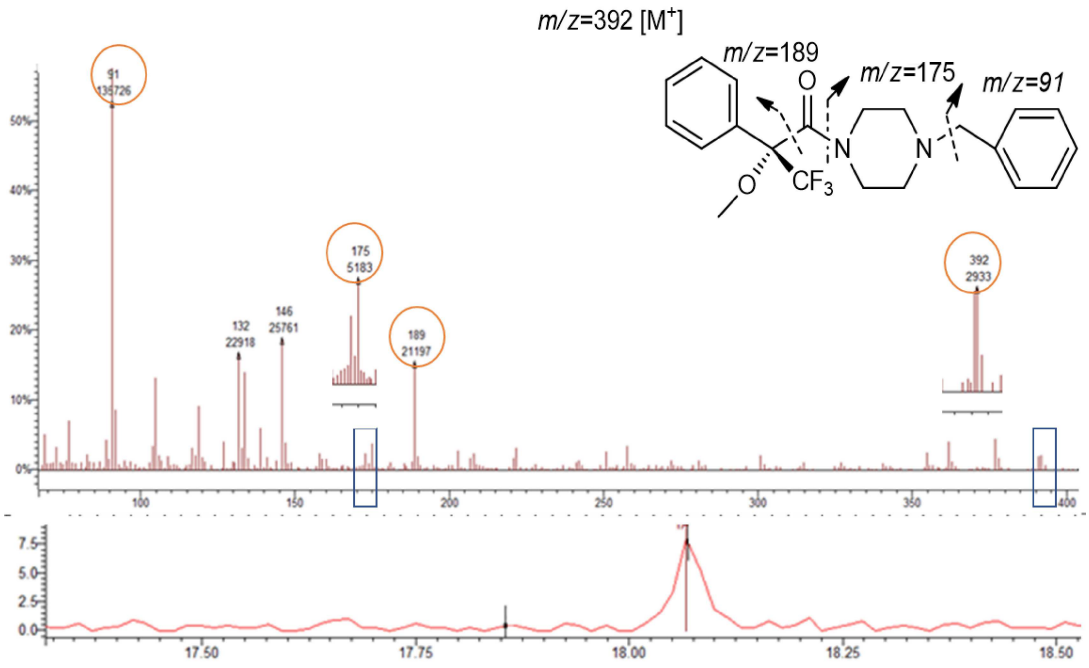


Figure 27 - Chromatogram of 1-BP derivative, mass spectrum and suggested fragmentation pattern.

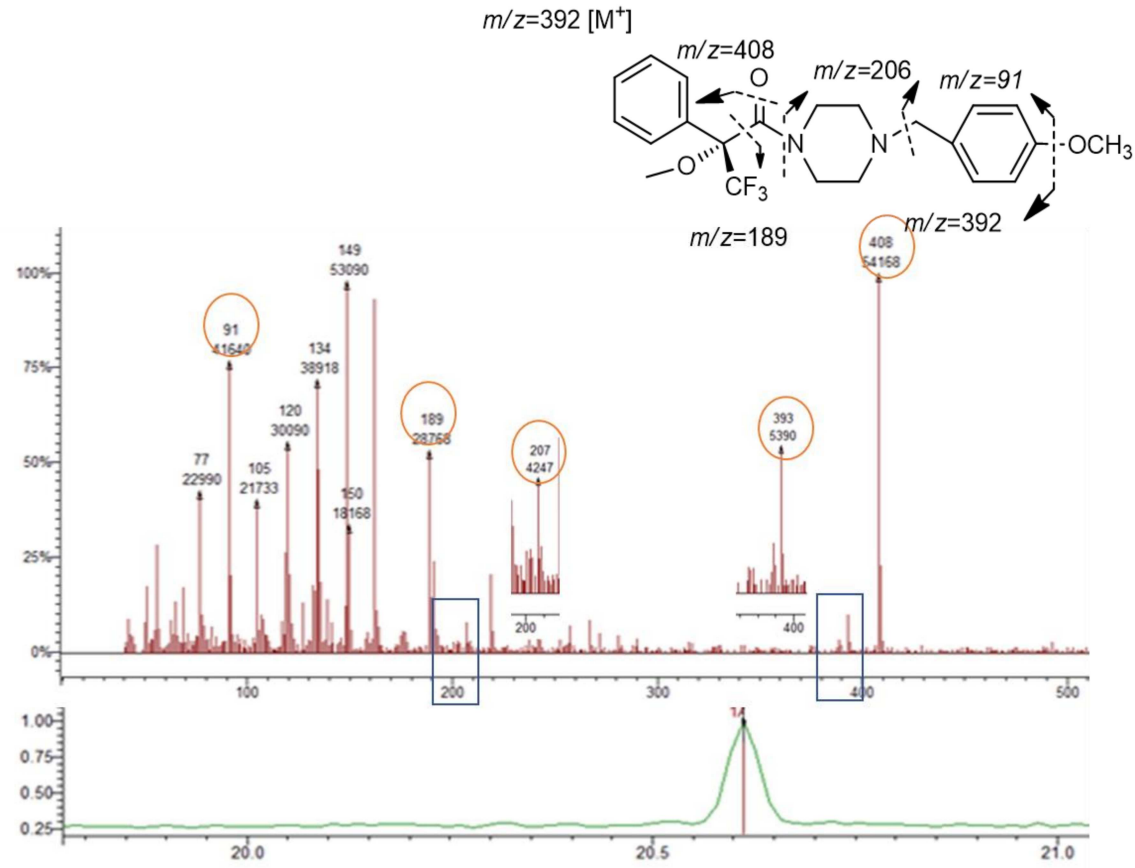


Figure 28- Chromatograms of 1,4-MPP derivative, mass spectrum and suggested fragmentation pattern.

The molecular ions $[M]^+$ of AMP, MAMP and MDMA were barely detectable and therefore of little qualitative and quantitative value.

It was possible to observe in the MS spectra from AMP, MAMP, MDMA, BPD, 3-MMC, 3,4-DMMC and BTL the presence of the fragments 91 and 119 m/z. These fragments could be a result of benzyl cation ($[C_7H_7]^+$) fragment at m/z 91. The mass spectra for both derivatives showed high relative abundance ions at m/z 119 corresponding to the phenylpropane hydrocarbon radical cation that is the pharmacophore of both amphetamine type substances and synthetic cathinones.

MS spectra of piperazine derivatives also showed the abundance of the fragment 91 *m/z* corresponding to the loss of the benzyl group (**Table 7**) and **Figures 27 and 28**.

The fragment at *m/z* 200 is present in all amphetamine type substances and synthetic cathinones (AMP, MAMP, MDMA, BPD, 3-MMC, 3,4-DMMC and BTL). This fragment results from the loss of the carbonyl group of the chiral reagent (*R*)-MTPA-Cl.

NK presented a different fragmentation pattern than other amphetamines due to the differences in their scaffold. The most abundant fragments were *m/z* 206 and 250 (**Figure 22** and **Table 7**).

Although the piperazines are not chiral, the presence of the ion 189 *m/z* was observed in both 1-BP (**Figure 27)** and 1,4-MPP (**Figure 28)** showing the reaction of piperazines with the derivatization reagent (*R*)-MTPA-Cl. Although there is no diastereomer formation, it allowed to improve the signal identification and detection of both compounds.

The chemical structure of both PP (1-BP and 1,4-MPP) are quite similar, differing on the methoxyl in *para* position of the phenyl group (1,4-MPP)

This similarity is justified by the presence of the fragment at *m/z* 392 for 1-BP derivative, and the fragment at *m/z* 393 corresponding to the loss of the methoxyl ($-OCH_3$) in the 1,4-MPP derivative.

5.4. Method validation

Validation of the analytical method was performed according to ICH guidelines and considering the following parameters: selectivity, linearity, accuracy, intra and inter-day precision, recovery, LOQ and LOD. Due to the coelution of AMP/AMP d3 (IS), BPD D2/3-MMC D1 and the matrix effect on 3,4-DMMC D2, no major gain in SIS mode selectivity and for LOD and LOQ was verified comparatively to FS mode for these compounds. Therefore, MS detection was conducted in both FS mode from which the quantification ions were selected and extracted (according to software program) or SIS (SIM) depending on the optimized conditions for each target PAS. Selectivity was verified by comparing the chromatograms of solvent standard mixtures, spiked and non-spiked extracted samples from spring water samples (used as blank matrix) from the source of Leça River. It was verified that the analytical method was selective for the quantification of all the target compounds. The following figure shows the comparison between the mixture of the standards and the blank (**Figure 29**).

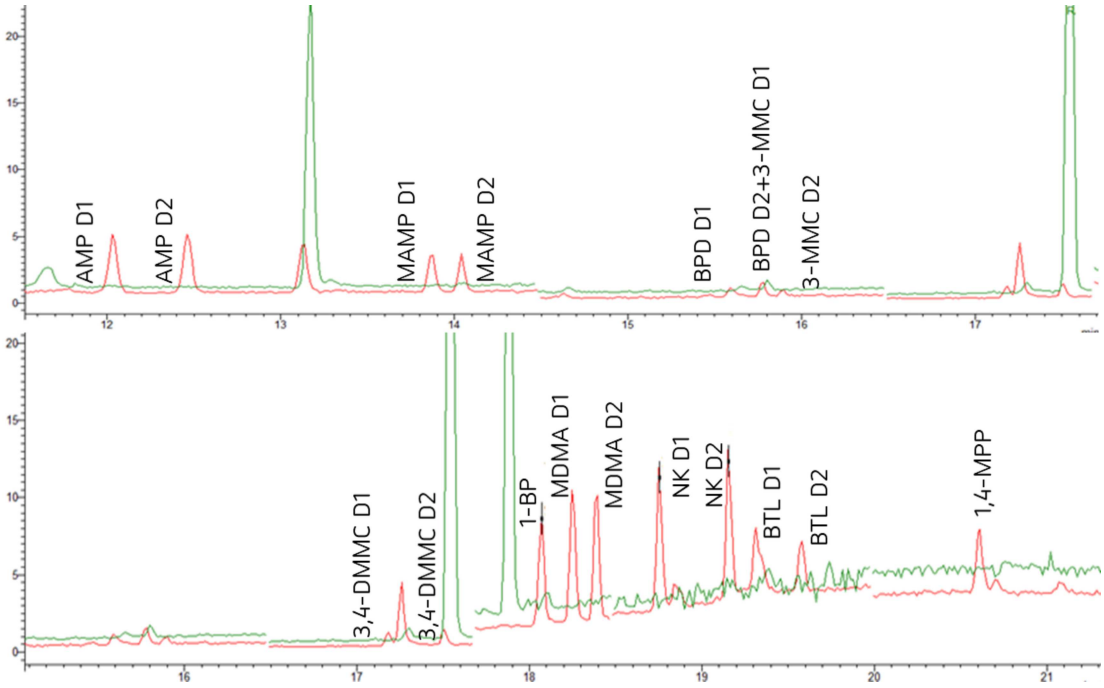


Figure 29 - Chromatogram with comparison of the blank (green) and the standard mixture (red) of 1 µg mL^{-1}.

For linearity determination a range of 6 concentrations levels for AMP, BPD, MDMA, NK, and BTL and 5 concentrations levels for MAMP, 3-MMC, 3,4-DMMC, 1,4-MPP and 1-BP were performed considering the LOQ as the first point of each calibration curve (**Table 8**)

The method showed to be linear with r^2 ranging from 0.9846 to 0.9972 for all target compounds and method LOD ranged from 14.2 to 89.5 ng L^{-1} and the method LOQ were between 50.0 and 250 ng L^{-1} (**Table 8**).

Table 8 - Linearity parameters and method LOD and LOQ.

	Concentration range (ng L^{-1})					
PAS	**Min**	**Máx**	**Equation**	**r^2**	**LOD (ng L^{-1})**	**LOQ (ng L^{-1})**
AMP D1	50.0	300	y=0.0071x + 0.5269	0.9891	31.8	50.0
AMP D2	50.0	300	y=0.0071x + 0.563	0.9846	38.0	50.0

Table 8 - *Continued*.

	Concentration range (ng L^{-1})					
PAS	**Min**	**Máx**	**Equation**	**r^2**	**LOD (ng L^{-1})**	**LOQ (ng L^{-1})**
MAMP D1	50.0	300	y=0.0081 - 0.0145	0.9968	18.0	50.0
MAMP D2	50.0	300	y=0.0081 + 0.1966	0.9935	25.0	50.0
BPD D1	125	425	y = 0.0017x + 0.0773	0.9938	35.9	175
BPD D2	125	425	y = 0.0018x + 0.0609	0.992	40.8	175
3-MMC D1	250	575	y = 0.001x - 0.1153	0.9887	89.5	250
3-MMC D2	250	575	y = 0.001x - 0.0899	0.9928	71.1	250
3,4-DMMC D1	250	625	y = 0.0018x - 0.2648	0.9928	70.3	250
3,4-DMMC D2	250	625	y=0,0018x-0,2585	0.9919	74.3	250
MDMA D1	75.0	375	y = 0.0108x − 0.23	0.9909	52.0	75.0
MDMA D2	75.0	375	y = 0.0108x − 0.1765	0.9949	38.0	75.0
NK D1	75	375	y = 53.535x - 1227.7	0.9972	14.2	75.0
NK D2	75	375	y = 56.272x - 1679.8	0.9903	26.4	75.0
BTL D1	75.0	375	y=0.0037x + 0.325	0.9926	24.7	75.0
BTL D2	75.0	375	y=0.0038x + 0.2954	0.9906	17.0	75.0
1-BP	250	625	y=70.682x - 7237.6	0.9882	88.0	250
1,4-MPP	75.0	250	y=44.495x - 2729.9	0.9928	29.0	75.0

Regarding accuracy determination the ICH guidelines recommend minimum ranges to be considered from 80 to 120 percent of the test concentration. In this study, accuracy values ranged from 82.4 to 116.9 showing that the method presents accuracy within acceptable values established by ICH (**Table 9**).

Intra-day and inter-day precision were also determined. The intra-day and inter-day precision were estimated by calculating the relative standard deviation (% RSD). Values were lower than 8.53%.

Table 9- Accuracy (%), intra-day precision (% RSD) and inter-day precision (% RSD).

PAS	1st QC			2nd QC			3rd QC		
	Accuracy	Intra day % RSD	Inter day % RSD	Accuracy	Intra day % RSD	Inter day % RSD	Accuracy	Intra day %RSD	Inter day % RSD
AMP D1	107.0	2.99	3.38	99.0	4.66	5.31	89.4	2.99	2.39
AMP D2	108.7	3.96	4.48	99.9	4.91	5.53	88.4	2.94	3.65
AMP D1	108.7	3.91	1.21	109.9	2.42	0.49	107.7	3.63	3.54
AMP D2	98.1	6.26	3.42	96.6	1.86	0.87	106.6	4.10	1.88
BPD D1	89.4	6.87	5.60	85.8	0.70	3.21	104.0	3.07	3.07
BPD D2	99.3	8.53	3.73	90.1	1.75	2.86	103.2	3.36	0.40
3-MMC D1	94.9	2.38	4.86	82.9	2.83	2.27	-	-	-
3-MMC D2	94.0	2.80	6.94	82.4	4.49	1.00	-	-	-
3,4-DMMC D1	90.9	2.43	4.41	89.5	2.10	1.02	89.8	2.91	2.82
3,4-DMMC D2	89.8	3.47	7.83	91.6	2.88	3.88	83.2	4.07	6.82
MDMA D1	112.4	3.40	2.22	111.0	3.54	4.92	115.2	3.05	1.97
MDMA D2	111.6	3.98	2.78	109.9	3.99	4.92	116.9	4.01	2.14
NK D1	87.3	2.46	5.64	108.4	1.46	3.90	92.6	1.07	4.57
NK D2	102.1	1.52	7.37	107.9	2.54	4.04	87.7	1.11	4.25
BTL D1	88.9	3.30	0.98	97.9	1.88	2.05	94.4	3.78	4.84
BTL D2	88.2	3.34	1.96	101.8	1.48	2.61	93.4	5.66	5.73
1,4-MPP	97.3	3.09	7.35	104.9	2.92	3.27	112.4	2.52	4.40
1BP	108.8	3.95	3.06	112.9	1.43	2.69	98.9	1.70	5.14

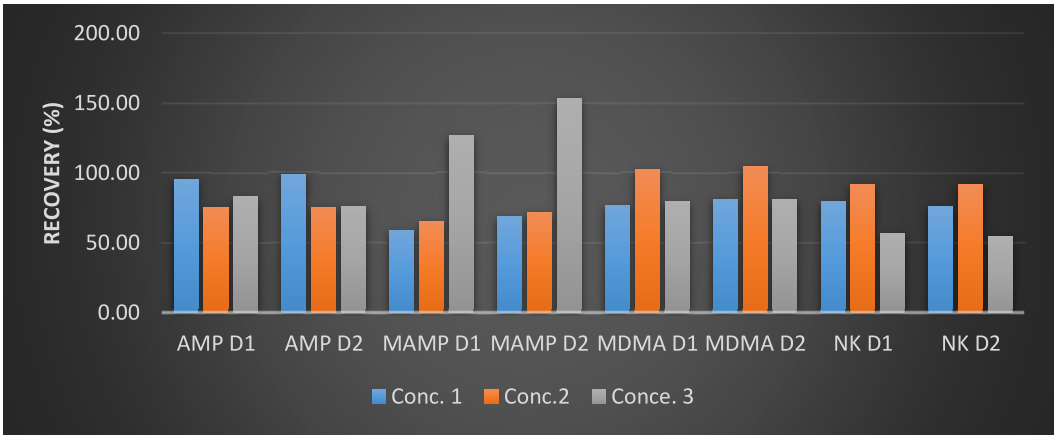


Figure 30 - Percentage of recovery obtained for amphetamines diastereomers: AMP (D1, D2), MAMP (D1, D2) MDMA (D1, D2) and NK (D1, D2) for the three QCs.

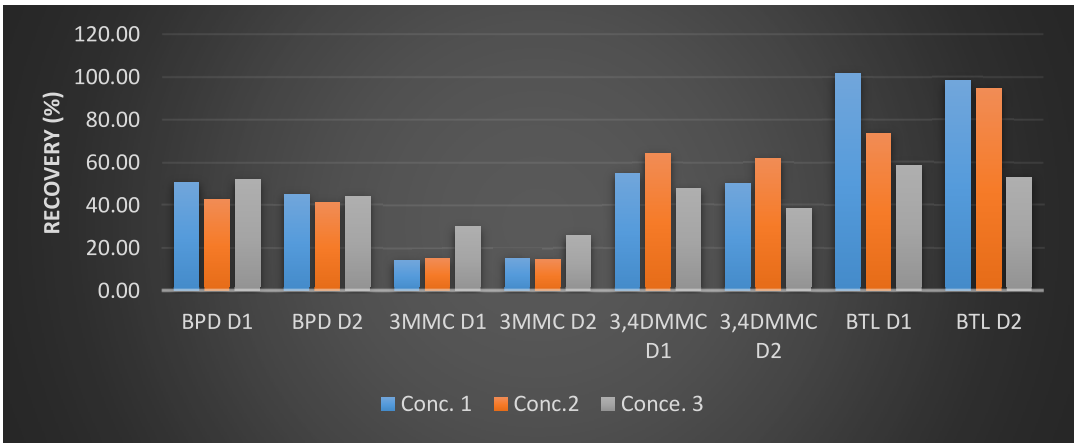


Figure 31 - Percentage of recovery obtained for synthetic cathinones diastereomers: BPD (D1, D2), 3-MMC (D1, D2) 3,4-DMMC (D1, D2) and BTL (D1, D2) for the three QCs.

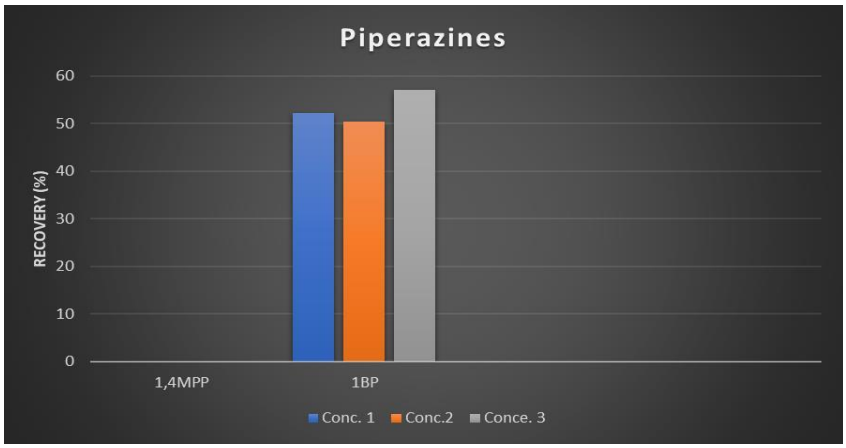


Figure 32 - Percentage of recovery obtained for piperazines derivatives, 1,4-MPP and 1-BP for the three QCs.

5.5. Application of the method

For method application, five sampling points along the estuary of the Douro river were selected according to previous studies (Coelho et al., 2019; Ribeiro et al., 2016b).Douro river is the third-longest river in the Iberian Peninsula, and it has a watershed shared between Spain (80%) and Portugal (20%)(Bordalo et al., 2006), receiving directly or indirectly effluents of eight WWTPs.

Composite samples of two WWTPs (WWTPA and WWTPB) that effluents discharge into a tributary of Douro River were collected on 09th July 2020.

Physico-chemical parameters were measured, and data are shown in **Table 10.** Physico-chemical parameters were within values found in previous monitoring studies and within expected values for estuarine water samples (Ribeiro et al., 2016b).

Table 10 - Water temperature and physico-chemical parameters (pH, EC and TDS) of estuarine water samples.

Sampling point	T (°C)	pH	EC (µS cm^{-1})	TDS (mg L^{-1})
S1	19	8.05	17.02	9.05
S2	21	8.30	16.90	8.89
S3	25	8.08	17.4	8.93
S4	24	8.32	16.86	8.90
S5	25	7.94	17.25	9.39

Regarding AMPs, both enantiomers of AMP (D1 and D2) were found though at < LOQ in S1 while only enantiomer of MAMP (D1) was found at S1 and S4). At sampling points S2 and S3 AMPs were not detected (concentration <LOD). Concentrations <LOQ were found for the MAMP D1 in S4 and MDMA D1 in S5

Regarding synthetic cathinones, one enantiomer of BPD (D1) was found in both S3 and S4 in concentration <LOQ. Neither BPD D2 or 3-MMC, 3,4-DMMC and BTL were found in this sampling collection.

Two composite samples of two WWTPs (WWTPA and WWTPB) with different treatment processes with their effluents discharging into a tributary of Douro River were also analysed (**Table 11**).

Table 11- Concentration and the EF for target PAS in effluent samples.

PAS	WWTPA		WWTPB	
	Conc. (ng L^{-1})	EF	Conc. (ng L^{-1})	EF
AMP (R)	<LOQ	n.d.	<LOD	n.d.
AMP (S)	<LOD		<LOD	n.d.
MAMP (S)	57.30	≅1	<LOQ	n.d.
MAMP (R)	<LOD		<LOD	n.d.
BPD D1	<LOQ	n.d.	<LOD	n.d.
BPD D2	<LOD		<LOD	n.d.
3-MMC D1	<LOD	n.d.	<LOD	n.d.
3-MMC D2	<LOD		<LOD	n.d.
3,4-DMMC D1	<LOQ	n.d.	<LOD	n.d.
3,4-DMMC D2	<LOD		<LOD	n.d.
MDMA (R)	<LOQ	n.d..	<LOD	n.d.
MDMA (S)	<LOQ		<LOD	n.d.
NK D1	<LOD	n.d.	<LOD	n.d.
NK D2	<LOD		<LOD	n.d.
BTL D1	<LOD	n.d.	<LOD	n.d.
BTL D2	<LOD		<LOD	n.d.
BP	<LOD	n.d.	<LOD	n.d.
1-MPP	<LOD	n.d.	<LOD	n.d.

Regarding to concentration of AMPs in effluents, AMP was found in concentration between <LOD and LOQ.

MAMP was also found in concentration between <LOD and 57.30 ng L^{-1}. Chromatogram and mass spectra showing the presence of one enantiomer of MAMP (D1) in WWTPA as well as the possible fragmentation pattern are shown in **Figure 33**. This result is similar to that found in previous work by Gonçalves et al (25.7 ng L^{-1}) and in Albany (3.82-6.22 ng L^{-1}), (Gonçalves et al., 2019a; Subedi and Kannan, 2014). Despite, low than that reported in Vietnam (120-420), U.S.A. (700 ng L^{-1}), Brazil (55.3-477.4 ng L^{-1}), and China (179 ng L^{-1})(Zheng et al., 2019).

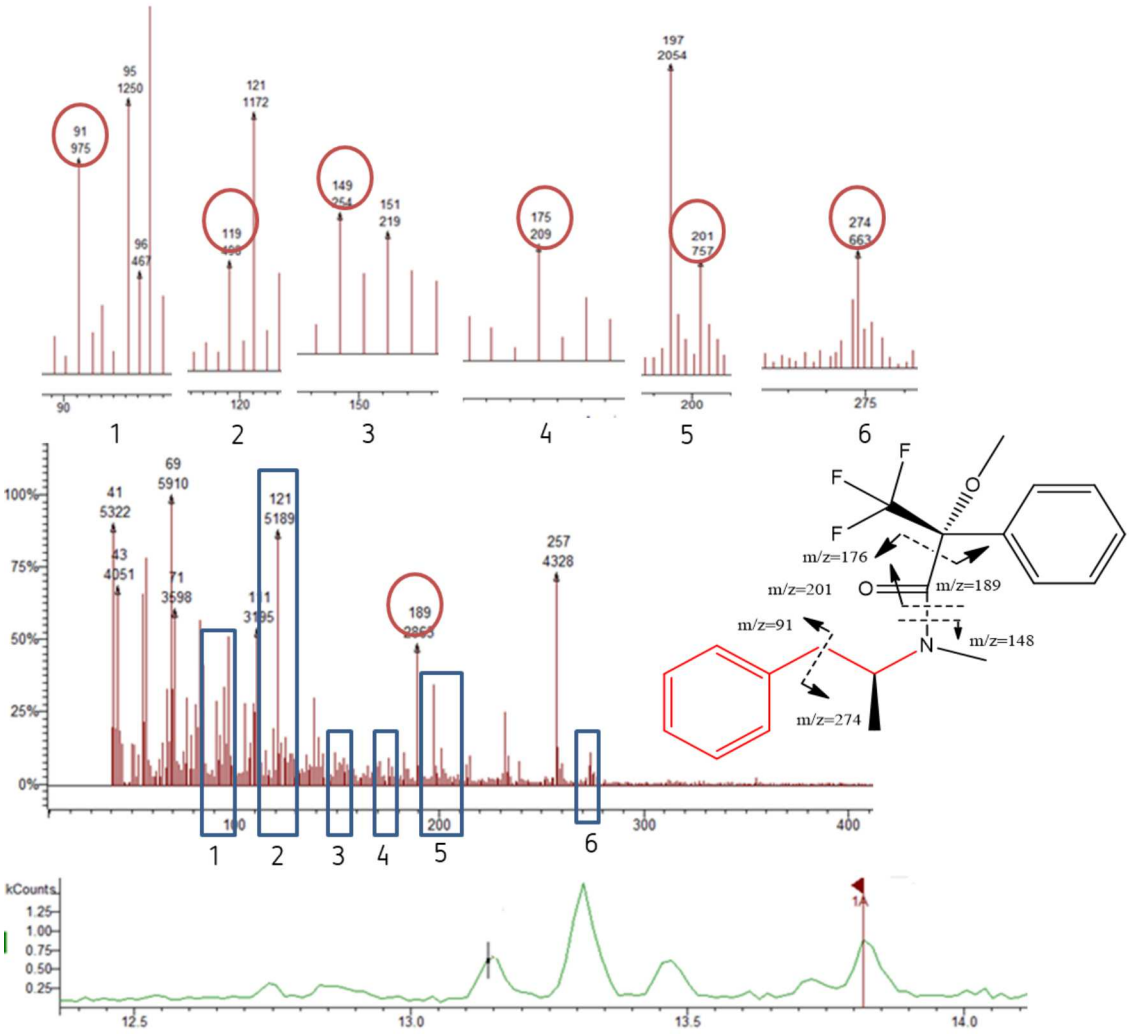


Figure 33 - Chromatograms showing MAMP D1 detected in WWTPA, mass spectrum and the possible fragmentation pattern.

According to our team previous work (*S*)-(+)-MAMP was the first enantiomer to elute and (*R*)-(-)-MAMP was the second enantiomer to elute and in this work we assume the same elution order.

Regarding to EF value is in accordance with that observed across Europe (EF≅1) with enrichment of (*S*)-(+)-MAMP. Furthermore (*S*)-(+)-MAMP is considered a chiral signature of European MAMP illegal market due use L-Ephedrine as starting material, resulting in a stereoselective production of (*S*)-(+)-MAMP besides being the most reported in several studies worldwide (Archer et al., 2018; Castrignanò et al., 2018; Evans et al., 2016; Gonçalves et al., 2019a; Xu et al., 2017)

Regarding MDMA, concentration were also found in range of <LOD to <LOQ somewhat similar to that found across Europe, (LOQ - 3.2 ng L^{-1}) in Greece, (21.7 ng L^{-1}) in Portugal, (< 62 ng L^{-1}) in Croatia and (0.5-24.8 ng L^{-1}) in U.K .(Baker and Kasprzyk-Hordern, 2013; Gatidou et al., 2016a; Gonçalves et al., 2019a; Krizman et al., 2016). MDMA also known as ecstasy, when in tablet form or MD in crystal form is a synthetic drug produced by clandestine laboratories and available in illegal market as racemate (EMCDDA, 2020).

Despite EF was not able to be determined in this work (concentrations <LOQ) MDMA metabolism is stereoselective favouring (*S*)-(+)-MDMA and enrichment of (*R*)-(-)-MDMA excretion leading to enrichment of this enantiomer in the environment (Baker and Kasprzyk-Hordern, 2013; Gonçalves et al., 2019a; Maia et al., 2017).

Regarding synthetic cathinones BPD as well as 3,4-DMMC were found in range of <LOD to <LOQ in WWTPA.

6. CONCLUSIONS

The main purpose of this study was to develop an enantioselective method by gas chromatography coupled to mass spectrometry (GC-MS) based on the formation of diastereomers for quantification of several classes of PAS in surface waters.

The derivatization method based on formation of diastereomers using the enantiopure derivatization reagent (*R*)-MTPA-Cl used elsewhere in Gonçalves et al (Gonçalves et al., 2019a) for enantiomeric quantification of PAS including AMPS (AMP, MAMP and MDMA) and β-blockers was optimized and allowed for formation of diastereomers of the synthetic cathinones (BTL, 3,4-DMMC, 3-MMC and BPD),

Piperazines are not chiral, however, their reaction with the chiral reagent were observed. Two illicit PP namely: 1-BP and 1,4-MPP were also included. Derivatization of PP with MTPA-Cl allowed to improve the signal identification and detection.

The chromatographic optimized conditions allowed the quantification of the target PS in surface waters as well as two effluents, a total of 18 diastereomers namely AMPs derivatives (AMP D1, AMP D2, MAMP D1, MAMP D2, MDMA D1 and MDMA D2), cathinones derivatives (BPD D1, BPD D2, 3-MMC D1, 3-MMC D2, 3,4-DMMC D1, 3,4-DMMC D2, BTL D1 and BTL D2), NK D1 and NK D2 as well as two PP namely: 1-BP and 1,4-MPP in less than 24.0 min.

The method was validated according to the International Conference on Harmonization (ICH) and showed to be linear (R^2 > 0.98) and LOD and LOQ allowed detection and quantification of the target PAS.

Validated method was applied to investigate the occurrence and spatial distribution of the selected PAS in Portuguese surface waters in the Greater Porto region and effluent samples from two WWTPs with different treatment technologies.

No quantifiable concentration was found in surface water samples (Conc. <LOQ) furthermore higher concentration was found for MAMP (57.30 ng L^{-1}) in WWTPA with EF ≅1.

The results obtained in this work allow to confirm that PAS are continuously consumed and discharged into the environment being a potential threat for non-target organisms.

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8. ANNEXES

Annex 1: Abstract and Poster communication presented in "XI Jornadas de Ciências and IV Congresso APCF"

XI JORNADAS CIENTÍFICAS DO INSTITUTO UNIVERSITÁRIO DE CIÊNCIAS DA SAÚDE

IV CONGRESSO DA ASSOCIAÇÃO PORTUGUESA DE CIÊNCIAS FORENSES

POSTER

MONITORIZAÇÃO DE DROGAS QUIRAIS PSICOATIVAS EM ÁGUAS DE SUPERFÍCIE

Ivan Marcelino Langa[1*], Maria Elizabeth Tiritan[1,2,3], Diana Dias da Silva[1,4], Cláudia Ribeiro[1,2*]

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Introdução: Segundo o Relatório Europeu sobre Drogas 2018 do Observatório Europeu da Droga e da Toxicodepêndencia, a Europa tem sido um dos principais mercados de drogas provenientes de várias partes do mundo, nomeadamente América latina, norte de África e Ásia do norte. Adicionalmente, dados recentes classificam também a Europa como região de produção, facilitando a comercialização e o acesso por parte do consumidor final.

Entre as drogas mais consumidas encontram-se fármacos e substâncias ilícitas, incluindo a canábis e as novas substâncias psicoativas (NSP) [1, 2]. A maioria destas substâncias é quiral podendo ser comercializada na forma de racemato ou enantiomericamente pura. Após consumo, estas substâncias podem ser excretadas em diferentes composições enantioméricas na sua forma inalterada ou na forma de metabolitos ativos e/ou inativos. Estes, através da rede de esgotos, entram nas estações de tratamento de águas que não têm capacidade para a sua remoção completa. Por conseguinte, resíduos destas substâncias e dos seus metabolitos são descartados pelos efluentes para as águas superficiais, sendo por isso considerados poluentes ambientais [2, 3]. Devido à sua ocorrência e persistência, estas substâncias biologicamente ativas podem causar efeitos adversos em organismos não-alvo [4]. Apesar da esterioquímica destas substâncias poder condicionar sobejamente os seus efeitos biológicos e toxicidade, os enantiómeros são frequentemente ignorados e a composição enantiomérica é frequentemente negligenciada, o que compromete a correta avaliação do risco ambiental [2, 4].

Objectivos: O presente estudo tem como objetivo desenvolver um método enantioseletivo por cromatografia gasosa acoplada a espectrometria de massa (GC-MS) baseado na formação de diastereoisómeros para quantificação de várias classes de NSP utilizadas para fins recreativos. O método será utilizado para avaliar a ocorrência, distribuição e a fração enantiomérica destas NSP.

Material e métodos: Para a formação dos diastereoisómeros utilizar-se-á o reagente enantiomericamente puro cloreto de (*R*)-(−)-α-Metoxi-α-(trifluorometil)-fenilacetilo (*R*-MTPA-Cl) e um protocolo desenvolvido para as anfetaminas e derivados, que será adaptado e otimizado para a inclusão de novas classes de NSP. Serão feitas colheitas de águas de superfície em diferentes estações do ano e pontos de colheita, de forma a avaliar a ocorrência e distribuição em amostras de águas de superfície da zona do grande Porto.

Resultados: Os resultados obtidos permitirão avaliar a ocorrência, distribuição e fração enantiomérica destas classes de NSP em águas de superfície portuguesas, importantes para uma correta avaliação de impacto ambiental, e determinar o perfil de consumo de drogas recreativas pela população.

Conclusões: O desenvolvimento de metodologias enantioseletivas é crucial para a monitorização de drogas quirais utilizadas para fins recreativos. Os dados obtidos permitirão determinar, pela primeira vez, a ocorrência de algumas classes de estimulantes em águas portuguesas, a utilização de drogas recreativas em Portugal e o risco e impacto da ocorrência destas substâncias no meio ambiente.

Agradecimentos: Projeto financiado pelo projeto: *BIOENVIROM-CESPU-2018.*

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XI JORNADAS DE CIÊNCIAS 2019
Os olhares clínico e forense sobre o melhoramento humano
"Uma visão interdisciplinar"

MONITORIZAÇÃO DE DROGAS QUIRAIS PSICOATIVAS EM ÁGUAS DE SUPERFÍCIE

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INTRODUÇÃO

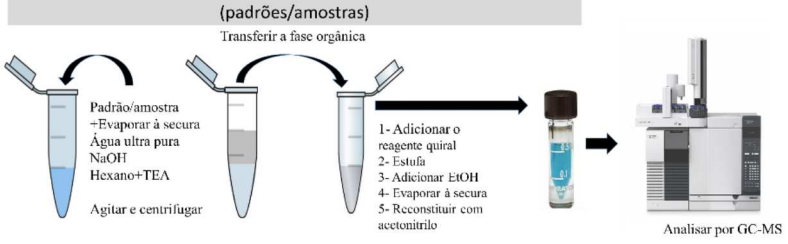
De acordo com o Relatório Europeu da Droga de 2018, a canabis encontra-se entre as drogas ilícitas mais consumida na Europa seguida da cocaína, anfetaminas e novas drogas sintéticas. Adicionalmente, dados recentes mostram a Europa como região de produção, facilitando a comercialização e o acesso por parte do consumidor final [1].

A maioria destas substâncias é quiral podendo ser comercializada na forma de racemato ou enantiomericamente pura. Após consumo, estas substâncias psicoativas (SP) podem ser excretadas em diferentes composições enantioméricas na sua forma inalterada ou na forma de metabolitos ativos e/ou inativos. Estes, através da rede de esgotos, entram nas estações de tratamento de águas que não têm capacidade para a sua remoção completa. Por conseguinte, resíduos de SP e dos seus metabolitos são descartados pelos efluentes para as águas superficiais, sendo por isso considerados poluentes ambientais [2, 3]. Devido à sua ocorrência e persistência, estas substâncias biologicamente ativas podem causar efeitos adversos em organismos não-alvo [4]. Apesar da estereoquímica destas substâncias poder condicionar os seus efeitos farmacológicos e/ou toxicidade, os enantiómeros são frequentemente ignorados e a composição enantiomérica é frequentemente negligenciada, o que compromete a correta avaliação do risco ambiental [2, 4].

O presente estudo tem como objetivo desenvolver um método enantiosseletivo por cromatografia gasosa acoplada a espectrometria de massa (GC-MS) baseado na formação de diastereoisómeros para quantificação de várias classes de SP utilizadas para fins recreativos. O método será utilizado para avaliar a ocorrência, distribuição e a fração enantiomérica destas SP.

MÉTODO

Para a formação dos diastereoisómeros utilizar-se-á o reagente enantiomericamente puro cloreto de (*R*)-(−)-α-Metoxi-α-(trifluorometil) fenilacetilo (*R*-MTPA-Cl). O procedimento de derivatização já ajustado para as anfetaminas e derivados que será adaptado e otimizado para a inclusão de novas classes de substâncias [5].



Serão testados diferentes procedimentos de SPE para otimizar o processo de preparação das amostras de água de superfície e os diferentes procedimentos serão avaliados considerando a melhor recuperação dos compostos e o menor efeito de matriz/ interferentes.

RESULTADOS

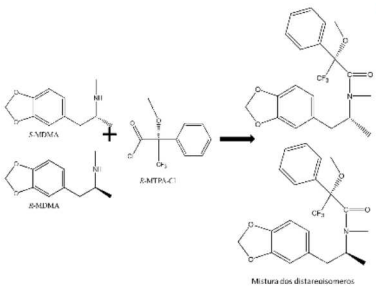


Figura 1: Reação dos enantiómeros *S* e *R* da MDMA com o reagente de derivatização *R*-MTPA-Cl para a formação dos diastereoisómeros.

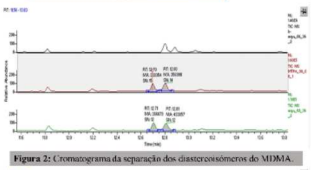


Figura 2: Cromatograma da separação dos diastereoisómeros do MDMA.

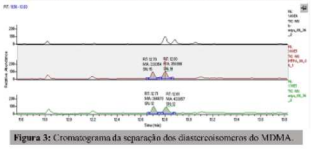


Figura 3: Cromatograma da separação dos diastereoisómeros do MDMA.

A **Figura 1** representa a reação dos enantiómeros do metilenodioximetanfetamina (MDMA) com o reagente enantiomericamente puro *R*-MTPA-Cl. As **Figuras 2 e 3** representam os cromatogramas mostrando a separação dos dois diastereoisómeros sendo que os dois apresentaram o mesmo espetro de massa (**Figura 4**). A metodologia vai ser aplicada para a derivatização de outras classes de SP e o método GC-MS otimizado a separação dos diastereoisómeros formados.

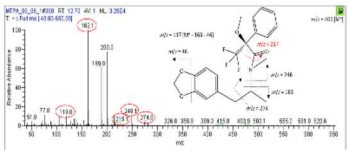


Figura 4: Espetro de massa do diastereoisomero formado e possíveis locais de fragmentação.

CONCLUSÕES

O desenvolvimento de metodologias enantiosseletivas é crucial para a monitorização de drogas quirais psicoativas. Os dados obtidos permitirão determinar, pela primeira vez, a ocorrência de algumas classes de estimulantes em águas portuguesas, a utilização de drogas recreativas em Portugal e o risco e impacto da ocorrência destas substâncias no meio ambiente.O método será utilizado para avaliar a ocorrência, distribuição espacial e sazonal e a FE em águas de superfície portuguesas na região do Grande Porto que permitirá: avaliar o seu impacto ambiental, determinar/verificar o consumo pela população de SP e as suas potenciais fontes.

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AGRADECIMENTOS

Projeto financiado pelo projeto: BIOENVIROM-CESPU-2018.

Annex 2: Abstract and Poster communication presented in "10º Encontro da Divisão de Química Analítica da SPQ, ANALITICA 2020".

DEVELOPMENT AND VALIDATION OF AN INDIRECT GC-MS METHOD FOR THE QUANTIFICATION OF PSYCHOACTIVE SUBSTANCES IN SURFACE WATERS

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The changes in the law to keep drug trafficking and consumption under control have boosted the synthesis and introduction of new psychoactive substances (PS) in the illegal market (Couto et al., 2018). Many of this NPS are chiral and available as racemate or enantiomerically pure. These substances reach the environment through different ways such as direct disposal by industry, illegal discharges and as humans excretion products (of parent compounds and their metabolites) being a potential threat to non-target organisms (Gatidou et al., 2016b; Ribeiro et al., 2017). Occurrence of these substances in surface waters may also give insights about their consumption in a specific region.
The aim of this work is the development and validation of indirect method based on the application of solid phase extraction (SPE) followed by gas chromatography-mass spectrometry (GC-MS) for enantiomeric quantification of 9 PS (5 synthetic cathinones and 3 amphetamine like substances) based on the formation of diastereomers using (R) - (-) - α-methoxy-α- (trifluoromethyl) phenylacetyl chloride (MTPA-Cl) as chiral derivatization reagent. Two illicit piperazines (PP) were also included. PP were also derivatized with MTPA-Cl improving signal identification and detection. The optimized conditions allowed the quantification of the target PS (a total of 18 diastereomers and two PP) in less than 23.0 min.
The method was validated according to the International Conference on Harmonization and showed to be linear ($R^2 > 0.98$). Limits of detection ranged from 17 to 100 ng/L and limit of quantification varied between 50 and 300 ng/L. The method will be used to evaluate the occurrence, spatial distribution, and the enantiomeric fraction of the PS in Portuguese surface waters in the Great Porto region. Data will allow to evaluate their environmental impact, determine/ verify the consumption of recreational drug by the population and their potential sources.

Acknowledgements: Projeto financiado pelo projeto MYCOBIOENV-PFT-IINFACTS-2019.

10º Encontro da Divisão de Química Analítica da SPQ, ANALITICA2020

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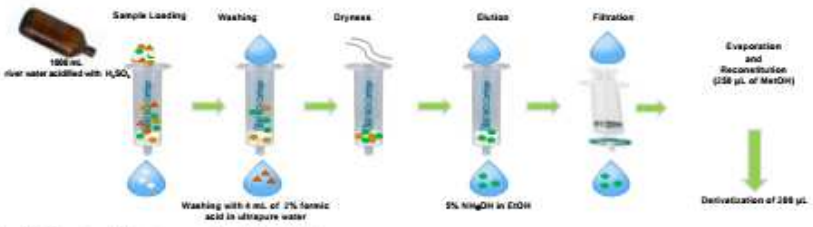
INTRODUCTION

The changes in the law to keep drug trafficking and consumption under control have boosted the synthesis and introduction of new psychoactive substances (PS) in the illegal market [1]. Many of this NPS are chiral and available as racemate or enantiomerically pure. These substances reach the environment through different ways such as direct disposal by industry, illegal discharges and as humans excretion products (of parent compounds and their metabolites) being a potential threat to non-target organisms [2, 3]. Occurrence of these substances in surface waters may also give insights about their consumption in a specific region.

The aim of this work is the development and validation of indirect method based on the application of solid phase extraction (SPE) followed by separation of the diastereomer using gas chromatography mass detector (GC-MS) for enantiomeric quantification of 8 PS (5 synthetic cathinones and 3 amphetamine like substances) based on the formation of diastereomers using (R) - (-) - α-methoxy-α- (trifluoromethyl) phenylacetyl chloride (*R*-MTPA-Cl) as chiral derivatization reagent. Two illicit piperazines (PP) were also included. PP were also derivatized with *R*-MTPA-Cl improving signal identification and detection. The optimized conditions allowed the quantification of the target PS (a total of 18 diastereomers and two PP) in less than 23.0 min.

METHOD

SOLID PHASE EXTRACTION - SPE



Upon arrival to the laboratory, all samples were immediately vacuum filtered and acidified to pH = 3 with H_2SO_4. SPE was performed using OASIS® MCX cartridges without cartridge conditioning as described else were in [4, 5] and a mixture of ammonium hydroxide/ethanol were used as eluent.

CHIRAL DERIVATIZATION (STANDARDS/SAMPLES)

For the formation of diastereomers the *R*-MTPA-Cl was used. The derivatization procedure established for amphetamines and derivatives was adapted and optimized for the inclusion of new classes of substances namely the synthetic cathinones and PP [5].

Analysis were performed using a GC-MS (Varian CP-3800 coupled with an ion trap mass spectrometer Saturn 2200 and an autosampler.

Chromatographic separation was performed using a Zebron (5% phenyl, 95% dimethylpolysiloxane) capillary column, 30 m x 0.25 mm I.D., 0.25 µm film thicknesses (Phenomenex, USA). The carrier gas was helium (99.999 % purity)

RESULTADOS

Table 1 show the ions used for the quantification of the diastereomers and the respective retention time. **Figures 1 and 2** represent the chromatograms showing the separation of the two diastereomers (AM3/1 and AM3/2).

The method was validated according to the International Conference on Harmonization and showed to be linear (R^2> 0.98) (**table 2**). Limits of detection ranged from 17 to 100 ng/L and limit of quantification varied between 50 and 300 ng/L (**Table 2**).

CONCLUSÕES

Development of enantioselective methods is crucial for monitorization of chiral PS. This method will be used to evaluate the occurrence, spatial distribution, and the enantiomeric fraction of the PS in Portuguese surface waters in the Great Porto region. Data will allow to evaluate their environmental impact, determine/ verify the consumption of recreational drug by the population and their potential sources.

Acknowledgements

Projeto financiado pelo projeto: IDIANTIBIOM-CESPU-2019.

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