Simultaneous Detection of Controlled Substances in Waste Water

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Abstract

This study presents a method of simultaneous detection of both traditional and newly emerged drugs of abuse in wastewater. The method is based on solid phase extraction (SPE) and gas chromatography- mass spectrometry (GC-MS) analysis. This analytical method separates 25 drugs from different classes including amphetamines, cathinones, tropane alkaloids, piperazines plus ketamine, amitriptyline, diazepam and morphine. In addition, newer compounds (methcathinone, mephedrone, butylone), and isomers (2-MEOPP, 4-MEOPP; 2-FPP, 4-FPP; 3-TFMPP, 4-TFMPP) have been separated, with greater sensitivity (x 100 order of magnitude). This work reports the detection of butylone, mephedrone, 4-MEOPP, 2-FPP and MBZP for the first time in waste water. This suggests that with changes in drug use patterns, constant monitoring of waste water entering treatment plants should be carried out and treatment processes need to be put in place for their removal.

KEY WORDS: Drugs, waste water, Simultaneous detection.

Introduction

The presence of chemical pollutants in water has led to increasing public awareness and concern, as well as scientific interest about the effects on the environment.[1,2] Historically, pesticides and endocrine-disrupting chemicals (EDCs) have been reported as water pollutants.[3-5] However, in recent years scientific interest in pharmaceutical products [6-9] and drugs of abuse as emerging pollutants has steadily increased.[10-12] These pollutants enter the aquatic environment mainly from treated and untreated waste water discharge [13,14] and direct disposal as a minor route. [11]

The efficiency of the treatment process in removing pharmaceuticals has been investigated by several studies and results vary depending on the chemical class and the treatment process used.[15-17] The removal efficiency range anything from 25 % to over 90 %, which still constitutes only partial removal, and therefore these drugs persist in treated waste water which is ultimately discharged into surface water. [15 18-20]

The knowledge that these pollutants are now being detected in water increases the global concern of sustainable water management. The Water Framework Directive (WFD) (2000/60/EC) of the European Union governs the quality of surface water through the Environmental Quality Standards Directive (EQSD) (2008/105/EC).[21] The EQSD (2008) acknowledges the threat to the aquatic environment that chemical pollution poses as this ultimately affects ecosystems and human health. Owing to this the EQSD identifies various priority substances that need to be regulated with regard to their discharge into surface water. On a national level, the treatment processes in England and Wales are regulated by the Environment Agency (EA) which governs the quality of waste water effluent before it is discharged.[22,23] In addition, a Chemical Investigations Program (CIP) has been set up by the UK Water Industry Research (UKWIR) and the EA to investigate how efficient the current treatment processes are in removing some of the priority substances as stipulated by the EQSD. The list of priority substances has recently been expanded to include a few pharmaceuticals such as oestrogen and ibuprofen, which have traditionally not been targeted for analysis. Although, only a few pharmaceutical compounds are being monitored in waste water effluent by CIP, this appears to be a step in the right direction in recognising the changing nature of emerging pollutants. Sources of pharmaceutical compounds found in waste water include manufacturers, hospitals, and household waste.[9,24] This leads to a wide spectrum of pharmaceutical-based pollutants not being monitored in waste water.[6]

Investigations into the presence of pharmaceuticals in treated and untreated waste water was taken a step further in 2005[25] by measuring illicit drugs in waste water, where measured levels of cocaine and its metabolites were used to estimate consumption by a population serviced by a particular waste water treatment plant (WWTP). Since then various research groups from different countries (e.g. USA, Belgium, Italy, Spain, U.K., Australia, Canada) have investigated illicit drugs in waste water and surface water.[14,26-32] The main difference between the groups has been the classes of drugs investigated, approaches to sampling, sample preparation, validation studies and interpretation of results. With such data gathered from different countries or at different times of the year, comparisons can be made regarding usage patterns of drugs of abuse in different locations or seasons.[28,29]

Moreover, sewage epidemiology as a means of estimating the consumption of drugs of abuse complements other forms of data such as criminal and medical records, drug monitoring, drug seizures and population surveys.[33] In many cases the drug consumption figures obtained from sewage epidemiology closely match figures obtained from socio-epidemiological studies.[34,35] Hence comparisons between the different forms of data can be made in order to obtain a better understanding of the trends in the use of drugs of abuse. One major advantage of the sewage epidemiology approach over socio-epidemiological methods is the production of real-time data since results from sewage can be obtained within hours or days while

socio-epidemiological studies take longer.[33,36] The sewage epidemiological approach has even been acknowledged by The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) as a feasible method for estimating community drug consumption.[37] In addition, month to month or seasonal variations can be tracked.[27,35] Although sewage epidemiology is anonymous and generalised, it can also be applied to specific geographic locations or timescales such as waste water from prisons[38] and after a major entertainment event.[39,40]

Although concentrations of drugs of abuse detected in surface waters are low, their potential risk to human and environmental health cannot be dismissed[17,41] and long term effects of exposure are not known. Based on the consumption levels of the common drugs of abuse globally and on a more local basis (U.K.)[42,-44] it can be expected that residues of these drugs and their metabolites will be present in waste water influent and effluent. Therefore these drugs and their metabolites were selected for this research (Figure 1). In addition, more novel and emerging drugs of abuse are also included such as piperazines and cathinones.[45]

Method

Chemicals and reagents

All drug standards were of analytical grade (purity >97 %), and were purchased as powders or as 1 mg/ml or 0.1 mg/ml standard solutions in methanol or acetonitrile. Amitriptlyne hydrochloride, amphetamine-d_6, amphetamine sulphate, cathinone hydrochloride, 1-(3-chlorophenyl)piperazeine (3-CPP), cocaine hydrochloride, diazepam, ecgnonine methyl ester hydrochloride hydrate (EME), ketamine, methcathinone hydrochloride, morphine sulphate pentahydrate, 1-(4-methylphenyl) piperazine (4-MPP), 1-methyl-4-benzylpiperazine (MBZP), 1-(4-methoxyphenyl) piperazine (4-MEOPP) were purchased from Sigma Chemical Company, U.K. 1-Benzylpiperazine (BZP), 1-(2-methoxyphenyl) piperazine (2-MEOPP), 3,4-methylenedioxymethamphetamine (MDMA), piperazine, 1-(4-trifluoromethylphenyl) piperazine (4-TFMPP) were purchased from Fluka, U.K. Butylone hydrochloride, 4-fluoromethamphetamine hydrochloride (4-FMA), 3-fluoromethcathinone hydrochloride (3-FMC) were purchased from National Measurement Institute, Australia. Mephedrone hydrochloride was purchased from Toronto Research Chemilab, Canada. Cocaine- d_3, methylbenzodioxolylbutanamine hydrochloride (MBDB), 3,4-methylenedioxymethamphetamine-d_5 and morphine- d_3 were purchased from Cerilliant, U.K. and 1-(3-trifluoromethylphenyl) piperazine (3-TFMPP) was purchased from Alfa Aesar, U.K. Acetone, 35% ammonium hydroxide, ethyl acetate, 37% hydrochloric acid, methanol, were purchased from Fisher Chemical Company, U.K. and sodium sulphate was purchased from Acros, U.K. Pentafluoropropionic anhydride (PFPA) was purchased from Sigma Chemical Company, U.K.

Instrumentation and chromatographic conditions

Gas Chromatography-Mass Spectrometry

A PerkinElmer Clarus 500 Gas Chromatograph-Mass Spectrometer (GC-MS) fitted with a Supelco Equity TM-5 capillary column (30 m x 0.25 µm x 0.25 mm i.d.) was used. The carrier gas was helium at a flow rate of 1 mL/min.

The GC oven program for quantification, repeatability and recovery studies was as follows: initial temperature of 50 ℃, held for 2 min, increased to 100 ℃ at 30 ℃/min then increased to 280 ℃ at 8 ℃/min and held for 5 min. The GC–MS transfer line and source temperatures were set at 280 ℃ and 230 ℃ respectively. The injector temperature was set at 250 ºC. The mass spectrometer was operated in the electron impact ionization mode (+70 eV) and the MS scan (where applicable) was (m/z 50–620). Selected ion monitoring was used for quantification and method validation. The total run time was 31.17 min and the solvent delay was 3.5 min. Data was collected, analysed and processed using TurboMass™ 5.4 GC/MS software.

For stability studies the oven program started at 80 ℃ and the transfer line was set at 260 ℃. A split ratio of 20:1 was used. All other parameters remained unchanged.

Preparation of standards and sample

Stock solutions as free base (1 mg/mL) were prepared in methanol or acetonitrile and were further diluted to individual or mixed working solutions. All standards were stored at -20 ℃ in the dark. Amphetamine-d_6, cocaine-d_3, 3,4-methylenedioxymethamphetamine-d_5 and morphine-d_3 were used as internal standards.

Mixed working standards were prepared from individual stock solutions and these were dried and stored at -20 ℃ until further analysis. For standard addition and recovery studies the standards were reconstituted in methanol for spiking. For linearity and stability studies standards were derivatised and then reconstituted in ethyl acetate.

Derivatisation

A mixed drug standard was derivatised with 0.1 mL of the PFPA:ethyl acetate (2:1 % v/v) at 90 ℃ for 30 minutes, dried and then reconstituted in ethyl acetate prior to analysis.

Sample Collection and Preparation

Waste water composite influent samples (72 hr) were collected from a treatment plant in Cambridge, U.K., in 1L polyethylene terephthalate (PET) containers. Samples were transported to the laboratory immediately after collection and vacuum filtered through a disposable 1000 mL capacity stericup funnel and receiver system with a 0.22 µm GP Millipore Express® Plus membrane (Millipore, UK). Once

filtered, samples were acidified with HCl to pH 2.4 to 2.7 and stored in high density polyethylene (HDPE) at -20 ℃ and extracted after 2 months of collection.

Extraction and recovery

Oasis MCX® (Waters, UK) cartridges were used for solid phase extraction (SPE) of waste water samples. Recovery was assessed by spiking 0.1mL mixed drug standard (1µg/mL) into 100mL treated waste water effluent. SPE protocol is given in Table 1.

INCLUDE TABLE 1

After sample loading and rinsing, the SPE sorbents were dried under vacuum and eluted on the same day of analysis. The eluents were evaporated to dryness, derivatised, reconstituted in ethyl acetate (0.1 mL) and analysed by GC-MS.

Linearity, LOD, LOQ, accuracy and precision

Instrumental linearity and range were determined by serial dilution of a mixed drug standard. Derivatised mixed drug standards were prepared in ethyl acetate at concentrations ranging from 0.0003 to 1 µg/mL

Intra-day repeatability of the analytical method was assessed over a short period under the same instrumental conditions. Six replicates of treated wastewater were spiked with 1 µg/mL of a mixed drug standard before extraction.

For autosampler stability mixed drug standard with individual drugs ranging from 15.8 to 28.8 µg/mL, except 2-FPP (40.7 µg/mL) were used. Internal standards were added as 1 µg/mL for MDMA-d_5, and morphine-d_3 and 10.2 µg/mL for cocaine-d_3.

Calibration standards and quantification

Six point standard addition calibration graphs of PAR against concentration of analyte, using the internal standards: amphetamine-d_6, MDMA-d5, cocaine-d_3, (0.06 µg/mL) and morphine-d_3 (1.25 µg/mL) was used for quantification.[46] A 500 mL, 72 hr composite waste water sample was diluted to 1 L and separated into 50 mL aliquots that were spiked with a mixed drug standards (0.0003 to 3.75 µg/mL) to enable matrix matching and standard addition to be carried out. SPE extractions were carried out according to the method described earlier.

Results and Discussion

Solvent blanks were used to ensure no carryover of the analyte in between injections. Solvent blanks and sample matrices (spiked with internal standards only) were used as negative controls. Positive controls of mixed drug standard solutions were used to generate reference standard chromatograms/spectra (TIC and/or SIM) for comparison with the analytes. All analysis (including extractions) was conducted in triplicate unless otherwise indicated.

Drug standards were simultaneously analysed (Figure 1) and identified using retention time (R_t), retention index (RI) and mass spectra and quantified using the ions (m/z) as shown in Table 2. All standards at various concentrations were analysed individually as well as a mixed standard following PFPA derivatisation. Linear range, lower detection limits (LOD) and lower quantification limits (LOQ) for each analyte were calculated (Table 2). All tables show the analytes in R_t order.

Insert Figure 1

Table 2 shows LOD and LOQs for the analytes, calculated using two methods: IUPAC[47] and signal to noise ratio (S:N)[48] The LODs and LOQs (calculated from S:N) from this research are lower compared to other publications (Jones-Lepp et al., 2004). As an example the LODs for amphetamine, methamphetamine and MDMA have been determined as 0.14, 0.14, 0.33 pg on column respectively, in comparison to 1.6, 2.9 and 5.9 pg using gas chromatography-ion trap-tandem mass spectrometry[33]. LOQs from this research for amphetamine, methamphetamine, MDMA, cocaine and morphine have been determined as 0.33, 0.33, 1.33, 0.67, 142 pg respectively and are significantly lower than reported values of 380, 208, 278, 18 and 250 pg using an LC-MS-MS analysis.[49]

Insert Table 2 here

Linearity was evaluated by plots of the deviation from the regression against the log of concentration (ISO 17025, QA/QC) [50]. The linear range (Table 2) varies from 3.0 x 10^{-1} to 1.3 µg/mL for cocaine to 5.0 x 10^{-4} to 1.0 µg/mL for amphetamine, which is analyte dependant and comparable to other publications where LC-MS-MS[49] was used.

Instrumental intra and inter-day repeatability was assessed using relative standard deviation (RSD) of the detector response, PAR (Table 3) of three concentrations (0.005, 0.1 and 1 µg/mL). Seven replicates were analysed for intra-day and three for the inter-day (three separate days) study. The intra-day relative standard deviation for 0.005 µg/mL ranges from 5.38% (amphetamine) to 24.19% (2-FPP); 0.1 µg/mL 1.76% (4-FPP) to 9.99% (4-MEOPP) and 1.0 µg/mL 0.86% (4-fluoroamphetamine) to 8.87% (ecgonine methyl ester). The inter-day relative standard deviation for 0.005 µg/mL ranges from 0.60% (mephedrone) to 15.99% (MBDB); 0.1 µg/mL 0.71% (amphetamine) to 7.57% (4-TFMPP) and 1.0 µg/mL 0.46% (methcathinone) to 10.79% (2-MEOPP). For the lowest concentration the relative standard deviation ranges are higher than those at higher concentrations. Generally there is little difference between the intra and inter-day repeatability. This shows the inherent stability of the method and instrumentation.

Autosampler stability using mixed drug standards was studied for 27 hrs. Data was analysed and interpreted using acceptance criteria of ±15% of target value[51]. A loss of <15% is considered acceptable, a loss of ≥15 - ≤30% is considered moderately stable and ≥30% loss is considered unstable. Most of the drugs were found to be stable. These include amitriptyline, amphetamine, BZP, butylone, cocaine, diazepam, 4-FMA, 3-FMC, 2 and 4-FPP, methamphetamine, mephedrone, MDMA,

3-TFMPP, 4-TFMPP, 4-MPP, ketamine, 2-MEOPP, 4-MEOPP, morphine, MBZP. However, ecgonine methyl ester was only stable for 11 hrs.

The percentage recovery values were all above 70%, except for MDMA (66%) and ketamine (58%). The method of extraction was optimized to enable simultaneous detection of this range of drugs, including the novel drugs. Therefore when compared to other publications some recoveries are improved, similar or slightly vary to enable the method to fit all the analytes of choice in this research. In addition, there are many different publications using different solid phase extraction methods and cartridges with different recovery ranges to the choice of analytes. Some of the lower recoveries could be due to the reduction of sorption efficiency of the SPE cartridge, owing to the complex sample matrix. [6]

Insert Table 3 here

The method of SPE followed by PFPA derivatisation and GC-MS analysis used in this research, was used to analyse real waste water samples collected from Cambridge U.K. Composite waste water samples (72 hrs, 25 mL x 3) were collected and analysed to enable matrix matched quantification using standard addition. The drugs detected in wastewater samples have been reported in Table 4. These include popular recreational drugs such as mephedrone, buytlone, methcathinone[45], and ketamine.

Results also show the detection of morphine (0.3033 ± 0.033 µg/mL), amitriptyline (0.0111 ± 0.006 µg/mL), ketamine (0.0972 ± 0.007 µg/mL); however, these could have been present in the waste water through disposal of prescribed pharmaceuticals, hospital waste, veterinary waste as well as illegal use. It is of interest that higher seizures of ketamine is reported in the media as well as by police officers involved in drugs seizures, Initial work by this research group with Cambridgeshire police have already found that 8 out of 35 seized samples have given positive results for ketamine.

Insert Table 4 here

Conclusion

In addition to the historically abused traditional drugs (amphetamine, methamphetamine, ketamine, amitriptyline, morphine) in this study we have reported the detection of newly emerging drugs of abuse in waste water. These include controlled cathinones (methcathinone, mephedrone, butylone) and piperazines (4-FPP, 2-MEOPP, 4-MEOPP, MBZP). Whilst the sample for this research came from Cambridgeshire, the method described can be used globally for waste waster analysis for the drugs outlined above. None of these drugs have any naturally occurring source in the U.K. and can only have been present in the water

as a consequence of controlled substance abuse or associated activities. The authors accept that amitriptyline, morphine and ketamine have legitimate medical and veterinary uses and the occurrence of these drugs in this sample could have come from either source or a combination of the two. The data obtained in this study suggest that in Cambridgeshire there is a shift in drug use patterns which include the use of the new amphetamine type stimulant drugs (piperazines and cathinones). It is of particular significance that ketamine has been recently detected within street samples of drugs in the Cambridge U.K. area (work carried out in collaboration with this research group and Cambridgeshire Constabulary) and this is also reflected by the occurrence of ketamine in the waste water.

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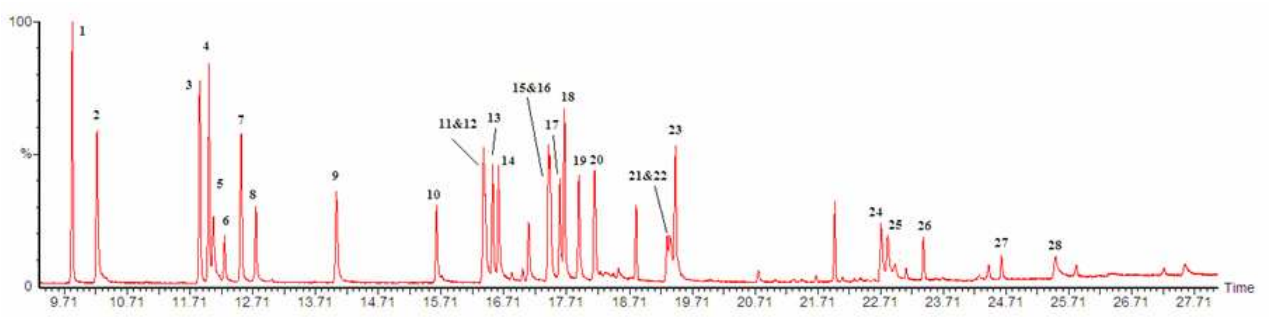

Figure 1 Total Ion Chromatogram of mixed drug standard

(1) piperazine, (2) amphetamine, (3) methamphetamine, (4) 4-fluoromethamphetamine, (5) cathinone, (6) 3-fluoromethcathinone, (7) methcathinone, (8) ecgonine methyl ester, (9) mephedrone, (10) 2-(fluorophenyl)piperazine, (11) 1-benzylpiperazine, (12) 4-(fluorophenyl)piperazine, (13) 1-(3-trifluoromethylphenyl) piperazine, (14) 3,4-methylenedioxymethamphetamine, (15) methylbenzodioxolylbutanamine, (16) 1-(4-trifluoromethylphenyl) piperazine, (17) 1-(4-methylphenyl)piperazine, (18) 1-methyl-4-benzylpiperazine, (19) 1-(2-methoxyphenyl) piperazine, (20) butylone, (21) 1-(3-chlorophenyl) piperazine, (22) 1-(4-methoxyphenyl) piperazine, (23) ketamine, (24) amitriptyline, (25) cocaine, (26) morphine, (27) diazepam, (28) heroin.

Table 1 Conditions for solid phase extraction

Parameters	
SPE CARTRIDGE	Oasis MCX® (60mg,3mL)
SAMPLE pH	2.5
CONDITION	2 mL methanol, 2 x 2 mL deionised water (pH 2.5)
FLOWRATE	5-8 mL/min
RINSE	1.5 mL deionised water (pH 2.5)
DRYING TIME	20 mins under high vacuum
ELUTION	a) 4 mL methanol (800 µL x 5); b) 5 x 800 µL 5% NH_4OH in acetone:ethyl acetate (1:1% v/v)
EVAPORATION	30-40 mins at 40 ℃ in MiVAC

Table 2 Major ions used for identification, limit of detection/quantification and linear range

Analyte	Retention index	Major ions	LOD / pg [a,b]	LOQ / pg [a,b]	LOD / pg [a,d]	LOQ / pg [a, e]	linear range / (µg/mL) [b]
Amphetamine	1252	91, 118, 190 [c]	3.67	12.24	0.14	0.33	5.0×10^{-4} - 1.0
Methampetamine	1408	118, 160, 204 [c]	2.48	8.26	0.14	0.33	1.0×10^{-3} - 0.8
4-Fluoroamphetamine	1413	136, 160, 204 [c]	5.25	17.51	0.14	0.33	1.0×10^{-3} - 1.0
Cathinone	1415	77, 105 [c] , 119	3.46	11.54	0.33	0.67	1.5×10^{-3} - 1.0
3-FMC	1421	123, 160, 204 [c]	2.24	7.46	0.14	2.67	1.0×10^{-1} - 1.0
Methcathinone	1431	105 [c] , 160, 204	6.46	21.54	0.14	0.67	2.0×10^{-3} - 1.0
Ecognine methyl ester	1438	82, 94 182 [c]	17.24	57.47	0.67	1.33	1.1×10^{-2} - 1.1
Mephedrone	1482	119 [c] , 160, 204	6.90	22.99	0.33	0.67	2.0×10^{-3} - 1.0
2-FPP	1638	150, 179, 326 [c]	23.86	79.52	0.67	2.67	1.5×10^{-3} - 1.0
BZP	1665	91 [c] , 146, 175	15.91	53.03	0.14	0.33	1.0×10^{-1} - 1.0
4-FPP	1666	150, 179, 326 [c]	21.35	71.18	0.67	2.67	1.5×10^{-3} - 1.0
3-TFMPP	1671	200 [c] , 229, 376,	28.04	93.46	0.67	2.67	2.0×10^{-3} - 0.8
MDMA	1674	160, 162, 204 [c]	17.64	58.79	0.33	1.33	1.0×10^{-1} - 1.0
MBDB	1804	160, 176, 218 [c]	22.87	76.25	0.33	0.67	2.0×10^{-3} - 0.8
4-TFMPP	1805	200 [c] , 229, 376	26.16	87.19	1.33	5.33	1.0×10^{-1} - 1.0
4-MPP	1811	146, 175, 322 [c]	21.30	71.01	2.67	5.33	1.5×10^{-3} - 1.0
MBZP	1813	105 [c] , 146, 189	14.82	49.40	0.33	0.67	1.0×10^{-1} - 0.8
2-MEOPP	1823	162, 191, 338 [c]	20.69	68.96	2.67	10.67	1.5×10^{-3} - 1.0
Butylone	1833	149 [c] , 160, 218	21.80	72.67	0.33	0.67	1.0×10^{-1} - 1.0
4-MEOPP	1880	162, 191, 338 [c]	16.97	56.57	5.33	10.67	1.5×10^{-3} - 1.0
Ketamine	1883	160 [c] , 312, 320	66.43	221.44	1.33	5.33	2.0×10^{-1} - 1.0
Amitriptyline	2226	58 [c] , 115, 202	51.70	172.33	10.67	21.33	1.0×10^{-1} - 1.0
Cocaine	2231	82 [c] , 105, 182	31.30	103.77	0.33	0.67	3.3×10^{-1} - 1.3
Morphine	2260	119, 146, 414 [c]	13.22	44.06	66.67	141.39	6.7×10^{-2} - 1.1
Diazepam	2459	256 [c] , 283, 285	89.20	297.33	10.67	21.33	2.0×10^{-1} - 0.8

[a] pg on column [b] n = 3 [c] Quantifying ion [d] calculated from 3*(S:N) [e] calculated from 10*(S:N)

Table 3 Intraday and interday repeatability and percentage recovery

Analyte	Intraday PAR [a] repeatability						Interday PAR [a] repeatability						% recovery
	0.005[b]	%[d]	0.1[b]	%[d]	1.0[b]	%[d]	0.005[c]	%[d]	0.1[c]	%[d]	1.0[c]	%[d]	
Amphetamine	0.146	5.38	3.37	2.70	36.79	1.33	0.193	1.80	3.69	0.71	38.24	1.50	79
Methampetamine	0.175	6.36	4.47	3.09	49.93	0.88	0.251	1.57	4.92	1.66	49.65	1.27	73
4-Fluoroamphetamine	0.159	5.79	4.31	3.10	48.62	0.86	0.229	2.90	4.58	1.25	47.79	0.99	73
Cathinone	0.069	14.31	1.20	2.69	6.97	0.70	0.075	4.24	1.32	2.73	7.39	0.49	133
Methcathinone	0.122	18.67	2.01	2.65	14.32	0.73	0.171	5.28	2.31	2.48	15.12	0.46	197
Ecgonine methyl ester	0.127	21.29	2.05	6.84	11.33	8.87	0.147	6.82	1.95	1.92	11.08	4.61	113
Mephedrone	0.189	7.77	4.31	2.91	34.20	1.09	0.263	0.60	4.92	2.46	37.04	0.47	149
2-FPP	0.026	24.19	1.10	2.55	9.29	2.73	0.048	3.09	1.04	5.24	9.60	8.11	111
BZP	0.595	8.80	4.25	6.05	34.66	2.02	0.872	4.29	4.95	3.83	41.85	9.54	75
4-FPP	*ND*		0.50	1.76	9.38	2.04	*ND*		0.47	4.94	9.83	8.87	148
3-TFMPP	*ND*		1.01	3.33	10.53	2.56	*ND*		0.93	6.88	10.70	9.38	150
MDMA	0.094	11.71	2.64	2.75	22.97	2.03	0.111	7.33	2.52	4.79	23.69	8.65	66
MBDB	0.094	9.97	2.23	2.59	21.01	2.84	0.117	15.99	2.14	4.59	21.12	8.00	77
4-TFMPP	*ND*		0.87	3.76	8.94	2.24	*ND*		0.81	7.57	9.26	9.62	108
4-MPP	*ND*		0.11	7.22	7.19	3.66	*ND*		0.09	7.34	5.75	10.21	153
MBZP	0.325	6.31	3.26	6.37	33.18	2.69	0.357	5.42	3.51	5.24	38.77	10.13	73
2-MEOPP	*ND*		0.28	6.72	5.68	3.65	*ND*		0.21	7.17	4.40	10.79	97
Butylone	0.389	7.97	6.18	1.80	46.69	2.95	0.448	4.46	6.26	4.68	50.93	7.26	94
3-CPP	*ND*		0.13	6.46	2.18	3.18	*ND*		0.11	3.14	1.81	9.48	96
4-MEOPP	*ND*		0.02	9.99	4.30	3.75	*ND*		*ND*		3.37	9.48	94
Ketamine	0.114	8.44	1.66	2.56	12.24	1.82	0.126	5.13	1.80	3.66	13.83	9.14	58
Amitriptyline	0.455	7.56	7.15	6.55	53.92	3.24	0.559	6.92	7.31	4.92	59.31	7.00	100
Cocaine	0.310	13.37	2.66	5.68	17.25	7.61	0.387	4.27	2.59	3.83	17.89	1.56	150
Morphine	*ND*		0.20	7.34	2.16	1.04	*ND*		0.21	3.74	2.33	1.92	71
Diazepam	*ND*		0.37	7.18	3.61	5.21	*ND*		0.38	5.39	3.49	6.83	83

[a] Peak Area Ratio [b] µg/mL (n = 3) [c] µg/mL (n = 7) [d] Relative Standard Error (%)

Table 4 Concentration of analytes from Cambridgeshire waste water

Analyte	µg/mL ± stdev [a]	pg [a,b]	notes
Amphetamine	0.0230 ± 0.0147	23.0	
Methampetamine	0.0715 ± 0.0401	71.5	
4-Fluoroamphetamine			*ND*
Cathinone			*ND*
Methcathinone	0.2531 ± 0.083	253.1	
Ecgonine methyl ester	0.1141 ± 0.056	114.1	
Mephedrone	0.5485 ± 0.046	548.5	
2-FPP			*ND*
BZP			*ND*
4-FPP	0.0980 ± 0.004	98.0	
3-TFMPP			*ND*
MDMA			*ND*
MBDB			*ND*
4-TFMPP			*ND*
4-MPP			*ND*
MBZP	0.0064 ± 0.0032	6.4	
2-MEOPP			*ND*
Butylone	0.0035 ±0.0023	3.5	
3-CPP			*ND*
4-MEOPP	0.0084 ± 0.006	8.4	
Ketamine	0.0972 ± 0.007	97.2	
Amitriptyline	0.0111 ± 0.006	11.1	
Cocaine			*ND*
Morphine	0.3033 ± 0.033	303.3	
Diazepam			*ND*

[a] n = 3 [b] on column ND = not detected