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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 deter-

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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

mine 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.

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^{TM} 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). Ultrapure 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)

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