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The detection of drugs of abuse and pharmaceuticals in drinking water using solid-phase extraction and liquid chromatography-mass spectrometry

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Abstract

Pharmaceuticals and drugs of abuse including novel psychoactive substances (NPS) are emerging as newer contaminants in the aquatic environment. The presence of such pollutants has implications on the environment as well as public health and therefore their identification is important when monitoring water quality. This research presents a new method for the simultaneous detection of 20 drugs of abuse and pharmaceuticals in drinking water, including 15 NPS, three traditional illicit drugs and two antidepressants. The developed method is based on the use of solid-phase extraction (SPE) followed by liquid chromatography-mass spectrometry (LC-MS). The SPE recoveries for the majority of target analytes ranged between 62-107%. The method detection and quantification limits ranged between 0.01-1.09 ng/L and 0.02-3.64 ng/L respectively. Both instrumental and method precisions resulted in relative standard deviations < 15.04%, with an accuracy of < ±8.66%. The results show that LC-MS can be an alternative to the more popular technique of liquid chromatography-tandem mass spectrometry for the analysis of drugs of abuse and pharmaceuticals in drinking water. This newly developed simultaneous detection method has been applied to drinking water collected

from the East Anglia region of the UK. Citalopram, cocaine, fluoxetine, ketamine, mephedrone, methamphetamine and methylone were detected at the range of 0.14 and 2.81 ng/L. This is the first time that the two NPS mephedrone and methylone, have been detected in UK drinking water.

Keywords: drugs of abuse, novel psychoactive substances, pharmaceuticals, drinking water, solid-phase extraction, liquid chromatography-mass spectrometry.

1. Introduction

Drugs of abuse and pharmaceuticals are emerging contaminants identified in the aquatic environment that have received increasing public concern and scientific interest (Peng, et al., 2016). These water contaminants are continuously introduced into waste water, either as parent compounds or metabolites, through human waste or improper disposal of unused or expired pharmaceuticals (Gros, et al., 2007). Previous studies have widely demonstrated that drugs of abuse and pharmaceuticals are ubiquitous in surface water and ground water, usually resulting from inefficient removal of these compounds by waste water treatment methods and the subsequent release of the resulting effluent into rivers and lakes (Cahill, et al., 2004; Kasprzyk-Hordern, et al., 2007). Aquifers are also reported to be similarly contaminated by either leakage from waste water systems or seepage from surface waters (Pal, et al., 2013).

With the presence of drugs of abuse and pharmaceuticals in drinking water and the fact that these contaminants are biologically active compounds, they could have associated impacts on human health (Peng, et al., 2016). Surface and ground waters, which are collectively known as raw water, are treated by drinking water treatment plants (DWTPs) for human consumption (Pal, et al., 2013). However, as drugs of abuse and pharmaceuticals are present in raw water and current drinking water treatments are not always able to completely remove them and therefore, have been reported in drinking water at part per trillion level (ng/L) (Huerta-Fontela,

et al., 2008). Such publications are limited probably due to the analytical sensitivity needed to quantify such compounds at ultra-trace levels in drinking water samples (Peng, et al., 2016). Therefore, we describe a developed, validated and sensitive methodology for the simultaneous determination of a broad range of drugs of abuse and pharmaceuticals in drinking water. Although liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is commonly used for the detection of drugs of abuse and pharmaceuticals in drinking water (Postigo, et al., 2008) and other methods such as capillary electrophoresis-ultraviolet detector (Castiglioni, et al., 2008) have also been reported. In this study liquid chromatography-mass spectrometry (LC-MS) is used. This is less expensive compared to LC-MS/MS and can have similar instrumental sensitivities (Díaz-Cruz, et al., 2003). Hence, LC-MS could be a cheaper method of choice and in light of this, here we report the use of LC-MS as an alternative to LC-MS/MS in the detection and quantification of drugs of abuse and pharmaceuticals in drinking water.

The 15 novel psychoactive substances (NPS), 3 drugs of abuse and 2 antidepressants were chosen in this study (Table 1) due to their frequency of use in the UK and limited studies regarding their presence in drinking water [Advisory Council on the Misuse of Drugs, 2010; Baker and Kasprzyk-Hordern, 2011; Mixmag, 2012; European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2014, 2015; Mwenesongole, et al., 2013; Health and Social Care Information Centre, 2014; United Nations Office of Drugs and Crime (UNODC), 2014]. According to Home Office (2012), NPS have gained popularity among drug users, as they are easily available over the internet and can be considered as alternatives to controlled drugs. Thus, the consumption of NPS has continuously grown in the UK. However, NPS have received minimal attention in the analysis of drinking water (Peng, et al., 2016). To date, only three NPS, ketamine, mephedrone and JWH-073 have been investigated in drinking water and only the presence of ketamine has been reported in Canada at 15 ng/L (Huerta-Fontela, et al., 2008; Boleda, et al., 2011; Mendoza, et al., 2016; Rodayan, et al., 2016; Asimakopoulos, et al., 2017).

Therefore, in this study we have analysed a larger selection of NPS in drinking water belonging to cathinones, piperazines and synthetic cannabinoids, which have never been studied before.

Drinking water samples were collected from the East Anglia region of the UK, which has never been investigated before with regards to the presence of drugs of abuse and pharmaceuticals.

2. Material and methods

2.1 Chemicals, equipment and materials

The suppliers of standards and internal standards are included in Table S1 in the supplementary data. Solvents for solid-phase extraction (SPE) were of HPLC grade from Sigma-Aldrich and Fisher Scientific (UK), with the exception of ultra-pure water, which was obtained from an Elga Purelab Ultra (Veolia, UK). SPE was carried out using a Biotage (UK) PRESSURE+48, positive pressure manifold with 48 wells and Strata-X-Drug B cartridges (60 mg, 6 mL) purchased from Phenomenex (UK). A miVac DNA concentrator (Genevac, UK) was used for evaporating samples. All solvents and reagents used for the LC-MS mobile phases were of LC-MS grade from Sigma-Aldrich (UK). Nitrogen for nebulising and drying was supplied by a nitrogen generator (Parker, UK). Silanised vials, LC-MS autosampler vials and inserts were purchased from Fisher Scientific (UK) and Hichrom (UK).

Preparation of stock solutions and working solutions

Individual stock solutions were prepared in methanol (1 mg/mL). Internal standard stock solutions of amphetamine-d_6, cocaine-d_3 and fluoxetine-d_6 were purchased as 0.1 mg/mL solutions in methanol or acetonitrile. All stock solutions were stored at -20°C. The internal standards were added to 1) mixed standards at the concentrations of 5, 0.1 and 0.75 ng/mL, respectively; 2) spiked waters at the concentrations of 50, 5, 25 ng/L, respectively.

2.2 Drinking water collection and preparation

Raw water (before treatment) and drinking water (after treatment) grab samples were collected from three DWTPs in the East Anglia region (UK) in 2L high-density polyethylene containers and transported to the laboratory immediately after collection. A further two drinking water samples were collected from taps in Cambridge (UK). All samples were stored at 5 °C and extracted within 24 h.

2.3 Solid-phase extraction

The SPE cartridges were conditioned with 2 mL methanol and equilibrated with 2 mL of 0.1 M hydrochloric acid. Then 200 mL of the water sample was acidified with 0.1 M hydrochloric acid (pH 2) and passed through the SPE cartridge, which was then washed with 2 mL 0.1 M hydrochloric acid followed by elution with 2 mL of 15% isopropanol/85% ethyl acetate and 2 x 2 mL of 10% ammonium hydroxide/20% isopropanol/70% ethyl acetate into silanised vials. The extracts were evaporated and reconstituted with 0.1 mL LC-MS injection solvent (0.5% formic acid/5% acetonitrile/94.5% water).

2.4 Liquid chromatography-mass spectrometry

Analysis was carried out using a Shimadzu Ultra High Performance Liquid Chromatography Nexera system, consisting of a pump (LC-20AD), an autosampler (SIL-20A), a photo diode array detector (SPD-M20A) and a column oven (CTO-20A), equipped with a LCMS-2020 single quadrupole mass spectrometer (MS) (Shimadzu, Japan). Two analytical columns were used, a C_{18} column (identification and quantification) and biphenyl column (confirmation). For both columns a flow rate of 0.2 mL/min and a 10 µL injection volume were used, with the column oven and autosampler set at 30 °C and 10 °C respectively.

2.4.1 Method for C_{18} column

An Acquity UPLC BEH C_{18} column (2.1 x 150 mm i.d., 1.7 µm particle size) coupled to a VanGuard pre-column (2.1 x 5 mm i.d., 1.7 µm particle size) (Waters, UK) was used. Mobile

phase A was 0.5% formic acid/99.5% acetonitrile and mobile phase B was 0.5% formic acid. The gradient programme started at 10% A for 1.5 min, then ramped until 60% A after 14 min and then ramped until 100% A after 15.5 min and held for 7 min. After the run, 10% A was restored and held for 20 min to equilibrate before the next injection.

2.4.2 Method for biphenyl column

A Kinetex biphenyl 100 Å LC column (4.6 x 100 mm i.d., 2.6 µm particle size) was used coupled to a SecurityGuard ULTRA cartridge UHPLC biphenyl (4.6 mm i.d.) (Phenomenex, UK). Mobile phase A consisted of 0.5% formic acid/59.7% methanol/39.8% acetonitrile and mobile phase B was 0.5% formic acid. The gradient programme started at 30% A for 4 min and then ramped until 60% after 19 min and then ramped until 100% A after 20 min and held for 9 min. After the run, 30% A was restored and held for 20 min to equilibrate before the next injection.

2.4.3 Mass spectrometry (MS)

The MS with an electrospray ionisation (ESI) source was used in positive ionisation mode. Interface conditions were fixed as: interface temperature 350°C; desolvation line (DL) temperature 250°C; heat block temperature 200°C; nebulising gas flow 1.5 L/min; drying gas flow 15 L/min. Data acquisition was carried out in selected ion monitoring (SIM) mode. Monitored ions of studied analytes are listed in Table S2 and S3 in the supplementary data and MS analysis time was divided into ten segments and their time intervals are shown. Event time was 0.03 min. Interface voltage was 4.5 kV and detector voltage was -1.4 kV. Other MS parameters, including DL voltage and lens system voltages (qarray DC and qarray RF), were optimised for each monitored ion and their voltage values are included in both tables. Data was collected, analysed and processed using LABSolutions software.

2.5 Method validation

Autosampler stability, instrumental linearity, instrumental precision, method precision and

accuracy, instrumental detection and quantification limits (IDL and IQL), method detection and quantification limits (MDL and MQL) and recovery were investigated using the C_{18} column for quantitative purposes. In addition, IDL was studied using the biphenyl column and used for confirmation.

2.6 Drinking water analysis

Three raw water and five drinking water samples were extracted by SPE. For each sample, three 200 mL aliquots were used as non-spiked samples and another three 200 mL aliquots were spiked with mixed standards, resulting in the added concentrations of 5, 50 and 100 ng/L. Each non-spiked and spiked samples were extracted by SPE in triplicate (Section 2.3). A blank (ultra-pure water) and a positive control (50 ng/L mixed standard) were also analysed during the run.

3 Results and discussion

3.1 Separation and selectivity using liquid chromatography-mass spectrometry

The use of formic acid to acidify the mobile phase not only improved the peak tailing by reducing the ionic interaction of basic analytes with the column, but was also beneficial for ionisation process (Sargent, 2013). ESI was used in positive ionisation mode, as all studied analytes showed maximum responses in this mode. The use of SIM mode, time segmentation and optimised DL and lens system voltages (Tables S2 and S3 in the supplementary data) improved selectivity and sensitivity. Figure 1 shows selected ion chromatograms of a mixed standard with internal standards using a C_{18} column (a) and biphenyl column (b), respectively. Based on diagnostic ions, retention times and retention indexes (the ratio of the retention time of analyte to the retention time of corresponding internal standard) shown in Table 1, the method is selective to distinguish studied analytes. Our results show that the majority of studied drugs of abuse and pharmaceuticals can be separated based on their retention times alone, including the separation of the positional isomers 3-TFMPP and 4-TFMPP. The

189 protonated molecular ions $[M+H]^+$ of the analytes were the most abundant ions and therefore
190 monitored as diagnostic ions in SIM mode for both analytical columns, as shown in Table 1 and
191 are also in agreement with other published literature (Baker and Kasprzyk-Hordern, 2011;
192 Sørensen, 2011; Ammann, et al., 2012; Asimakopoulos, et al., 2017).

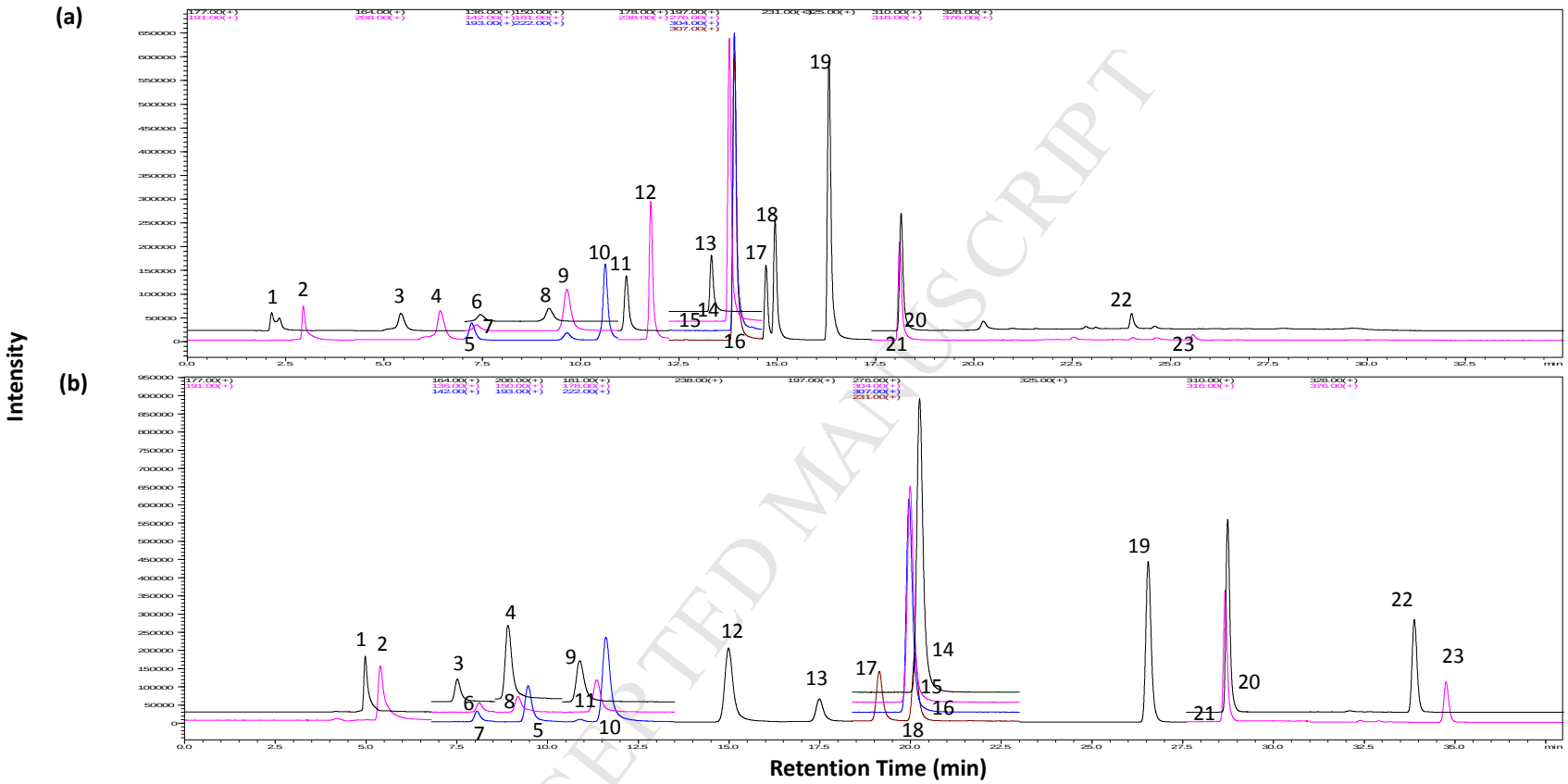

Fig. 1 Selected ion chromatograms of a mixed standard with internal standards for LC-MS analysis using (a) a C_{18} column and (b) a biphenyl column

(1) m/z 177 BZP, (2) m/z 191 MBZP, (3) m/z 164 methcathinone, (4) m/z 208 methylone, (5) m/z 193 4-MeOPP, (6) m/z 136 amphetamine, (7) m/z 142 amphetamine-d_6, (8) m/z 150 methamphetamine, (9) m/z 181 4-FPP, (10) m/z 222 butylone, (11) m/z 178 mephedrone, (12) m/z 238 ketamine, (13) m/z 197 3-CPP, (14) m/z 276 MDPV, (15) m/z 304 cocaine, (16) m/z 307 cocaine-d_3, (17) m/z 231 3-TFMPP, (18) m/z 231 4-TFMPP, (19) m/z 325 citalopram, (20) m/z 310 fluoxetine, (21) m/z 316 fluoxetine-d_6, (22) m/z 328 JWH-073, (23) m/z 376 JWH-398

193 **Table 1**

194 **Retention times (RT), retention indexes (RI), diagnostic ions and instrumental linear ranges for using a C_{18} and biphenyl column**

Analytes	C_{18} Column		Biphenyl Column		C_{18} Column	Diagnostic Ion [d]
	RT (min)	RI	RT (min)	RI	Linear Range (ng/mL)	(m/z)
BZP	2.14	0.29 [a]	4.98	0.62 [a]	0.5-1000	177
MBZP	2.95	0.40 [a]	5.39	0.67 [a]	0.1-1000	191
Methcathinone	5.42	0.74 [a]	7.52	0.93 [a]	0.25-1000	164
Methylone	6.43	0.87 [a]	8.91	1.11 [a]	0.5-1000	208
4-MeOPP	7.26	0.99 [a]	9.46	1.18 [a]	5-1000	193
Amphetamine-d_6	7.35	–	8.05	–	–	142
Amphetamine	7.42	1.01 [a]	8.12	1.01 [a]	2.5-1000	136
Methamphetamine	9.20	1.25 [a]	9.18	1.14 [a]	0.75-1000	150
4-FPP	9.65	1.31 [a]	10.89	1.35 [a]	0.25-1000	181
Butylone	10.63	1.45 [a]	11.60	1.44 [a]	0.05-500	222
Mephedrone	11.16	0.80 [b]	11.36	1.41 [a]	0.05-1000	178
Ketamine	11.78	0.85 [b]	14.99	0.75 [b]	0.05-500	238
3-CPP	13.33	0.96 [b]	17.49	0.88 [b]	0.25-1000	197
MDPV	13.78	0.99 [b]	20.25	1.01 [b]	0.1-1000	276
Cocaine-d_3	13.91	–	19.96	–	–	307
Cocaine	13.91	1.00 [b]	19.99	1.00 [b]	0.05-500	304
3-TFMPP	14.66	1.05 [b]	19.14	0.96 [b]	0.05-1000	231
4-TFMPP	15.00	1.08 [b]	20.12	1.01 [b]	0.05-1000	231
Citalopram	16.31	0.90 [c]	26.55	0.93 [c]	0.025-500	325
Fluoxetine-d_6	18.11	–	28.67	–	–	316
Fluoxetine	18.15	1.00 [c]	28.74	1.00 [c]	0.5-1000	310
JWH-073	24.01	1.33 [c]	33.00	1.15 [c]	5-1000	328
JWH-398	25.57	1.41 [c]	34.76	1.21 [c]	5-1000	376

195 [a] Amphetamine-d_6; [b] Cocaine-d_3; [c] Fluoxetine-d_6; [d] Quantifier ions using both columns

196

197 For the analysis of drugs of abuse and pharmaceuticals in aqueous samples, normally at least two
198 confirmation ions are monitored along with the quantifier ion in order to improve the reliability of
199 confirmation (Rivier, 2003). As this was not possible with the fragmentation in ESI mode, a biphenyl
200 column with different selectivity was used for further confirmation (López de Alda and Barceló, 2000),
201 after the studied analytes were first separated using a C_{18} column for quantification and initial
202 identification. This therefore allowed our method to use a single quadrupole mass spectrometer,
203 particularly as light fragmentation was observed for some analytes, e.g. butylone, citalopram, cocaine,
204 cocaine-d_3, ketamine and MDPV resulting in only one or two predominant ions in their mass spectra.
205
206 **3.2 Solid-phase extraction recoveries of drugs of abuse and pharmaceuticals**
207 To evaluate SPE recovery, three raw water samples (200 mL) were spiked with a mixed standard
208 pre-extraction. Another set of three samples were spiked post-extraction (final concentration of 200
209 ng/mL). Strata-X-Drug B was used providing mixed-mode cation-exchange sorbent and reverse-phase
210 retentions. The applied SPE method (Section 2.3) was optimised based on the generic protocol of
211 Strata-X-Drug B (Phenomenex, 2011). 0.1 M hydrochloric acid was added to convert basic groups of the
212 target analytes to their ionised form to interact with the SPE sorbent. As the Strata-X-Drug B has acidic
213 and non-polar groups on its sorbent surface, two elution solvents were used in tandem to elute the
214 desired analytes from the cartridge. The 15% isopropanol/85% ethyl acetate was first used to elute two
215 synthetic cannabinoids which are more hydrophobic and 10% ammonium hydroxide/20% isopropanol/70%
216 ethyl acetate for the other more basic compounds. In addition, with a sample loading of 200 mL and the
217 resulting eluant evaporated and reconstituted in 0.1 mL of solvent, this resulted in an enrichment factor
218 of 2000 to increase the sensitivity of the method.
219
220 The assessment of the SPE method and hence, its extraction recoveries (Table 2) were calculated using
221 Eq. 1a and 1b and these are comparable to other published recoveries using similar matrices. The results
222 also indicate good repeatability (Table 2) as shown by the relative standard deviation (RSD).
223 $$\% \text{ Absolute Recovery} = (PA_{\text{sample spiked before extraction}}/PA_{\text{sample spiked after extraction}}) \times 100 \quad \text{Eq. 1a}$$

224 % Relative Recovery = ($PAR_{\text{sample spiked before extraction}}$/$PAR_{\text{sample spiked after extraction}}$) x 100 Eq. 1b

225 Where, PA represents the peak area of analyte. PAR is the peak area ratio of the analyte to the internal

226 standard.

Table 2

SPE recoveries for studied drugs of abuse and pharmaceuticals using Strata-X-Drug B column

Analytes	Absolute Recovery (%, n = 3)	RSD (%, n = 3)	Relative Recovery (%, n = 3)	RSD (%, n = 3)	Reported in other academic papers	
					Absolute Recovery (%)	Relative Recovery (%)
3-CPP	76	4.7	79	8.6	–	–
3-TFMPP	83	3.4	86	7.9	79 [a]	101 [a]
4-FPP	86	2.9	81	2.8	–	–
4-MeOPP	39	14.5	39	14.9	–	–
4-TFMPP	62	8.7	65	14.1	–	–
Amphetamine	102	4.4	97	0.9	82 [a]; 90.5 [b]; 72 [e]; 23.2 [f]; 32.0 [g]; 33.1 [h]	99 [a]; 121.4 [b]; 101 [c]; 92 [d]; 92 [e]
Butylone	73	6.6	67	12.9	–	–
BZP	79	5.1	72	11.2	76 [a]	99 [a]
Citalopram	88	10.1	98	13.8	52.4 [f]; 50.8 [g]; 28.1 [h]	97 [i]
Cocaine	96	3.1	100	0.3	89 [a]; 70.1 [b]; 86 [e]; 0.3 [f]; 0.1 [g]; 0.0 [h]	102 [a]; 98.5 [b]; 105 [c]; 91 [d]; 86 [e]
Fluoxetine	94	14.7	103	2.6	53 [a]; 35.1 [f]; 40.2 [g]; 24.9 [h]; 33.13 [k]	101 [a]; 102 [j]; 102.44 [k]
JWH-073	96	4.8	107	14.5	22.0 [f]; 35.6 [g]; 0.0 [h]	
JWH-398	82	14.1	99	14.9	–	–
Ketamine	87	8.3	90	13.7	90 [a]; 84.4 [f]; 66.5 [g]; 68.3 [h]	100 [a]; 93 [d]
MBZP	72	9.7	65	14.5	–	–
MDPV	93	2.7	96	7.1	–	–
Mephedrone	45	11.1	47	14.6	14.3 [f]; 8.78 [g]; 23.0 [h]	
Methamphetamine	102	4.9	97	7.4	81 [a]; 93 [e]; 53.6 [f]; 22.2 [g]; 30.1 [h]	92 [a]; 108 [c]; 75 [d]; 98 [e]
Methcathinone	31	12.9	30	13.3	62 [a]	71 [a]
Methylone	72	5.4	70	12.0	–	–

[a] Surface water, Oasis MCX (Baker and Kasprzyk-Hordern, 2011); [b] Surface water, Oasis MCX (Kasprzyk-Hordern, et al., 2007); [c] Surface water, Oasis MCX (Zuccato, et al., 2008); [d] Drinking water, Oasis HLB (Boleda, et al., 2011); [e] Drinking water, PLRP-s (Valcárcel, et al., 2012); [f] Drinking water, Oasis HLB (Asimakopoulos, et al., 2017); [g] Drinking water, Oasis MCX (Asimakopoulos, et al., 2017); [h] Drinking water, Supelclean ENVI-Carb (Asimakopoulos, et al., 2017); [i] Raw water, Oasis HLB (Gros, et al., 2012); [j] Drinking water, Oasis HLB (Vanderford and Snyder, 2006); [k] Drinking water, HySphere Resin GP (López-Serna, et al., 2010)

For the absolute recoveries from raw water, as shown in Table 2, 17 out of 20 analytes had recoveries between 62-102% with < 15% RSDs, indicating good repeatability (Peters, et al., 2007). As co-extracted matrix is the common contributor to signal suppression during ESI, relative recoveries (accounting for internal standards loss) could correct for these matrix effects (Petrie, et al., 2016). In Table 2, 17 of the analytes exhibiting absolute recoveries in the range 62-102% also showed moderate and high relative recoveries (65-107%). This illustrates the applied SPE method removed undesired interferences from the water samples. When developing and validating a simultaneous detection method not all recoveries are high; however, the values were repeatable in this study and therefore precise (RSD < 15%). Although various extractions were investigated, the method reported here resulted in the highest recoveries for most of the 20 drugs of abuse and pharmaceuticals.

In Table 2, 11 of the recoveries are compared with those reported in other academic papers with similar sample matrices (surface, raw and drinking waters). We were unable to compare the majority of the NPS recoveries included in this simultaneous method as these are not reported as yet. The recovery (absolute and relative) results of amphetamine, citalopram, cocaine, fluoxetine, JWH-073, ketamine, mephedrone and methamphetamine obtained in this study are close or higher to those values published previously.

3.3 Method validation

3.3.1 Autosampler stability

Autosampler stability was evaluated at low and high concentrations of a mixed standard including internal standards (10 and 500 ng/mL) and all were stable ($p > 0.05$) in LC-MS injection solvent for up to five d when stored at 10°C. These were assessed using plots of PAR against injection time and the slopes of all the plots were not significantly different from zero ($p > 0.05$) (Saar, et al., 2010).

3.3.2 Instrumental linearity

Instrumental linearity (Table 1) was determined by linear regression (0.001-10000 ng/mL). The coefficients of determinations (R^2) for all analytes were above 0.9992, indicating good linearity ($R^2 \geq$

0.9900) (UNODC, 2009). This was evaluated further using plots of relative response (mean PAR/standard concentration) against log of concentration, which resulted in all data points within ±5% of the mean relative response which fulfilled the acceptance criterion of linearity (Huber, 2007). Table 1 shows all analytes have a linear range over four to five orders of magnitude, which also covers the expected and good working range for sample analysis.

3.3.3 Instrumental intra-assay and intermediate precisions

Instrumental intra-assay and intermediate precisions were determined by repeating the analysis of mixed standards at low (5 ng/mL), medium (50 ng/mL) and high concentrations (500 ng/mL). Intra-assay precision results showed that the RSDs of intraday replicates (n = 6) for studied drugs of abuse and pharmaceuticals ranged from 2.51 to 15.04% at low concentration, 0.33 to 6.61% for both medium and high concentrations. These values did not exceed 15% for medium and high concentrations and 20% for low concentration, indicating good repeatability of LC-MS method (Peters, et al., 2007). Moreover, intermediate precision on three separate d resulted in RSDs of 2.60-8.70% at low concentration, 1.03-6.87% at medium concentration and 0.54-3.27% at high concentration, which were all below the 20% and 15% acceptance criteria, respectively. Thus, good intermediate precision was obtained for all analytes, proving the repeatability and suitability of the simultaneous method developed in this research.

3.3.4 Method precision and accuracy

Quality control standards (mixed standard) at low (10 ng/L), medium (40 ng/L) and high concentrations (80 ng/L) of the target analytes were analysed in triplicate by LC-MS for calculating the method precision and accuracy. Method precision was evaluated by the RSDs of three replicates and ranged as 1.79-8.32% for low concentration, 0.67-7.57% for medium concentration and 0.63-7.15% for high concentration indicating good method precision. Method accuracy was assessed by the biases of calculated concentrations from their nominal concentrations (10, 40 and 80 ng/L). Concentrations were calculated using a calibration curve of the mean PARs against concentrations (5, 30, 50, 70 and 100 ng/L). Biases for

studied drugs of abuse and pharmaceuticals were below ±8.66% at low concentration (10 ng/L), ±7.98% at medium concentration (40 ng/L) and ±7.69% at high concentration (80 ng/L). Bias values were all within ±20% for low concentration and ±15% for medium and high concentrations, which indicates good accuracy obtained for all analytes (Peters, et al., 2007). This also proves the suitability of the simultaneous method developed using SPE and LC-MS in this research for the quantification of studied drugs of abuse and pharmaceuticals in drinking water.

3.3.5 Instrumental detection and quantification limits

IDL and IQL are used to specify the capabilities of the LC-MS method for detection and quantification and the results are shown in Table 3, which were calculated by root mean square error method according to Eq. 2 and 3 (Corley, 2003). Table 3 also shows comparison of IDL and IQL with other published methods.

$$IDL = (3/m) \times [(E^2/(n-2)]^{1/2} \quad \text{Eq. 2}$$

$$IQL = (10/m) \times [(E^2/(n-2)]^{1/2} \quad \text{Eq. 3}$$

Where, m represents the slope of the linear regression fit of a plot of mean PARs of five standards against corresponding concentrations. E^2 is the sum of the square of errors (difference between calculated PAR and measured PAR) for all standards. n = 5 (the number of standards).

Table 3
Instrumental detection and quantification limits for studied drugs of abuse and pharmaceuticals using a C_{18} column and biphenyl column

Analytes	This Study (LC-MS)			Literature (LC-MS/MS)	
	C_{18}	C_{18}	Biphenyl		
	IDL (ng/mL)	IQL (ng/mL)	IDL (ng/mL)	IDL (ng/mL)	IQL (ng/mL)
3-CPP	0.08	0.28	0.30	–	–
3-TFMPP	0.03	0.09	0.08	0.025 [a]	0.1 [a]
4-FPP	0.09	0.28	0.06	–	–
4-MeOPP	0.85	2.84	0.08	–	–
4-TFMPP	0.03	0.09	0.08	–	–
Amphetamine	0.53	1.78	0.48	0.1 [a]; 0.3 [b]; 0.03 [c]	0.5 [a]; 1 [b]; 0.1 [c]
Butylone	0.01	0.04	0.02	–	–
BZP	0.12	0.41	0.03	0.5 [a]	1 [a]
Citalopram	0.01	0.04	0.03	0.03 [c]	0.1 [c]
Cocaine	0.01	0.04	0.02	0.025 [a]; 0.05 [b]; 0.003 [c]	0.1 [a]; 0.2 [b]; 0.01 [c]
Fluoxetine	0.13	0.42	0.03	0.075 [a]; 0.3 [c]	0.5 [a]; 1.0 [c]
JWH-073	0.88	2.94	0.28	0.003 [c]	0.01 [c]
JWH-398	0.93	3.08	0.42	–	–

Ketamine	0.01	0.04	0.03	0.025 [a]; 0.15 [c]	0.1 [a]; 0.5 [c]
MBZP	0.05	0.17	0.03	–	–
MDPV	0.03	0.09	0.02	–	–
Mephedrone	0.03	0.08	0.11	0.03 [c]	0.1 [c]
Methamphetamine	0.27	0.89	0.23	0.025 [a]; 0.003 [c]	0.1 [a]; 0.01 [c]
Methcathinone	0.07	0.22	0.10	0.075 [a]	0.5 [a]
Methylone	0.13	0.42	0.01	–	–

IDL was only calculated for the biphenyl column due to it use for identification only [a] Baker and Kasprzyk-Hordern, 2011; [b] Kasprzyk-Hordern, et al., 2007; [c] Asimakopoulos, et al., 2017

In Table 3, the IDLs of studied drugs of abuse and pharmaceuticals determined by LC-MS in this study are lower than or similar to the reported values using LC-MS/MS (Kasprzyk-Hordern, et al., 2007; Baker and Kasprzyk-Hordern, 2011; Asimakopoulos, et al., 2017). Higher IDLs and IQLs were only observed for amphetamine, JWH-073 and methamphetamine. Thus, these results show the potential of LC-MS for the analysis of drugs of abuse and pharmaceuticals at ultra-trace level in drinking water.

3.3.6 Method detection and quantification limits

MDL and MQL defines the limitations of whole analytical method including sample preparation and instrument analysis and are shown in Table 4, these were calculated using Eq. 4 and 5. The relative recovery results used were those presented in Table 2 and the IDLs and IQLs results are those presented in Table 3. During sample preparation (200 mL) concentrations of samples were enriched by 2000, which is into consideration in the results below.

$$MDL = (IDL/Relative\ Recovery \times Concentration\ Factor) \times 100 \quad \text{Eq. 4}$$

$$MQL = (IQL/Relative\ Recovery \times Concentration\ Factor) \times 100 \quad \text{Eq. 5}$$

Table 4
Method detection and quantification limits for studied drugs of abuse and pharmaceuticals using a C_{18} column

Compound	This Study		Reported in Literature	
	MDL (ng/L)	MQL (ng/L)	MDL (ng/L)	MQL (ng/L)
3-CPP	0.05	0.18	–	–
3-TFMPP	0.02	0.05	0.05 [a]	0.10 [a]
4-FPP	0.06	0.17	–	–
4-MeOPP	1.09	3.64	–	–
4-TFMPP	0.02	0.07	–	–
Amphetamine	0.27	0.92	0.50 [a]; 0.2 [b]; 0.19 [c]; 2 [d]; 1.33 [e]	1.00 [a]; 1 [b]; 0.65 [c]; 4.0 [e]; 1.0 [j]; 4.28 [k]
Butylone	0.01	0.03	–	–

BZP	0.08	0.28	1.00 [a]	5.00 [a]
Citalopram	0.01	0.02	1.33/1.11 [e]; 0.24 [f]	4.0/3.3 [e]; 0.76 [f]; 10 [l]
Cocaine	0.01	0.02	0.05 [a]; 0.1 [b]; 0.04 [c]; 0.8 [d]; 0.13/0.11 [e]; 2 [g]	0.10 [a]; 0.3 [b]; 0.13 [c]; 0.4/0.33 [e]; 6 [g]; 0.1 [j]; 0.13 [k]; 2.5 [m]
Fluoxetine	0.06	0.20	1.00 [a]; 13.3 [e]; 0.04 [f];18 [h]; 20 [i]	5.00 [a]; 40 [e]; 0.12 [f]; 66 [i]; 10 [l]
JWH-073	0.41	1.37	0.11 [e]	0.33 [e]
JWH-398	0.47	1.56	–	–
Ketamine	0.01	0.02	0.08 [a]; 6.7/5.0 [e]	0.50 [a]; 20/16.7 [e]; 1.5 [j]
MBZP	0.04	0.13	–	–
MDPV	0.02	0.05	–	–
Mephedrone	0.03	0.09	1.33/1.11 [e]	4.0/3.3 [e]
Methamphetamine	0.14	0.46	0.05 [a]; 0.12 [c]; 0.6 [d]; 0.13 [e]	0.10 [a]; 0.41 [c]; 0.4 [e]; 0.5 [j]; 1.28 [k]
Methcathinone	0.12	0.37	0.10 [a]	1.00 [a]
Methylone	0.09	0.30	–	–

[a] Baker and Kasprzyk-Hordern, 2011; [b] Kasprzyk-Hordern, et al., 2007; [c] Zuccato, et al., 2008; [d] Bijlsma, et al., 2009; [e] Asimakopoulos, et al., 2017 (used two different sample preparation protocols, see the footnote f and g of Table 2); [f] Paíga and Delerue-Matos, 2016; [g] Campestrini and Jardim, 2017; [h] Cahill, et al., 2004; [i] Gros, et al., 2006; [j] Boleda, et al., 2011; [k] Valcárcel, et al., 2012; [l] Alonso, et al., 2010; [m] López-Doval, et al., 2017

Table 4 shows that the MDLs and MQLs of the drugs of abuse and pharmaceuticals obtained in this study are comparable or in some cases lower (3-TFMPP, BZP, ketamine and methadrone as examples) than those reported previously from other studies showing again the potential of this developed simultaneous method for the detection and quantification of studied drugs of abuse and pharmaceuticals in drinking water.

3.4 Analysis of drugs of abuse and pharmaceuticals in water samples

The drugs of abuse and pharmaceuticals detected in the raw and drinking water samples were identified by using the validated LC-MS method. Three identification points were used as recommended by the Commission Decision 2002/657/EC (Commission Decision 2002/657/EC; Rivier, 2003): (1) one RI obtained from a C_{18} column, (2) one RI obtained from a biphenyl column and (3) one quantifier ion monitored in SIM mode for both the C_{18} and biphenyl column (Table 1). The difference of RI between the water sample and positive control for all detected analytes were within ±1% for both the C_{18} and biphenyl columns which fulfilled the identification criterion published by World Anti-doping Agency (2010).

3.4.1 Presence of drugs of abuse and pharmaceuticals in drinking water

Standard addition was used for the initial quantification of the analytes in the drinking water using this new validated LC-MS method (Frenich, et al., 2009). Of the 20 drugs of abuse and pharmaceuticals analysed for seven compounds were detected, namely citalopram, cocaine, fluoxetine, ketamine, mephedrone, methamphetamine and methylone (Table 5). These samples were drinking water samples collected from the East Anglia region of the UK. The concentrations of these analytes are shown in Table S4 in the supplementary material and were all detected in the ng/L range and above their MQL values. Their detection frequencies (number of positive samples/number of total samples) are also presented in Table S4.

Table 5

Concentrations of drugs of abuse and pharmaceuticals detected in drinking water from this research (UK) and other countries

Analytes	Concentration range detected in this study (ng/L)	Range of concentrations from other studies (ng/L)
Citalopram	2.26-2.80	< 1.3-1.5 [a]
Cocaine	0.19-0.84	< 0.1-85.67 [b-f]
Fluoxetine	0.27	0.1-19.2 [g-l]
Ketamine	0.14-1.12	15.0 [c]
Mephedrone	0.77-2.81	–
Methamphetamine	2.21	< 0.5-3.13 [d, f]
Methylone	1.37	–

[a] Giebułtowicz and Nałęcz-Jawecki, 2014; [b] Campestrini and Jardim, 2017; [c] Rodayan, et al., 2016; [d] Boleda, et al., 2011; [e] Mendoza, et al., 2014; [f] Mendoza, et al., 2016; [g] Wu, et al., 2015; [h] Paíga and Delerue-Matos, 2016; [i] López-Serna, et al., 2010; [j] Benotti, et al., 2009; [k] Padhye, et al., 2014; [l] Vanderford and Snyder, 2006

3.4.1.1 Traditional illicit drugs detected

The presence and concentration of cocaine in drinking water analysed in this study (UK, 0.19-0.84 ng/L) is comparable to Japan (< 0.1 ng/L), European countries (0.1 ng/L), Spain (0.11-2.3 ng/L) and Latin American countries (0.6 ng/L) (Boleda, et al., 2011; Mendoza, et al., 2014; Mendoza, et al., 2016). Higher concentrations were reported in Canada (4.3 ng/L) and Brazil (< 6-22 ng/L), which could be due to low removal efficiency of clarification and post-chlorination treatment methods used (Rodayan, et al., 2016; Campestrini and Jardim, 2017), in comparison to the methods of treatment used for drinking water samples in this study, which consisted of pre-ozonation, clarification, post- ozonation, granular activation filtration and post-chlorination. An even higher cocaine concentration (85.67 ng/L) was detected in a study from Aranjuez of Spain (Mendoza, et al., 2016), which is explained as accidental/illegal disposal of cocaine at/near the sampling site, as the ratio of cocaine to its metabolite benzoylecgonine was 1.62. This is considered as an abnormal ratio (> 0.75), suggesting the measured value may not result from human consumption (Castiglioni, et al., 2008; van Nuijs, et al., 2009).

The concentration of methamphetamine found in the UK from this study (2.21 ng/L) is higher than that reported in Latin American countries (< 0.5-0.6 ng/L) and Spain (< 0.5-0.6 ng/L) (Boleda, et al., 2011). A possible reason may lie in different study periods, where our study was conducted in 2016 compared to the older studies of 2008 and 2009. According to the EMCDDA (2014) report, European countries have seen an increase in the use of methamphetamine since 2012. This may explain the concentration of

methamphetamine in the UK in this study (2.21 ng/L) and correlates with the concentration of 3.13 ng/L reported in Spain from 2013 (Mendoza, et al., 2016).

3.4.1.2 Antidepressants detected

Both citalopram and fluoxetine were detected in drinking water in this research and their presence is not surprising as they are the most prescribed antidepressants (Health and Social Care Information Centre, 2016), with the UK listed as the sixth highest consumer of antidepressants worldwide in 2013 (Organisation for Economic Co-operation and Development, 2015). Citalopram was detected at the concentrations between 2.26-2.80 ng/L in this study, which is slightly higher than that found in Poland (< 1.3-1.5 ng/L) (Giebułtowicz and Nałęcz-Jawecki, 2014). In addition, fluoxetine was detected at 0.27 ng/L, which is lower than 1.90-1.97 ng/L in Portugal, 2.74 ng/L in Spain, < 0.5-19.2 ng/L in the USA, but similar to 0.1-0.2 ng/L in China (Vanderford and Snyder, 2006; Benotti, et al., 2009; López-Serna, et al., 2010; Padhye, et al., 2014; Wu, et al., 2015; Paíga and Delerue-Matos, 2016) and probably due to different prescribing patterns of antidepressants across countries in the world.

3.4.1.3 Novel psychoactive substances detected

15 NPS were analysed in this study and three of these (ketamine, mephedrone and methylone) were detected in drinking water. The presence of NPS in drinking water is most likely related to their increased consumption in the UK. According to EMCDDA report (2015), there has been a seven-fold increase in the seizure of NPS across Europe between 2008 and 2013.

Ketamine concentrations ranged between 0.14-1.12 ng/L in this research. The detection of ketamine in drinking water has also been reported in Canada at a higher concentration of 15.0 ng/L (Rodayan, et al., 2016). This could be associated with the less efficient water treatment methods of clarification and post-chlorination. The treatment method used for our drinking water sample is the same as described above in section 3.4.1.1 and with a secondary amine functional group present in ketamine is a potential reaction breakdown site for ozone and chlorine treatment (Westerhoff, et al., 2005), which could explain

409 the low concentrations determined in our study.

410

411 Mephedrone and methylone were also detected in this study at the concentrations of 0.77-2.81 and 1.37
412 ng/L, respectively. The results are in agreement with patterns of drug consumption in the UK, where
413 mephedrone was the most abused cathinone, followed by methylone (Mixmag, 2012). This is the first
414 time that these two NPS have been reported to be present in drinking water, thus no data is available for
415 comparison.

416

417 **4 Conclusions**

418 A novel LC-MS based method has been developed and validated for the monitoring of 20 drugs of abuse
419 (traditional illicit drugs and NPS) and pharmaceuticals (antidepressants) from drinking water. This is the
420 first time that 15 NPS have been investigated in drinking water. We have used SPE for sample preparation
421 followed by LC-MS using a C_{18} column for detection and quantification and a biphenyl column for further
422 confirmation. The mixed mode cation-exchange SPE cartridge (Strata-X-Drug B, 6 mL) resulted in the
423 obtainment of high and repeatable recoveries (62-107%) for the majority of studied drugs of abuse and
424 pharmaceuticals. Precision and accuracy for all 20 analytes were determined at three concentration
425 levels and RSDs and biases are within the acceptance criteria of 20% RSD (Peters, et al., 2007). MDLs and
426 MQLs (0.01-1.09 ng/L and 0.02-3.64 ng/L respectively) are also comparable to other studies using
427 LC-MS/MS. Thus, this research shows that LC-MS can be a good alternative to popularly used LC-MS/MS
428 in the detection and quantification of drugs of abuse and pharmaceuticals in drinking water.

429

430 The method was applied for the evaluation of the presence of the 20 analytes in drinking water from the
431 East Anglia region of the UK, which has never been reported before. Five drugs of abuse and two
432 pharmaceuticals were detected at the range of 0.14-2.81 ng/L, including cocaine, ketamine, mephedrone,
433 methamphetamine, methylone, citalopram and fluoxetine. Two NPS (mephedrone and methylone) have
434 been reported for the first time in drinking water, which proves the newer emerging drugs of abuse are
435 present in drinking water owing to their increased consumption in the UK. It is hoped that this study will

436 inform drinking water regulatory bodies of the presence of drugs of abuse and pharmaceuticals, as they
437 are currently not included within the regulatory framework. In addition, this study may support future
438 development of early monitoring strategies for such compounds in drinking water, as little is known of
439 the possible accumulation and the health impact. In this study, drugs of abuse and pharmaceuticals were
440 detected in drinking water at trace levels (sub ng/L), which are not sufficient to induce pharmacological
441 and toxicological effects to humans. However, there are still concerns with the long-term exposure to
442 these contaminants causing a chronic human health risk, as some of these compounds are lipophilic and
443 therefore can bio-accumulate in human body. Another concern to be considered is the possible reaction
444 with other compounds which might cause synergistic or antagonistic effects. Further information
445 regarding human health impacts can be found in Peng, et al. (2016).
446

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454

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Highlights:

- A simultaneous and sensitive LC-MS method for detecting drugs in drinking water.
- Detection method for 20 drugs of abuse and pharmaceuticals including 15 NPSs.
- 5 drugs of abuse and 2 antidepressants detected in samples from East Anglia, UK.
- Mephedrone and methylone have been detected in drinking water for the first time.