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Solid-phase extraction followed by liquid chromatography-high resolution mass spectrometry to determine synthetic cathinones in different types of environmental water samples

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ABSTRACT

Synthetic cathinones have become popular in recent years, which would explain why their determination in influent sewage samples has already been documented. In the present study a method based on solid-phase extraction followed by liquid chromatography and high resolution mass spectrometry is developed, validated and applied to determine twelve cathinones and one of their metabolites in different environmental samples including influent and effluent sewage and river water. Two cation-exchange sorbents (Oasis MCX and Oasis WCX) were compared, with better results achieved with Oasis WCX in terms of apparent recoveries (70–100%) and matrix effects (lower than −34%).

The method was validated with effluent sewage samples providing suitable figures of merit, with method quantification limits ranging from 1 ng/L to 5 ng/L and method detection limits from 0.1 ng/L to 0.5 ng/L for all the compounds. Of the different cathinones studied, three, namely methylone, mephedrone metabolite and methylenedioxypyrovalerone, were quantified at concentration levels of low ng/L in each of the different samples analysed, while a number of the other cathinones were also detected in some of the samples.

1. Introduction

Novel psychoactive substances (NPS), which include a wide range of compounds such as synthetic cathinones, cannabinoids, phenethylamines, tryptamines, piperazines, and ketamine, continue to appear on the recreational drug market as an alternative to controlled stimulants (i.e. amphetamines, cocainics and opioid derivatives) because they have similar sympathomimetic effects [1]. Between 2008 and 2015, more than 600 NPS were reported by the United Nations Office on Drug Crime (UNODC) early warning advisory on NPS. Although data collection for 2015 is still in progress, 75 new substances have been reported to the UNODC for the first time, compared to a total of only 66 in 2014 [2]. Synthetic cathinones, which are a family of derivatives of cathinone (the active ingredient of the khat plant *Catha edulis*) typically purchased as "bath salts" or incense [3], were the largest NPS category identified by the European Monitoring Centre for Drug Addiction (EMCDA). In fact, the data reported in 2015 by the UNODC showed the appearance of 20 new synthetic cathinones, pointing to a different pattern in worldwide drug consumption [2]. As a result of this increased presence, the most commonly consumed synthetic cathinones – 3,4-methylenedioxypyrovalerone (MDPV), mephedrone, and methylone – were banned in many European countries [4] and the USA [1]. Additional cathinones were temporarily scheduled as Class I drugs and new laws have been developed to accommodate the emergence of new cathinones with their health risks and consequences [4,5].

To document cathinone consumption, an analysis of synthetic cathinones in biological fluids (blood, urine, hair, etc.) is essential. Several studies have monitored the presence of certain synthetic cathinones along with other NPS in different biological fluids such as urine [6–9], plasma [10], serum [11], blood [8,12,13] and hair [14,15]. These studies also indicate that cathinones are extensively metabolised in humans, but part of them remains unchanged when excreted in urine.

The determination of synthetic cathinones along with their metabolites in wastewater was also proposed as a complementary tool for assessing the consumption of these drugs within a particular population, and this would eventually take the place of other traditional monitoring methods such as consumer interviews, medical records and population surveys [16]. In view of this, some recent studies have been published that aim to determine certain cathinones alone [17] but most determine their presence with other drugs or NPS [16,18–22] in influent wastewater. So far, however, few studies have been carried out with the aim of monitoring the presence of some cathinones so as to assess their occurrence in the environment and thus their impact as potential emerging contaminants. In detail, in these studies [20,22,23] three cathinones – mephedrone [20,22,23] and its metabolite (4-methylephedrine) [23], methylone [22], MDPV [16,22,23] and alpha-pyrrolidinopentiophenone (α-PVP) [20,23] –have been included in the analysis of effluent wastewater [16,20,23] and river water [23] samples.

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Liquid chromatography (LC) followed by tandem mass spectrometry (MS/MS) using triple quadrupole has already been described to determine these substance in wastewater [16,17,19,20] because it is considered to offer the greatest sensitivity. More recently, high resolution mass spectrometry (HRMS) has been used to determine cathinones in urine [7,9,24], blood [8], plasma [10] and hair [14]. HRMS instruments such Orbitrap™ provide improved mass accuracy, enhanced selectivity and the opportunity for retrospective analysis, this latter point being a distinct advantage with such a rapidly evolving drug type. these instrumental methods need to include a sample preparation step in order to preconcentrate the sample and eliminate matrix interferences because of the low levels of concentration and the complexity of the samples. Solid-phase extraction (SPE) using mixed-mode ion-exchange sorbents are a suitable option since it exploits the capacity features (both reversed-phase and ionic interactions) with a clean-up based on organic solvent (removal of interferences while the target analytes remain ionically retained) [25]. A mixed-mode type of sorbent has already been used in some studies in which cathinones were extracted from influent wastewater. González-Mariño et al. [17], for instance, chose Oasis MCX as an SPE sorbent but they did not perform the clean-up step, so the removal of interferences was not completed. Bade et al. [19] used the same sorbent and included a clean-up step based on 5 mL of MeOH. However, interference removal was not fully accomplished since high values of matrix effect (ME) were obtained if internal standards were not used to correct them.

The present study aims to develop and validate a highly sensitive and selective method based on SPE followed by LC-HRMS using Orbitrap as analyser for the quantitative determination of relevant synthetic cathinones along with one metabolite in environmental samples including surface water and effluent and influent sewage samples. The cathinones selected, which are shown in Table 1, were chosen based on data detailing their occurrence in analytical, forensic and toxicological studies. A substantial list of cathinones has not yet been determined in these types of samples where it is expected their concentration to be at low ng/L levels. In viewing so, special attention has focused on the SPE step, and, two mixed-mode ion-exchange materials (Oasis WCX and Oasis MCX) were compared in terms of recovery and ME.

2. Experimental part

2.1. Standards and materials

The standards of cathinones and one metabolite were flephedrone, 3,4-methylenedioxymethcathinone HCl (methylone), N-Ethylcathinone HCl (ethcathinone), 4′-methoxymethcathinone HCl (methedrone), buphedrone HCl (buphedrone), 4-methylephedrine HCl (4-MEP), butylone HCl (butylone), 4′-methoxymethcathinone HCl (mephedrone or 4-MMC), 4-methylethylcathinone HCl (4-MEC), beta-ethylmethcathinone HCl (pentedrone), 3,4-dimethylmethylcathinone (3,4-DMMC), alpha-pyrrolidinovalerophenone HCl (α-PVP) and methylenedioxypyrovalerone HCl (MDPV) and were purchased from LGC Standards (Luckenwalde, Germany). The structure of these analytes (Fig. 1S) and the exact mass are shown in Table 1. Stock standard solutions of analytes were prepared by dissolving the weighed solid standard in MeOH at a concentration of 1000 mg/L or 2000 mg/L depending on the compound. For purchased standards available as solution in glass ampoules, the content of the ampoule was diluted with MeOH to obtain solution at a concentration of 100 mg/L. These solutions were kept in the freezer at −20 °C and were stable for several months. They were further diluted with mobile phase to obtain working solutions.

The solvents methanol (MeOH) and acetonitrile (ACN) of HPLC-grade purity were from J.T. Baker (Deventer, Netherlands). Ultra-pure water was obtained from a water purification system (Veolia Water, Sant Cugat del Vallès, Spain). Formic acid (HCOOH) was from J.T. Baker and ammonium hydroxide (NH_4OH) from Sigma-Aldrich. Oasis WCX and Oasis MCX (500 mg/6cc) extraction cartridges from Waters Corp. (Milford, MA, USA) were used in the SPE.

2.2. Sampling

River water samples were collected from the River Ebre in Catalonia, while influent and effluent sewage samples were collected from sewage treatment plants (STPs) in the Tarragona area that include primary and secondary treatments in their processes. The samples were collected in pre-cleaned bottles and were stored at −20 °C until the day of the analysis. Before being analysed, the samples were filtered through a 1.2 μm glass-fibre membrane filter and then through a 0.45 μm nylon membrane filter, both purchased from Whatman (Maidstone, UK)

Table 1
Retention time, diagnostic ions (formula, exact mass and calculated accurate mass) and the two fragments (postulated formula and accurate mass).

		Diagnotic ion $[M + H]^+$			Fragment 1		Fragment 2	
Compound	Rt (min)	Formula	Exact mass *m/z*	Accurate mass *m/z*	Formula	Accurate mass *m/z*	Formula	Accurate mass *m/z*
Flephedrone	6.59	$C_{10}H_{13}FNO$	182,09757	182,09740	$C_{10}H_{11}FN$	164,08714	C_9H_8FN	149,0638
Methylone	6.89	$C_{11}H_{14}NO_3$	208,09682	208,09663	$C_{10}H_{10}NO$	160,07579	$C_{11}H_{12}NO_2$	190,08652
Ethcathinone	7.06	$C_{11}H_{16}NO$	178,12264	178,12241	$C_{11}H_{14}N$	160,11218	$C_9H_{10}N$	132,08157
Methedrone	8.34	$C_{11}H_{16}NO_2$	194,11755	194,11729	$C_{11}H_{14}NO$	176,10699	$C_{10}H_{11}NO$	161,08377
Buphedrone	8.49	$C_{11}H_{16}NO$	178,12264	178,12219	$C_{11}H_{14}N$	160,11217	C_9H_9N	131,07355
4-MEP	9.25	$C_{11}H_{18}NO$	180,13829	180,13786	$C_{11}H_{16}N$	162,12778	$C_{10}H_{13}N$	147,10443
Butylone	9.53	$C_{12}H_{16}NO_3$	222,11247	222,11192	$C_{11}H_{12}NO$	174,09154	$C_{12}H_{14}NO_2$	204,10205
Mephedrone	9.65	$C_{11}H_{16}NO$	178,12264	178,12219	$C_{11}H_{14}N$	160,11201	$C_{10}H_{11}N$	145,08875
4-MEC	11.05	$C_{12}H_{18}NO$	192,13829	192,13797	$C_{12}H_{16}N$	174,12775	$C_{10}H_{11}N$	145,08881
Pentedrone	11.84	$C_{12}H_{18}NO$	192,13829	192,13795	$C_{12}H_{16}N$	174,12785	$C_9H_{10}N$	132,08138
3,4-DMMC	13.38	$C_{12}H_{18}NO$	192,13829	192,13792	$C_{12}H_{16}N$	174,12773	$C_{11}H_{13}N$	159,10431
α-PVP	13.73	$C_{15}H_{22}NO$	232,16959	232,16891	C_7H_7	91,05506	C_7H_5O	105,03424
MDPV	14.75	$C_{16}H_{22}NO_3$	276,15942	276,15959	$C_8H_{16}N$	126,12852	$C_8H_7O_2$	135,04462

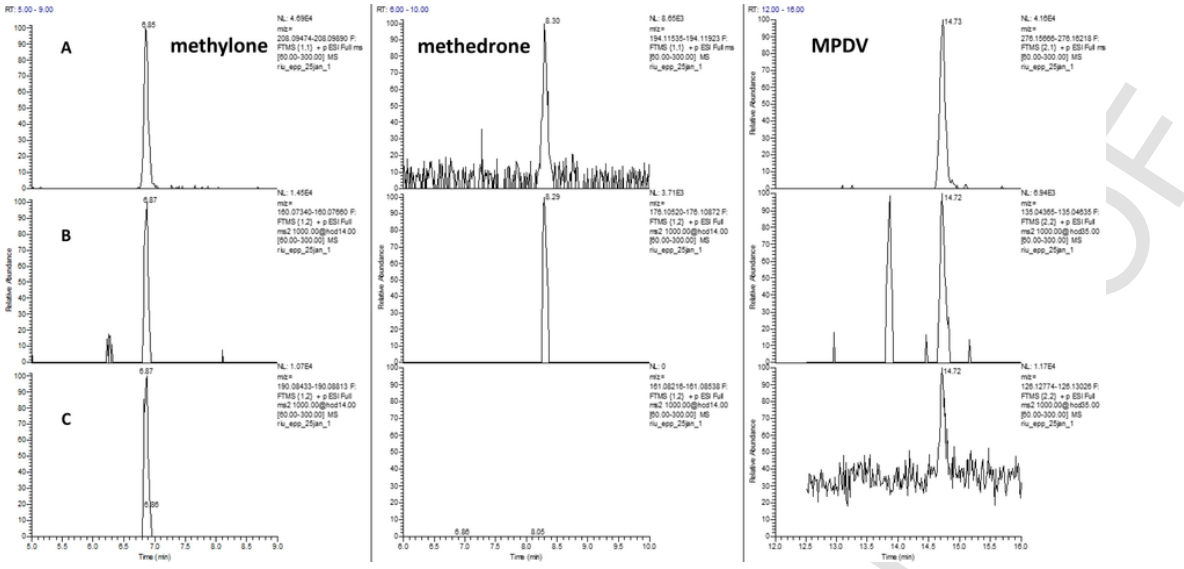

Fig. 1. An extracted ion chromatogram of the molecular ion (A) and the two fragments (B, C) of an analysed River Ebre sample.

2.3. Solid-phase extraction

Oasis WCX and Oasis MCX cartridges (500 mg) were used during the optimisation. The optimal loading volume were 500 mL for river water, 250 mL for effluent and 100 mL for influent sewage samples.

Both cartridges (Oasis MCX and Oasis WCX) were conditioned with 10 mL of MeOH, followed by 10 mL of ultrapure water adjusted to the same pH as the sample. Samples were adjusted to pH 3 for Oasis MCX and pH 7 for Oasis WCX before being loaded into the cartridge. The washing step consisted of 10 mL pure MeOH. Finally, the analytes were eluted with 5 mL of 5% NH_4OH in MeOH for Oasis MCX and 5 mL of 5% HCOOH in MeOH for Oasis WCX.

The extracts obtained after elution were evaporated to dryness using a MiVac concentrator (Genevac, Ipswich, UK) and reconstituted to a final volume of 1 mL H_2O:MeOH (90:10, v/v), filtered through a 0.45 μm PTFE syringe filter and injected into the LC-HRMS.

2.4. LC-Orbitrap-HRMS ANALYSIS

An Accela 1250 UHPLC system coupled to an Exactive Orbitrap™ mass spectrometer from Thermo Scientific (Bremen, Germany) was used for the LC-HRMS analysis. The UHPLC instrument includes an automatic injector (refrigerated at 10 °C), a quaternary pump (1250 bar) and a column oven (thermostatised at 35 °C). The HRMS instrument is also equipped with a heated electrospray ionisation (HESI) source and a higher-energy collisional dissociation (HCD) cell to fragment the analytes for their confirmation. The chromatographic column used was an Acquity UPLC HSS T3 (100 × 2.1 mm, 1.8 μm) supplied by Waters. The mobile phase was a mixture of solvent A (0.1% HCOOH in H_2O) and solvent B (0.1% HCOOH in ACN). The gradient profile started with 2% of B that increased to 20% in 15 min, then to 100% B in 2 min and then held for 1 min before returning to the initial conditions in 2 min and being maintained for 5 min. The flow rate was 400 μL/min and the injection volume 25 μL.

In the HRMS instrument, the signal of the molecular ion $[M + H]^+$ of each analyte was monitored to optimise the interface conditions so as to obtain the highest response for all the analytes. This optimisation was performed in full scan at high resolution (50,000 FWHM) in a mass range of 100–500 *m*/*z*. The optimal parameters for positive ionisation were: spray voltage, 2 kV; skimmer voltage, 20 V; capillary voltage, 40 V; and tube lens voltage, 90 V. The sheath gas was set at 40 AU (adimensional units) and the auxiliary gas at 5 AU. The heater and capillary temperatures were 300 °C and 350 °C respectively. The probe position adjustment was side to side 0, vertical C and micrometre 1.

For data acquisition, two time windows were used both in positive mode (0–13 min and 13–17 min) with two alternating scan events in each window. The first scan event was a full scan at 50,000 FWHM with 250 ms of injection time, while the second was a fragmentation scan at 10,000 FWHM with 50 ms of injection time, using an optimum collision voltage of 14 eV in the HCD cell in the first window and 14 eV and 35 eV in the second. For quantification, the molecular ions were measured (with a mass extraction window of 5 ppm) and the selected fragments and ion ratios were taken into account for confirmation.

2.5. Validation

Instrumental limits of detection (ILDs) were the concentrations whose signal-to-noise ratio (S/N) was greater than 3 and whose molecular ion and at least one fragment provided a signal intensity higher than 1×10^3 in the Orbitrap analyser. Instrumental limits of quantification (IQLs) adopted were the lowest concentration in the calibration curve which also accomplished a S/N higher than 10.

The extraction recovery ($\%R_{SPE}$) was defined as the recovery obtained in the SPE procedure alone and was calculated as the ratio between the concentration obtained from a sample spiked before the SPE procedure and that obtained from direct injection of the standard. The ME was calculated from the concentration obtained when the extract from the sample was spiked after the SPE and just before injection into the LC-HRMS ($C_{POST\text{-}SPIKED}$), which was introduced in the expression $\%ME = -[100-(C_{POST\text{-}SPIKED}/C_{STD})x100]$, where C_{STD} is the concentration of the standard injected in the LC-HRMS instrument. The apparent recovery ($\%R_{app}$) was defined as the recovery of the whole method and calculated from the concentration obtained from a sample spiked for the SPE at the beginning of the analysis. All

of the experimental concentrations mentioned were calculated using a calibration curve prepared in pure standard.

Method limits of quantification (MQLs) were defined as the lowest point of the matrix-matched calibration curve, while method limits of detection (MDLs) corresponded to a concentration whose S/N ratio was greater than 3, with a signal intensity higher than 1×10^3 in the Orbitrap analyser. In cases where the compounds were already present in the blank sample, the MDLs were estimated from the instrumental limits (IQLs and IDLs) and the%R_{app} values.

Repeatability (intra-day) and reproducibility (inter-day) expressed as% relative standard deviation (%RSD) were obtained with five replicated samples performed the same day and different days, respectively.

3. Results and discussion

3.1. LC-Orbitrap-HRMS

A challenging feature of the cathinone class is the number of isobaric compounds (positional isomers), since many of these isomers share the same diagnostic ions and fragmentation pathways, and mass separation is not always feasible. In this particular study, two trios of cathinones – ethcathinone, buphedrone and mephedrone; and 4-methylethcathinone, pentedrone and 3,4-DMMC – shared the same molecular weight. In order to achieve the chromatographic separation, different stationary phases – Ascentis Express Fused-Core C_{18} (100 mm x 4.6 mm, 2.7 μm), Kinetex PFP column (100 × 2.1 mm, 2.6 μm), Ascentis Express RP Amide (100 × 2.1 mm, 2.7 μm) and Acquity UPLC HSS T3 (100 × 2.1 mm, 1.8 μm) – were tested along with different types of mobile phase that consisted of H_2O with 0.1% HCOOH combined with either pure ACN, ACN with 0.1% HCOOH or MeOH with 0.1% HCOOH, applying different gradients. All four phases tested provided different selectivity, but only Acquity UPLC HSS T3 provided the separation of the whole group of cathinones studied when the optimum gradient was applied, regardless of the mobile phase used. However, selection of the optimum mobile phase will be further subject to the results of the ionisation of the analytes.

A mixture of all the compounds in solution under the chromatographic flow and mobile phase composition was infused in order to optimise the parameters that affect ionisation and transfer to the Orbitrap analyser. According to the structure of the cathinones, all showed better performance when ionised in positive mode. Furthermore, sheath gas (5–60 AU) and auxiliary gas (0–15 AU), capillary and heater temperature (200–400 °C), spray voltage (2–5 kV), capillary voltage (30–60 V), tube lens voltage (40–160 V), skimmer voltage (10–60 V) and prove position adjustment − side to side (-1 to +1), vertical (A, B, C or D) and micrometre (0–2)- were evaluated in the ranges shown in brackets. Optimal HRMS parameters (described in section 2.4) were established as a compromise between the highest responses achieved for each analyte. ACN with 0.1% HCOOH was selected as the organic component of the mobile phase, although the analytes' ionisation is similar when MeOH with 0.1% HCOOH was used in the mobile phase. Nonetheless, ACN provided better chromatographic performance.

Collision energies (10–60 eV) in HCD were optimised by infusing each analyte individually in order to observe two known fragment ions for each compound in all ion fragmentation spectra for confirmatory purposes. The selection of the two ions was also strengthened due to the fact that some of the cathinones share the same fragments. All the cathinones, with the exception of PVP and MDPV, showed the compromise in the emergence of two fragments when the collision energy was 14 eV. This HCD energy was chosen during the whole analysis, and in the last 3 min (when PVP and MDPV appeared) a stronger energy (35 eV) was also adopted to obtain two fragments with high response from PVP and MDPV. The stronger energy is justified since these two cathinones belong to the same class of pyrrolidinyl-substituted cathinones. Fig. S1 shows the structure of the 13 analytes studied along with their proposed fragments.

Table 1 details the exact and accurate mass of the diagnostic ions of the analytes studied, which in all instances is the $[M + H]^+$, and also the accurate mass of the two fragments selected together with the formula postulated. All the cathinones apart from PVP and MDPV presented an ion corresponding to the neutral loss of a molecule of water $[M + H\text{-}H_2O]$. The loss of water is consistent with the β-keto reduction of the ketone group shared in all the cathinone structure where this fragment arose [26]. The loss of methyl radical groups, consistent with *N*-de-alkylation, was also observed and the fragments derived were selected for the identification of certain cathinones (see Table 1). In the case of α-PVP, the fragments correspond to the benzoyl cation (*m*/*z* 105) and tropylium ion (*m*/*z* 91) [19]. As for MDPV, the *m*/*z* 126 fragment corresponds to the alpha-cleavage between positions 1 and 2, while the *m*/*z* 135 corresponds to the dimethoxyphenyl and carbonyl motiety [27]. All the above fragmentation patterns agree with those selected in previous studies where Q-Exactive [26], QqTOF [22] or QqQ [16,17,20] analysers were used.

Under these conditions, the ILDs ranged from 0.01 μg/L to 0.05 μg/L, and IQLs s were 0.1 μg/L for all the analytes with the exception of flephedrone (0.25 μg/L) and α-PVP (0.5 μg/L). Satisfactory linearity was achieved in the range between IQLs and 250 μg/L.

3.2. Comparison of cation-exchange sorbents

As cathinones and ephedrines possess an amino group, the cation-exchange interactions can be promoted either with strong-cation (Oasis MCX) or weak-cation (Oasis WCX) exchange sorbents. Therefore both sorbents were compared in terms of promoting the recovery of the cathinones and also in diminishing the ME. In addition, each type of sorbent should be evaluated using an explicit protocol so that the ionic interactions can be promoted/disrupted during the SPE steps. First of all, we tested Oasis MCX and Oasis WCX when the compounds were in ultrapure water, using common protocols. That is, after conditioning, 100 mL of ultrapure water adjusted at pH 3 (Oasis MCX) or at pH 7 (Oasis WCX) and spiked with analyte mixture at 0.5 μg/L was loaded, then the clean-up step consisted of two fractions of 2 mL of MeOH each, and finally the elution of the analytes was achieved in the case of Oasis MCX with three fractions of 5 mL of 5% NH_4OH in MeOH each (the basic additive deprotonates the cathinones and the ionic interactions are disrupted); or in the case of Oasis WCX, with three fractions of 5 mL of 5% HCOOH in MeOH each (the acidic additive protonates the carboxylic acid moieties of the sorbent and the ionic interactions are disrupted). All the washing and elution fractions were collected, evaporated to dryness and reconstituted in 1 mL of H_2O:MeOH (90:10, v/v) (see section 2.3).

From this comparison, we observed similar trends for both sorbents, since all the analytes were completely recovered in the first 5 mL elution fraction and therefore no loss of analytes was observed in any of the washing fractions. Moreover, the loss of analytes during evaporation and the filtration with the PTFE filter was no higher than 10% for all the analytes in both the basic (5% NH_4OH) and acidic (5% HCOOH) MeOH. In the next experiments the elution volume was fixed at 5 mL, the volume of MeOH in the washing step was raised to 10 mL and the volume of ultrapure water loaded was tested at 250 mL and 500 mL. In all the tests, no loss of analytes was ob-

served during the washing step. As for the elution, the recovery results achieved with the Oasis WCX are higher (≈ 100%) than those obtained with the Oasis MCX, which were not 100% in some instances, as with flephedrone (64%), ethcathinone (69%), buphedrone (59%), pentedrone (56%) and PVP (66%) as it can be seen in Table 2.

Before selecting one of the two sorbents, we decided to move on to environmental water and evaluate the%ME and%R_{app} in each type of matrix. The matrices and volumes analysed (according to complexity) were: 250 mL and 500 mL of river water spiked at 0.2 μg/L and 0.1 μg/L respectively, with the analyte mixture; and 100 mL and 250 mL of effluent sewage spiked at 0.5 μg/L and 0.2 μg/L respectively. The final evaporated extracts of all these samples were then reconstituted at 1 mL. For influent sewage, the volume was fixed at 100 mL spiked at 0.5 μg/L and 1 μg/L, depending on the final extract, which was reconstituted at 1 mL and 2 mL respectively. The protocol followed for these samples is the same as shown above and described in section 2.3. In all cases, the response of the analytes present in non-spiked samples was subtracted from the response in the spiked samples. Although all the recoveries were calculated at all concentrations and volumes, Table 2 shows only the%R_{app} and%ME when the larger volume of each type of sample was percolated through Oasis MCX and Oasis WCX. The results achieved comparing both volumes in each type of sample are similar in all instances.

The%ME is always in the form of ion suppression and, as a general trend, the values obtained for Oasis MCX are higher than those for Oasis WCX. For influent sewage samples, for instance, the%ME in the Oasis MCX ranged from −19% to −43, whereas in the Oasis WCX they ranged from −3 to −22., being these last values considered acceptable. This low%ME is certainly attributable to the effective washing step that consisted of 10 mL of MeOH. The difference in%ME values and thus recovery values could be attributed to the type of interferences present in the samples, which at the loading pH (pH3 for Oasis MCX or pH7 for Oasis WCX) may be differently retained to the sorbent and later in the elution step eluted along with the analytes. Furthermore, the%R_{app} for the Oasis WCX are higher than those for the Oasis MCX.

In view of the good results when larger volumes of samples are percolated, these volumes (i.e. 500 mL of river sample, 250 mL of effluent sewage and 100 mL of influent sewage, all of them finally reconstituted to 1 mL) were selected for further analysis since the decrease in%ME (and the consequent gain in%R_{app}) is not justified for the two-fold dilution factor in these samples. Furthermore, comparing the performance of both sorbents, the best values in terms of%ME and%R_{app} were achieved when Oasis WCX was used, and this was selected for further analysis. In fact, Oasis WCX has never been used as a sorbent to extract cathinone, while Oasis MCX [17,19,21,23] and other strong cation-exchanger sorbents such as SOLA SCX [9] and Drug Prep I [11] have indeed been applied to extract certain cathinones along with other illicit drugs from influent sewage [17,19,21] and other less complex environmental samples [23], and also from urine [9] and serum [11]. However, the results and protocols used in these studies present substantial differences mainly regarding to the type and volume of the washing solvent. Nevertheless, the results in the present study are better to those obtained in previous studies [17,19,21,23]. For example, when 100 mL of influent sewage sample were percolated through Oasis MCX values of ion enhancement from 26 to 48% where obtained for mephedrone, methedrone, methylone and MDPV [19], or ion suppression from −37 to −60% for mephedrone and MDPV [23], while values up to −22% were found in the present study when using Oasis WCX.

3.3. Validation in environmental samples

The method using Oasis WCX was validated with effluent sewage samples by evaluating the linear range, the MQLs, the MDLs, repeatability (intra-day) and reproducibility (inter-day). The linear range was evaluated using a matrix-matched calibration curve with samples spiked at different concentrations from 1 to 500 ng/L prior to SPE extraction. In all instances, a non-spiked sample was also analysed to subtract any analyte signal present in the sample. The linear range was from MQL to 500 ng/L for each compound, with R^2 higher than 0.9996 for all compounds. All the MQLs and MDLs are shown in Table 3. The MQLs range from 1 to 5 ng/L and the MDLs from 0.1 to 0.5 ng/L. These values are similar to those found when the QqQ analyser (claimed to be more sensitive) was used to determine this type of analytes [17,20]. For instance, the MDLs for mephedrone and α-PVP were reported at 10 ng/L and 1.9 ng/L respectively when 50 mL of sewage sample was analysed using SPE followed by LC–MS/MS with QqQ as analyser [20]. In another study in which a similar analytical method based on SPE (Oasis MCX) fol-

Table 2
% R_{SPE} when 500 mL of ultrapure water were percolated and, % ME and%R_{app} obtained when 500 mL of river water, 250 mL of effluent sewage and 100 mL of influent sewage spiked with the analyte mixture (see the concentration as footnote) were percolated through the Oasis MCX and Oasis WCX.

	Ultrapure water[a]		River[a]				Effluent[b]				Influent[c]			
	MCX	WCX	MCX		WCX		MCX		WCX		MCX		WCX	
Analyte	%R_{SPE}	%R_{SPE}	% ME	%R_{app}	% ME	%R_{app}	% ME	%R_{app}	% ME	%R_{app}	% ME	%R_{app}	% ME	%R_{app}
Flephedrone	64	100	−26	39	−19	77	−21	15	−34	59	−35	32	−22	67
Methylone	92	101	−32	66	−18	96	−39	24	−11	83	−43	43	−20	72
Ethcathinone	69	100	−22	55	−14	89	−26	25	−11	87	−30	40	−16	73
Methedrone	92	105	−26	73	−16	84	−31	29	−19	76	−34	51	−3	77
Buphedrone	59	100	−23	39	−15	82	−26	23	−21	74	−28	43	−18	75
4-MEP	104	97	−25	79	−20	92	−24	53	−14	80	−19	69	−17	75
Butylone	95	101	−30	63	−20	82	−36	36	−18	73	−39	50	−17	83
Mephedrone	87	100	−23	63	−14	88	−26	21	−15	79	−32	43	−16	77
4-MEC	81	101	−18	68	−13	97	−22	35	−11	88	−28	51	−11	89
Pentedrone	56	97	−18	30	−15	76	−23	20	−18	75	−32	38	−15	74
3,4-DMMC	96	96	−18	65	−17	88	−25	21	−9	82	−31	45	−9	81
α-PVP	66	97	−12	13	−11	56	−13	49	−7	71	−23	55	−10	72
MDPV	81	101	−12	27	−13	71	−15	62	−3	86	−23	66	−6	85

[a] at 0.1 μg/L.
[b] at 0.2 μg/L.
[c] at 0.5 μg/L.

Table 3
Performance of the method based on SPE with Oasis WCX followed by LC—HRMS for the determination of the studied compounds in environmental samples.

	River				Effluent						Influent			
Analyte	% ME	%R_{app}	MQLs[a]	MDLs[a]	% ME	%R_{app}	MQLs[a]	MDLs[a]	Rep.[b]%RSD	Repro.[b]%RSD	% ME	%R_{app}	MQLs[a]	MDLs[a]
Flephedrone	−19	69	0.8	0.08	−28	55	2	0.2	1	8	−22	61	5	0.5
Methylone	−16	88	0.25	0.08	−11	83	1	0.2	2	5	−20	82	2	0.5
Ethcathinone	−19	77	1.5	0.15	−16	87	5	0.5	2	4	−16	75	10	1
Methedrone	−20	78	1.5	0.15	−23	77	5	0.5	1	7	−13	72	10	1
Buphedrone	−18	84	0.25	0.08	−27	72	2	0.2	3	7	−12	77	2	0.5
4-MEP	−15	86	0.25	0.08	−14	81	1	0.2	3	15	−17	81	2	0.5
Butylone	−28	73	0.8	0.15	−18	67	2	0.5	2	8	−17	73	5	1
Mephedrone	−3	91	0.25	0.08	−15	81	1	0.2	2	7	−16	74	2	0.5
4-MEC	−11	87	0.25	0.04	−11	85	1	0.1	1	4	−11	99	2	0.5
Pentedrone	2	82	0.25	0.08	−18	78	1	0.2	2	7	−19	72	2	0.5
3,4-DMMC	−12	75	0.25	0.08	−19	81	1	0.2	2	7	−12	81	2	0.5
α-PVP	−14	54	1.5	0.08	−20	63	2	0.2	4	5	−11	76	10	0.5
MDPV	−15	67	0.25	0.08	−15	81	1	0.1	7	12	−16	90	2	0.2

[a] concentrations in ng/L.

[b] spiked at 40 ng/L.

lowed by LC-Orbitrap-HRMS was reported, the MQLs obtained were 2.8 and 3.5 ng/L for mephedrone and MDPV, respectively, when 250 mL of river water was analysed [23]. Thus, the limits reported in the present study could be attributed to the satisfactory results in the SPE step that provided a high pre-concentration factor as well as a suitable%R_{app}. Repeatability (intra-day) and reproducibility (inter-day) were calculated via five spiked samples at two concentration levels (40 and 200 ng/L) and are expressed as%RSD (n = 5). For repeatability the%RSD ranged from 1% to 7% for the two levels assayed, while in the case of reproducibility the%RSD values ranged from 4% to 15%.

Additionally, %ME and%R_{app} were also evaluated when the three types of environmental samples (500 mL of river water, 250 mL of effluent and 100 mL of influent sewage) were spiked at a low concentration level (20 ng/L, 40 ng/L and 100 ng/L respectively) with the mixture of analytes. Table 3 shows the%ME and%R_{app} values, which were very similar to those obtained when spiking at a higher level during optimisation (see Table 2). The analytes showed the ME in the form of ion suppression and in all instances the values were lower than 20% except for butylone (−28) in river water, flephedrone (−28), methedrone (−23) and buphedrone (−27) in effluent, and flephedrone (−22) in influent. The%R_{app} values were also acceptable and comparable between the different matrices analysed. Therefore, considering that the%R_{app} values were satisfactory and that repeatability of the method was fine, quantification in the environmental water was proposed using an external calibration method and taking into account the%R_{app}. This was further proved by quantifying the response obtained in a non-spiked effluent sewage by matrix-matched calibration curve and comparing the concentration obtained by external calibration and applying the%R_{app}. The accuracy of both approaches was between 85% and 96%, and therefore the analytes found in all the matrices analysed were quantified using the external calibration curve and applying the%R_{app}. The same approach was applied for the calculation of the limits. Thus they were first of all estimated from the IDLs and IQLs (section 3.1) and, whenever possible (i.e. the analyte was not present in the non-spiked sample), they were calculated by spiking the sample at the limit concentration level and checking that they fulfilled the S/N criteria and that the response was higher than 1×10^3. Table 3 shows the limits in each type of matrix. Thus for river water (the largest volume percolated) the MDLs are up to 0.15 ng/L, whereas for influent (the lowest percolated volume and the lowest%R_{app}) the MDLs ranged from 0.2 ng/L to 1 ng/L.

3.4. Analysis of environmental samples

The method was applied to analyse different samples from the River Ebre and influent and effluent sewage from STPs in the Tarragona area (north-east Spain). The compounds were identified based on their retention time (± 0.1 min) and an exact mass (error < 5 ppm) of their diagnostic and fragment ions following the SANCO guidelines [28]. Furthermore, the ion ratio between the fragment and the molecular ion was also checked, and overall, ensuring at least 4 identification points depending on the compound. Table 4 shows the concentrations of analytes found and its frequency in the three types of sample along with the error obtained for the molecular ions.

Three of the 13 compounds evaluated (ethcathinone, methedrone and 4-methylethcathinone) were not detected in any sample. However, methylone, mephedrone and its metabolite and MPDV were detected in all the samples analysed, suggesting a considerable consumption of these substances since they were even found in river samples. The cathinones that were found are in agreement with those

Table 4
Concentration (ng/L) found in ranges of the target compounds and Δ m error (ppm) when the environmental samples were analysed.

	River (n = 5)		Effluent (n = 5)		Influent (n = 5)	
Analyte	Conc. (ng/L)	Δm error (ppm)	Conc. (ng/L)	Δm error (ppm)	Conc. (ng/L)	Δm error (ppm)
Flephedrone	< MQL	<2.8 ppm	n.d.	–	3.6–7.9	<3.5 ppm
Methylone	1.8 – 4.7	<0.8 ppm	3.0–17.9	<0.6 ppm	7.4 – 13.5	<4.3 ppm
Ethcathinone	n.d.	–	n.d.	–	n.d.	–
Methedrone	n.d. – 1.8	<2.3 ppm	n.d.	–	n.d.	–
Buphedrone	n.d.	–	n.d.	–	n.d. – 4.1	<3.8 ppm
4-MEP	< MQL – 0.6	<1.7 ppm	0.9 – 3.2	<2.3 ppm	MQL –2.2	<4.5 ppm
Butylone	< MQL	<3.2 ppm	< MQL	<2.1 ppm	< MQL	<3.9 ppm
Mephedrone	< MQL	<2.1 ppm	< MQL	<1.7 ppm	MQL –3.1	<4.1 ppm
4-MEC	n.d.	–	n.d.	–	n.d.	–
Pentedrone	n.d.	–	< MQL	<1.4 ppm	n.d. – 3.1	<3.9 ppm
3,4-DMMC	< MQL	<2.7 ppm	< MQL	<1.3 ppm	n.d. – 3.3	<2.7 ppm
α-PVP	< MQL	<1.3 ppm	3.3–5.1	<3.1 ppm	n.d. –<MQL	<3.6 ppm
MDPV	1.4–1.6	<0.6 ppm	2.8 –25.0	<0.8 ppm	5.6 – 12.0	<2.9 ppm

found in influent sewage samples from different countries. Mephedrone, for instance, was found at 5 ng/L in Copenhagen [19] but also at a higher level (up to 100 ng/L) in different STPs in the United Kingdom [17,19] and at a lower level (below 2 ng/L) in China [16]. Another example is MDPV, whose concentrations in the literature are more uniform (below 6 ng/L in Italy [17,19], Norway [19], Switzerland [17,19] and China [16]) but lower than the concentration found (5.6–12.0 ng/L) in the present study. Indeed these levels of MDPV in influent are lower than those in effluent (2.8–25.0 ng/L), which might be attributed in part to the fact that they are grab samples. Moreover, a poor removal rate (ranging from 6.8% to 21.2%) of MDPV has been reported, suggesting that it might enter receiving waters in large quantities [16]. It has also been reported that MDPV as such has been detected in urine with no free excreted metabolites, since both the pyrrolidine ring and methylenedioxy groups inhibit carbonyl reduction [29].

As for the analytes and the levels found in effluent, only few studies have analysed this type of sample, and α-PVP were found in Spain at 12.6 ± 30.6 ng/L but not MDPV [22]; MDPV was only found at 1.6 ng/L in China when this compound and mephedrone were the target compounds [16]. In a previous study by our research group [23] in which mephedrone, 4-MEP and MDPV were determined from sewage and river water, none of these compounds were found, presumably because of the higher MQLs of the method developed.

As expected, the levels of the compounds found in river water were the lowest of all the samples analysed: few ng/L for methylone, 4-MEP and MDPV and below MQL in all other cases. tThe quantification of methedrone at 1.8 ng/L in one sample was unexpected, whereas this compound was not detected in any of the other samples analysed. Fig. 1 shows the extracted ion chromatogram for this sample in which methylone, methedrone and MDPV were found. It can be seen that, for methedrone, just one fragment appeared, and although the other identification points matched, it can only be tentatively confirmed.

Table 4 also details the mass error for confirmation purposes that in all instances are lower than 5 ppm, which is considered an accurate determination. Only the fragment ion error mass for flephedrone, pentedrone and 3,4-DMMC was not within ± 5 ppm, when these compounds were present at very low concentrations. In addition, the ratios of the fragment ions for these compounds when present at low concentrations were outside the accepted ratio (± 30%) and therefore confirmation of these compounds cannot be supported, presumably due to the low concentration found.

In general, the low ng/L concentration levels can be explained by the fact that cathinones undergo extensive metabolism in the body, leading to low levels of parent compounds to be excreted and emitted to the environment [10,13,14]. This is supported by the concentration levels found for mephedrone in comparison to those found for its metabolite (4-MEP), where in both river and effluent samples the concentration level of mephedrone (below 0.25 and 1 ng/L respectively) is lower than the concentration of 4-MEP (up to 0.6 and 3.2 ng/L, respectively). In influent samples, on the contrary, the concentration of mephedrone is up to 3.1 ng/L while the metabolite concentration is lower (up to 2.2 ng/L). This might suggest that the parent compounds suffer further transformation as a result of the treatment process. Nonetheless, both the effluent and influent samples analysed are grab samples that do not correspond to the same source. Thus, further studies on this issue should be conducted to confirm this hypothesis as well as to find their metabolites in those environmental samples.

4. Conclusions

A method based on SPE using weak cation-exchange sorbent followed by LC-HRMS was successfully developed, validated and applied to determine a substantial list of cathinones in sewage and surface water samples.

Comparing the performance of Oasis MCX and Oasis WCX, Oasis WCX was selected since it provided better recoveries and the ME encountered in the method was lower when complex samples were analysed. Indeed the ME for all the environmental samples analysed was lower than −20%, which is considered acceptable, and attributed to the effective washing that consisted of 10 mL of MeOH.

Some of the cathinones studied were determined in the influent, effluent and even in river water for the first time at low ng/L concentration levels. In all the quantifications, the mass error obtained was lower than 5 ppm, which showed an accurate determination. In view of the success of this study, future research should deal with the quantification of cathinone metabolites and transformation products in similar environmental samples.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.chroma.2017.10.002.

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