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Simultaneous determination of drugs of abuse and their main metabolites using pressurized liquid extraction and liquid chromatography–tandem mass spectrometry

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ABSTRACT

An analytical method based on pressurized liquid extraction (PLE) and liquid chromatography–(electrospray)–tandem mass spectrometry was developed for the simultaneous determination of nicotine, four drugs of abuse (opiates and alkaloids) and four of their main metabolites in sewage sludge. The optimum PLE conditions were: cell volume 11 mL, dichloromethane as extraction solvent, 5 min preheating time, 100 °C temperature, 1500 psi pressure, 60% flush volume, 1 cycle, 15 min static extraction time, 120 s purge time and sample weight 1 g. Absolute recoveries for all compounds were between 25% and 65%. Data acquisition was done by selective reaction monitoring and the two most abundant product ions were used for confirmation. Limits of detection were lower than 10 μg/kg dry weight (d.w.) and limits of quantification were between 2.5 and 25 μg/kg (d.w.).

The highest concentrations found in sludge samples from two sewage treatment plants were for nicotine and cocaine in the range of 23–173 μg/kg (d.w.) and 9–232 μg/kg (d.w.) respectively.

1. Introduction

In the last few years the determination of drugs of abuse and their metabolites in the aquatic environment has attracted considerable attention, and the measured analyte concentration has also been used to back-calculate drug usage in local communities [1]. As is well known, drugs of abuse may undergo partial degradation in the human organism and can be excreted through urine into sewage as an intact form or as metabolites. They are then released to the sewage treatment plants (STPs), where they are partially removed via degradation or sorption into the sludge.

Although there are several studies in which drugs of abuse have been determined in influent and effluent sewage [2,3], surface water [1,4] and even air samples [5–7], less attention has been devoted to their determination in sludge, which is generated during the treatment process in the STPs. The presence of these contaminants in sludge may limit its re-use due to public health and environmental protection requirements, although they are not included in current legislation.

Recent studies [8,9] related to the presence of these contaminants in sewage include their determination not only in sewage but also in sewage-suspended particulate matter (SPM), and some of these contaminants such as cocaine and its metabolite benzoylecgonine are definitively found in SPM. This confirms that some of these contaminants tend to be retained in particulate matter and hence they can also be found in sludge, although drugs of abuse are relatively polar and not expected to be much retained in the sludge.

Therefore only a few studies evaluate the presence of drugs of abuse in sludge, and most of these studies only a limited number of drugs. Kaleta et al. [10], for instance, study the presence of amphetamine in sewage sludge, Langford et al. [11] develop a multiresidue method for sludge samples in which some drugs of abuse are included although none were found in the samples, and Jones-Lepp et al. [12] determined metamphetamine in sludge. Very recently, Mastroianni et al. [13] developed a method to determine 20 drugs of abuse (cocainics, amphetamines, opioids, benzodiazepines, LSD and cannabinoids) and 16 compounds were found in sludge samples at concentrations from 0.4 to 579 ng/g of dry weight (d.w.).

Several extraction techniques have been applied to extract drugs of abuse from sludge [10–12], the most popular being pressurized liquid extraction (PLE) and ultrasonic solvent extraction (USE). In some cases solid-phase extraction (SPE) is further applied as a clean-up technique. For instance USE followed by SPE has been used to determine amphetamine [10] and PLE has been applied to determine methamphetamine [12] and a group of various drugs [11]. As regards SPM, once the sample is filtered, the suspended matter is extracted in a similar way to sludge, with PLE [8,9,14] being the most used technique.

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After extraction, liquid chromatography–tandem mass spectrometry (LC–MS/MS) is usually applied [15,16], although gas chromatography tandem mass spectrometry (GC–MS/MS) after derivatization has also been used [17]. As regards LC–MS/MS, ultra-high performance liquid chromatography (UHPLC) is increasingly used [8,11] and different analyzers have been used to determine these compounds in water, SPM and sludge samples, with triple quadrupole (QqQ) being the most frequent [4,18,19], although quadrupole-linear trap (QTRAP) [20,21] and quadrupole-time of flight (QTOF) [22] have also been used.

The aim of this study is to develop an analytical method for the simultaneous determination of nicotine, four drugs of abuse (morphine, codeine, cocaine and methadone) and some of their main metabolites (2-ethylidene-1,5 dimethyl-3,3-diphenylpyrrolidine, dihydrocodeine, 6-acetylmorphine and benzoylecgonine) in sewage sludge. Although nicotine is a legal drug, it was included in this study due to the high consumption of tobacco worldwide. The method is based on PLE and liquid chromatography–(electrospray)−tandem mass spectrometry (LC–(ESI)MS/MS) and was applied to determine these drugs of abuse in several sludge samples from two STPs.

2. Experimental

2.1. Reagents and standards

Standards of nicotine (NIC), codeine (COD), dihydrocodeine (DIC), 6-acetylmorphine (6-AM), cocaine (COC), benzoylecgonine (BE), 2-ethylidene-1,5 dimethyl-3,3-diphenylpyrrolidine (EDDP), methadone (MTD), morphine (MOR), 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) and the surrogates EDDP-d_3, COD-d_6 and MOR-d_6 were acquired from Cerilliant (Round Rock, TX, USA), available as solution in 1 mL of methanol or acetonitrile at 1000 mg/L. Stock solutions of individual standards were prepared by diluting each compound in methanol at 100 mg/L and storing them at −20 °C in the dark. A mixture of all compounds in methanol:water (1:1 v/v) at 100 μg/L was prepared weekly. Working solutions were prepared daily by appropriate dilution of this solution with methanol:water (1:1 v/v).

Ultra pure water was obtained using a purelab ultra purification system (Veolia water, Sant Cugat del Vallés, Spain). The acetonitrile, acetone, dichloromethane and methanol (HPLC-grade) came from SDS (Peypin, France), the nitrogen from Carburos Metálicos (Tarragona, Spain) and the acetic acid from Prolabo (Bois, France). Diatomaceous earth was supplied by Sigma-Aldrich (St. Louis, USA).

2.2. Sample pretreatment

Sewage sludge samples were collected from two urban STPs in two cities of about 130,000 inhabitants located in southern Catalonia. They were then homogenized, frozen and lyophilized using the freeze-dry system (Labconco, Kansas City, MO, USA). The lyophilized samples were homogenized by mortar and pestle, sieved through a 125 μm screen and stored at room temperature.

To optimize the method, the pretreated sludge samples were spiked with the analytes dissolved in acetone, and then the solvent was evaporated at room temperature while submitted to vigorous shaking. The volume of acetone was enough to completely cover the sludge.

2.3. Pressurized liquid extraction

Pretreated sludge samples were extracted by PLE using an ASE 200 (Dionex, Sunnyvale, CA, USA). Deuterated compounds (MOR-d_3, COD-d_6 and EDDP-d_3) at a concentration of 125 μg/kg (d.w.) were added to 1 g of pretreated sludge. This was then thoroughly mixed with 2 g of diatomaceous earth to remove possible traces of water from the sludge and maximize effectiveness in the extraction process, especially in the case of solvents immiscible with water. The mixture was then transferred to the inside of the extraction cell and a Whatman glass fiber filter (Ahlstrom, PA, USA) was put at the top and bottom of the cell.

The extraction solvent was dichloromethane and the operating conditions were: preheating period 5 min, extraction temperature 100 °C, extraction pressure 1500 psi with a static period of 15 min in one cycle, flush volume 60% of cell volume and nitrogen purge time 120 s. The final extraction volume was approximately 15 mL, which was evaporated to dryness under a nitrogen stream and redissolved in 5 mL of methanol:water (1:1 v/v), filtered with a microfilter of 0.20 μm (Teknokroma, Barcelona, Spain), and then analyzed by LC–MS/MS.

2.4. Liquid chromatography–tandem mass spectrometry (LC–MS/MS)

The determination was performed using an HP 1200 series liquid chromatography–triple quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany) with electrospray ionization (ESI). The chromatographic column was a fused core Ascentis Express C_{18} (4.6 × 50 mm) with a 2.7 μm particle size (Sigma-Aldrich, Madrid, Spain) and the volume injected was 50 μL. The flow-rate was 0.4 mL/min and the column temperature was kept at 30 °C.

A binary mobile phase with a gradient elution was used. Solvent A was acidified water with acetic acid (pH 2.8) and solvent B was acetonitrile. The gradient was initially 5% B, which was increased to 15% in 3.5 min, to 50% in 2.5 min, to 100% in 6 min, kept constant for 2 min and finally returned to 5% B in 1 min. All the compounds were eluted within 13 min.

The optimized conditions for the ESI interface in positive mode were: 45 psi nebulizer pressure, 12 L/min drying gas flow-rate, 350 °C drying gas temperature and 3000 V spray potential. Cone voltage values and collision energy voltages were within 60–140 V and 15–60 V respectively. Two selective reaction monitoring (SRM) transitions for each compound were monitored and the values are shown in Table 1. The most abundant transition was used for quantification, while the other was used for confirmation. Relative ion intensities and retention time were also used as confirmation criteria.

3. Results and discussion

3.1. LC–(ESI)MS/M

Chromatographic separation was performed on a C_{18} column based on fused-core particle technology, which provides more than twice the speed and efficiency of traditional columns at half the backpressure of sub-2-μm columns [23]. The binary gradient elution enabled the ten compounds to be separated in 13 min.

LC–(ESI)MS/MS conditions were based on a previous paper by Pedrouzo [24], in which these compounds were determined in water samples. EDDP-d3, COD-d6 and MOR-d6 were used as surrogates to compensate the adverse ion suppression given in ESI. The surrogate used for each compound was chosen taking into account similar structure, proximity in retention time and similar behavior with regard to ion supression.

Due to the positive charge provided by the amino group present in the analyte's structure, the compounds were analyzed in positive ionization mode producing abundant $[M+H]^+$ ion.

Table 1
SRM conditions and ions selected for determination of drugs of abuse.

Compound	Precursor ion	Product ions		Cone Voltage (V)	Collision energy (eV)
		Quantification	Confirmation		
Nicotine (NIC)	163	130	117	60	15
Morphine (MOR)	286	152	128	100	60
Morphine-d6 (MOR-d6)	292	152	128	60	60
Dihidrocodeine (DIH)	302	141	165	100	60
Codeine (COD)	300	165	181	100	60
Codeine-d6 (COD-d6)	306	165	153	100	45
6-acetylmorphine (6-AM)	328	165	181	100	45
Benzoylecgonine (BE)	290	168	82	100	15
Cocaine (COC)	304	182	82	60	15
EDDP (EDDP)	278	234	186	140	30
EDDP-d3	281	234	249	140	30
Methadone (MET)	310	265	223	60	15

Table 2
PLE absolute recoveries ($n=3$) using different solvents. For other conditions see text.

Compound	Water	Methanol	Water:Methanol (1:1)	Dichloromethane	Acetone
NIC	–	–	–	35	29
MOR	–	–	–	10	–
DIH	20	28	23	36	30
COD	18	16	20	40	32
6-AM	19	20	16	19	16
BE	47	40	33	15	18
COC	36	32	45	50	40
EDDP	16	–	13	19	–
MET	14	–	10	16	23
THC-COOH	–	–	–	–	–

– < 10%.
%RSD ($n=3$) < 12%.

Quantification was performed under SRM mode and two product ions were used for confirmation (Table 1).

Instrumental calibration curves were obtained by injection of a standard solution at concentration levels ranging from 0.5 to 50 μg/L, except for COD and DIC (1–50 μg/L). Determination coefficients (r^2) were higher than 0.9986.

The instrumental repeatability values, expressed as RSD% ($n=5$, 10 μg/L), were lower than 5% and the reproducibility values ($n=5$, 10 μg/L) were lower than 8%. The instrumental detection (LOD) and quantification limits (LOQ) were determined by direct injection of decreasing amounts of each substance. The LODs were calculated as the concentrations where the signal-to-noise ratio (S/N) was 3 and were found between 0.01 and 0.5 μg/L; the LOQs were defined as the lowest concentration of the calibration curve and ranged from 0.5 to 1 μg/L.

3.2. PLE optimization

The lyophilized sludge was spiked with 125 μg/kg (d.w.) of each compound to perform the PLE optimization. This concentration was selected due to the high concentration of some compounds in the sample. The four parameters that have the most influence on PLE were optimized: extraction solvent, extraction temperature, static time and number of cycles. Parameters such as pressure, preheating time, flush volume and purge time are usually minor variables and were not optimized. The initial conditions were taken in accordance with our previous experience [25–27]: 1500 psi pressure, 80 °C temperature, 5 min preheating time, 5 min static extraction time, 60% flush volume, 120 s purge nitrogen, cell size 11 mL, 1 cycle and 1 g sample weight. In the PLE optimization, a non-spiked sample (blank) was analyzed to test for the presence of these compounds in the sample.

The first parameter optimized was the extraction solvent, and water, methanol, water–methanol mixture (1:1 v/v), dichloromethane and acetone were tested. Extracts with dichlorometane and acetone were evaporated to dryness under nitrogen and redissolved with 25 mL of water–methanol (1:1 v/v). Extracts of water, methanol or a mixture of the two were diluted to 25 mL with the appropriate solvent in order to obtain a solution of water-methanol (~1:1 v/v). Table 2 shows the absolute recoveries of each compound. Dichloromethane provided the best recoveries for all compounds, except BE (15%) and MET (16%). BE showed a better recovery when a polar solvent such as water (47%) was used, and MET was slightly better extracted with acetone. NIC and MOR were recovered lower than 10% with water, methanol or water: methanol (1:1 v/v). THC-COOH was barely extracted with any of the solvents tested. Recoveries with acetone were quite similar to those obtained with dichloromethane except for NIC, MOR, COD, COC and EDDP, whose recoveries were higher with dichloromethane. Although dichloromethane did not extract all compounds quantitatively, it was selected as the extracting solvent because it gave the best recoveries for most of the analytes. Previous studies have reported water–methanol adjusted to pH 2 as the optimum extracting solvent in PLE [11,14,20] for some drugs of abuse. However, some of the drugs of abuse determined in this study (alkaloids) are weak bases poorly soluble in water but readily soluble in non-polar solvents.

The second parameter optimized was temperature. Three temperatures at 60 °C, 80 °C and 100 °C were tested to improve the extraction while all other conditions were the same as the initial ones, but using dichloromethane as the extracting solvent.

As can be seen in Table 3, recoveries were similar at 60 °C and 80 °C and slightly better at 100 °C, although for some compounds such as MOR, BE and COC the recoveries did not increase significantly. Therefore temperatures higher than 100 °C were not tested, and 100 °C was selected for further optimization.

Static time was the third parameter optimized. Four static times between 5 and 20 min were tested and the recovery results are summarized in Table 3. Although there are no significant differences between the results obtained, 15 min was selected since this involves slightly higher recoveries for some compounds such as DIH and EDDP.

The last parameter optimized was the number of cycles. We tested two cycles with dichloromethane at 100 °C and 15 min of static time. Recovery values did not increase with two cycles and for this reason one cycle was selected, which decreased extraction time.

To summarize, the final conditions were: dichloromethane as extraction solvent, preheating period 5 min, extraction temperature 100 °C, extraction pressure 1500 psi with a static period of 15 min in one cycle, flush volume 60% of cell volume, nitrogen purge time 120 s and 1 g of sample (d.w.). As shown in Table 3, absolute recoveries at optimum conditions were higher than 50% for DIH, COD, NIC and COC, between 36% and 41% for EDDP, 6-AM and MET, between 17% and 25% for MOR and BE, and lower than 5% for THC-COOH.

As mentioned earlier, the PLE extract (about 15 mL) was evaporated to dryness and redissolved to 5 mL with methanol:water (1:1 v/v). The loss by evaporation was evaluated as follows: a mix of each compound at 25 μg/L was dissolved in dichloromethane, subsequently evaporated under N_2 stream and reconstituted with water:methanol (1:1 v/v). The response was compared with the signal of each compound at 25 μg/L dissolved in water:methanol (1:1 v/v) without being subjected to evaporation. Loss by evaporation was not observed for any compound.

To exclude any possible causes of the low recoveries of some compounds, especially THC-COOH, the retention of the compounds in both nylon and polytetrafluoroethylene (PTFE) membrane filters was evaluated by comparing the responses of unfiltered and filtered standard solution. Strong retention of THC-COOH (85%) was observed when using a nylon filter, which was used for PLE optimization (Tables 2 and 3), but this was substituted by PTFE since retention of THC-COOH in this filter was negligible.

The matrix effect was then studied by comparing the responses of analytes in spiked sludge samples after PLE extraction with the areas obtained with a direct injection of a standard solution in water:methanol (1:1 v/v) at a concentration of 25 μg/L. As can be seen in Table 4, the compounds least affected by ion suppression were COC, NIC, COD, DIH and 6-AM, with an ion suppression between 15% and 35%. The signal was suppressed by 53–61% for all other compounds except THC-COOH, whose signal was suppressed by 91%. THC-COOH was then excluded from the study.

Relative recovery percentages (Table 4) for sample spiked at 125 μg/kg (d.w.) were calculated with calibration curves generated by spiking blank extracts of PLE with different amounts of each compound between 0.25 and 50 μg/L. Additional experiments at lower concentration (25 μg/kg (d.w.)) were done for those compounds present at low concentration in the sample and similar recoveries were obtained. Three isotopically labeled standards (MOR-d6, COD-d6 and EDDP-d3) were used as surrogate to minimize matrix effects and compensate variations occurring during sample preparation, the deuterated compound assigned to each compound was selected according to three criteria: close elution, similar recoveries and similar behavior in the source. Recoveries of each deuterated compound were similar to those corresponding to their analog compounds. Thus recovery of MOR-d_6, COD-d6 and EDDP-d3 were 20%, 68% and 38% respectively.

3.3. Method validation

To validate the method calibration curves were generated by spiking sludge samples before PLE with 125 μg/kg (d.w.) of each surrogate and different amounts of each compound between 2.5 μg/kg (d.w.) and 500 μg/kg (d.w.).The value of signal generated

Table 3
Absolute recoveries ($n=3$) using differents temperatures, extraction times and numer of cycles.

Compound	1 cycle. 5 min			100 °C. 1 cycle			15 min. 100 °C
	60 °C	80 °C	100 °C	10 min	15 min	20 min	2 cycles
NIC	30	35	42	42	50	48	53
MOR	11	10	14	20	17	16	15
DIH	41	36	50	46	61	56	66
COD	38	41	53	58	63	65	64
6-AM	22	19	34	41	40	43	29
BE	12	15	17	13	25	30	25
COC	55	50	57	78	65	69	72
EDDP	19	19	28	24	36	19	26
MET	12	16	30	27	41	41	38
THC-COOH	11	–	–	–	–	10	–

– < 10%.
%RSD ($n=3$) < 9%.

Table 4
Method validation parameters.

Compound	Relative Recovery (%)	Linear range (μg/kg)	LOD (μg/kg)	%RSD[a]	%RSD[b]	Ion suppression (%)	SR_2/SR_1 spiked sample
NIC	77	5–500	2	14	20	35	40
MOR	44	25–500	10	7	16	61	19
DIH	84	10–500	5	12	19	27	87
COD	81	5–500	1	11	20	22	26
6-AM	59	5–500	1	7	18	32	28
BE	71	2.5–500	0.5	11	12	65	5
COC	76	2.5–500	0.5	8	14	15	7
EDDP	77	10–500	2.5	10	12	53	20
MET	95	2.5–500	0.5	12	17	57	18
THC-COOH	–	–	–	–	–	91	–

– Not calculated.
SR_2/SR_2 relative ion intensities (%).
[a] Repeatability ($n=5$).
[b] Reproducibility between days ($n=5$).

by each compound present in the sample blank (NIC, COD, BE, COC, EDDP and MET) was subtracted to each point of the matrix-matched calibration curve. Determination coefficients (r^2) were higher than 0.994.

The labeled surrogate MOR-d_6 was used to quantify its corresponding analog MOR; the surrogate COD-d_6 was used to quantify NIC, DIH, COD and COC; and lastly EDDP-d_3, was used to quantify EDDP, BE, 6-AM and MET. Table 4 shows the validation data obtained for the whole method. It can be seen that the use of deuterated compounds and matrix-matched calibration enables a good level of quantification.

Repeatability and reproducibility were evaluated by five replicate extractions of spiked sludge at a concentration of 125 μg/kg (d.w.) for NIC and EDDP and 25 μg/kg (d.w.) for the rest of compounds, injected the same day and different days, respectively. The repeatability RSD% values were lower than 14% and the reproducibility values were lower than 20%. Detection limits (LODs) were calculated by analyzing a sludge sample spiked with decreasing concentrations of each compound. When the compounds were not found in the blank sample, the LODs were calculated as the concentrations giving peaks for which the signal-to-noise ratio was 3; when analytes were present in the sample, the LODs were estimated as the concentrations giving a signal average of plus three times the standard deviation of the blank signal. For all compounds the LODs were between 0.5 and 10 μg/kg (d.w.). For some compounds, such as COC and BE, the LODs (0.5 μg/kg) were lower than those reported by Langford et al. [11] (20 μg/kg for COC and 5 μg/kg for BE), the extraction for which was carried out using PLE and determination by LC–(ESI)–MS/MS. LOQs were defined as the concentrations of the

Table 5
Results in μg/kg (d.w) of sewage sludge samples analyzed.

Compound	Sample 1		Sample 2		Sample 3		Sample 4	
NIC	23.9	(46)	120	(37)	51.8	(39)	173.1	(45)
COD	< LOQ	(28)	< LOQ	(21)	7.9	(31)	< LOQ	(24)
BE	3.5	(6)	9.4	(6)	15.6	(5)	19	(4)
COC	4.7	(6)	3.9	(6)	4.2	(7)	3.8	(6)
EDDP	9.7	(25)	232.2	(21)	144.3	(24)	113.9	(18)
MET	7.7	(23)	31.8	(13)	14.7	(16)	21.3	(18)

% RSD ($n=3$) < 16.
Relative ion intensities in brackets (%).

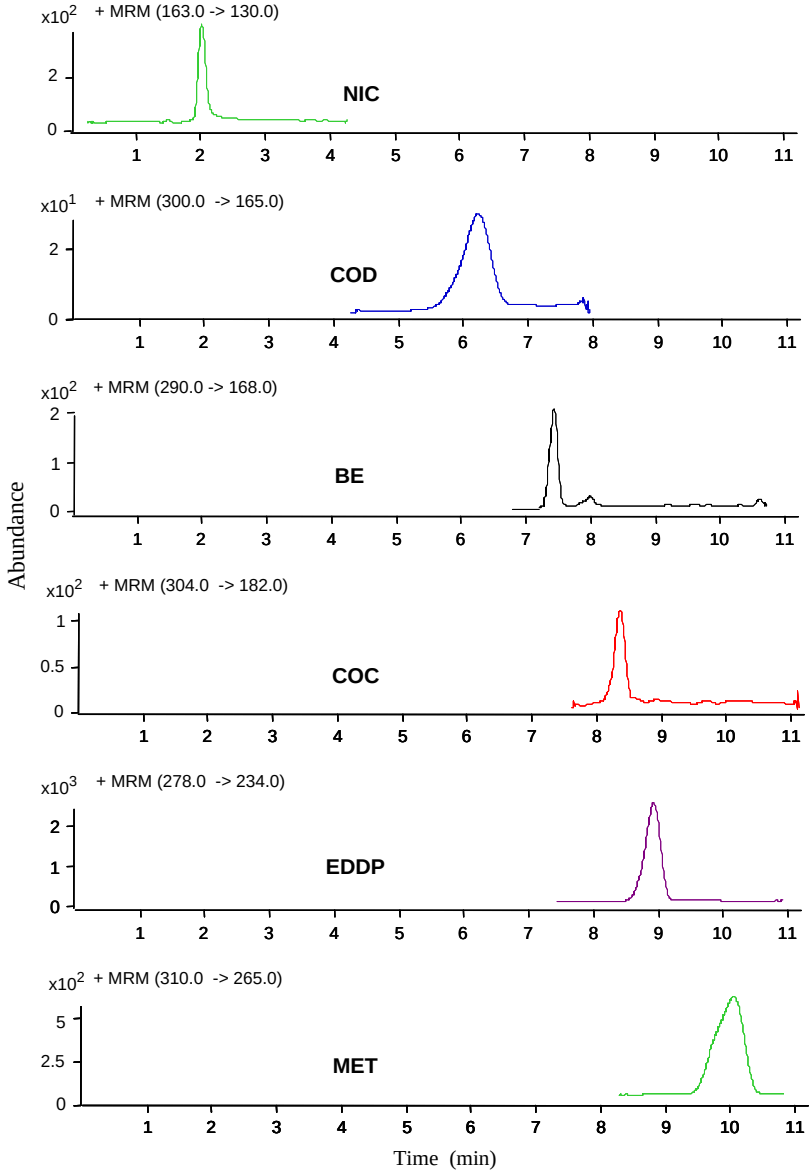

Fig. 1. SRM chromatogram obtained by PLE/LC–MS/MS. For experimental conditions see text.

lowest point of the calibration curve and were between 2.5 and 25 μg/kg (d.w.).

3.4. Method application

The method was used to analyze different sewage sludge samples collected from two different treatment plants in the Tarragona region in Spain. The identification and confirmation criteria for each compound were based on retention time, presence of two transitions, and relative ion intensities between the signal (peak height) of the qualifier ion (SRM_2) and the quantifier ion (SRM_1). Table 4 also shows relative ion intensities of a sludge sample spiked at a concentration of 125 μg/kg (d.w.). Thus the maximum permitted tolerances obtained under the guidelines of Commission Decision 2002/657/EC [28] to confirm the presence of these compounds in the sample were ±20% for DIH, ±25% for NIC, COD and 6-AM, ±30% for MOR, EDDP and MET, and finally ±50% for BE and COC. The values of concentration and relative ion intensities of the positive results are shown in Table 5. MOR, DIH and 6-AM were not detected in any sample. For the other compounds detected, relative ion intensities were within the limits permitted by Commission Decision 2002/657/EC [28].

The highest levels in both STPs were observed for NIC and EDDP, with maximum values of 173 μg/kg (d.w) and 232 μg/kg (d.w) respectively. High values are expected for the stimulatory non-controlled drug nicotine due to the large, widespread consumption of tobacco. MET was found at lower concentrations (31.8 μg/kg (d.w)) compared to its principal metabolite EDDP (232.2 μg/kg (d.w)). According to the literature, 20–60% of the MET dose is excreted in urine in 24 h, with up to 33% as the uncharged drug and up to 43% as EDDP [29]. A similar metabolism and excretion pattern occurs when COC is consumed [30]. COC is extensively metabolized to its major metabolite BE. Hence COC is excreted without change in the urine at around 1–14% and BE is excreted at around 45% [30]. Our results showed lower but still significant levels of COC and its metabolite BE at concentrations ranging from 3.8 to 4.7 μg/kg (d.w) and 3.5 to 19 μg/kg (d.w) respectively. However, the degradation process during sewage treatment also needs to be taken into account. COD was found at below the LOQ in all samples except one, where it was found at 7.9 μg/kg (d.w). Fig. 1 is an SRM chromatogram of the compounds present in sewage sludge sample number 3. This shows that six of the nine drugs of abuse monitored were determined. Their respective concentrations are shown in Table 5.

The occurrence of these contaminants in sludge depends on the concentration in influent water, removal efficiency of STPs and also their degradation. For instance in Pedrouzo's study [24], where sewage samples from the same STPs were analyzed, COC and BE showed the highest values for influent samples, with maximum concentrations of 3300 ng/L and significant removal during treatment. However, these were not found at a higher concentration in the sludge. Partition coefficients for COC and BE suggest that little will partition from the dissolved phase to the sludge [31]. Unlike our results with sludge, Pedrouzo detected MOR, 6-AM and DIH at low levels of ng/L in influent samples. With regard to effluent samples, the values reported by Pedrouzo show 99% removal for most compounds after tertiary treatment, although DIH, present at low concentrations, was not removed and was reported at constant values in all the sampling points between 9 and 14 ng/L.

In the only study [11] on the occurrence of COD, COC, and BE in sludge samples, from three STPs located in Scotland, none of these drugs were found. Amphetamine [10] and methamphetamine [12] were found in sludge, but these drugs of abuse were not included in this study.

When some drugs of abuse were analyzed in SPM, which can provide information about their retention in solid particles, COC, BE and metamphetamine were those found at higher concentrations [8,14]. In another study in Croatia [9], MOR, COD, BE, EDDP, COC, EDDP and MET were found in wastewater samples at concentrations of between 5 and 200 ng/g (d.w), with EDDP being the one with the highest concentration.

4. Conclusions

A method based on PLE and LC–MS/MS was developed for the determination of nicotine, 4 drugs of abuse and 4 metabolites in sewage sludge at low levels (μg/kg) (d.w.). Although the method was suitable for the determination of all compounds, THC-COOH had to be excluded due to the high ion suppression observed. The use of a chromatographic column with fused-core particle technology gave us satisfactory separation of all compounds in 13 min. Significant PLE parameters such as solvent, temperature and number of extraction cycles were optimized in order to improve extraction efficiency for the simultaneous determination of target analytes from sludge samples. Extraction efficiency was improved with the use of non-polar solvent (dichloromethane) for most analytes. Matrix-matched calibration and deuterated compounds as surrogate were used to compensate matrix effect and ensure accurate quantification. LOQs lower than 25 μg/kg (d.w.) and %RSD ($n=5$) were found between 7% and 20%, these values being lower than others reported in the published literature concerning drugs of abuse in sewage sludge. The method was successfully applied to determine these compounds in two STPs. High levels of NIC and EDDP were found in sewage sludge. 6-AM, DIH and MOR were not detected in any samples.

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