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Determination of drugs of abuse in water by solid-phase extraction, derivatisation and gas chromatography–ion trap-tandem mass spectrometry

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ABSTRACT

An alternative method for the sensitive determination of several drugs of abuse and some of their metabolites in surface and sewage water samples is proposed. Analytes are concentrated using a solid-phase extraction (SPE) sorbent, converted into the corresponding trimethylsilyl derivatives and selectively determined by gas chromatography (GC) with tandem mass spectrometry (MS/MS) detection. Parameters affecting the performance of extraction, derivatisation and determination steps are systematically investigated. Moreover, the stability of target analytes in sewage water samples is discussed. Under final working conditions, water samples were adjusted at pH 8.5 and concentrated using a 200 mg OASIS HLB SPE cartridge. Analytes were sequentially eluted with ethyl acetate followed by acetone and silylated using *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA). The reaction was completed in 60 min at 80 °C and the mixture injected directly in the GC–MS/MS system without further purification. In most cases, analytes presented a poor stability in sewage water samples; however, once they are submitted to the SPE process, cartridges can be stored at −20 °C for at least 3 months without significant degradation and/or inter-conversion reactions of illicit drugs. The proposed method provided recoveries over 74% and LODs between 0.8 and 15 ng/L for river and treated wastewater samples. In the case of raw wastewater slightly worse recoveries, between 63 and 137%, and similar LODs were attained. Analysis of a limited number of waste and surface water samples confirmed the presence of several illicit drugs in the aquatic environment, with the highest levels and frequency corresponding to benzoylecgonine, the main metabolite of cocaine.

1. Introduction

Abuse of illicit drugs has become a serious global problem in our contemporary society. According to the United Nations Office of Drugs and Crime (UNODC), 200 million people consumed any illicit substance during 2005, 110 million used them regularly and 25 million were considered addicted. Facing these data with appropriate solutions requires reliable figures about local consumption and trends. Currently, the above information is inferred from socio-epidemiological studies integrated with population surveys, crime and medical records, drug production and drug seizures. These indicators may lead to inaccurate conclusions since they are too general and most of the information is obtained from the consumers themselves. Moreover, data collection and analysis are time consuming, so updated figures and changing patterns cannot be properly estimated [1,2].

In the past decade, several pharmaceuticals and their metabolites were detected in the water environment. These data were employed by several authors to monitor the consumption of pharmaceuticals in a specific location. Loads of these compounds, and their major metabolites, in waste- and surface waters are calculated and are then related to the local population equivalents (i.e. the number of people served by a given sewage treatment plant (STP) or living in a river's catchment basin). In 1999, Daughton [3] suggested that residues of illicit drugs may be similarly detected in the aquatic environment and, as their metabolism patterns are mostly known, correlated to their consumption. In this way, the first report concerning the presence of illicit substances in water appeared in 2004 [4], but it was not until 2005 when Zuccato et al. [5] related measured levels to local consumption, what was named as *sewage epidemiology*, beginning a trend that has been followed by many other researchers [6–10].

In contrast to classical strategies of screening drugs consumption, the analysis of water samples is cheaper, anonymous (avoiding potential conflicts over privacy) and provides real-time data, which would enable to detect changes in drugs usage if a

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long-term monitoring program was carried out. In addition, the obtained data are also valuable to evaluate the removal efficiency of illicit substances in STPs and to identify the percentage of them that reach natural waters, where their effects remain mostly unknown. According to the recent studies [5–14], some illicit drugs occur in this media at concentrations similar to other emerging contaminants (i.e. the psychiatric drug carbamezapine and the anti-inflammatory diclofenac) so, in future, they might be included in the list of *priority substances* of the EU Water Framework Directive (WDF).

Developed methods for the analysis of drugs of abuse in waters are based on a solid-phase extraction step (SPE) followed by a subsequent determination by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) [5–21]. SPE provides adequate enrichment factors, it is robust and well established in most analytical laboratories. However, with a few exceptions [22], the selectivity of SPE is rather limited; consequently complex extracts, which normally lead to signal suppression in LC–MS measurements, and even unreliable results in the most critical situations, are obtained [18].

On the other hand, gas chromatography–mass spectrometry (GC–MS) is an inexpensive alternative not suffering from ionization matrix effects, as compared to LC–MS; it is accessible to most laboratories and has a long tradition for the determination of drugs of abuse in clinical and forensic sciences [18]; however, to the best of our knowledge, it had not been applied yet with environmental purposes. Thus, the goal of this work was the development of a SPE–GC–MS/MS method for the simultaneous determination of several illicit drugs, belonging to four different chemical families, and some of their metabolites in environmental water samples. Experimental parameters were optimised to achieve the maximum efficiency during extraction and derivatisation steps. Moreover, the stability of the analytes during sample storage was discussed. Finally, after validation of the proposed method, it was applied to several river and sewage water samples collected in the NW of Spain.

2. Experimental

2.1. Chemicals and stock solutions

The illicit drugs and metabolites studied were the following: (±)-amphetamine (AMP), (±)-methamphetamine (MAMP), (±)-3,4-methylenedioxyamphetamine (MDA), (±)-N-methyl-3,4-methylenedioxymethamphetamine (MDMA), (±)-N-ethyl-3,4-methylenedioxyethylamphetamine (MDEA), cocaine (COC), cocaethylene (COE), benzoylecgonine (BE), morphine (MOR), codeine (COD), heroine (HER), (±)-methadone (MET), (−)-Δ^9-tetrahydrocannabinol (THC) and (±)-11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH). All of them were purchased from Cerilliant (Round Rock, TX, USA) as 1 mg/mL solutions in acetonitrile (COC, COE and HER) or methanol (the remaining ones), except THCCOOH (0.1 mg/mL in methanol). Deuterated compounds were also obtained from Cerilliant (0.1 mg/mL in acetonitrile or methanol) and were used as surrogated internal standards (ISs) for quantification of their analogue native analytes. In the case of COE and HER, COC-d3 and MOR-d3 were used as ISs as there was no labelled compound available in the lab. The structures of analytes and labelled ISs are compiled in Table 1.

Mixed working solutions containing all target analytes or their deuterated analogues were prepared in methanol:water (1:1), when used to fortify water samples, and in ethyl acetate, when considered to evaluate the performance of the GC–MS system. The above solutions were stored in the dark at −20 °C. Calibration standards with increasing concentrations of analytes and 250 ng/mL of ISs were prepared in ethyl acetate containing the appropriate amount of derivatisation reagent.

Methanol and acetonitrile, as well as ammonia solution (25%) and hydrochloric acid (37%), for pH adjustments, were supplied by Merck (Darmstadt, Germany). Acetone and ethyl acetate were form Prolabo (Cerdanyola del Vallès, Spain). Anhydrous sodium sulphate was from Panreac (Castellar del Vallès, Spain).

Standards and sample extracts, both in ethyl acetate, were derivatised before GC–MS determination. Different derivatisation agents were tested in this work: *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetimide (MTBSTFA) and *N*-methyl-bis-(trifluoroacetamide) (MBTFA) were purchased from Sigma–Aldrich (Milwaukee, WI, USA); *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Supelco (Bellefonte, Bellefonte, PA, USA); and finally, acetic anhydride was provided by Across Organics (Geel, Belgium).

A key parameter to guarantee reliable results in the analysis of environmental samples is insuring the stability of the analytes during sample storage. Series of experiments were carried out with spiked aliquots of filtered sewage samples, stored under different conditions before being submitted to the analytical procedure. In some cases, sodium azide (Riedel-deHaën, Seelze, Germany) was added to the samples in order to reduce their microbiological activity

2.2. Samples

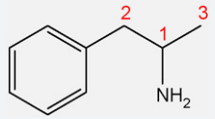
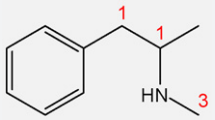
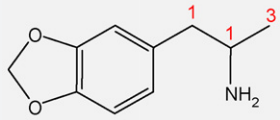
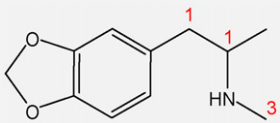
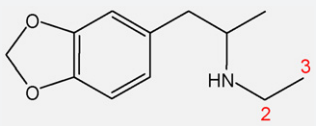
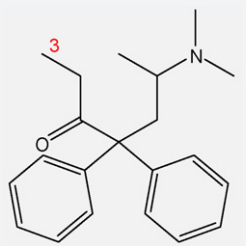
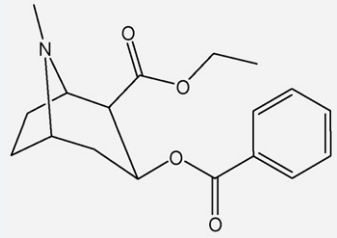
Three surface water samples and several STP influents and effluents were employed in this study. The first ones were taken from the rivers Sar, Dos Pasos and Lengüelle in Galicia (NW Spain). Raw and treated wastewaters were collected at five STPs which receive the discharges from the same number of small and medium size cities (from 15,000 to 125,000 inhabitants) located in the NW Spain. All samples were collected in amber glass bottles, previously rinsed with methanol and ultrapure water, and extracted (SPE) within 24 h of sampling.

2.3. Sample preparation and derivatisation

A SPE procedure was developed to isolate and to concentrate target compounds from waters. Prior to extraction, samples (500 mL for river water, 200 mL for STP effluent and 100 mL for the influent) were passed through a combination of glass fibre prefilters and 0.45 μm nitrocellulose filters (Millipore, Bedford, MA, USA) to remove particulate matter. The filtrate was then adjusted to the desired pH, using either HCl or ammonia solutions, spiked with isotopically labelled standards (50 ng each) and, in the case of recovery studies, also with analytes. Oasis HLB 60 mg (3 mL) and 200 mg (6 mL) cartridges (Waters, Milford, MA, USA), were tested.

In the final method, the Oasis HLB 200 mg cartridges were employed. They were sequentially conditioned with 5 mL of ethyl acetate, 5 mL of acetone and 5 mL of ultrapure water. Samples (pH 8.5) were passed through at a flow rate of approximately 10 mL/min and, then, sorbents were dried by a continuous nitrogen stream for a minimum of 30 min, wrapped in aluminium foil and stored at −20 °C until desorption. Just before this step, cartridges were dried again and analytes were eluted in two separated fractions: the first one with 2 mL ethyl acetate and the second one using 8 mL acetone. The acetone fraction was evaporated to dryness at 25 °C, under a gentle stream of nitrogen, and the residue was mixed with the ethyl acetate fraction. The reconstituted extract was finally concentrated to 0.1 mL and derivatised, in a closed vial equipped with a 0.25 mL insert, by adding 0.1 mL of MSTFA and by heating the mixture at 80 °C for 60 min.

Table 1
Structures, physicochemical data and experimental parameters employed for the MS/MS determination of target compounds.

Compound	Structure[a]	Empirical formula	Monoisotopic MW	pK_a	Log Kow	Precursor (m/z)	Scan range (m/z)	Products[b] (m/z)	Excit. stor. level (m/z)	Excit. amplitude (V)
AMP		$C_9H_{13}N$	135.10	10.1[c]	1.76[c]	116	70–125	73	40	0.35
AMP-d6		$C_9H_7D_6N$	141.1	–	–	120	70–125	73	40	0.35
MAMP		$C_{10}H_{15}N$	149.12	9.87[c]	2.07[c]	130	70–135	73	40	0.35
MAMP-d5		$C_{10}H_{10}D_5N$	154.12	–	–	134	70–135	73	40	0.35
MDA		$C_{10}H_{13}NO_2$	179.09	9.67[c]	1.64[c]	116	70–125	73	40	0.35
MDA-d5		$C_{10}H_8D_5NO_2$	184.09	–	–	120	70–125	73	40	0.35
MDMA		$C_{11}H_{15}NO_2$	193.11	10.32[d]	1.806[d]	130	70–135	73	40	0.32
MDMA-d5		$C_{11}H_{10}D_5NO_2$	198.11	–	–	134	70–135	73	40	0.32
MDEA		$C_{12}H_{17}NO_2$	207.13	10.34[d]	2.337[d]	144	70–150	73	40	0.32
MDEA-d5		$C_{12}H_{12}D_5NO_2$	212.13	–	–	149	70–150	73	40	0.32
MET		$C_{21}H_{27}NO$	309.21	8.94[c]	3.93[c]	296	180–305	281, 206, 191	130	0.59
MET-d3		$C_{21}H_{24}D_3NO$	312.21	–	–	299	180–305	284, 209, 191	130	0.59
COE		$C_{18}H_{23}NO_4$	317.16	9.04[d]	3.610[d]	196	75–250	168, 150, 82	70	0.42

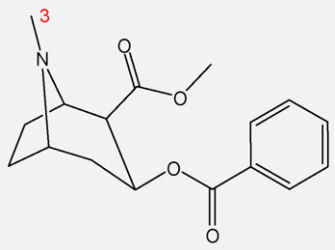
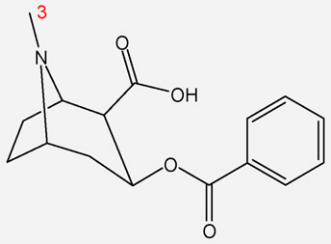
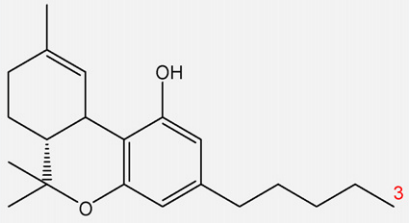
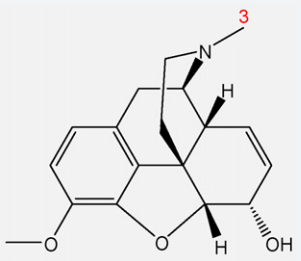
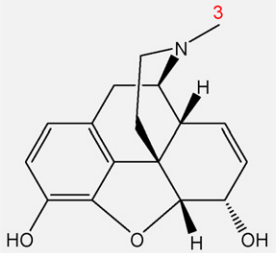
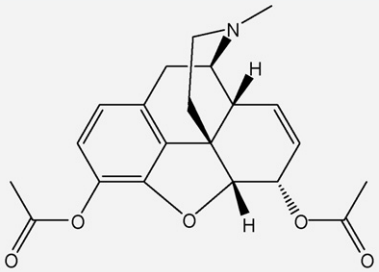
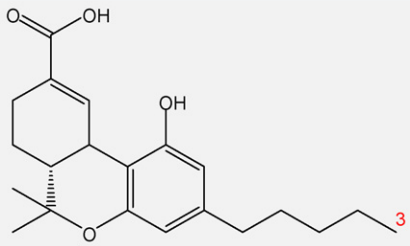
COC		$C_{17}H_{21}NO_4$	303.15	8.61[c]	2.30[c]	182	75–190	150, 122, 82	70	0.42
COC-d3		$C_{17}H_{18}D_3NO_4$	306.15	–	–	185	75–190	153, 125, 85	70	0.42
BE		$C_{16}H_{19}NO_4$	289.13	10.8[d]	2.715[d]	240	75–250	150, 108, 82	60	0.33
BE-d3		$C_{16}H_{16}D_3NO_4$	292.13	–	–	243	75–250	153, 111, 85	60	0.35
THC		$C_{21}H_{30}O_2$	314.22	10.6[c]	7.60[c]	386	280–395	371, 330, 315	170	0.98
THC-d3		$C_{21}H_{27}D_3O_2$	317.22	–	–	389	280–395	374, 330, 315	170	1.14
COD		$C_{18}H_{21}NO_3$	299.15	8.21[c]	1.19[c]	371	175–380	355, 281, 234	140	1.05
COD-d3		$C_{18}H_{18}D_3NO_3$	302.15	–	–	374	175–380	358, 284, 237	140	1.05

Table 1 (*Continued*)

Compound	Structure[a]	Empirical formula	Monoisotopic MW	pK_a	Log Kow	Precursor (*m/z*)	Scan range (*m/z*)	Products[b] (*m/z*)	Excit. stor. level (*m/z*)	Excit. amplitude (V)
MOR		$C_{17}H_{19}NO_3$	285.14	8.21[c]	0.89[c]	429	230–440	414, 401, 287	180	1.1
MOR-d3		$C_{17}H_{16}D_3NO_3$	288.14	–	–	432	230–440	417, 404, 290	180	1.1
HER		$C_{21}H_{23}NO_5$	369.16	7.95[c]	1.58[c]	327	200–335	284, 268	160	1.05
THCCOOH		$C_{21}H_{28}O_4$	344.20	4.68[d]	6.213[d]	473	280–480	355	240	1.75
THCCOOH-d3		$C_{21}H_{25}D_3O_4$	347.20	–	–	476	280–480	358	240	1.75

[a] The numbers indicate the position and number of deuterium atoms in the labelled internal standard.
[b] Most intense product ions, with the quantification ion being underlined.
[c] Experimental values provided by PhysProp database (Syracuse Research Corporation).
[d] Software estimated values obtained from SciFinder 2007 database.

2.4. Gas chromatography–mass spectrometry

Analytes were determined by GC–MS/MS using a Varian CP 3900 gas chromatograph (Walnut Creek, CA, USA) connected to a Varian Saturn 2100 ion trap-mass spectrometer. Injections (2 μL) were made in the splitless mode with a splitless time of 1 min and a split ratio of 50. The injector port was set at 280 °C.

Separations were carried out in a HP-5MS type capillary column (30 m × 0.25 mm i.d., d_f 0.25 μm) supplied by Agilent (Wilmington, DE, USA). Helium (99.999%) was employed as carrier gas using an initial pressure pulse of 25 psi for 1.1 min and then keeping the flow at a constant value of 1.3 mL/min. The GC oven was programmed as follows: the initial temperature of 90 °C was held for 1 min; next, it was increased to 130 °C at 25 °C/min and, finally, to 280 °C at 4 °C/min (held for 5 min). The total run time was 45.10 min and the solvent delay 4.5 min. The GC–MS interface and the ion trap temperatures were set at 280 and 220 °C, respectively.

The mass spectrometer was operated in the electron impact ionization mode (70 eV) and MS scan (70–500 *m/z*) in the preliminary experiments, and in resonant MS/MS in the final method, with a filament emission current of 60 μA and a multiplier offset of 100 V. The MS/MS detection conditions, as well as *m/z* ratios corresponding to the parent and product ions for each compound, and their deuterated analogues are given in Table 1.

3. Results and discussion

3.1. Derivatisation

3.1.1. Selection of the derivatisation agent

The compounds included in this study contain polar functionalities (hydroxyls, carboxylic acids and amines) that have to be transformed prior to their GC–MS analysis. The aim of an ideal derivatisation reaction is not only decreasing the polarity of the native substances and increasing their volatility, but also improving their stability, chromatographic separation and detectability. To this end, several derivatisation strategies have already been considered in the literature for the GC–MS determination of drugs of abuse and their metabolites in biological samples [23,24]. Among them, silylation and acylation seemed to be the most efficient reactions.

So, in order to obtain the silyl derivatives, the first derivatising agents contemplated in this study were MSTFA, BSTFA and MTBSTFA. Among them, the last two reagents failed to derivatise some of the aliphatic hydroxyl and the amine groups, even when they were added to the sample at a 40% (v/v) concentration and the mixture was heated at 80 °C for 2 h. On the other hand, MSTFA silylated all the reactive groups (amine, aromatic and aliphatic hydroxyl and carboxyl moieties) including the enolate form of MET. This reaction was performed in softer conditions (20%, v/v, 60 °C, 1 h), with the only drawback of leading to relatively low *m/z* ions in the MS spectra of amphetamines and a single product ion in MS/MS experiments (see Section 3.2). Thus, a mixed derivatisation strategy consisting of a first acylation step, in order to derivatise amine and phenolic groups, followed by silylation of the remaining reactive moieties with MSTFA was also considered. In situ acetylation, by the addition of acetic anhydride to the aqueous samples and extraction of the derivatised analytes into an organic solvent [25], produced still broad, tailing peaks for amphetamines, so it was discarded. The combination between acylation in organic medium (ethyl acetate), using MBTFA, and then silylation with MSTFA (both added at a 20%, v/v level and heated at 60 °C for 45 min) produced goods results for all the compounds in terms of peak shape and MS spectra, particularly for amphetamines. Unfortunately, the stability of the acyl-derivatives worked out to be lower than 48 h, even when they were kept at −20 °C.

Table 2
Experimental domain and relative importance (with their sign) of the main effects associated to each factor in the Box-Behnken design.

Factor	MSTFA volume (μL)	Temperature (°C)	Time (min)
Low level	20	40	20
Central level	60	60	60
High level	100	80	100
AMP	+ + +	–	–
MAMP	+ + +	–	+
MDA	+ + +	–	–
MDMA	+ + +	+	–
MDEA	+ + +	+ + +	+ + +
MET	+ + +	+ + +	+ + +
BE	+ + +	– –	–
THC	+ + +	–	–
COD	+ + +	– – –	– – –
MOR	+ + +	– –	– –
THCCOOH	+	–	–

+ + + or – – – indicate a statistically significant effect (95% confidence level), positive or negative, respectively; ++ or – – indicate that the effect was close to the statistically significance boundary; + or - indicate that the effect was far from being statistically significant.

In view of these results, MSTFA was selected as the sole derivatising agent and the derivatisation conditions were studied in further details.

3.1.2. Optimisation of the MSTFA derivatisation

Silylation conditions (volume of MSTFA, temperature and time) were simultaneously evaluated using a Box-Behnken response surface design [26]. Derivatisation assays were carried out with aliquots from a pooled SPE extract corresponding to spiked aliquots of wastewater samples, operating in a similar way as described elsewhere for acidic pharmaceuticals [27]. The Box-Behnken design allows the optimisation of three factors with the lowest number of experiments [28]. Thus, all the variables were tested at three different levels: 20, 60 and 100 μL of MSTFA (final volume was always made to 200 μL); 40, 60 and 80 °C; and 20, 60 and 100 min respectively, resulting in a total set of 15 experiments.

The analysis of the obtained results (Table 2) showed that the volume of the derivatising agent was the most important factor, affecting positively to the response of all the derivatised compounds with the exception of THCCOOH (on which it did not show to have a significant effect at a 95% of confidence level). MET and MDEA silylation was also influenced positively by time and temperature. At last, second order interactions presented small and non-significant effects, meaning that the three variables play independent roles on the derivatisation reaction. Finally, the optimum conditions were selected using a desirability function [26,27], calculated taking into account only the derivatisation reactive compounds (Fig. 1). The optimum values predicted by this function were 100 μL of MSTFA, 80 °C and 60 min, which resulted in a complete derivatisation reaction. The derivatives obtained with this optimised procedure turned to be stable for at least 1 week (see Section 3.4).

3.2. GC–MS/MS

In order to improve the sensitivity and selectivity of the method, fragmentation and MS/MS detection conditions were optimised using a resonant waveform. These parameters are presented in Table 1. Also, Fig. 2 shows the main MS/MS fragmentation pathways for some representative compounds included in this study.

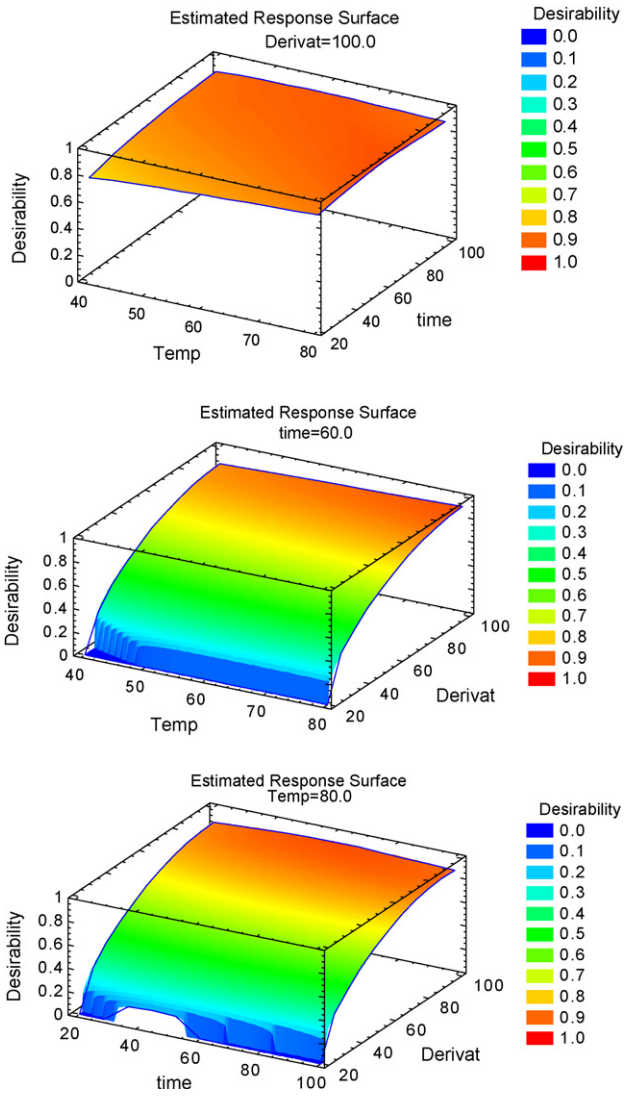


Fig. 1. Desirability function plots.

The MS spectra of silylated amphetamines showed a predominant signal at *m/z* $[M-91]^{+\bullet}$, corresponding to the loss of the $PhCH_2$ group. The above parent ion was isolated in the ion trap and submitted to collision induced dissociation (CID), resulting in an only intense product ion at *m/z* 73 $[Si(CH_3)_3]^{+\bullet}$ (Fig. 2a). In the case of COC and its derivatives (COE and BE), electron impact ionization produced a precursor ion at *m/z* $[M\text{-}121]^{+\bullet}$ as a result of the loss of the benzoic group and then, the CID fragmentation of this parent yielded three intense signals at *m/z* 150, 122 and 82 (Fig. 2b). Electron impact ionization of the opioids (HER, COD and MOR) caused the loss of one of the acetyl, methyl or trimethylsilyl groups attached to the alcohol moieties and then, the CID of the resulting ion originated a further cleavage in the second alcohol group and, finally, the loss of one of the two OH groups (Fig. 2c). The most intense signal in the MS spectra of the trimethylsylilated THC and THCCOOH (Fig. 2d) was the molecular ion, whose MS/MS resulted in three intense signals at *m/z* $[M-15]^{+\bullet}$, corresponding to the loss of one of the methyl groups, *m/z* $[M-56]^{+\bullet}$ (loss of C_4H_8 from the alkyl chain) and *m/z* $[M-71]^{+\bullet}$ (replacement of the whole alkyl chain by hydrogen). Finally, the mass spectra of MET showed a main fragment at *m/z* 296, whose CID originated three intense ions at *m/z* 281, 206 and 291, following the fragmentation path proposed in Fig. 2e.

3.3. Solid-phase extraction

Optimisation of the solid-phase extraction procedure was made with the aim of reaching good extraction recoveries for all the target analytes with different physicochemical properties. In order to achieve this goal, the Oasis HLB sorbent was selected because its hydrophilic–lipophilic balance provides a good efficiency in the extraction of compounds with a wide range of polarities and acidic characters, as it has been already proved for the multi-residue determination of pharmaceuticals and other emerging contaminants [29], including drugs of abuse [11,12].

Initially, the effect of sample pH (3, 6, 7, 8.5 and 12) on the retention of analytes was investigated with 500 mL aliquots of ultrapure water, spiked at the 1 ng/mL level, passed through SPE cartridges containing 60 mg of the Oasis HLB sorbent. Compounds were eluted with 5 mL of ethyl acetate. As it is shown in Fig. 3, acidic media produced a dramatic reduction on the recoveries of amphetamines (represented by AMP), COD and MOR, which were protonated in a considerable extent (pK_a values between 8.21 and 10.34) and, consequently, became too polar to be retained on the sorbent. On the other hand, a pH of 12 was satisfactory for extracting most of the aforementioned compounds (excepting MOR), but not for isolating COC, HER (likely due to hydrolysis of the ester bonds) and THCCOOH (bearing two negative charges at this pH value). Intermediate pHs enabled the best overall recoveries, so, for further experiments, samples were adjusted at a pH value of 8.5. Anyway, non-quantitative recoveries were still observed for some of the compounds (e.g. MOR, MET or THCCOOH). So the possibility of analytes breakthrough or incomplete elution was investigated.

Possible breakthrough problems were evaluated by passing spiked aliquots of ultrapure water, adjusted at pH 8.5, through two cartridges connected in series. Considering 60 mg of sorbent, the breakthrough volumes of AMP, MAMP, BE and MOR remained below 500 mL. On the other hand, at least 1 L volume samples could be concentrated using the 200 mg cartridges (data not given), which were selected to continue with the study.

Finally, different solvents and volumes were considered for the elution step, bearing in mind that aprotic polar solvents are the best suited to perform the further silylation reaction [30]. All compounds could be recovered using just 5 mL of ethyl acetate or acetonitrile. The only exception was THCCOOH. This species showed a low affinity to both solvents, requiring elution volumes over 10 mL. On the other hand, THCCOOH was completely eluted with 6 mL of acetone, but the trimethylsilyl derivatives of the amphetamines could not be found in the extract after derivatisation. The same happened when a standard of amphetamines in acetone was mixed with MSTFA. Likely, there is a reaction between the amino group of these compounds and the carbonyl moiety of the solvent, which prevents their further silylation with MSTFA. In view of these results, a two-step elution strategy was adopted: first, amphetamines were eluted with 2 mL of ethyl acetate and, subsequently, the remaining compounds were recovered in a separated fraction with 8 mL of acetone. This second eluate was evaporated to dryness prior to its combination with the first one, avoiding by this way the contact between amphetamines and acetone.

3.4. Analytes stability

In order to achieve accurate results in the determination of drugs of abuse in the aqueous environment, it is essential to evaluate the stability of these compounds in water samples. In this context, Castiglioni et al. [16] investigated the stability of some illicit drugs and their metabolites in raw wastewater (stored in the dark at 4 °C for 3 days) and found a substantial decrease in the concentrations of COC and COE, accompanied by a parallel upsurge in the amount of their metabolite BE. The same pattern was observed for MOR,

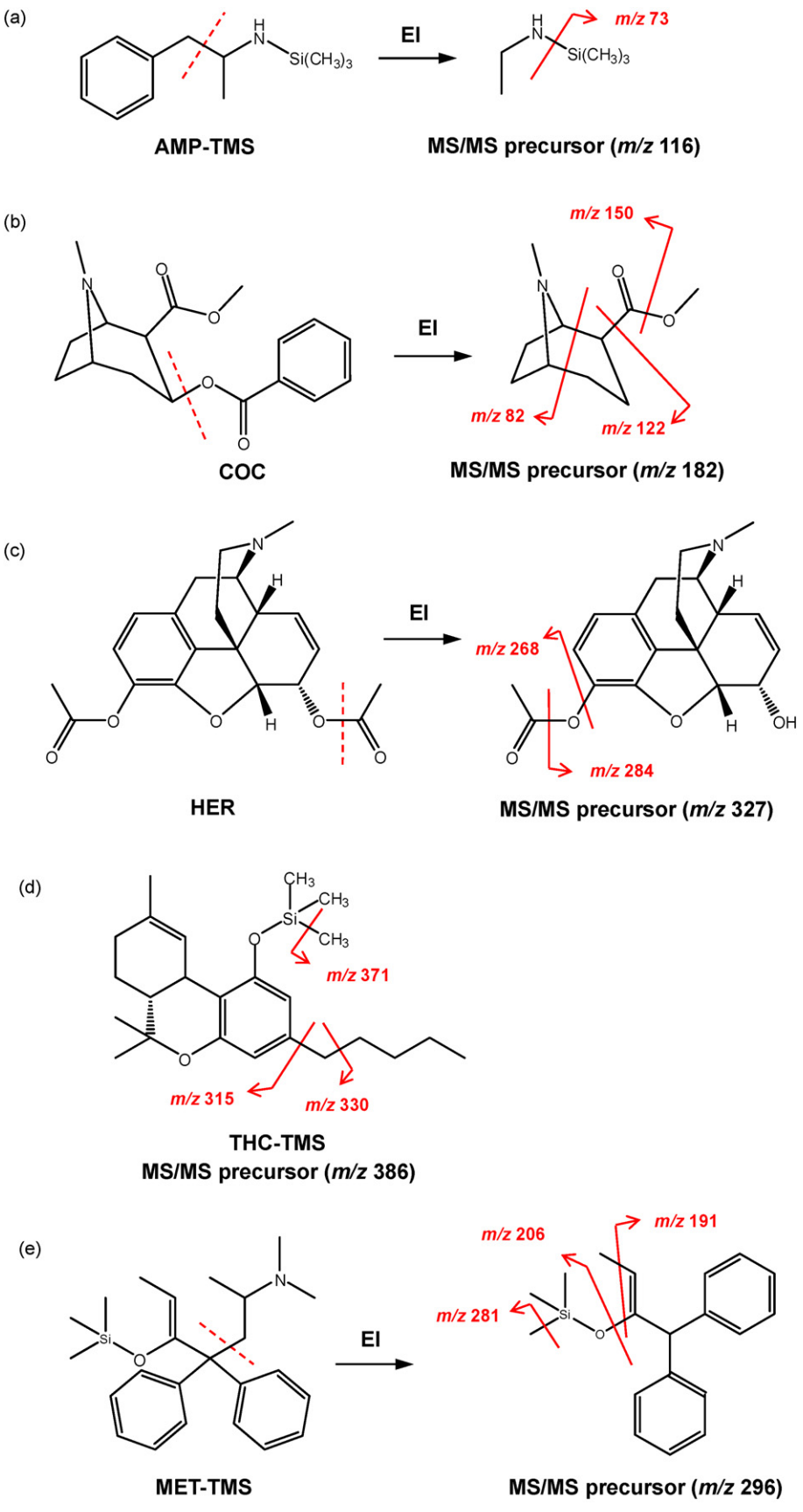


Fig. 2. EI-MS(/MS) fragmentation pathways for five representative analytes; TMS means trimethylsilyl.

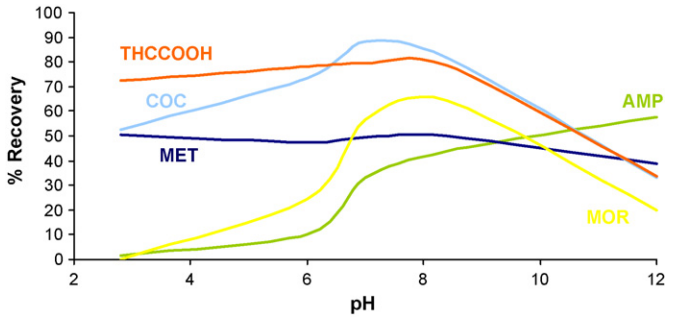


Fig. 3. Influence of the pH on the SPE extraction efficiency with Oasis HLB 60 mg cartridges ($n = 2$).

whose level was increased as a consequence of the degradation of other opioids. Gheorghe et al. [15] proved that acidification of water samples (pH 2) improved the stability of COC and its metabolite, BE. However, that report did not consider other drug classes and, also, acidified samples would need to be basified again before SPE, leading to a cumbersome procedure, so that was discarded in our study.

Instead, the preservative agent NaN_3 was considered in this research. To this end, raw wastewater was filtered through a combination of glass fibre prefilters and 0.45 μm nitrocellulose filters, spiked with target compounds at 100 ng/mL and divided in two fractions. One of them was then poisoned with NaN_3 (0.2%) and both groups of samples were stored in the dark at 4 °C. Every 2 days, an aliquot was submitted to the above described sample preparation procedure and analyzed. Amphetamines and COD did not undergo any apparent degradation either in presence or in absence of NaN_3. On the contrary, MET concentration decreased severely in the sample without azide (Fig. 4a), whereas it presented a better stability in the poisoned one (Fig. 4b). A high concentration diminution was also detected in the case of COC, COE, THC and THCCOOH (Fig. 4a), whose degradation was slowed down but not completely stopped by NaN_3 (Fig. 4b). HER levels fell down remarkably in both samples and, finally, MOR and BE concentrations experimented an increase (mainly in the not poisoned sample) as a consequence of the degradation of COD and HER, and COC and COE, respectively. In view of these results, the possibility of extracting the samples as soon as received, followed by storage of the frozen SPE cartridges was considered. In order to assess the feasibility of this approach, aliquots of the same raw wastewater were spiked with target analytes, processed immediately and the dried SPE cartridges were then kept at −20 °C for different periods: 1–3 weeks (typical analysis time) and additionally after 12 weeks. Fig. 4c shows the results (average values for duplicate assays) obtained as function of storage time. Within the first 3 months an acceptable stability was observed

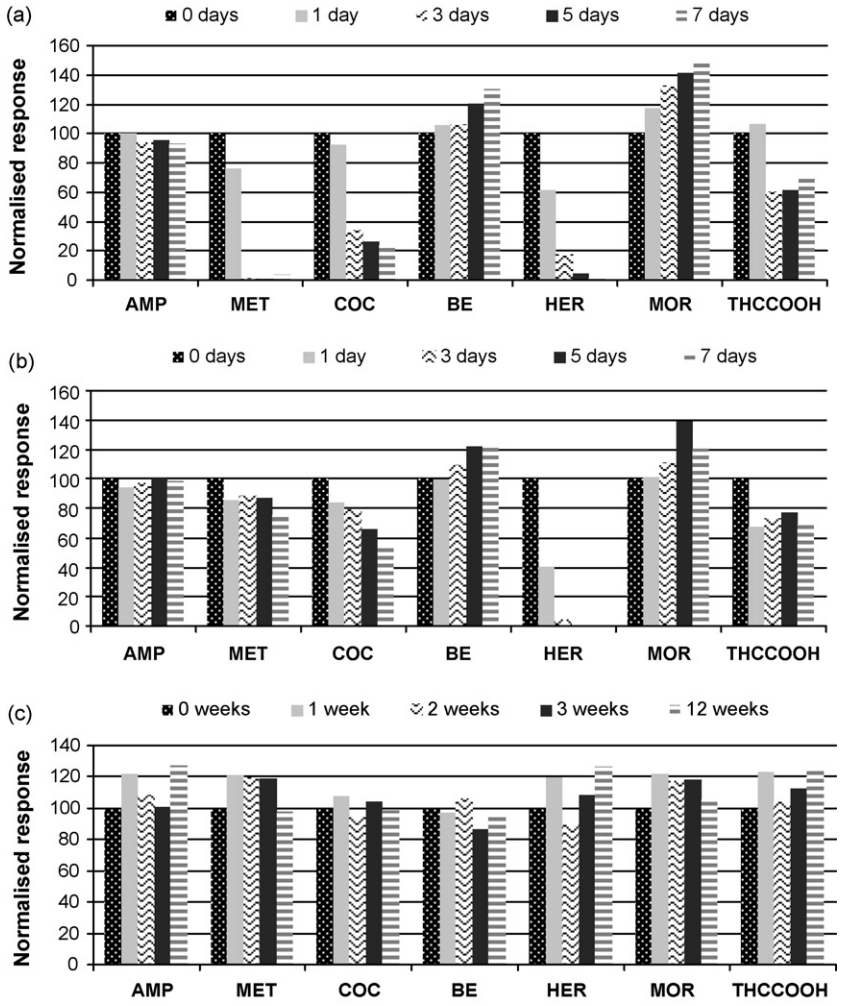


Fig. 4. Stability data, mean of two replicates, for some of the illicit drugs involved in this research: (a) wastewater stored at 4 °C; (b) wastewater stored at 4 °C after addition of NaN_3 (0.2%); and (c) aliquots of the same matrix concentrated on Oasis HLB SPE cartridges, which were dried and stored at −20 °C.

Table 3
Instrumental performance, overall internal standard-corrected recoveries ($n = 4$) and LODs for all the compounds.

Compound	IS	GC–MS/MS			SPE–GC–MS/MS					
		R^2 [a]	%RSD[b]	LOD (pg)[c]	%Recovery (%RSD)			LOD (ng/L)[c]		
					River water[d]	Treated wastewater[e]	Raw wastewater[f]	River water	Treated wastewater	Raw wastewater
AMP	AMP-d6	0.9981	1.7	1.6	112.1 (2.1)	102.7 (3.5)	106.4 (4.5)	0.8	3	7
MAMP	MAMP-d5	0.9993	5.3	2.9	110.0 (1.2)	99.2 (3.4)	119.2 (3.9)	2	7	7
MDA	MDA-d5	0.9958	1.7	4.5	124.7 (6.2)	107.4 (7.6)	103.9 (10.2)	2	5	7
MDMA	MDMA-d5	0.9996	4.6	5.9	119.1 (1.7)	85.4 (6.6)	137.2 (2.5)	2	6	8
MDEA	MDEA-d5	0.9980	2.0	4.8	107.1 (1.2)	93.2 (2.1)	135.9 (8.7)	2	11	12
MET	MET-d3	0.9943	2.3	7.2	121.1 (4.4)	114.1 (3.2)	134.2 (3.2)	2	6	6
COE	COC-d3	0.9911	9.1	34.5	98.7 (7.1)	107.0 (19.0)	98.4 (8.0)	3	6	6
COC	COC-d3	0.9976	7.7	2.8	100.9 (7.9)	115.8 (7.2)	100.9 (10.2)	1	3	12
BE	BE-d3	0.9910	10.0	2.1	120.4 (20.0)	125.1 (5.7)	124.1 (9.4)	4	4	8
THC	THC-d3	0.9972	1.2	0.7	96.8 (18.6)	108.4 (19.4)	106.7 (3.4)	0.9	3	3
COD	COD-d3	0.9960	3.8	2.4	96.9 (12.5)	112.9 (10.0)	63.4 (10.8)	1	4	6
MOR	MOR-d3	0.9990	3.8	4.1	91.7 (8.1)	134.2 (17.7)	68.8 (6.7)	3	11	11
HER	MOR-d3	0.9900	17.6	34.5	73.9 (7.7)	107.9 (25.0)	97.5 (16.3)	13	15	15
THCCOOH	THCCOOH-d3	0.9968	3.4	0.1	93.3 (13.6)	96.7 (5.0)	66.6 (4.9)	1	1	1

[a] 5–500 ng/mL calibration.
[b] Five injections of a 50 ng/mL standard in a 48 h period.
[c] S/N = 3.
[d] SPE of 500 mL samples spiked at 100 ng/L level ($n = 4$).
[e] SPE of 200 mL samples spiked at 250 ng/L level ($n = 4$).
[f] SPE of 100 mL samples spiked at 500 ng/L level ($n = 4$).

for all species (Fig. 4c). Consequently, this procedure was selected as the best alternative to avoid analytes' degradation.

Finally, the stability of the silylated compounds was also evaluated. Four replicates standards of 200 ng/mL of all analytes were prepared in ethyl acetate, derivatised with 50% MSTFA (heating at 80 °C during 60 min) and stored at −20 °C for 1, 2, 4 and 7 days. Then, their GC–MS/MS signals were compared to the ones obtained with a fresh derivatised standard of the same concentration. No significant variations were observed, proving that silylated drugs are stable at −20 °C for, at least, 1 week (data not shown).

3.5. Method performance

Table 3 summarises some data related to the performance of the method. Linearity was investigated by injection of standards solutions at six different concentration levels between 5 and 500 ng/mL. The R^2 values for the corresponding graphs varied from 0.9900 to 0.9996. Instrumental precision studies were carried out by five injections of the same standard (50 ng/mL level) over a 48 h period, resulting in relative standard deviations (% RSD) between 1.7 and 17.6%. Absolute limits of detection of the GC–MS/MS method (S/N = 3) ranged from 0.1 pg (for THCCOOH) to 34.5 pg (for COE and HER).

Recoveries of the whole procedure were evaluated with spiked aliquots (from 100 to 500 mL) of different environmental water samples: river water (100 ng/L), treated (250 ng/L) and raw (500 ng/L) wastewater. A chromatogram of a spiked river is presented in Fig. 5. The obtained recoveries ranged from 73.9 to 124.7% in the first matrix, between 85.4 and 134.2% in the second one and between 63.4 and 137.2 in raw wastewater. Finally, the estimated LODs of the whole method varied from 0.6 to

Table 4
Concentrations ($n = 4$) found in different surface water and municipal wastewater samples. None of the analytes was found above the LODs in River Lengüelle and Dos Pasos Creek.

Mean concentration in ng/L (%RSD)										
Compound	Sar River [a]	Treated STP-A[b]	Raw STP-A[c]	Treated STP-B[b]	Raw STP-B[c]	Treated STP-C[b]	Raw STP-C[c]	Treated STP-D[b]	Raw STP-D[c]	Treated STP-E[b]
AMP	n.d.[d]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MAMP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MDA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MDMA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MDEA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MET	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
COE	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
COC	30 (11)	43 (25)	104 (21)	n.d.	472 (10)	n.d.	39 (22)	n.d.	37.2 (7)	61 (26)
BE	316 (11)	653 (18)	571 (10)	122 (11)	2153 (8)	n.d.	866 (9)	164 (7)	36 (7)	689 (8)
THC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	23 (7)	n.d.	n.d.	n.d.
COD	149 (49)	n.d.	115 (26)	129 (8)	536 (15)	n.d.	168 (8)	426 (32)	n.d.	n.d.
MOR	89 (3)	140 (10)	182 (9)	103 (10)	194 (4)	n.d.	101 (6)	76 (3)	69 (14)	90 (11)
HER	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
THCCOOH	31 (5)	77 (9)	148 (6)	n.d.	74 (7)	n.d.	401 (7)	49 (3)	36 (6)	13 (6)

[a] SPE of 500 mL samples ($n = 4$).
[b] SPE of 200 mL samples ($n = 4$).
[c] SPE of 100 mL samples ($n = 4$).
[d] n.d.: not detected (<LOD).

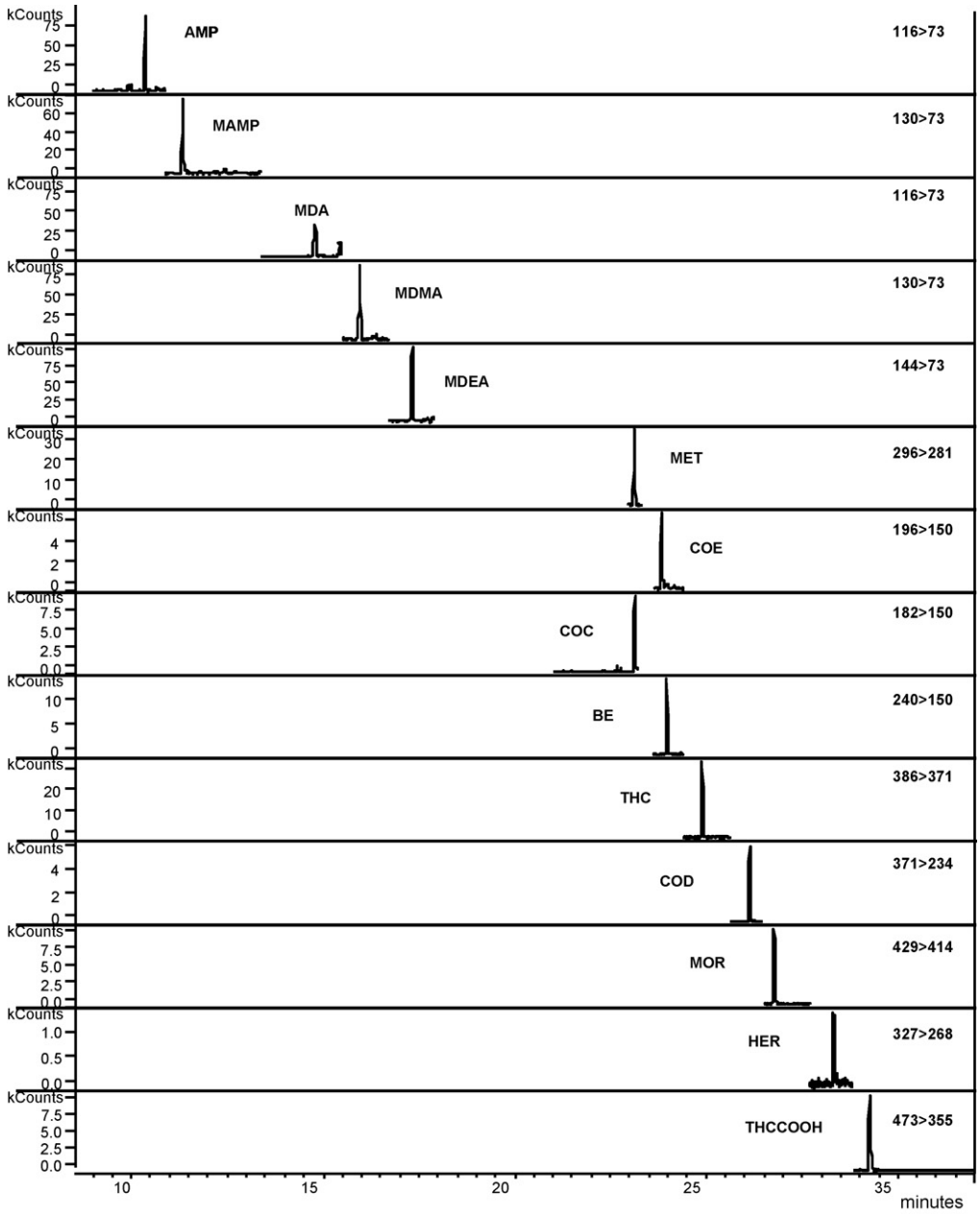


Fig. 5. Chromatogram of a river water sample spiked with 100 ng/L of each compound.

18.2 ng/L (Table 3). These values were calculated considering the signal to noise (S/N) ratios of chromatographic peaks for target compounds in the extracts from the above-mentioned samples, and the volume of each matrix submitted to the SPE step. In the same manner, LOQs (S/N = 10) ranged between 2 and 60 ng/L (not given in Table 3, but they can be calculated as 3.3 times the LODs).

3.6. Application to real samples

The developed method was applied to determine the levels of the selected illicit drugs in waters from 3 rivers and 5 different STPs in the northwest of Spain. Grab samples were taken in each of these locations, without considering the residence time of the plants. Fig. 6 shows the chromatogram of one of the raw wastewater samples and the MS/MS spectrum of COC as compared to that of a pure standard. From this comparison it is evident that one of the advantages of ion trap-MS/MS systems is their capability to provide unambiguous confirmation of positive samples.

As shown in Table 4, COC, BE, COD, MOR and THCCOOH were found in most samples, whereas the rest of the compounds normally remained below the LOD. Usually, BE, the metabolite of COC, was the species at higher concentrations (up to 2 ng/mL) in the samples. This finding matches with the data reported by other authors [6,12,14,16,19] and, highlights the widespread consumption of illicit drugs in developed countries.

Although results obtained for grab sewage water are useless to evaluate the efficiency of STPs, the presence of some of the investigate drugs of abuse in one of the processed river samples confirms their capability to reach surface water sources and to migrate into the aquatic environment.

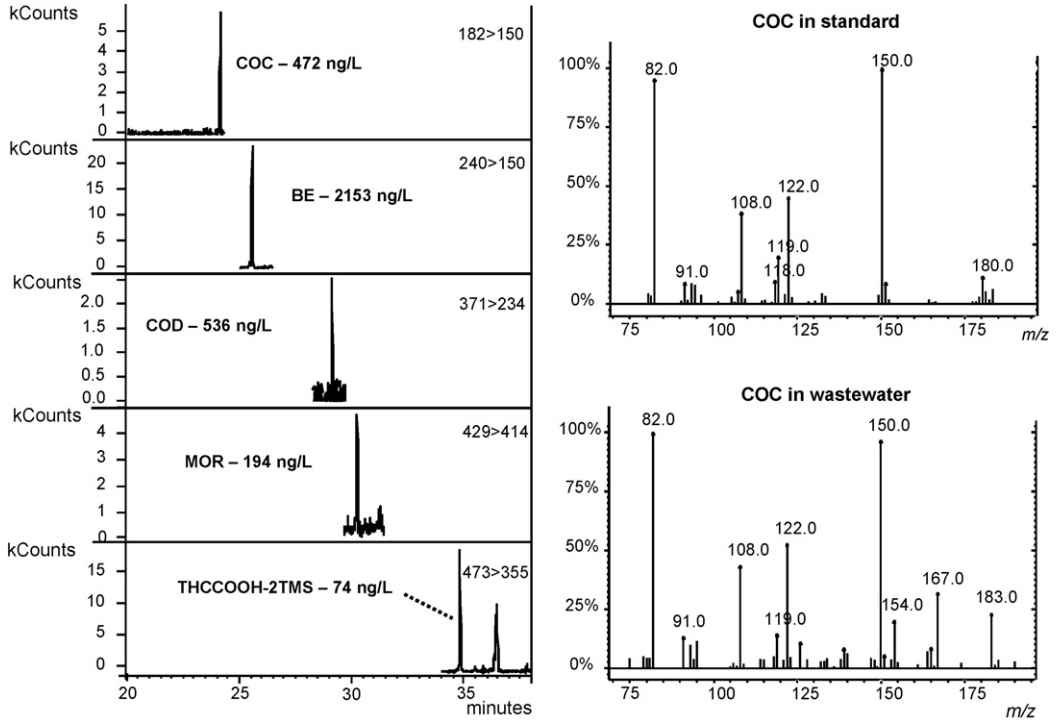


Fig. 6. Chromatogram of a (non-spiked) raw wastewater sample and MS/MS spectra corresponding to the peak of cocaine in the sample and in a standard.

4. Conclusions

A method for the determination of drugs of abuse in water samples by GC–MS/MS has been developed for the first time. Samples were extracted by SPE and the SPE cartridges, loaded with the analytes, can then be stored at −20 °C for at least 12 weeks, avoiding the problems of drugs degradation in the sample. After extraction, elution was performed sequentially with two solvents and the concentrated extract was derivatised by silylation with MSTFA. Once optimised, this reaction was capable of derivatising the whole set of analytes considered and the SPE–GC–MS/MS method provided recoveries (74–134%) and LODs (0.8–15 ng/L) similar to those reported by SPE–LC–MS/MS, but at a lower cost and without the inconvenient of matrix effects reported with the last methodology. A further advantage of the ion trap GC–MS/MS instrument is its capability to record full scan MS/MS spectra, which can be used for the unequivocal confirmation of positives. On the other hand, the main drawbacks of the method are that it is not suitable for the analysis of the main metabolite of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), which is a quaternary amine, and, to a minor extent, the fact the method is slower than those procedures based on LC–MS, as it requires 90 min for derivatisation of the analytes.

The final application of the method to grab surface water and sewage samples confirmed COC, BE, THCOOH, MOR and COD as common emerging contaminants entering the aquatic environment.

Acknowledgements

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