Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography-positive electrospray ionisation tandem mass spectrometry

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A new-multi residue method was developed for the environmental monitoring of 65 stimulants, opiod and morphine derivatives, benzodiazepines, antidepressants, dissociative anaesthetics, drug precursors, human urine indicators and their metabolites in wastewater and surface water. The proposed analytical methodology offers rapid analysis for a large number of compounds, with low limits of quantification and utilises only one solid-phase extraction-ultra performance liquid chromatography-positive electrospray ionisation tandem mass spectrometry (SPE-LC-MS/MS) method, thus overcoming the drawbacks of previously published procedures. The method employed solid phase extraction with the usage of Oasis MCX sorbent and subsequent ultra-performance liquid chromatography-positive electrospray ionisation tandem mass spectrometry. The usage of a 1.7 µm particle size column (1 x 150 mm) resulted in very low flow rates (0.04 mL min^{-1}), and as a consequence gave good sensitivity, low mobile phase consumption and short retention times for all compounds (from 2.9 to 23.1 min). High SPE recoveries (>60 %) were obtained for the majority of compounds. The mean correlation coefficients of the calibration curves were typically higher than 0.997 and showed good linearity in the range 0 – 1000 µg L^{-1}. The method limits of detection ranged from 0.1 ng L^{-1} for compounds including cocaine, benzoylecgonine, norbenzoylecgonine and 2-oxo-3-hydroxy-LSD to 100 ng L^{-1} for caffeine. Method quantification limits ranged from 0.5 to 154.2 ng L^{-1}. Intra- and inter-day repeatabilities were on average less than 10 %. The method accuracy range was within -33.1 to 30.1 %. The new multi-residue method was used to analyse drugs of abuse in wastewater and river water in the UK environment. Of the targeted 65 compounds, 46 analytes were detected at levels above the method quantification limit (MQL) in wastewater treatment plant (WWTP) influent, 43 in WWTP effluent and 36 compounds in river water.

KEYWORDS: illicit drugs, drugs of abuse, precursors, UPLC, wastewater, surface water, environment, LC-MS/MS, sewage, crack cocaine, SPE, pharmaceuticals, multi-residue

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1. INTRODUCTION

Abuse of both illicit drugs and legally prescribed drugs is a significant problem in societies across the world. Due to the quantity of these compounds produced and consumed, they are now established as emerging environmental contaminants. Drug residues reach natural surface waters primarily due to the insufficient removal of these compounds at wastewater treatment plants. These residues are present in the sewage water due to excretion of pharmacologically active compounds by humans after consumption; mainly via urine or faeces, and also due to the direct disposal of drug compounds from households into the sewage system.

Currently, the presence of abused compounds in the environment has been studied to a somewhat limited extent, with few publications detailing relatively comprehensive monitoring results for detection of illicit/abused drugs in surface water and/or wastewater [1-8]. As observed for some pharmaceuticals, for example diclofenac and fluoxetine [9], the presence of compounds in the environment that have been specifically designed to have an impact on humans can also have a negative impact on ecosystems, even at relatively low concentrations. Therefore, research into the presence of these biologically active compounds in the environment is vital in order to improve knowledge on the occurrence, fate and exposure of these compounds and their potential impact on aquatic and human life.

According to statistics from the United Nations Office on Drugs and Crime 'World Drug Report 2008' [10], there were an estimated 208 million illicit drugs users in the last year, with 26 million users considered severely drug dependant. Official statistics for this so called 'hidden' problem are currently generated mainly through the use of population surveys, as well as indicators such as medical figures and crime statistics [10,11]. However, as the information is collected mainly from the drug users themselves, data generated may be inaccurate, estimates are difficult to compare between different communities and due to the time consuming nature of population surveys new trends cannot be monitored rapidly [12]. Therefore, as recently recommended by the Commission on Narcotic Drugs of the United Nations [13] a novel approach is needed in order to provide more accurate and comparable drug estimates, as well as providing these estimates more rapidly in order to detect changing drug trends.

With this in mind, an approach to provide direct quantitative estimates, in a non-invasive manner and in almost real-time was first suggested by Daughton in 2001 [14] and later put into practice for the first time by Zuccato and co-workers in 2005 [15] to estimate cocaine consumption. This approach is based on the assumption that the concentration of drug residues in wastewater, before treatment, is proportional to the quantity of drug consumed by the local population from which the wastewater originated. The potential and limitations of this monitoring approach are discussed in a recent EMCDDA report [16] and a review by van Nuijs et al [17].

Liquid chromatography coupled with tandem mass spectrometry is the method of choice for the analysis of drugs of abuse in the aqueous environment due to the high signal to noise ratio and the selectivity offered [3,18-32]. The majority of recently published papers now employ the use of smaller particle size columns, termed UPLC or UHPLC depending on manufacturer, and provide well established advantages in terms of sensitivity, speed of analysis and resolution of analytes compared to HPLC. Recently, Kasprzyk-Hordern et al. [33] were able to determine R- and S- enantiomers of several amphetamines, ephedrines and venlafaxine in the aqueous environment simultaneously using a chiral HPLC column, albeit with relatively long run times. All environmental papers published employ off-line SPE for the concentration and clean-up of samples. An exception to this is the work by Postigo et al. [19] in which online SPE was utilised and the work by Chiaia et al [27] and Berset et al [30] in which large volume injection replaced the need for SPE.

From an analytical perspective the methods published to date have been fit for purpose and have successfully monitored the concentration of target residues in the environment. However the analytical methods published to date suffer from limitations; in terms of studying a relatively small number of compounds, with all methods studying 23 compounds or less [3,18-32]. Additionally, some methods

have further shortcomings, requiring more than one LC column [27], more than one LC mobile phase [18,27], more than one SPE sorbent [19] and more than one sample diluent [26,27].

In this context, the main objectives of this work were:

(1) To present a new, fast and sensitive analytical method for the detection of abused compounds overcoming many of the limitations of previous work, including (i) the use of one SPE procedure (ii) one LC method and (iii) reconstitution of sample in one diluent.

(2) To incorporate a greater number of compounds into the methodology in order to improve the value of results, understanding of concentration levels in the environment and the cost-effectiveness of each sample.

(3) To apply the validated method to river water and sewage water in various locations in order to collect the first comprehensive results from the UK.

This method is the first to provide simultaneous analysis of this quantity of drugs of abuse. This includes eight compounds (anhydroecgonine methyl ester, ecgonidine, methcathinone, 3,4-(methylenedioxyphenyl)-2-butanamine (BDB), mescaline, norbuprenorphine, benzoylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP)) that to the authors' knowledge have been analysed in the aqueous environment for the first time. Among the compounds studied are stimulants, opiod and morphine derivatives, benzodiazepines, antidepressants, dissociative anaesthetics, drug precursors, human urine indicators and associated metabolites. In addition, the proposed analytical methodology offers rapid analysis for a large number of compounds, with low limits of quantification and one extraction and one LC method, thus overcoming the drawbacks of previously published procedures.

2. EXPERIMENTAL

2.1 Chemicals and materials

Analyte names, CAS number, molecular formula, log K_{ow}, pKa and supplier are shown in Table S1. All standards and internal standards were of the highest purity available (>97 %). Mobile phase solvents and additives were all of LC-MS quality and purchased from Sigma-Aldrich, with the exception of H_2O which was purchased from Fisher. Hydrochloric acid (37 %), 5 % dimethylchlorosilane (DMDCS) in toluene and ammonium hydroxide (30 %) were purchased from Sigma-Aldrich.

Surrogate/internal standards were purchased from LGC (UK), at a purity >97 %, as solutions in methanol or acetonitrile at a concentration of 1 or 0.1 g L^{-1}: amphetamine-d11, methamphetamine-d14, nicotine-d4, buprenorphine-d4, diazepam-d5, heroin-d9, cocaine-d3, fentanyl-d5, codeine-d6, ketamine-d4, fluoxetine-d6, propoxyphene-d11, oxycodone-d6, norpropoxyphene-d5, MDMA-d5, oxazepam-d5, mescaline-d9, PCP-d5, morphine-d6, benzoylecgonine-d8, LSD-d3, methadone-d9, EDDP-d3, methaqualone-d7, dihydrocodeine-d6, MBDB-d5, cocaethylene-d8, MDEA-d5, temazepam-d5, and MDA-d5. Caffeine-d9 was purchased from Sigma-Aldrich. All surrogate/internal standards were added to samples before extraction and were used for the quantification of samples.

Individual stock solutions were purchased or prepared from powdered substance in either acetone or methanol at a concentration of 1 or 0.1g L^{-1} and stored in the dark at -20 °C. Mixed standard solutions were prepared at 10 mg L^{-1} in methanol and diluted as necessary to prepare working solutions on a daily basis. The surface of glassware was deactivated in order to minimise loss of polar compounds through absorption onto –OH sites present on the surface of glass. The procedure to deactivate the glassware consisted of rinsing (once) with reagent (5 % DMDCS/toluene) for 15 seconds, toluene (twice) and finally methanol (thrice).

Different water and wastewater samples were used for method development and validation. These were:

- UHQ water: ultra-high quality water (UHQ-PS, ELGA, UK),

- surface water: collected in Marsden (UK) close to the source of the river Calder and before discharge from industry or treatment plants,
- WWTP wastewater: wastewater influent and effluent collected from a treatment plant within the UK in April 2010 (population served 308,000, flow 55296 m^3 day^{-1}).

2.2 Sample collection and preparation

All samples were collected in amber silanised bottles with Teflon faced caps (Fisher, UK). River water and wastewater samples were vacuum filtered, firstly through GF/D 2.7 μm glass fibre filter (Whatman, UK) and subsequently through GF/F 0.7 μm glass fibre filter (Whatman, UK). After filtration, samples were acidified with 31 % HCl to pH 1.8 – 1.9. Samples were stored in the dark at 4 °C and extracted within 20 hours. Grab samples were collected from 7 WWTP and 6 river locations and transported back to the laboratory in a dark and iced cool box.

2.3 Solid-phase extraction

Solid phase extraction (SPE) of samples was carried out with Gilson SPE, Aspec XL4 (Anachem, UK). A TurboVap LV concentration workstation (Caliper, UK) was utilised for evaporation of SPE extracts. The method was optimised through several preliminary experiments involving the following variables: type of sorbent, sample volume, elution conditions, evaporation temperature and filter membrane prior to LC-MS/MS injection.

Initially the efficiency of various different sorbents was assessed, including: Isolute, HCX, 200 mg (Kinesis, UK); Oasis HLB, 60 mg (Waters, UK); Oasis MCX, 60 mg (Waters, UK); Chromabond C18ec, 200 mg (Anachem, UK); Supelclean ENVI-Carb, 250 mg (Sigma, UK) and Isolute ENV+, 100 mg (Kinesis, UK). After selection of sorbent, Oasis MCX, sample volume was optimised by assessing the extraction efficiency of spiked river water matrix (sample volumes 100 – 250 mL for wastewater and 250 – 1000 mL for river water). The loss of analytes during the evaporation step (temperatures 30, 35, 40, 45, 50 °C) and as a result of the usage of silanised/non-silanised tubes was also evaluated to ascertain whether the loss of analytes during the SPE procedure was caused by low SPE recovery, evaporation, or due to interactions of basic analytes with –OH groups present on glass surfaces. Several 0.2 μm filter membranes prior to LC injection were also investigated: Phenex – RC, PTFE, NY (Phenomenex, UK); Millex – LG, GV (Millipore, UK) and Whatman – PTFE (Whatman, UK).

The final SPE procedure was as follows. Initially the Oasis MCX was conditioned with MeOH (2 mL) and equilibrated with 2%HCOOH/H_2O (2 mL, pH 2) both at a flow rate of 3 mL min^{-1}. Acidified river water (500 mL), wastewater influent (100 mL) or effluent (100 mL) were spiked with 50 ng of each surrogate/internal standard (except amphetamine-d11, nicotine-d4 and temazepam-d5 at 75 ng; heroin-d9 and morphine-d6 at 150 ng; norpropoxyphene-d5 and fluoxetine-d6 at 100 ng and mescaline-d9 at 62.5 ng) and then passed through the MCX cartridge at a rate of 6 mL min^{-1}. Immediately following loading, cartridges were washed with 2%HCOOH/H_2O (2 mL, pH 2) at a flow rate of 3 mL min^{-1} and subsequently wrapped in aluminium foil and stored at –20 °C no longer than one week before being eluted. Cartridges were washed with 0.6%HCOOH/MeOH (2 mL, pH 2) at a flow rate of 3 mL min^{-1} followed by elution with 7%NH_4OH/MeOH (3 mL) at a flow rate of 1 mL min^{-1} into silanised vials. Extracts were evaporated to dryness (40 °C, N_2, 2-10 psi) and reconstituted with 0.3%CH_3COOH/5%MeOH/H_2O (500 μL). All samples were filtered through 0.2 μm PTFE filters (Whatman, Puradisc, 13 mm) before being transferred to maximum recovery deactivated vials with PTFE septa (Waters, UK).

SPE recoveries for studied compounds in UHQ water, surface water and wastewater were calculated as the ratio of the analyte peak area in the sample extract spiked before extraction with analytes (the peak area of analyte in unspiked sample extract was subtracted) to the analyte peak area in the non-extracted standard solution. SPE recoveries were determined at environmentally relevant

concentrations: in UHQ water, WWTP effluent and WWTP influent concentrations studied were 100, 500 and 1000 ng L^{-1}, and in surface water concentrations determined were 20, 100 and 200 ng L^{-1}.

2.4 Ultra performance liquid chromatography - tandem mass spectrometry

2.4.1 Ultra performance liquid chromatography

Analyses were carried out with the usage of Waters ACQUITY UPLC™ system (Waters, UK) consisting of ACQUITY UPLC™ binary solvent manager and ACQUITY UPLC™ sample manager. Several parameters were investigated to improve peak shape and resolution, and also to improve ESI+ performance.

Preliminary investigations involved the usage of three different columns at various flow rates, ranging from an initial pressure of 3000 psi to a maximum initial pressure of 10000 psi, and temperatures of 30, 40 and 50 °C. The columns evaluated were all ACQUITY UPLC BEH C18, 1.7 µm, columns (Waters, UK), with the following dimensions; 2.1 mm x 100 mm, 1 mm x 100 mm and 1 mm x 150 mm. Other LC parameters studied included: injection volume and mobile phase composition. The first mobile phase tested was gradient water/methanol. However, this composition caused broad peak shapes and poor separation for nearly all compounds. To solve this issue, several additives were investigated including acidic compounds: acetic acid and formic acid; and basic additives ammonium acetate and ammonium hydroxide.

The final UPLC method employed mobile phase A (pH 2.9): 79.7%H_2O, 20%MeOH, 0.3%CH_3COOH and mobile phase B (pH 3.30): 99.7%MeOH, 0.3%CH_3COOH. The gradient programme was a follows: 0min-100%A, 17min-41.3%A, 17.2min-0%A, 20.2min-0%A, 20.3min-100%A, 34.0min-100%A. An injection volume of 20 µL was injected into the system. The column, an AQUITY UPLC BEH C18 (1.7µm; 1 mm x 150 mm), was maintained at 30 °C and the temperature of the sample manager was 4 °C. The flow rate was 0.04 mL min^{-1}, which gave an initial pressure of ~6500 psi.

2.4.2 Mass spectrometry

A triple quadrupole mass spectrometer (TQD, Waters, UK) with an orthogonal electrospray ionisation (ESI) source Z-spray was used. Analysis was performed in positive mode with an optimised capillary voltage of 3 kV, source temperature of 150 °C and a desolvation temperature of 400 °C. A cone gas flow of 100 L h^{-1} and desolvation gas flow of 550 L h^{-1} were selected. Nitrogen, used as a nebulising and desolvation gas, was supplied by a high purity nitrogen generator (Peak Scientific, UK). Argon (99.999%) was used as a collision gas. Argon pressure in the collision cell was maintained at $2.5e^{-3}$ mbar.

The mobile phase flow rate of 0.04 mL min^{-1} was directly introduced in the ion source without splitting. The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode, measuring the fragmentation of the protonated pseudo-molecular ions of each compound. Data acquisition was carried out with the use of time windows, with an optimised dwell time of 20 ms per ion pair.

The choice of fragmentation ion for each compound was based on the most intense signal. The optimisation of cone voltages and collision energies was made individually for each compound by infusion of 100 µg L^{-1} standards into the stream of mobile phase. Mobile phase flow was 0.05 mL min^{-1} and the syringe pump introduced the sample at 15 µL min^{-1}. All 100 µg L^{-1} standards were prepared by spiking stock solution into methanol.

2.5 Quantification and method validation

Each compound was quantified by MRM, with the protonated molecular ion employed as the precursor. The most abundant transition product ion was typically used for quantification with a second transition, for nearly all compounds, used for confirmation. 31 deuterated internal standards were used to compensate for signal suppression or enhancement of analytes in the ESI source and low SPE recoveries. Table S2 shows the corresponding surrogate/internal standard used for each compound. A deuterated internal standard for all analytes was not possible due to lack of commercial availability, hence an internal standard that was similar in structure and gave similar analytical responses was selected as a surrogate for those compounds.

Instrumental validation parameters: linearity and range, accuracy, instrumental precision, instrumental detection and instrumental quantification limits (IDL and IQL, respectively) were determined using spiked sample diluent (0.3%CH_3COOH/5%MeOH/H_2O). Method quantification and detection parameters: linearity and range, accuracy, precision of analytical method, method detection and method quantification limits (MDL and MQL, respectively) were determined using surface water and wastewater spiked with known concentrations of analytes and then extracted according to the procedure described in section 2.3.

15-point multi-component internal standard calibration curves were prepared in surface water spiked before extraction and used for the quantification of environmental samples. The calibration curve was prepared by calculating the ratios between the peak area of each substance and the peak area of the internal standard. Masslynx 4.1 software was used to analyse and process all data.

Linearity and range of the analytical method were determined by serial dilution of a stock solution of compounds (10 mg L^{-1}). Sample diluent was spiked at concentrations typically found after extraction: 0 - 1000 µg L^{-1} of each compound, apart from creatinine which due to the high levels present in the environment was measured from 0 – 7500 µg L^{-1}. Surface water spiked before extraction was also spiked at concentrations typically found in the environment: 0 - 1000 ng L^{-1} of each compound, apart from creatinine which due to the high levels present in the environment was measured from 0 – 7.5 µg L^{-1}.

Accuracy of the method was assessed as the percentage of deviation from the known amount of analyte added to the sample. Precision was evaluated as the relative standard deviation (RSD) of replicate measurements. Both intra- and inter-day reproducibilities of the analytical method were assessed.

Instrumental intra-day precision was determined over a short period of time under the same instrumental conditions. Twenty-four determinations covered four concentrations (0.5, 50, 500 and 1000 µg L^{-1}) in sample diluents, six replicates of each. Instrumental inter-day precision was verified by determinations that covered four concentrations (5, 50, 500 and 1000 µg L^{-1}) in sample diluent, three replicates on three different days.

Intra-day precision of the analytical method was evaluated over a short period of time under the same instrumental conditions. Nine determinations covered three concentrations (0.5, 50 and 1000 ng L^{-1}) of surface water spiked before extraction, three replicates each. Inter-day precision of the analytical method was verified by determinations that covered three concentrations (0.5, 50 and 1000 ng L^{-1}) of surface water spiked before extraction, three replicates each analysed on three different days.

Quantification and detection limits were determined using a signal-to-noise approach. Standard solutions diluted with sample diluents (0.3%CH_3COOH/5%MeOH/H_2O) were used for instrumental detection and instrumental quantification limit determinations (IDL and IQL, respectively). Surface water spiked before extraction was used for method detection and method quantification limit determinations (MDL and MQL, respectively). The detection limit was selected as the concentration of compound that gave a signal-to-noise ratio of 3:1. The quantification limit corresponded to the concentration of compound that gave a signal-to-noise of 10:1. Method detection limits (MDL_{calc}) and

quantification limits (MQL_{calc}) for wastewater influent and effluent were calculated using Eqs. (1) and (2) respectively:

$$MDL_{calc} = \left(\frac{IDL \times 100}{Rec \times CF} \right) \quad (1)$$

$$MQL_{calc} = \left(\frac{IQL \times 100}{Rec \times CF} \right) \quad (2)$$

where: IDL is the instrumental detection limit (ng L^{-1}), IQL is the instrumental quantification limit (ng L^{-1}), Rec is the absolute recovery of the analyte (%) in matrix, and CF is the concentration factor, which in this method denotes 200 for wastewater.

3. RESULTS AND DISCUSSION

3.1 Choice of compounds

A total of 65 stimulants, opiod and morphine derivatives, benzodiazepines, antidepressants, dissociative anaesthetics, drug precursors and human urine indicators were selected and incorporated into the method. This includes eight compounds (anhydroecgonine methyl ester, ecgonidine, methcathinone, 3,4-(methylenedioxyphenyl)-2-butanamine (BDB), mescaline, norbuprenorphine, benzoylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP)) that to the authors' knowledge have been analysed in the aqueous environment for the first time.

The range of illicit drugs selected was based mainly on their usage from UK [34,35], Europe [36] and global statistics [10]. Abused prescription compounds were chosen based on their reputation/potential for abuse [37-39] and prescription data in England [40]. Also selected were a range of metabolites that are excreted in man after the parent drugs are consumed. The inclusion of both parent compound and metabolite gives the possibility, if the parent compound is extensively metabolised, of distinguishing between a drug which has been consumed and a drug which has been directly disposed.

For the first time, to the authors' knowledge, a method has been developed to monitor the aqueous environment to potentially differentiate the use of powder cocaine (intranasal or intravenous) to that of crack cocaine (smoked). Crack cocaine, when smoked, causes the thermal degeneration of cocaine into many compounds including anhydroecgonine methyl ester (AEME) [41-44]. Thus, when crack cocaine is smoked AEME will be inhaled. AEME is subsequently metabolised in the body to ecgonidine (ECD) [42-44]. The powdered form of cocaine cannot be vaporized or smoked to create euphoric effect [44]. Both AEME and ECD are excreted mainly in the urine, with average concentration levels according to one study of cocaine users of 341 and 3030 ng mL^{-1} respectively (AEME was detected in 457 samples and ECD in 540 sample out of 730 samples that tested positive for cocaine) [44]. AEME and ECD have been shown to be suitable for forensic urine testing to determine crack cocaine use [44]. AEME has been detected in urine after intranasal or intravenous administration of cocaine [41].

Ephedrine, pseudoephedrine and norephedrine, which are amphetamine and methamphetamine drug precursors were monitored in an attempt to develop a method which could potentially indicate drug manufacture in a local area. Although all three of these compounds have legal uses, such as in bronchodilators and nasal decongestants, their distribution is highly regulated around the globe [45].

Human urine indicators continine, 1,7-dimetylxanthine and creatinine were measured in order to potentially use these compounds to index mass loads. This could overcome variables in drug estimation with regards to fluctuating population numbers and dilution of wastewater samples. Creatinine was used to index loads of illicit drugs by Chiaia and co-workers [27].

3.2 UPLC-MS/MS Method Optimization

In LC-MS/MS an efficient separation is desired in order to minimise matrix effects and improve sensitivity. In order to optimise chromatographic performance (reduction of peak tailing and better resolution) and increase response with ESI+, different mobile phases were investigated containing several different basic and acidic additives (see section 2.4.1). Acidic additives are known to promote protonation of basic compounds [23,46] thus an increase in signal is observed in ESI+, which was also the case in this work. Acetic acid at a concentration of 0.3 % was selected as the optimal mobile phase additive due to increased sensitivity, good separation and good peak shapes.

Sufficient chromatographic separation was obtained with an AQUITY UPLC BEH C18 column (1.7 μm; 1 mm x 150 mm) at 30 °C and with relatively simple gradient (see section 2.4.1). Separation was not achieved between ephedrine and pseudoephedrine, and due to these two compounds possessing identical MRM transitions they were quantified as one, as previously reported by Postigo et al [19]. In order to separate ephedrine and pseudoephedrine chiral chromatography has to be applied [33]. Chromatograms of WWTP influent spiked with analytes before extraction are presented in Fig 1. The usage of a column with a small particle size, a small internal diameter and long length allowed for the establishment of low mobile flow rates (0.04 mL min^{-1}) and short retention times (from 2.9 to 23.1 min) for all compounds and internal standards analysed. High speed of analysis, simplicity of the gradient, and low mobile phase consumption are some of the advantages of the method. As a result a fast and cost effective method was developed.

The ESI+ parameters were optimised as discussed in section 2.4.2, with final operational conditions compiled in Table S2. All compounds showed maximum sensitivity in positive ionisation mode, with the response from analytes varying significantly due to the different functionalities present in the molecules. Data acquisition was carried out in MRM with two transition ions, one for quantification and one for confirmation. In this manner, the method fulfilled EU guidelines with four identification points for the confirmation of analytes with LC-MS/MS detection [47]. Additionally, the ratio between the two transitions was used as an identification criterion with values in environmental samples required to be within ±20 – 50 % those determined by spiked standards [47]. For five compounds only one transition was possible due to low response in the mass spectrometer, including fluoxetine, norfluoxetine, nortramadol, norpropoxyphene and 1,7-dimethylxanthine. As only one transition does not achieve four identification points, analysis of these compounds will be on a semi-quantitative basis. The ion ratios of MRM transitions in surface water spiked before extraction are provided in Table S2.

3.3 Solid-phase extraction

One of the greatest challenges with multi-residue analysis is the selection of sorbent able to give acceptable recoveries for all compounds characterised by different physicochemical properties. In this work, several different sorbents were investigated, amongst them were polymer and silica-based sorbents capable of non-polar and/or ion-exchange interactions (see section 2.3), with the aim of achieving one extraction step for all compounds. Of the sorbents investigated, the Oasis HLB and Oasis MCX were found to give the highest recoveries for the majority of compounds. The HLB gave superior recoveries most notably for heroin and the antidepressants, whereas the MCX provided improved recoveries for ecgonidine. An advantage of the MCX is the need to ionise basic compounds which requires an acidic pH (~pH 2.0) for extraction, a pH that promotes the stability of many compounds studied in this work [21], compared to an optimum neutral pH for the HLB sorbent. Additionally, as basic compounds are retained by the MCX through mixed mode interactions, an organic solvent washing step may be incorporated to remove acidic and neutral compounds, hence providing visibly cleaner extracts in comparison to the HLB. For these reasons, the Oasis MCX sorbent was selected.

Besides SPE sorbent, several other factors were also investigated. The evaporation step was investigated (temperatures 30, 35, 40, 45, 50 °C) with an evaporation temperature of 40 °C providing

an optimal compromise between analyte recovery and time taken to evaporate SPE extract to dryness. Recovery of some compounds during the evaporation step was also shown to increase with the use of silanised vials, which prevent the adsorption of basic compounds onto –OH sites present on the surface of glassware. Sample volume was investigated in order to select the optimum volume to achieve the highest possible recoveries and limits of detection, while also being mindful that an increase in sample volume brings with it difficulties in terms of an increased time for extraction, possible matrix effects and difficulties in the collection, handling and storage of samples. Sample volumes selected for wastewater influent, effluent and river water were 100, 100 and 500 mL respectively. Two wash steps were employed to remove matrix and provide cleaner extracts, firstly acidified water followed by acidified organic solvent. The acidified water did not result in the loss of any compounds, however acidified methanol resulted in the breakthrough of temazepam, caffeine and 1,7-dimethylxanthine and subsequently lower recoveries of these compounds. Due to the significantly cleaner extracts provided with the washing step it was concluded that the washing step should remain. An elution volume of 2 mL was shown to elute nearly 100 % of all compounds, thus to err on the side of caution an elution volume of 3 mL was selected. Prior to UPLC-MS/MS injection, samples must be filtered through a 0.2µm filter to ensure the removal of particles which could potentially block the column pre-filter, thus several filter membranes were investigated (see section 2.3). PTFE membrane (Whatman) was selected as it provided recoveries of over 90 % for all compounds, apart from nordiazepam (88 %) and nitrazepam (83 %).

High recoveries were obtained for the majority of compounds studied in UHQ water, river water, and WWTP influent and effluent. The variety of structured compounds with different physicochemical properties resulted in different recoveries, which for the majority of compounds were > 60 % in WWTP influent. Recovery values obtained for heroin and its deuterated analogue were unacceptably low and did not permit quantification. However, in addition to the three compounds lost during the wash step (caffeine, 1,7-dimethylxanthine and temazepam), results for heroin may be deemed 'semi-quantitative' due to internal standards able to compensate for the loss of compounds during the extraction procedure. EDDP and EMDP showed highly variable recoveries across the different matrices studied.

Deuterated internal standards were added prior to SPE extraction in order to compensate for losses or enhancement of compounds during both the sample preparation procedure and resulting from matrix effects. Absolute and relative (relative to deuterated internal standard) recoveries are presented in Table 2.

3.4 Quantification and method validation parameters

Concentrations of compounds were calculated using the standard calibration curve for the surface water spiked with compounds before extraction, which were constructed using a detector response defined as the ratio of the peak ion (the specific product ion of the highest intensity) to the base peak ion of the internal standard.

The mean correlation coefficients (R^2) of the calibration curves prepared in sample diluent were typically higher than 0.997 and showed good linearity in the range 0 – 1000 µg L^{-1}. Similarly the R^2 values of the calibration curves prepared in surface water spiked before extraction were typically higher than 0.997 and showed good linearity in the range 0 – 1000 ng L^{-1} for the majority of compounds (Table 1). An exception to this was creatinine, with an unacceptable R^2 value (0.929) when spiked in surface water before extraction. This value is likely to be a result of poor SPE recovery at higher concentrations as an excellent R^2 value was obtained in spiked sample diluent. As high concentrations of creatinine are found is wastewater, direct injection of samples could be conducted to analyse creatinine.

The instrumental and method limits of detection and quantification are presented in Table 1. Method limits of quantification were in general similar or in some cases lower than those reported by previous authors, leading to advancement on current methods due to the number of compounds incorporated into

this multi-residue method. The instrumental limits of quantification varied from 0.1 μg L^{-1} for several compounds including cocaine, benzoylecgonine and EDDP to 10 μg L^{-1} for creatinine. The method limits of detection were at low nanogram per litre levels and ranged from 0.1 ng L^{-1} for compounds including cocaine, benzoylecgonine, norbenzoylecgonine and 2-oxo-3-hydroxy-LSD to 100 ng L^{-1} for caffeine. Method quantification limits ranged from 0.6 to 154.2 ng L^{-1} in effluent and from 0.5 to 139.9 ng L^{-1} in influent.

The instrumental intra-day and inter-day repeatabilities, as indicated by standard deviation, were below 7 % and 8 %, respectively for nearly all samples (Table S3). The method intra-day repeatability was less than 8 % for nearly all samples, with inter-day repeatability's in general less than 10 % (Table S4). The method accuracy range was within -33.1 to 30.1 % (Table S5).

3.5 Environmental application

The new multi-reside method was used to analyse stimulants, opiod and morphine derivatives, benzodiazepines, antidepressants, dissociative anesthetics, drug precursors and human urine indicators in wastewater and river water in the UK environment.

Wastewater grab samples, both influent and effluent, were collected from seven WWTPs in the UK in June 2010 during a period of low rainfall. Three of the wastewater plants sampled served small rural communities (population 10,000 - 15,000), with the remaining four plants serving large urban populations (population 200,000 – 308,000). River water was sampled along a major river in the UK at six locations near to discharge points of the sampled WWTPs. Maximum and average concentration values of all three matrices are shown in Table 3.

Of the targeted 65 compounds, 47 compounds were detected at levels above the MQL in WWTP influent, 46 in WWTP effluent and 38 compounds in river water. Unusually high concentrations of amphetamine in relation to other monitoring campaigns by other authors were detected in two of the seven WWTP influent samples (1583 and 2300 ng L^{-1}). Similarly, concentrations of tramadol and its metabolite nortramadol were found to be unusually high in all three studied matrices. Many of the compounds present in wastewater influent are not effectively removed by the treatment systems and compounds are consequently discharged into the rivers.

4. CONCLUSIONS

A new-multi residue method was developed for the environmental monitoring of 65 stimulants, opiod and morphine derivatives, benzodiazepines, antidepressants, dissociative anaesthetics, drug precursors and human urine indicators. The methodology enabled all compounds to be analysed through the usage of one extraction step, one reconstitution step and one LC-MS/MS method, thus overcoming many limitations of previously published work in which extra steps were required or a relatively small number of compounds were analysed. The method employed solid phase extraction with the usage of a strong cation-exchange mixed-mode polymeric sorbent (Oasis MCX, 60 mg) and subsequent ultra performance liquid chromatography positive electrospray ionisation tandem mass spectrometry. The usage of a 1.7 μm particle size column, 1 mm in diameter and 150 mm in length led to the use of very low flow rates (0.04 mL min^{-1}), and as a consequence gave good sensitivity, low mobile phase consumption and short retention times for all compounds (from 2.9 to 23.1 min).

This work, for the first time to the authors' knowledge, has developed an analytical method to measure crack cocaine metabolites, anhydroecgonine methyl ester and ecgonidine, in the aqueous environment. Thereby potentially measuring the trends of crack cocaine use and differentiating the use of powder cocaine to that of crack cocaine. Although these crack cocaine tracers were not detected in this work, it should be noted that the number of environmental samples was relatively small. It may also be the case that these compounds are present at concentrations lower than the method detection limit, hence may be the subject of further analytical development. Additionally, this paper describes the

potential to monitor drug manufacturing precursors in order to indicate the manufacture of illicit drugs in a local area.

The method was sensitive enough to detect many compounds in the aqueous environment, providing high SPE recoveries for nearly all compounds and limits of quantification in the low nano gram per litre range. The developed method was applied to wastewater (influent and effluent) and river water in the UK. The results confirmed that the multi-residue method is suitable, with the detection of many compounds in all matrices studied. Many drugs of abuse were found not only in WWTP influent, but also WWTP effluent and at lower levels in UK river water.

The developed methodology presented will be employed within the UK to monitor wastewater at various geographical locations throughout the year in order to provide information on drug consumption and drug trends. Effluent from WWTPs with various different treatment systems will be monitored to assess the removal efficiency of the studied compounds. Additionally, river water will be monitored in order to assess the impact of the discharge from WWTPs into receiving waters.

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Table 1 – Performance data for studied compounds in sample diluent, surface water, wastewater effluent and wastewater influent

Compound	t_R[d]	Sample diluent[a]						Surface water[b]						WWTP effluent[b]		WWTP influent[b]	
	(min)	Linearity range			R^2	$IDL_{S/N}$	$IQL_{S/N}$	Linearity range			R^2	$MDL_{S/N}$	$MQL_{S/N}$	MDL_{calc}[c]	MQL_{calc}[c]	MDL_{calc}[c]	MQL_{calc}[c]
		(µg L^{-1})				(µg L^{-1})	(µg L^{-1})	(ng L^{-1})				(ng L^{-1})	(ng L^{-1})	(ng L^{-1})	(ng L^{-1})	(ng L^{-1})	(ng L^{-1})
Stimulants and their metabolites																	
Cocaine	11.8	0.025	-	1000	0.999	0.025	0.10	0.05	-	750	0.999	0.05	0.10	0.2	0.6	0.2	0.7
Benzoylecgonine	10.7	0.025	-	1000	0.999	0.025	0.10	0.05	-	500	0.992	0.05	0.10	0.2	0.6	0.2	0.7
Norbenzoylecgonine	11.4	0.025	-	750	0.998	0.025	0.10	0.05	-	500	0.996	0.05	0.10	0.2	0.8	0.2	0.8
Norcocaine	12.8	0.025	-	1000	0.999	0.025	0.10	0.05	-	1000	0.998	0.05	0.10	0.2	0.7	0.2	0.8
Cocaethylene	13.9	0.025	-	750	0.999	0.025	0.10	0.05	-	750	0.998	0.05	0.10	0.1	0.6	0.2	0.9
Anhydroecgonine methyl ester	3.6	0.100	-	750	0.995	0.100	0.50	0.50	-	750	0.995	0.50	5.00	2.5	12.3	2.8	14.2
Ecgonidine	3.0	0.100	-	750	0.997	0.100	0.50	1.00	-	750	0.994	1.00	5.00	6.3	31.5	3.0	15.2
Amphetamine	7.5	0.100	-	1000	0.999	0.100	0.50	0.50	-	1000	0.999	0.50	1.00	0.4	2.1	1.0	5.1
Methamphetamine	7.9	0.025	-	1000	0.999	0.025	0.10	0.05	-	1000	0.999	0.05	0.10	0.1	0.6	0.1	0.6
Methcathinone	5.6	0.075	-	1000	0.999	0.075	0.50	0.10	-	1000	0.992	0.10	1.00	0.8	5.4	1.5	9.7
Benzylpiperazine (BZP)	4.3	0.500	-	1000	0.998	0.500	1.00	1.00	-	750	0.994	1.00	5.00	4.2	8.3	4.8	9.6
Trifluoromethylphenylpiperazine (TFMPP)	14.5	0.025	-	1000	0.999	0.025	0.10	0.05	-	500	0.998	0.05	0.10	0.3	1.3	0.2	0.7
Hallucinogens and their metabolites																	
MDA	8.1	0.050	-	1000	0.999	0.050	0.50	0.10	-	1000	0.999	0.10	0.50	0.4	4.2	0.4	4.2
MDMA (Ecstasy)	8.3	0.025	-	1000	1.000	0.025	0.10	0.10	-	1000	0.998	0.10	0.50	0.2	0.8	0.2	0.7
MDEA (MDE)	9.4	0.025	-	1000	1.000	0.025	0.10	0.05	-	1000	0.999	0.05	0.10	0.5	1.8	0.3	1.1
MBDB	10.3	0.025	-	1000	0.998	0.025	0.10	0.05	-	1000	1.000	0.05	0.10	0.3	1.2	0.2	0.7
BDB	10.3	0.075	-	100	0.993	0.075	0.50	0.50	-	100	0.995	0.50	1.00	0.5	3.4	0.3	2.0
Mescaline	7.0	0.500	-	1000	0.999	0.500	1.00	1.00	-	1000	0.997	1.00	5.00	22.8	45.6	26.5	52.9
LSD	13.8	0.025	-	1000	0.999	0.025	0.10	0.05	-	1000	0.998	0.05	0.10	0.2	0.7	0.2	0.7
O-H-LSD	9.1	0.025	-	1000	0.999	0.025	0.10	0.05	-	250	0.999	0.05	0.10	0.2	0.7	0.2	0.9
Human indicators																	
Caffeine	9.0	0.100	-	1000	0.999	0.100	0.50	50.00	-	10000	0.991	50.00	100.00	19.2	96.2	23.8	119.0
1,7-dimethylxanthine	6.1	0.100	-	1000	0.998	0.100	0.50	1.00	-	1000	0.939	1.00	5.00	9.6	48.1	16.7	83.3
Nicotine	3.3	0.075	-	1000	0.992	0.075	0.50	1.00	-	1000	0.995	1.00	5.00	2.0	13.5	0.7	4.7
Continine	3.6	0.075	-	750	0.999	0.075	1.00	0.50	-	250	0.991	0.50	5.00	4.0	53.9	2.6	34.0
Creatinine	2.9	0.500	-	7500	0.996	0.500	10.00	-	-	-	-	-	-	-	-	-	-
Opiods, morphine derivatives and their metabolites																	
Heroin	11.4	0.075	-	1000	0.999	0.075	0.50	5.00	-	1000	0.997	5.00	10.00	23.1	154.2	21.0	139.9
6-acetylmorphine	6.4	0.075	-	1000	0.999	0.075	0.50	0.10	-	1000	1.000	0.10	0.50	0.3	2.1	0.4	2.6
Codeine	4.8	0.075	-	1000	0.999	0.075	0.50	0.10	-	1000	0.999	0.10	0.50	0.5	3.2	0.6	3.7
Norcodeine	5.1	0.100	-	1000	0.998	0.100	0.50	0.50	-	1000	0.998	0.50	1.00	0.6	3.0	0.7	3.4
Oxycocodone	5.4	0.075	-	750	0.999	0.075	0.50	0.10	-	750	0.998	0.10	0.50	0.7	4.3	0.7	4.9
Oxymorphone	3.6	0.075	-	750	0.998	0.075	0.50	0.10	-	750	0.997	0.10	0.50	1.7	11.1	1.6	10.5
Morphine	3.5	0.100	-	1000	1.000	0.100	0.50	0.50	-	1000	0.995	0.50	1.00	2.5	12.5	13.0	64.8
Normorphine	3.5	0.100	-	1000	0.998	0.100	0.50	1.00	-	1000	0.994	1.00	5.00	2.2	11.1	6.5	32.6
Dihydrocodeine	4.6	0.075	-	1000	0.999	0.075	0.50	0.10	-	1000	0.999	0.10	0.50	0.5	3.6	0.8	5.1
Buprenorphine	17.3	0.100	-	1000	0.999	0.100	0.50	0.50	-	1000	0.999	0.50	1.00	0.7	3.5	1.8	8.9
Norbuprenorphine	15.2	0.100	-	500	0.998	0.100	0.50	0.50	-	500	0.998	0.50	1.00	0.7	3.6	1.0	5.1
Methadone	20.2	0.025	-	750	1.000	0.025	0.10	0.08	-	750	0.998	0.08	0.50	0.2	0.7	0.2	0.8
EDDP	16.7	0.025	-	1000	0.999	0.025	0.10	0.10	-	1000	0.998	0.10	0.50	0.2	0.7	0.5	2.0
EMDP	20.8	0.025	-	500	0.999	0.025	0.10	0.10	-	500	0.999	0.10	0.50	0.2	0.8	0.2	1.0

Fentanyl	15.3	0.025	-	750	0.997	0.025	0.10	0.08	-	750	0.994	0.08	0.50	0.1	0.6	0.2	0.8
Norfentanyl	11.0	0.025	-	750	0.998	0.025	0.10	0.10	-	750	0.992	0.10	0.50	0.2	0.8	0.3	1.2
Propoxyphene	19.6	0.075	-	1000	0.999	0.075	1.00	0.10	-	1000	0.998	0.10	5.00	0.3	4.5	0.4	5.2
Norpropoxyphene	20.1	1.000	-	1000	0.997	1.000	5.00	1.00	-	1000	0.992	1.00	5.00	4.1	20.4	4.2	21.2
Tramadol	11.4	0.050	-	1000	0.995	0.050	0.50	0.10	-	1000	0.995	0.10	1.00	0.3	2.8	0.5	5.1
Nortramadol	12.5	1.000	-	500	0.998	1.000	5.00	1.00	-	500	0.993	1.00	5.00	2.4	12.0	1.5	7.4
Benzodiazpines and their metabolites																	
Temazepam	22.2	0.025	-	750	0.998	0.025	0.10	0.50	-	750	0.996	0.50	1.00	5.2	20.9	4.2	16.6
Diazepam	23.1	0.075	-	1000	0.999	0.075	0.50	0.10	-	1000	0.997	0.10	0.50	0.5	3.3	0.9	6.0
Nordiazepam	22.8	0.075	-	1000	0.997	0.075	0.50	0.10	-	1000	0.999	0.10	0.50	0.4	2.8	0.7	4.9
Nitrazepam	19.8	0.075	-	500	0.995	0.075	0.50	0.10	-	500	0.999	0.10	1.00	0.5	3.3	0.5	3.2
7-aminonitrazepam	6.2	0.075	-	500	0.996	0.075	0.50	0.50	-	500	0.997	0.50	1.00	1.2	7.9	1.9	12.9
Oxazepam	21.7	0.075	-	1000	0.999	0.075	0.50	0.10	-	1000	0.998	0.10	0.50	0.9	5.7	0.9	5.9
Chlordiazepoxide	17.2	0.075	-	1000	0.997	0.075	0.50	0.10	-	1000	0.999	0.10	0.50	0.5	3.0	0.5	3.1
Antidepressants and their metabolites																	
Dosulepin	19.2	0.075	-	1000	0.999	0.075	0.50	0.10	-	500	0.991	0.10	0.50	0.6	4.1	0.7	4.5
Amitriptyline	20.8	0.075	-	1000	0.999	0.075	0.50	0.10	-	500	0.993	0.10	0.50	0.5	3.4	0.7	4.8
Nortriptyline	21.3	0.075	-	1000	0.999	0.075	0.50	0.10	-	500	0.994	0.10	0.50	0.6	4.0	0.7	4.9
Fluoxetine	21.6	0.075	-	1000	0.999	0.075	0.50	1.00	-	1000	0.998	1.00	5.00	0.5	3.3	0.5	3.0
Norfluoxetine	21.8	0.075	-	250	0.996	0.075	0.50	0.50	-	250	0.999	0.50	5.00	0.5	3.4	0.4	2.7
Venlafaxine	15.4	0.075	-	1000	0.999	0.075	0.50	0.10	-	250	0.992	0.10	0.50	0.5	3.6	0.5	3.5
Dissociative anesthetics and their metabolites																	
Phencyclidine	14.7	0.025	-	1000	0.999	0.025	0.10	0.08	-	1000	0.999	0.08	0.50	0.1	0.6	0.1	0.5
Ketamine	10.4	0.025	-	1000	0.999	0.025	0.10	0.08	-	1000	0.999	0.08	0.50	0.3	1.3	0.3	1.0
Norketamine	10.2	0.075	-	1000	0.999	0.075	0.50	0.10	-	1000	1.000	0.10	0.50	1.0	6.5	0.7	5.0
Other																	
Methaqualone	20.2	0.075	-	50	0.995	0.075	0.50	0.10	-	50	0.999	0.10	0.50	0.4	2.9	0.5	3.1
Sildenafil (viagra)	18.8	0.500	-	250	0.997	0.500	1.00	1.00	-	250	0.998	1.00	5.00	2.3	4.7	2.3	4.6
Drug precursors																	
Ephedrine/Pseudoephedrine	5.9	0.500	-	1000	0.999	0.500	1.00	5.00	-	1000	0.995	5.00	10.00	2.6	5.2	2.8	5.6
Norepehedrine	5.2	1.000	-	500	0.995	1.000	5.00	5.00	-	500	0.992	5.00	10.00	1.7	8.4	1.9	9.5

[a] Sample diluent spiked with compounds
[b] Surface water and wastewater spiked with compounds before extraction
[c] MDL_{calc} and MQL_{calc} calculated for the lowest recorded SPE recovery
[d] Retention time determined in wastewater influent spiked before extraction

Table 2 – SPE recovery for studied compounds

Compound	SPE recovery (%) (n=6)																			
	WWTP influent[b]				WWTP effluent[b]				Surface water[d]				UHQ water[e]			
	Absolute		Relative[a]		Absolute		Relative[a]		Absolute		Relative[a]		Absolute		Relative[a]	
Stimulants and their metabolites																
Cocaine	91 ± 2		96 ± 6		93 ± 4		102 ± 6		89 ± 3		102 ± 2		86 ± 7		103 ± 4	
Benzoylecgonine	103 ± 19		106 ± 20		91 ± 4		100 ± 2		91 ± 3		102 ± 1		88 ± 2		101 ± 3	
Norbenzoylecgonine	93 ± 4		96 ± 5		92 ± 3		101 ± 2		95 ± 3		106 ± 4		77 ± 2		89 ± 3	
Norcocaine	88 ± 5		92 ± 9		87 ± 2		95 ± 5		95 ± 6		109 ± 5		83 ± 9		100 ± 5	
Cocaethylene	88 ± 2		94 ± 5		95 ± 5		99 ± 5		95 ± 3		101 ± 2		88 ± 6		101 ± 3	
Anhydroecgonine methyl ester	74 ± 7		77 ± 10		92 ± 16		100 ± 16		74 ± 3		85 ± 3		92 ± 7		111 ± 15	
Ecgonidine	77 ± 5		79 ± 5		93 ± 80		101 ± 82		42 ± 7		47 ± 8		115 ± 32		131 ± 28	
Amphetamine	70 ± 17		78 ± 16		82 ± 13		95 ± 11		82 ± 4		99 ± 5		98 ± 29		100 ± 18	
Methamphetamine	76 ± 2		90 ± 3		81 ± 2		94 ± 3		81 ± 4		92 ± 3		84 ± 6		97 ± 1	
Methcathinone	33 ± 3		39 ± 3		56 ± 0		65 ± 3		62 ± 1		71 ± 4		64 ± 7		74 ± 12	
Benzylpiperazine (BZP)	101 ± 18		130 ± 29		84 ± 7		101 ± 9		76 ± 12		99 ± 22		52 ± 10		68 ± 17	
Trifluoromethylphenylpiperazine (TFMPP)	79 ± 9		102 ± 16		77 ± 11		92 ± 6		79 ± 10		101 ± 14		63 ± 5		81 ± 7	
Hallucinogens and their metabolites																
MDA	90 ± 3		97 ± 8		81 ± 4		105 ± 7		87 ± 10		99 ± 7		89 ± 4		102 ± 9	
MDMA (Ecstasy)	86 ± 3		94 ± 6		84 ± 6		102 ± 10		85 ± 4		104 ± 6		91 ± 4		103 ± 6	
MDEA (MDE)	86 ± 6		94 ± 7		89 ± 7		101 ± 10		87 ± 5		102 ± 3		88 ± 1		103 ± 2	
MBDB	88 ± 5		95 ± 8		85 ± 2		101 ± 4		85 ± 2		101 ± 4		88 ± 3		104 ± 4	
BDB	89 ± 5		96 ± 7		87 ± 3		103 ± 7		86 ± 3		102 ± 5		89 ± 2		104 ± 1	
Mescaline	85 ± 18		89 ± 15		103 ± 22		108 ± 10		89 ± 3		96 ± 3		86 ± 3		103 ± 2	
LSD	80 ± 2		97 ± 8		99 ± 23		106 ± 7		51 ± 8		101 ± 6		66 ± 10		103 ± 3	
O-H-LSD	78 ± 5		95 ± 13		84 ± 4		91 ± 17		76 ± 5		153 ± 38		68 ± 1		108 ± 11	
Human indicators																
Caffeine	36 ± 17		166 ± 120		7 ± 3		100 ± 28		10 ± 4		179 ± 82		18 ± 3		113 ± 5	
1,7-dimethylxanthine	15 ± 26		48 ± 113		3 ± 3		44 ± 42		6 ± 1		95 ± 20		55 ± 1		348 ± 50	
Nicotine	76 ± 9		90 ± 11		82 ± 31		84 ± 28		95 ± 36		104 ± 16		68 ± 4		110 ± 5	
Continine	37 ± 2		105 ± 6		48 ± 8		70 ± 12		53 ± 4		125 ± 22		105 ± 12		179 ± 23	
Opiods, morphine derivatives and their metabolites																
Heroin	3 ± 0		124 ± 6		2 ± 0		130 ± 18		1 ± 0		129 ± 25		1 ± 0		141 ± 26	
6-acetylmorphine	139 ± 16		141 ± 20		119 ± 5		129 ± 2		102 ± 7		113 ± 7		120 ± 8		130 ± 5	
Codeine	103 ± 4		105 ± 8		98 ± 10		106 ± 9		92 ± 2		103 ± 5		96 ± 7		104 ± 3	
Norcodeine	91 ± 3		92 ± 6		91 ± 5		98 ± 3		84 ± 2		94 ± 1		92 ± 12		100 ± 6	
Oxycocodone	57 ± 3		97 ± 9		75 ± 11		108 ± 10		82 ± 20		120 ± 24		62 ± 16		101 ± 4	
Oxymorphone	68 ± 6		115 ± 19		80 ± 6		117 ± 4		69 ± 9		102 ± 13		71 ± 23		114 ± 9	
Morphine	75 ± 6		85 ± 8		90 ± 2		99 ± 2		96 ± 5		153 ± 9		124 ± 4		120 ± 21	
Normorphine	92 ± 6		105 ± 8		103 ± 17		114 ± 17		68 ± 6		109 ± 15		96 ± 18		91 ± 5	
Dihydrocodeine	96 ± 7		93 ± 3		98 ± 7		99 ± 2		88 ± 1		102 ± 2		94 ± 8		98 ± 2	
Buprenorphine	77 ± 5		86 ± 6		93 ± 3		99 ± 3		84 ± 4		97 ± 2		80 ± 19		100 ± 3	
Norbuprenorphine	85 ± 4		96 ± 6		93 ± 3		100 ± 5		74 ± 2		86 ± 4		86 ± 13		109 ± 9	
Methadone	97 ± 6		99 ± 5		90 ± 2		102 ± 4		89 ± 4		106 ± 4		79 ± 25		97 ± 7	
EDDP	38 ± 4		107 ± 3		83 ± 13		120 ± 20		38 ± 5		88 ± 3		54 ± 4		92 ± 4	
EMDP	55 ± 3		57 ± 4		66 ± 3		75 ± 3		36 ± 3		43 ± 4		6 ± 2		7 ± 1	
Fentanyl	96 ± 7		98 ± 7		95 ± 5		100 ± 6		87 ± 1		102 ± 5		86 ± 13		102 ± 0	

Norfentanyl	85	±	2	86	±	3	86	±	5	91	±	4	74	±	7	87	±	5	80	±	6	96	±	19
Propoxyphene	90	±	4	96	±	7	87	±	7	97	±	6	106	±	2	108	±	4	79	±	24	93	±	10
Norpropoxyphene	99	±	9	93	±	8	100	±	4	111	±	21	94	±	4	112	±	13	117	±	41	165	±	39
Tramadol	96	±	7	98	±	13	105	±	21	113	±	19	91	±	7	101	±	5	87	±	8	96	±	16
Nortramadol	101	±	13	102	±	11	78	±	20	84	±	22	80	±	15	89	±	15	104	±	14	114	±	21
Benzodiazpines and their metabolites																							
Temazepam	15	±	8	80	±	41	10	±	5	48	±	27	21	±	2	100	±	6	46	±	24	98	±	5
Diazepam	105	±	13	103	±	10	90	±	4	99	±	3	89	±	2	100	±	2	80	±	1	101	±	2
Nordiazepam	71	±	4	70	±	3	110	±	4	122	±	7	93	±	7	105	±	11	67	±	14	84	±	17
Nitrazepam	92	±	3	91	±	4	95	±	5	105	±	7	79	±	14	89	±	19	44	±	19	55	±	23
7-aminonitrazepam	36	±	8	104	±	30	44	±	6	64	±	10	50	±	5	116	±	20	21	±	2	36	±	6
Oxazepam	54	±	5	99	±	7	51	±	3	96	±	4	62	±	2	205	±	5	66	±	16	107	±	5
Chlordiazepoxide	86	±	4	85	±	5	93	±	3	102	±	5	90	±	6	101	±	9	75	±	8	95	±	8
Antidepressants and their metabolites																							
Dosulepin	67	±	3	190	±	14	76	±	4	110	±	5	79	±	0	185	±	23	56	±	14	94	±	18
Amitriptyline	80	±	6	226	±	4	86	±	2	124	±	6	86	±	12	204	±	52	66	±	23	111	±	33
Nortriptyline	64	±	8	181	±	7	76	±	9	109	±	12	55	±	7	131	±	32	64	±	20	107	±	27
Fluoxetine	61	±	4	88	±	12	74	±	5	104	±	14	53	±	17	101	±	11	80	±	66	99	±	1
Norfluoxetine	62	±	9	89	±	5	60	±	11	83	±	10	47	±	21	87	±	9	71	±	60	87	±	3
Venlafaxine	91	±	15	92	±	15	85	±	8	90	±	6	97	±	8	113	±	7	91	±	11	108	±	6
Dissociative anesthetics and their metabolites																							
Phencyclidine	80	±	2	103	±	7	85	±	0	103	±	8	86	±	2	110	±	8	81	±	6	103	±	2
Ketamine	70	±	5	90	±	6	84	±	2	97	±	4	90	±	2	100	±	5	92	±	2	99	±	4
Norketamine	67	±	7	86	±	13	86	±	3	99	±	5	83	±	3	91	±	5	77	±	7	82	±	7
Other																							
Methaqualone	98	±	5	97	±	4	96	±	10	103	±	15	105	±	4	101	±	8	74	±	4	95	±	6
Sildenafil (viagra)	81	±	23	98	±	26	97	±	4	106	±	16	87	±	19	170	±	16	61	±	29	92	±	34
Drug precursors																							
Ephedrine/Pseudoephedrine	73	±	13	82	±	11	85	±	12	99	±	12	102	±	12	123	±	27	99	±	2	103	±	9
Norepehedrine	95	±	3	108	±	10	90	±	11	104	±	10	98	±	16	116	±	7	110	±	27	112	±	17
Internal standards																							
Cocaine-D3	96	±	4				91	±	3				87	±	2				84	±	5			
Benzoylecgonine-D8	97	±	1				91	±	4				89	±	2				87	±	4			
Cocaethylene-D8	94	±	7				96	±	4				94	±	5				87	±	3			
Amphetamine-D11	89	±	5				86	±	3				84	±	9				97	±	10			
Methamphetamine-D14	85	±	4				86	±	5				88	±	6				87	±	5			
MDA-D5	93	±	5				77	±	2				88	±	6				88	±	4			
MDMA-D5	92	±	2				82	±	3				82	±	6				89	±	3			
MBDB-D5	93	±	3				84	±	6				84	±	2				85	±	1			
MDEA-D5	92	±	3				89	±	2				86	±	6				85	±	3			
Caffeine-D9	24	±	5				7	±	0				6	±	1				16	±	3			
Nicotine-D4	85	±	1				97	±	13				96	±	50				62	±	3			
LSD-D3	83	±	7				94	±	17				51	±	11				64	±	7			

Heroin-D9	2	±	0	2	±	0	1	±	0	1	±	0
Oxycodone-D6	59	±	5	69	±	4	68	±	4	61	±	18
Morphine-D6	89	±	1	91	±	1	63	±	4	106	±	20
Methadone-D9	98	±	3	88	±	2	84	±	0	80	±	19
EDDP-D3	35	±	3	69	±	1	43	±	5	59	±	5
Fentanyl-D5	98	±	1	94	±	4	86	±	4	84	±	13
Codeine-D6	99	±	6	93	±	2	90	±	2	92	±	7
Dihydrocodeine-D6	103	±	7	99	±	7	86	±	3	95	±	10
Buprenorphine-D4	89	±	1	94	±	3	86	±	2	80	±	19
Propoxyphene-D11	95	±	3	90	±	4	98	±	2	83	±	18
Norpropoxyphene-D5	107	±	11	93	±	17	85	±	7	70	±	12
Temazepam-D5	18	±	2	23	±	2	21	±	2	48	±	26
Diazepam-D5	102	±	2	91	±	3	89	±	3	79	±	2
Oxazepam-D5	55	±	2	53	±	4	30	±	1	63	±	18
Fluoxetine-D6	70	±	8	71	±	5	53	±	19	81	±	66
PCP-D5	78	±	3	83	±	7	78	±	6	79	±	6
Ketamine-D4	78	±	3	87	±	5	91	±	6	93	±	6
Mescaline-D9	95	±	6	96	±	12	93	±	3	83	±	1
Methaqualone-D7	101	±	1	94	±	5	104	±	5	78	±	4

[a] Relative recovery to surrogate/internal standard

[c] Average recovery from WWTP influent and effluent spiked before extraction (two replicates at three concentrations: 100, 500 and 1000 ng L^{-1})

[d] Average recovery from surface water spiked before extraction (two replicates at three concentrations: 20, 100 and 200 ng L^{-1})

[e] Average recovery from UHQ water spiked before extraction (two replicates at three concentrations: 100, 500 and 1000 ng L^{-1})

Table 3 – Concentration of compounds in influent, effluent and river water from 7 WWTPs and 6 river locations in the UK

Compound	WWTP influent (n=7)			WWTP effluent (n=7)			River water (n=6)		
	samples (>LOQ)[a]	Conc. Max (ng L^{-1})[b]	Conc. Mean (ng L^{-1})[c]	Samples (>LOQ)[a]	Conc. Max (ng L^{-1})[b]	Conc. Mean (ng L^{-1})[c]	Samples (>LOQ)[a]	Conc. Max (ng L^{-1})[b]	Conc. Mean (ng L^{-1})[c]
Stimulants and their metabolites									
Cocaine	7	109.0	70.9	6	65.2	29.2	6	14.0	6.0
Benzoylecgonine	7	368.3	243.1	7	293.3	115.9	6	52.5	26.8
Norbenzoylecgonine	7	15.2	7.5	7	12.0	7.0	5	2.8	1.8
Norcocaine	1	1.0	1.0	0	<MDL	<MDL	1	0.1	0.1
Cocaethylene	7	5.4	3.0	4	5.4	3.8	5	1.4	0.6
Anhydroecgonine methyl ester	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
Ecgonidine	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
Amphetamine	7	2300.1	829.6	7	24.4	7.7	4	4.3	3.3
Methamphetamine	6	3.8	2.0	3	1.2	1.0	0	<MDL	<MDL
Methcathinone	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
Benzylpiperazine (BZP)	4	37.6	24.5	7	66.3	30.9	5	65.3	26.0
Trifluoromethylphenylpiperazine (TFMPP)	6	9.8	4.8	7	9.4	4.6	5	1.1	0.6
Hallucinogens and their metabolites									
MDA	2	15.2	10.4	2	24.5	15.4	0	<MDL	<MDL
MDMA (Ecstasy)	7	137.9	39.0	7	155.7	37.5	5	24.8	8.7
MDEA (MDE)	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
MBDB	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
BDB	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
Mescaline	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
LSD	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
O-H-LSD	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
Human indicators									
Caffeine	7	13759.1	9902.3	5	7137.0	2048.3	6	437.4	265.2
1,7-dimethylxanthine	7	15285.8	9466.9	7	12456.1	3168.6	6	421.4	277.0
Nicotine	7	7042.6	4446.0	6	89.9	52.1	6	56.4	32.3
Continine	1	43.7	43.7	0	<MDL	<MDL	0	<MDL	<MDL
Opiods, morphine derivatives and their metabolites									
Heroin	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
6-acetylmorphine	5	69.7	22.2	0	<MDL	<MDL	0	<MDL	<MDL
Codeine	7	2703.5	2121.2	7	1206.2	436.9	6	341.7	128.2
Norcodeine	7	150.9	111.7	5	64.6	32.8	5	19.9	8.9
Oxycocodone	6	17.1	12.2	6	25.3	12.0	5	7.1	3.0
Oxymorphone	3	23.5	19.9	0	<MDL	<MDL	0	<MDL	<MDL
Morphine	7	819.2	481.2	4	243.8	130.7	1	35.8	35.8
Normorphine	7	364.2	202.6	1	61.9	61.9	0	<MQL	<MQL
Dihydrocodeine	7	526.1	320.7	7	316.7	145.5	6	90.1	36.4
Buprenorphine	2	34.4	33.2	2	24.2	13.9	0	<MDL	<MDL
Norbuprenorphine	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
Methadone	7	171.1	87.9	7	68.8	49.6	6	18.4	10.0
EDDP	7	342.2	193.0	7	161.6	89.1	6	38.2	19.1
EMDP	1	5.7	5.7	3	2.3	1.7	2	1.0	0.8

Fentanyl	3	2.2	1.7	0	<MDL	<MDL	0	<MDL	<MDL
Norfentanyl	2	9.8	6.9	1	1.1	1.1	0	<MDL	<MDL
Propoxyphene	1	10.7	10.7	2	8.6	7.1	0	<MDL	<MDL
Norpropoxyphene	0	<MDL	<MDL	7	142.7	91.3	5	38.2	22.9
Tramadol	7	6278.1	2758.7	7	1536.7	1225.6	6	441.8	221.4
Nortramadol	7	7192.8	2457.3	7	538.6	432.6	5	265.8	148.5
Benzodiazpines and their metabolites									
Temazepam	7	278.3	167.0	7	251.1	135.3	5	52.9	27.8
Diazepam	0	<MDL	<MDL	2	6.2	5.1	3	1.1	0.8
Nordiazepam	7	51.5	25.2	7	14.2	9.9	5	5.5	3.2
Nitrazepam	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
7-aminonitrazepam	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
Oxazepam	7	113.8	49.5	7	82.8	57.9	5	17.4	11.4
Chlordiazepoxide	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
Antidepressants and their metabolites									
Dosulepin	7	418.7	227.6	7	121.0	57.2	6	32.2	9.7
Amitriptyline	7	1055.5	659.0	7	222.7	129.8	6	71.6	29.5
Nortriptyline	6	184.5	114.1	7	53.8	32.9	6	19.0	6.8
Fluoxetine	7	175.9	86.1	6	43.3	29.3	3	13.5	9.0
Norfluoxetine	7	118.0	62.8	3	20.1	13.2	7	<MQL	<MQL
Venlafaxine	7	343.8	249.0	7	269.6	187.5	6	71.6	35.1
Dissociative anesthetics and their metabolites									
Phencyclidine	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
Ketamine	7	160.0	79.4	7	228.4	129.6	6	51.0	21.3
Norketamine	6	96.4	26.5	7	54.3	28.0	5	14.4	5.8
Other									
Methaqualone	0	<MDL	<MDL	0	<MDL	<MDL	0	<MDL	<MDL
Sildenafil (viagra)	7	49.8	24.9	5	10.2	7.0	0	<MDL	<MDL
Drug precursors									
Ephedrine/Pseudoephedrine	7	1032.1	685.2	2	126.9	70.2	3	16.5	11.7
Norepehedrine	7	134.0	85.9	1	59.2	59.2	0	<MDL	<MDL

[a] Number of samples with concentration level >MQL
[b] Maximum concentration measured
[c] Average concentration measured in those samples with levels >MQL

Fig 1 - UPLC-MS/MS separations for compounds spiked into WWTP influent before extraction (concentration, 500 ng L^{-1})

Table S1 - Selected pharmaceuticals and their properties

Compound	CAS	Formula	MW	Pka		LogP		Supplier
				Experimental[a]	Calculated[b]	Experimental[a]	Calculated[b]	
Stimulants and their metabolites								
Cocaine	50-36-2	$C_{17}H_{21}NO_4$	303.4	8.6 (20°)	8.9	2.3	2.3	LGC
Benzoylecgonine	519-09-5	$C_{16}H_{19}NO_4$	289.3		10.8, 3.3	−1.3	2.3	LGC
Norbenzoylecgonine	60426-41-7	$C_{15}H_{17}NO_4$	275.3		10.4, 3.4		2.6	LGC
Norcocaine	N/A	$C_{16}H_{19}NO_4$	289.3		9.0		3.1	LGC
Cocaethylene	529-38-4	$C_{18}H_{23}NO_4$	317.4		9.0		2.8	LGC
Anhydroecgonine methyl ester	43021-26-7	$C_{10}H^{15}NO_2$	181.2		8.0		0.4	LGC
Ecgonidine	74242-55-0	$C_8H_{11}NO_2$	153.2		9.6, 3.8		1.5	LGC
Amphetamine	300-62-9	$C_9H_{13}N$	135.2	10.1	9.9	1.8	1.8	LGC
Methamphetamine	R-(-):33817-09-3, S-(+):537-46-2	$C_{10}H_{15}N$	149.2	10.1	10.4	2.1	2.2	LGC
Methcathinone	49656-78-2	$C_{10}H_{13}NO$	163.2		7.1		0.4	Sigma-Aldrich
Benzylpiperazine (BZP)	N/A	$C_{11}H_{16}N_2$	176.3		9.3, 3.4		1.1	LGC
Trifluoromethylphenylpiperazine (TFMPP)	N/A	$C_{11}H_{13}F_3N_2$	230.2		8.8, 2.1		1.3	LGC
Hallucinogens and their metabolites								
MDA	4764-17-4	$C_{10}H_{13}NO_2$	179.2		9.9	1.64	1.6	LGC
MDMA (Ecstasy)	4254210-9	$C_{11}H_{15}NO_2$	193.2	(benzene, pH 9.0) 9.4	10.3		2.1	LGC
MDEA (MDE)	82801-81-8	$C_{12}H_{17}NO_2$	207.3		10.3		2.6	LGC
MBDB	145225-00-9	$C_{12}H_{17}NO_2$	207.3		10.5		2.6	LGC
BDB	N/A	$C_{11}H_{15}NO_2$	193.2		10		2.2	LGC
Mescaline	832-92-8	$C_{11}H_{17}NO_3$	211.3	9.6	9.6	0.8	0.5	LGC
LSD	50-37-3	$C_{20}H_{25}N_3O$	323.4	7.5	7.4	2.9	2.7	LGC
O-H-LSD	N/A	$C_{20}H_{25}N_3O_3$	355.4		11.7, 6.8		-1.9	LGC
Human indicators								
Caffeine	58-08-2	$C_8H_{10}N_4O_2$	194.2	14.0 (25°), 10.4 (40°)	0.5	−0.07	-0.6	Sigma-Aldrich
1,7-dimethylxanthine	611-59-6	$C_7H_8N_4O_2$	180.2		8.5, 0.2		-0.9	Sigma-Aldrich
Nicotine	54-11-5	$C_{10}H_{14}N_2$	162.2	7.9, 3.2, (25°)	8.0, 3.2	1.2	0.6	Sigma-Aldrich
Continine	486-56-6	$C_{10}H_{12}N_2O$	176.2		4.7		0.07	Sigma-Aldrich
Creatinine	60-27-5	$C_4H_7N_3O$	113.1		6.9		-0.8	Fisher-Acros
Opiods, morphine derivatives and their metabolites								
Heroin	561-27-3	$C_{21}H_{23}NO_5$	369.4	7.6 (23°)	7.9	1.58	1.6	LGC
6-acetylmorphine	2784-73-8	$C_{19}H_{21}NO_4$	327.4		9.4, 8.0		1.6	LGC
Codeine	76-57-3	$C_{18}H_{21}NO_3$	299.4	8.2 (20°)	13.4, 8.2	0.6	1.4	Sigma-Aldrich
Norcodeine	467-15-2	$C_{17}H_{19}NO_3$	285.3	9.2 (25°)	13.3, 9.3	0.7	0.5	LGC
Oxycocodone	76-42-6	$C_{18}H_{21}NO_4$	315.4	8.9 (20°)	13.1, 7.6	0.7	1.6	LGC
Oxymorphone	76-41-5	$C_{17}H_{19}NO_4$	301.3	9.3, 8.5	13.5, 9.2, 7.6	0	1.2	LGC
Morphine	57-27-2	$C_{17}H_{19}NO_3$	285.3	9.9, 8.0 (20°)	13.5, 9.5, 8.3	–0.1	0.9	LGC
Normorphine	466-97-7	$C_{16}H_{17}NO_3$	271.3	9.8 (25°)	13.4, 9.5, 9.2	−2.8	0.0	LGC
Dihydrocodeine	125-28-0	$C_{18}H_{23}NO_3$	301.4	8.8 (25°)	8.4		0.6	LGC
Buprenorphine	52485-79-7	$C_{29}H_{41}NO_4$	467.6	8.5, 10.0	9.5, 8.3	5.0	2.8	LGC
Norbuprenorphine	78715-23-8	$C_{25}H_{35}NO_4$	413.6		9.8, 9.1		1.2	LGC
Methadone	76-99-3	$C_{21}H_{27}NO$	309.4	8.94 (25°), 8.3 (20°)	9.1	3.9	3.9	Sigma-Aldrich
EDDP	66729-78-0	$C_{21}H_{25}N$	291.4		8.4		5.0	LGC
EMDP	N/A	$C_{20}H_{23}N$	277.4		8.1		5.8	LGC
Fentanyl	437-38-7	$C_{22}H_{28}N_2O$	336.5		8.9, 0.3	2.3	3.7	LGC

Norfentanyl	N/A	$C_{14}H_{20}N_2O$	232.3		9.8, 0.3		1.7	LGC
Propoxyphene	469-62-5	$C_{22}H_{29}NO_2$	339.5	6.3	9.2	4.2	4.1	LGC
Norpropoxyphene	159208-83-0	$C_{21}H_{27}NO_2$	325.4		10.1		3.7	LGC
Tramadol	36282-47-0	$C_{16}H_{25}NO_2$	263.4	9.4, 8.3	9.6	3.0	2.3	Sigma-Aldrich
Nortramadol	N/A	$C_{15}H_{23}NO_2$	249.4		10.6		1.7	LGC
Benzodiazpines and their metabolites								
Temazepam	846-50-4	$C_{16}H_{13}ClN_2O_2$	300.7	1.6	11.7, 1.6	2.2	2.2	LGC
Diazepam	439-15-5	$C_{16}H_{13}ClN_2O$	284.7	3.3 (20°)	3.4	2.7	2.8	LGC
Nordiazepam	1088-11-5	$C_{15}H_{11}ClN_2O$	270.7	12.0, 3.5	11.7, 3.2	2.9	2.8	LGC
Nitrazepam	146-22-5	$C_{15}H_{11}N_3O_3$	281.3	10.8, 3.2 (20°)	11.4, 2.6	2.1	2.4	Sigma-Aldrich
7-aminonitrazepam	4928-02-3	$C_{15}H_{13}N_3O$	251.3		12.3, 4.3, 2.3		1.1	LGC
Oxazepam	604-75-1	$C_{15}H_{11}ClN_2O_2$	286.7	11.6, 1.7 (20°)	12.8, 10.9, 1.2	2.2	2.2	LGC
Chlordiazepoxide	58-25-3	$C_{16}H_{14}ClN_3O$	299.8	4.8	8.6, 6.5	2.4	2.8	LGC
Antidepressants and their metabolites								
Dosulepin	113-53-1	$C_{19}H_{21}NS$	295.4		9.1	2.8	4.3	LGC
Amitriptyline	549-18-8	$C_{20}H_{23}N$	277.4	9.4 (25°)	9.2	5.0	4.4	Sigma-Aldrich
Nortriptyline	894-71-3	$C_{19}H_{21}N$	263.4	9.7	10.0	1.7	4.0	Sigma-Aldrich
Fluoxetine	59333-67-4	$C_{17}H_{18}F_3NO$	309.3		10.1	1.	3.9	LGC
Norfluoxetine	N/A	$C_{16}H_{16}F_3NO$	295.3		9.1		3.8	LGC
Venlafaxine	99300-78-4	$C_{17}H_{27}NO_2$	277.4		9.3	0.4	2.5	Sigma-Aldrich
Dissociative anesthetics and their metabolites								
Phencyclidine (PCP)	77-10-1	$C_{17}H_{25}N$	243.4	8.5	8.2	4.7	4.3	LGC
Ketamine	1867-66-9	$C_{13}H_{16}ClNO$	237.7	7.5	6.5	3.1	3.0	Sigma-Aldrich
Norketamine	N/A	$C_{12}H_{14}ClNO$	223.7	6.7	6.3		2.4	LGC
Other								
Methaqualone	72-44-6	$C_{16}H_{14}N_2O$	250.3	2.5	3.0	4.3	2.5	LGC
Sildenafil (viagra)	139755-83-23	$C_{22}H_{30}N_6O_4S$	474.6	8.7	6.0, 0.6		1.6	LGC
Drug precursors								
Ephedrine	50-98-6	$C_{10}H_{15}NO$	165.2	9.6 (25°)	9.5	1.1	1.0	LGC
Norepehedrine	154-41-6	$C_9H_{13}NO$	151.2		12.1, 8.5		0.4	Sigma-Aldrich
Pseudoephedrine	345-78-8	$C_{10}H_{15}NO$	165.2	9.8	9.5	0.9	1.0	Sigma-Aldrich

[a] Moffat, A.C.; Osselton, D. M.; Widdop, B. Clarke's analysis of drugs and poisons, pharmaceutical press 2004, http://www.medicinescomplete.com/mc/clarke/current/, accessed June 2009

[b] ACD/I-lab accessed via ACD/chemsketch, version 12.0, Advanced chemistry development Inc. Toronto, ON, Canada. www.acdlabs.com

Table S2. Optimised MRM conditions and surrogate/internal standard used for quantification

Compound	CV/CE[a]	MRM1 (quantification)	CV/CE[a]	MRM2 (confirmation)	MRM ratio[b] ± % RSD	Internal standard
Stimulants and their metabolites						
Cocaine	40/20	304.2 > 182.1	40/31	304.2 > 82.1	2.56 ± 0.04	Cocaine-D3
Benzoylecgonine	38/19	290.2 > 168.1	38/30	290.2 > 105.1	2.67 ± 0.04	Benzoylecgonine-D8
Norbenzoylecgonine	32/16	276.1 > 154.0	32/21	276.1 > 136.1	1.53 ± 0.03	Benzoylecgonine-D8
Norcocaine	40/15	290.2 > 168.1	40/24	290.1 > 136.1	1.76 ± 0.10	Cocaine-D3
Cocaethylene	38/20	318.2 > 196.2	38/30	318.2 > 82.1	1.68 ± 0.03	Cocaethylene-D8
Anhydroecgonine methyl ester	39/23	182.1 > 118.0	39/21	182.1 > 122.1	0.83 ± 0.09	Cocaine-D3
Ecgonidine	37/23	168.1 > 91.1	37/20	168.1 > 122.1	2.68 ± 0.22	Benzoylecgonine-D8
Amphetamine	18/8	136.2 > 119.1	18/16	136.2 > 91.1	4.55 ± 0.16	Amphetamine-D11
Methamphetamine	24/19	150.2 > 91.1	24/10	150.2 > 119.1	2.82 ± 0.11	Methamphetamine-D14
Methcathinone	28/19	164.1 > 131.0	28/12	164.1 > 146.1	0.55 ± 0.03	Methamphetamine-D14
Benzylpiperazine (BZP)	35/20	177.1 > 91.1	35/15	177.1 > 85.1	6.18 ± 0.11	PCP-D5
Trifluoromethylphenylpiperazine (TFMPP)	46/23	231.0 > 188.0	46/35	231.0 > 118.3	2.42 ± 0.06	PCP-D5
Hallucinogens and their metabolites						
MDA	21/11	180.0 > 163.1	21/22	180.0 > 105.1	3.05 ± 0.07	MDA-D5
MDMA (Ecstasy)	24/13	194.1 > 163.1	24/24	194.1 > 105.1	2.51 ± 0.08	MDMA-D5
MDEA (MDE)	28/13	208.1 > 163.1	28/27	208.1 > 105.1	3.18 ± 0.05	MDEA-D5
MBDB	26/20	208.1 > 135.1	26/11	208.1 > 177.1	2.52 ± 0.14	MBDB-D5
BDB	20/9	194.1 > 177.1	20/16	194.1 > 135.1	4.03 ± 0.51	MBDB-D5
Mescaline	46/12	212.3 > 195.1	46/18	212.3 > 180.1	1.83 ± 0.15	Mescaline-D9
LSD	41/24	324.2 > 223.2	41/28	324.2 > 208.1	1.62 ± 0.04	LSD-D3
O-H-LSD	41/24	356.2 > 237.1	41/24	356.2 > 74.1	1.46 ± 0.04	LSD-D3
Human indicators						
Caffeine	38/15	195.1 > 138.0	38/23	195.1 > 110.0	2.93 ± 0.06	Caffeine-D9
1,7-dimethylxanthine	54/21	181.0 > 124.1	none	none	- -	Caffeine-D9
Nicotine	37/20	163.1 > 130.0	37/24	163.1 > 117.0	0.95 ± 0.04	Nicotine-D4
Continine	34/21	177.1 > 80.0	34/22	177.1 > 98.1	3.15 ± 0.08	EDDP-D3
Creatinine	31/11	114.0 > 86.1	31/16	114.0 > 72.1	2.54 ± 0.08	EDDP-D3
Opiods, morphine derivatives and their metabolites						
Heroin	51/50	370.2 > 165.1	51/29	370.2 > 268.1	1.39 ± 0.04	Heroin-D9
6-acetylmorphine	52/39	328.1 > 165.1	52/26	328.1 > 211.1	1.77 ± 0.04	Codeine-D6
Codeine	49/57	300.2 > 152.1	49/25	300.2 > 215.1	1.22 ± 0.03	Codeine-D6
Norcodeine	46/40	286.1 > 165.1	46/20	286.1 > 268.2	0.87 ± 0.03	Codeine-D6
Oxycocodone	36/29	316.2 > 241.1	36/26	316.2 > 256.1	1.46 ± 0.04	Oxycodone-D6
Oxymorphone	40/19	302.1 > 284.1	40/28	302.1 > 227.1	3.59 ± 0.09	Oxycodone-D6
Morphine	53/56	286.1 > 152.1	53/38	286.1 > 165.1	1.33 ± 0.05	Morphine-D6
Normorphine	45/49	272.1 > 152.1	45/43	272.1 > 165.0	1.34 ± 0.03	Morphine-D6
Dihydrocodeine	53/33	302.1 > 199.1	53/60	302.1 > 128.1	1.48 ± 0.04	Dihydrocodeine-D6
Buprenorphine	69/45	468.3 > 84.1	69/43	468.3 > 101.0	1.69 ± 0.04	Buprenorphine-D4
Norbuprenorphine	60/47	414.3 > 83.0	60/39	414.3 > 101.1	1.26 ± 0.03	Buprenorphine-D4
Methadone	31/15	310.2 > 265.1	31/28	310.2 > 105.1	2.26 ± 0.08	Methadone-D9
EDDP	50/29	278.2 > 234.1	50/24	278.2 > 249.1	1.15 ± 0.11	EDDP-D3
EMDP	47/21	264.2 > 235.1	47/31	264.2 > 220.2	1.04 ± 0.02	Methadone-D9
Fentanyl	44/38	337.2 > 105.1	44/23	337.2 > 188.2	1.01 ± 0.03	Fentanyl-D5
Norfentanyl	27/20	233.2 > 84.0	27/15	233.2 > 177.1	17.68 ± 0.44	Fentanyl-D5
Propoxyphene	19/8	340.2 > 266.2	19/22	340.2 > 143.0	24.40 ± 1.28	Propoxyphene-D11
Norpropoxyphene	15/7	326.2 > 252.1	none	none	- -	Norpropoxyphene-D5
Tramadol	24/17	264.2 > 58.1	24/11	264.2 > 246.3	92.34 ± 3.07	Codeine-D6
Nortramadol	21/8	250.2 > 232.1	none	none	- - -	Codeine-D6
Benzodiazpines and their metabolites						
Temazepam	37/21	301.1 > 255.1	37/14	301.1 > 283.1	1.97 ± 0.07	Temazepam-D5
Diazepam	54/27	285.0 > 154.1	54/31	285.0 > 193.1	1.32 ± 0.02	Diazepam-D5
Nordiazepam	51/29	271.1 > 140.1	51/29	271.1 > 165.0	1.26 ± 0.06	Diazepam-D5
Nitrazepam	44/24	282.1 > 236.1	44/37	282.1 > 180.1	6.12 ± 0.29	Diazepam-D5
7-aminonitrazepam	48/25	252.1 > 121.1	48/40	252.1 > 94.1	3.05 ± 0.09	EDDP-D3
Oxazepam	38/21	287.1 > 241.1	38/15	287.1 > 269.0	1.15 ± 0.04	Oxazepam-D5
Chlordiazepoxide	32/15	300.1 > 283.1	32/25	300.1 > 227.1	2.04 ± 0.08	Diazepam-D5
Antidepressants and their metabolites						
Dosulepin	34/24	296.1 > 218.1	34/24	296.1 > 223.1	0.81 ± 0.02	EDDP-D3
Amitriptyline	37/26	278.2 > 91.1	37/18	278.2 > 233.2	1.10 ± 0.02	EDDP-D3

Nortriptyline	33/16	264.2 > 233.1	33/23	264.2 > 91.0	1.30	±	0.03	EDDP-D3
Fluoxetine	25/8	310.3 > 148.1	none	none	-		-	Fluoxetine-D6
Norfluoxetine	17/7	296.2 > 134.1	none	none	-		-	Fluoxetine-D6
Venlafaxine	27/12	278.2 > 260.1	27/32	278.2 > 121.0	3.93	±	0.11	Fentanyl-D5
Dissociative anesthetics and their metabolites								
Phencyclidine	18/14	244.2 > 159.1	18/34	244.2 > 91.1	1.24	±	0.03	PCP-D5
Ketamine	31/27	238.1 > 125.0	31/15	238.1 > 220.1	2.71	±	0.04	Ketamine-D4
Norketamine	23/27	224.0 > 125.0	23/12	224.0 > 207.1	1.02	±	0.03	Ketamine-D4
Other								
Methaqualone	58/27	251.1 > 132.1	58/42	251.1 > 91.1	5.53	±	0.59	Methaqualone-D7
Sildenafil (viagra)	60/28	475.3 > 100.2	60/40	475.3 > 283.2	4.46	±	0.07	PCP-D5
Drug precursors								
Ephedrine/Pseudoephedrine	23/12	166.1 > 148.1	23/21	166.1 > 133.0	43.37	±	5.41	Amphetamine-D11
Norepehedrine	21/11	152.1 > 134.1	21/19	152.1 > 117.1	64.26	±	7.56	Amphetamine-D11
Internal standards								
Cocaine-D3	40/20	307.2 > 185.1						
Benzoylecgonine-D8	38/19	298.2 > 171.1						
Cocaethylene-D8	38/20	326.2 > 204.2						
Amphetamine-D11	18/8	147.2 > 130.1						
Methamphetamine-D14	24/19	164.2 > 98.1						
MDA-D5	21/11	185.1 > 168.1						
MDMA-D5	26/13	199.1 > 165.1						
MBDB-D5	26/20	213.1 > 136.1						
MDEA-D5	28/13	213.1 > 163.0						
Caffeine-D9	38/15	204.2 > 144.1						
Nicotine-D4	37/20	167.1 > 134.1						
LSD-D3	41/24	327.2 > 226.1						
Heroin-D9	51/50	379.2 > 165.8						
Oxycodone-D6	36/29	322.2 > 247.1						
Morphine-D6	53/38	292.2 > 171.1						
Methadone-D9	31/15	319.3 > 268.2						
EDDP-D3	50/29	281.2 > 234.1						
Fentanyl-D5	44/38	342.2 > 105.1						
Codeine-D6	52/28	306.2 > 218.1						
Dihydrocodeine-D6	53/33	308.2 > 202.1						
Buprenorphine-D4	69/45	472.4 > 88.1						
Propoxyphene-D11	19/8	351.3 > 277.2						
Norpropoxyphene-D5	15/7	331.2 > 257.2						
Temazepam-D5	37/21	306.7 > 260.1						
Diazepam-D5	54/27	290.1 > 154.1						
Oxazepam-D5	38/21	292.0 > 246.0						
Fluoxetine-D6	25/8	316.2 > 154.1						
PCP-D5	18/14	249.2 > 164.1						
Ketamine-D4	31/27	242.1 > 129.1						
Mescaline-D9	46/12	221.2 > 204.2						
Methaqualone-D7	58/27	258.2 > 139.1						

[a]CV, cone voltage (V); CE, collision energy (eV)

[b] MRM ratio : MRM1/MRM2 ratio calculated in surface water spiked before extraction with the ratio over the calibration range averaged

Table S3 – Performance data for compounds (intra- and inter-day instrumental repeatability)

Compound	Instrumental precision[a]															
	Intra-day RSD% (n=6)												Inter-day RSD% (n=3)			
	0.5 (µg L^{-1})			50 (µg L^{-1})			500 (µg L^{-1})			1000 (µg L^{-1})			0.5 (µg L^{-1})	50 (µg L^{-1})	500 (µg L^{-1})	1000 (µg L^{-1})
	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3				
Stimulants and their metabolites																
Cocaine	3.8	3.4	3.5	0.4	0.8	1.8	1.6	2.9	0.4	1.6	1.8	0.8	5.0	0.7	0.9	2.0
Benzoylecgonine	2.7	4.4	7.3	2.2	2.8	1.3	1.4	1.5	1.2	0.9	1.0	1.2	6.2	1.7	0.7	0.3
Norbenzoylecgonine	3.4	2.9	7.5	2.1	2.1	2.0	1.5	1.0	1.4	Out of linearity range[b]			1.2	0.8	0.7	OLR[b]
Norcocaine	4.0	2.9	5.4	3.1	2.1	0.8	1.0	1.9	1.7	2.0	2.9	1.5	6.3	1.2	0.6	3.4
Cocaethylene	5.1	2.8	4.1	2.3	2.2	1.5	0.7	1.0	1.6	Out of linearity range[b]			4.6	0.6	0.3	OLR[b]
Anhydroecgonine methyl ester	7.0	10.9	8.7	1.4	2.6	3.2	1.4	1.2	3.2	Out of linearity range[b]			2.9	2.6	1.4	OLR[b]
Ecgonidine	6.0	8.8	7.9	1.5	3.5	1.8	1.2	0.9	2.4	Out of linearity range[b]			3.0	2.6	0.3	OLR[b]
Amphetamine	3.9	2.5	3.8	4.1	3.4	3.2	1.4	2.5	4.1	1.8	3.8	1.7	2.0	4.9	0.8	3.0
Methamphetamine	5.7	3.7	3.9	4.7	3.9	1.9	2.0	2.8	2.9	1.2	2.7	2.7	1.5	2.3	1.0	1.2
Methcathinone	6.2	4.5	4.3	4.4	4.9	4.5	1.9	3.6	2.0	2.2	3.5	1.4	2.5	3.6	0.1	0.1
Benzylpiperazine (BZP)	<IQL	< IQL	< IQL	4.3	1.7	4.1	1.9	1.1	2.8	2.4	1.0	2.6	<IQL	6.1	7.3	5.6
Trifluoromethylphenylpiperazine (TFMPP)	5.7	4.5	4.6	2.6	3.0	2.8	1.8	1.3	1.0	2.7	2.5	2.0	3.0	2.4	4.4	4.5
Hallucinogens and their metabolites																
MDA	7.7	4.5	3.4	5.9	2.4	2.5	2.3	2.0	2.4	1.1	2.0	2.5	3.9	2.3	1.1	1.1
MDMA (Ecstasy)	3.9	3.8	3.6	2.7	3.3	3.8	1.5	2.4	2.7	1.9	1.5	1.2	4.4	2.0	1.8	0.4
MDEA (MDE)	2.3	3.2	5.1	1.1	1.4	2.9	1.6	1.1	1.5	2.9	1.0	0.8	5.1	0.4	1.2	1.8
MBDB	7.9	4.8	4.3	2.1	1.2	1.9	1.3	0.9	1.7	1.9	1.7	3.0	7.2	9.6	10.3	10.8
BDB	3.7	3.9	6.0	1.8	1.6	2.7	Out of linearity range[b]			Out of linearity range[b]			9.5	1.4	OLR[b]	OLR[b]
Mescaline	< IQL	< IQL	< IQL	1.9	1.0	2.3	2.0	1.7	1.1	2.4	1.8	2.4	<IQL	1.0	0.4	2.5
LSD	7.7	4.5	3.0	1.5	1.5	1.1	3.2	1.5	1.4	0.8	2.2	2.5	5.5	1.3	0.7	1.2
O-H-LSD	7.7	4.8	2.9	1.2	1.0	0.9	2.0	1.4	0.9	2.1	1.9	1.5	2.3	2.2	2.5	0.2
Human indicators																
Caffeine	3.9	6.0	5.5	4.8	2.4	2.6	1.8	2.9	2.5	2.4	4.8	1.8	0.6	4.5	2.6	3.5
1,7-dimethylxanthine	6.2	2.1	4.2	4.9	1.6	3.2	3.6	4.3	1.0	2.7	4.8	1.5	0.8	7.9	4.7	2.6
Nicotine	5.9	4.3	8.9	2.2	3.3	2.6	2.4	2.5	3.0	3.0	3.0	1.1	5.6	2.2	2.4	0.4
Continine	< IQL	< IQL	< IQL	2.1	1.2	3.0	2.9	3.0	2.2	Out of linearity range[b]			<IQL	0.9	1.6	OLR[b]
Creatinine	< IQL	< IQL	< IQL	2.0	2.1	3.2	2.2	3.4	2.5	0.9	3.1	2.7	<IQL	1.7	2.3	2.9
Opiods, morphine derivatives and their metabolites																
Heroin	6.1	4.7	3.0	3.1	1.3	1.1	1.3	1.2	1.3	1.4	1.1	1.7	4.8	2.2	1.4	0.4
6-acetylmorphine	2.1	4.2	4.8	2.4	1.8	1.1	2.4	1.8	1.1	2.5	0.5	2.6	0.8	0.2	1.1	2.6
Codeine	9.1	4.6	5.8	1.9	2.1	2.3	1.4	2.4	1.7	1.9	2.0	3.1	1.3	2.9	0.9	2.0
Norcodeine	8.6	4.4	5.0	1.8	1.7	3.1	1.9	2.0	2.0	2.2	1.1	3.5	4.7	2.5	1.6	1.6
Oxycocodone	2.7	5.2	4.0	2.6	2.5	2.9	2.8	1.7	2.7	Out of linearity range[b]			4.2	0.9	2.0	OLR[b]
Oxymorphone	7.9	3.5	4.6	1.2	3.2	2.4	2.1	2.1	2.8	Out of linearity range[b]			2.8	1.8	1.2	OLR[b]
Morphine	6.6	5.0	3.8	3.4	2.8	2.6	3.4	1.3	3.8	2.7	4.3	3.2	4.5	1.9	0.7	3.1
Normorphine	7.7	8.5	12.7	6.0	2.6	2.0	3.2	2.4	3.0	3.8	4.4	2.6	10.0	2.9	0.4	1.2
Dihydrocodeine	8.6	3.1	4.8	1.4	1.6	1.1	0.7	1.1	1.0	1.1	1.2	1.3	3.2	0.3	0.6	0.3
Buprenorphine	8.2	6.9	3.6	2.0	0.9	3.8	1.1	1.0	2.2	1.2	1.5	0.8	4.4	2.4	1.5	0.8
Norbuprenorphine	5.2	5.6	6.1	2.1	2.6	1.3	1.4	1.6	3.6	Out of linearity range[b]			4.8	1.2	0.1	OLR[b]
Methadone	2.5	4.9	3.9	1.3	1.1	1.8	2.6	1.6	1.9	Out of linearity range[b]			5.9	1.4	1.6	OLR[b]
EDDP	3.3	4.7	8.4	2.8	1.0	1.8	3.7	3.2	3.3	0.4	3.0	2.0	5.3	0.2	1.4	1.6

EMDP	3.1	4.0	3.4	2.6	4.0	2.0	0.9	0.2	1.0	Out of linearity range[b]			5.1	2.3	1.2	OLR[b]
Fentanyl	2.7	3.6	5.2	2.1	2.3	2.2	2.1	1.7	2.6	Out of linearity range[b]			0.2	0.9	0.8	OLR[b]
Norfentanyl	4.1	4.2	2.9	1.2	3.2	2.9	2.6	1.7	3.3	Out of linearity range[b]			2.0	2.6	1.8	OLR[b]
Propoxyphene	6.2	9.0	3.9	5.8	0.8	4.6	1.7	1.8	1.5	2.0	3.8	1.9	5.7	1.8	0.7	0.4
Norpropoxyphene	< IQL	< IQL	< IQL	4.0	5.6	3.2	2.6	5.1	3.5	2.4	1.5	3.8	<IQL	2.4	2.2	2.3
Tramadol	6.0	4.5	2.6	1.9	2.3	2.0	2.3	2.0	1.7	2.7	1.4	3.9	4.7	1.2	0.7	2.5
Nortramadol	< IQL	< IQL	< IQL	5.4	4.3	4.3	4.7	4.5	4.4	Out of linearity range[b]			<IQL	3.9	2.6	OLR[b]
Benzodiazpines and their metabolites																
Temazepam	5.9	7.1	3.9	1.6	2.4	0.9	3.8	3.5	1.5	Out of linearity range[b]			2.4	1.3	2.3	OLR[b]
Diazepam	4.1	7.1	2.7	1.8	1.8	1.1	2.2	1.5	1.5	0.8	0.4	2.2	4.4	0.4	0.4	1.7
Nordiazepam	7.1	8.3	4.8	2.2	1.7	1.6	1.1	2.0	1.6	1.9	1.5	1.1	7.3	6.5	6.0	3.0
Nitrazepam	7.8	9.2	7.0	2.6	1.6	4.8	1.4	1.2	3.4	Out of linearity range[b]			7.3	1.0	3.9	OLR[b]
7-aminonitrazepam	8.8	9.0	3.9	2.7	1.8	1.8	3.7	3.6	2.6	Out of linearity range[b]			7.2	10.4	6.8	OLR[b]
Oxazepam	4.8	2.7	3.5	1.5	2.9	2.0	1.2	1.4	1.9	1.2	1.1	1.0	4.5	3.1	1.8	0.4
Chlordiazepoxide	6.8	8.4	4.9	4.5	2.5	3.6	1.1	1.5	1.1	0.9	2.5	1.8	5.8	6.2	0.8	0.2
Antidepressants and their metabolites																
Dosulepin	4.1	7.2	5.7	3.6	3.9	3.5	4.3	3.1	4.2	1.8	2.5	2.6	8.0	0.5	1.5	2.1
Amitriptyline	5.6	3.6	6.1	2.0	1.4	1.8	3.4	4.2	3.8	1.6	3.6	2.8	6.6	1.5	0.2	0.6
Nortriptyline	4.0	2.1	6.9	2.5	1.4	1.8	3.7	3.6	4.4	1.4	2.1	2.9	6.9	0.8	1.8	3.1
Fluoxetine	9.8	9.8	11.9	1.5	4.2	3.3	1.6	1.0	1.0	2.7	2.4	1.8	10.8	2.0	2.4	2.8
Norfluoxetine	5.5	5.4	3.7	5.3	2.4	3.7	Out of linearity range[b]			Out of linearity range[b]			6.9	3.7	OLR[b]	OLR[b]
Venlafaxine	9.1	2.9	2.8	2.5	3.1	4.0	3.5	2.7	1.8	1.7	2.4	2.7	3.9	2.2	2.8	1.1
Dissociative anesthetics and their metabolites																
Phencyclidine	7.2	5.6	5.5	2.9	3.5	2.3	1.1	1.3	3.0	1.8	1.9	2.8	2.9	1.5	0.5	3.3
Ketamine	5.2	6.7	4.2	1.7	0.8	0.9	1.6	1.1	1.6	1.9	2.3	1.4	3.3	2.2	0.6	1.3
Norketamine	4.3	5.8	6.9	2.0	1.0	1.9	1.4	1.1	0.5	2.4	2.3	1.2	4.3	0.8	1.0	1.4
Other																
Methaqualone	2.1	3.4	2.3	2.3	4.6	1.9	Out of linearity range[b]			Out of linearity range[b]			4.3	2.6	OLR[b]	OLR[b]
Sildenafil (viagra)	< IQL	< IQL	< IQL	3.4	1.9	0.8	Out of linearity range[b]			Out of linearity range[b]			<IQL	0.9	0.9	OLR[b]
Drug precursors																
Ephedrine/Pseudoephedrine	< IQL	< IQL	< IQL	6.2	1.6	3.3	2.4	1.9	2.4	2.9	3.0	3.1	<IQL	3.2	1.5	1.9
Norepehedrine	< IQL	< IQL	< IQL	5.7	3.7	3.3	3.1	2.6	1.1	Out of linearity range[b]			<IQL	3.6	1.5	OLR[b]

[a] Sample diluent spiked with all compounds

[b] Out of linearity range (OLR): Concentration higher than calibration range, see table 1

Table S4 – Performance data for compounds (intra- and inter-day method repeatability)

Compound	Method Precision[a]											
	Intra-day RSD% (*n*=3)									Inter-day RSD% (*n*=3)		
	0.5 (ng L^{-1})			50 (ng L^{-1})			1000 (ng L^{-1})			0.5 (ng L^{-1})	50 (ng L^{-1})	1000 (ng L^{-1})
	D1	D2	D3	D1	D2	D3	D1	D2	D3			
Stimulants and their metabolites												
Cocaine	6.5	5.4	7.6	2.1	3.9	1.7	Out of linearity range[b]			4.5	3.7	OLR[b]
Benzoylecgonine	5.5	5.1	3.8	1.4	5.6	1.8	Out of linearity range[b]			5.8	2.8	OLR[b]
Norbenzoylecgonine	5.7	3.7	6.9	2.7	1.8	2.2	Out of linearity range[b]			6.5	1.3	OLR[b]
Norcocaine	9.2	8.6	10.9	2.3	2.8	4.4	2.2	3.0	2.4	5.9	3.4	1.9
Cocaethylene	6.3	5.3	5.4	1.8	2.1	2.0	Out of linearity range[b]			5.7	2.9	OLR[b]
Anhydroecgonine methyl ester	<MQL	<MQL	<MQL	2.6	3.7	2.7	Out of linearity range[b]			<MQL	2.5	OLR[b]
Ecgonidine	<MQL	<MQL	<MQL	8.2	9.9	4.5	Out of linearity range[b]			<MQL	5.6	OLR[b]
Amphetamine	<MQL	<MQL	<MQL	3.4	8.4	6.6	3.6	2.9	6.3	<MQL	10.0	5.1
Methamphetamine	12.8	13.4	12.0	3.2	5.5	3.9	3.1	2.0	4.0	8.3	4.1	4.1
Methcathinone	<MQL	<MQL	<MQL	3.8	4.7	4.9	5.8	6.3	5.5	<MQL	7.2	3.3
Benzylpiperazine (BZP)	<MQL	<MQL	<MQL	3.4	3.5	4.3	Out of linearity range[b]			<MQL	3.8	OLR[b]
Trifluoromethylphenylpiperazine (TFMPP)	12.0	6.5	8.9	7.3	4.0	4.6	Out of linearity range[b]			13.3	5.3	OLR[b]
Hallucinogens and their metabolites												
MDA	11.6	7.0	10.6	3.2	3.5	2.3	4.4	3.5	3.0	11.0	2.1	3.0
MDMA (Ecstasy)	5.6	8.9	6.7	2.9	1.7	3.5	2.8	1.9	2.7	9.4	3.9	3.2
MDEA (MDE)	7.1	8.2	6.9	1.5	1.9	1.3	1.6	2.1	1.7	7.8	1.9	3.8
MBDB	5.5	7.8	4.6	11.2	1.7	3.3	1.3	1.9	1.0	7.2	8.7	10.4
BDB	<MQL	<MQL	<MQL	3.2	2.8	3.4	1.1	1.1	1.6	<MQL	7.9	3.0
Mescaline	<MQL	<MQL	<MQL	1.6	2.1	2.4	0.9	5.2	1.6	<MQL	3.4	1.4
LSD	14.1	12.1	7.8	3.0	1.5	3.7	2.1	1.8	1.2	12.7	1.9	2.1
O-H-LSD	11.9	7.2	10.7	6.5	6.2	6.1	Out of linearity range[b]			8.3	9.8	OLR[b]
Human indicators												
Caffeine	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	12.7	13.0	13.3	<MQL	<MQL	13.5
1,7-dimethylxanthine	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	13.4	16.4	12.3	<MQL	<MQL	15.2
Nicotine	<MQL	<MQL	<MQL	2.9	3.6	3.2	2.1	1.9	2.7	<MQL	2.5	2.1
Continine	<MQL	<MQL	<MQL	4.8	3.0	3.7	Out of linearity range[b]			<MQL	2.2	OLR[b]
Opiods, morphine derivatives and their metabolites												
Heroin	<MQL	<MQL	<MQL	2.9	2.8	1.8	2.2	2.7	1.8	<MQL	4.1	2.1
6-acetylmorphine	10.8	11.6	12.9	2.7	4.4	3.7	1.9	2.4	3.3	9.2	5.4	3.6
Codeine	10.5	13.2	10.6	1.8	1.5	2.4	1.8	2.0	2.5	8.4	1.7	2.2
Norcodeine	<MQL	<MQL	<MQL	2.6	3.5	2.2	2.6	2.3	3.5	<MQL	3.9	4.4
Oxycocodone	14.8	12.4	13.4	3.6	4.4	2.3	Out of linearity range[b]			10.7	1.9	OLR[b]
Oxymorphone	11.9	7.3	10.0	6.9	5.3	8.9	Out of linearity range[b]			10.4	9.3	OLR[b]
Morphine	<MQL	<MQL	<MQL	5.2	6.5	7.7	2.6	3.9	6.0	<MQL	8.3	5.6
Normorphine	<MQL	<MQL	<MQL	5.3	5.4	7.4	3.8	4.4	6.2	<MQL	7.4	6.4
Dihydrocodeine	9.0	5.9	13.3	1.9	1.9	0.9	1.1	2.1	1.2	8.2	1.2	1.8
Buprenorphine	<MQL	<MQL	<MQL	4.2	3.4	3.2	2.5	1.0	1.5	<MQL	3.8	1.4
Norbuprenorphine	<MQL	<MQL	<MQL	4.4	5.0	4.7	Out of linearity range[b]			<MQL	4.3	OLR[b]
Methadone	3.7	5.1	3.6	2.4	2.0	3.4	Out of linearity range[b]			3.1	2.4	OLR[b]
EDDP	7.9	2.9	5.2	3.4	3.1	4.7	2.9	2.3	3.7	5.7	5.9	4.1

EMDP	6.7	7.3	5.4	2.2	3.6	3.7	Out of linearity range[b]			6.8	6.5	OLR[b]
Fentanyl	4.7	6.5	11.4	1.1	2.4	3.0	Out of linearity range[b]			6.2	2.2	OLR[b]
Norfentanyl	6.3	6.2	10.4	2.4	3.1	3.8	Out of linearity range[b]			6.0	2.7	OLR[b]
Propoxyphene	<MQL	<MQL	<MQL	3.3	1.8	3.2	3.0	3.5	1.9	<MQL	1.7	2.1
Norpropoxyphene	<MQL	<MQL	<MQL	6.6	6.7	5.5	2.1	3.4	3.1	<MQL	9.3	5.4
Tramadol	<MQL	<MQL	<MQL	1.9	2.0	3.0	1.7	4.0	3.1	<MQL	3.1	3.9
Nortramadol	<MQL	<MQL	<MQL	12.5	7.1	14.5	Out of linearity range[b]			<MQL	16.5	OLR[b]
Benzodiazpines and their metabolites												
Temazepam	<MQL	<MQL	<MQL	5.9	4.7	4.7	Out of linearity range[b]			<MQL	5.7	OLR[b]
Diazepam	5.1	5.9	6.7	2.2	1.1	1.2	1.4	1.4	1.4	6.4	2.2	1.6
Nordiazepam	7.6	9.5	13.1	3.3	3.1	2.8	1.9	2.4	1.9	9.9	11.9	6.2
Nitrazepam	<MQL	<MQL	<MQL	10.8	12.6	11.4	Out of linearity range[b]			<MQL	14.1	OLR[b]
7-aminonitrazepam	<MQL	<MQL	<MQL	2.1	6.9	4.5	Out of linearity range[b]			<MQL	9.9	OLR[b]
Oxazepam	6.4	14.8	14.3	2.6	1.5	3.5	1.4	1.4	1.8	12.5	3.2	1.6
Chlordiazepoxide	12.1	11.9	10.7	3.0	3.8	3.1	2.8	3.2	2.0	12.0	7.1	2.2
Antidepressants and their metabolites												
Dosulepin	12.2	14.7	7.7	5.9	7.7	7.7	Out of linearity range[b]			12.5	11.2	OLR[b]
Amitriptyline	12.9	7.7	15.0	7.6	8.0	8.6	Out of linearity range[b]			8.5	12.0	OLR[b]
Nortriptyline	8.0	9.9	9.6	6.5	8.5	8.4	Out of linearity range[b]			5.6	12.7	OLR[b]
Fluoxetine	<MQL	<MQL	<MQL	3.4	5.0	2.3	2.2	2.5	1.4	<MQL	3.5	1.6
Norfluoxetine	<MQL	<MQL	<MQL	3.2	3.9	6.9	Out of linearity range[b]			<MQL	7.6	OLR[b]
Venlafaxine	12.2	13.1	7.1	3.0	3.1	2.9	Out of linearity range[b]			9.6	3.8	OLR[b]
Dissociative anesthetics and their metabolites												
Phencyclidine	8.9	10.3	8.2	7.7	5.1	4.9	2.9	2.2	3.7	9.7	5.7	2.5
Ketamine	5.5	5.2	6.6	1.4	2.1	1.4	1.5	1.8	0.6	6.1	1.5	1.8
Norketamine	12.7	8.9	13.4	1.5	1.8	1.8	2.3	2.1	2.0	11.1	2.4	1.7
Other												
Methaqualone	5.1	3.7	4.9	3.0	3.5	2.6	Out of linearity range[b]			4.3	4.6	OLR[b]
Sildenafil (viagra)	<MQL	<MQL	<MQL	5.0	3.7	6.7	Out of linearity range[b]			<MQL	4.5	OLR[b]
Drug precursors												
Ephedrine/Pseudoephedrine	<MQL	<MQL	<MQL	8.2	11.9	6.4	2.9	5.0	5.5	<MQL	10.7	4.4
Norepehedrine	<MQL	<MQL	<MQL	6.6	10.1	11.2	Out of linearity range[b]			<MQL	13.1	OLR[b]

[a] Surface water spiked with compounds before extraction

[b] Out of linearity range (OLR): Concentration higher than calibration range, see table 1

Table S5 – Performance data for compounds (method accuracy)

Compounds	Method accuracy RSD%[a]								
	0.5 (ng L^{-1})			50 (ng L^{-1})			1000 (ng L^{-1})		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
Stimulants and their metabolites									
Cocaine	4.7	5.8	0.3	1.0	0.9	-0.7	Out of Linearity range[b]		
Benzoylecgonine	10.0	7.5	8.1	5.9	5.6	1.9	Out of Linearity range[b]		
Norbenzoylecgonine	-1.7	10.1	10.1	0.6	0.1	0.8	Out of Linearity range[b]		
Norcocaine	21.5	14.5	8.3	8.3	5.4	8.2	-7.1	-6.3	-4.4
Cocaethylene	9.5	11.0	10.3	6.5	4.9	2.4	Out of Linearity range[b]		
Anhydroecgonine methyl ester	<MQL	<MQL	<MQL	10.0	8.6	9.0	Out of Linearity range[b]		
Ecgonidine	<MQL	<MQL	<MQL	4.1	6.3	4.6	Out of Linearity range[b]		
Amphetamine	1.1	-2.0	-1.8	-7.8	-6.4	-18.0	-2.7	-5.8	-7.2
Methamphetamine	1.6	17.1	-6.3	-0.5	-1.7	-1.4	-4.3	-4.0	-5.1
Methcathinone	-15.7	-19.9	-19.5	17.3	18.9	20.4	-5.7	4.7	9.2
Benzylpiperazine (BZP)	<MQL	<MQL	<MQL	3.8	0.6	6.8	Out of Linearity range[b]		
Trifluoromethylphenylpiperazine (TFMPP)	-0.3	18.2	23.7	28.7	27.6	22.9	Out of Linearity range[b]		
Hallucinogens and their metabolites									
MDA	-0.1	-4.7	2.3	-4.7	-3.7	-3.8	0.6	0.2	-3.0
MDMA (Ecstasy)	-3.0	-0.4	-3.1	0.4	-3.0	-3.2	-2.1	-3.3	-3.9
MDEA (MDE)	2.3	7.7	-1.3	-2.1	-2.8	-5.3	-4.6	-8.1	-8.7
MBDB	-3.5	-6.9	-11.5	-4.5	-4.7	-7.9	-0.1	-0.5	-1.4
BDB	<MQL	<MQL	<MQL	-22.6	-20.6	-25.0	1.4	-2.5	-6.7
Mescaline	<MQL	<MQL	<MQL	8.3	5.4	1.1	-1.9	-1.7	-3.9
LSD	3.5	4.1	1.1	-5.8	-5.8	-8.7	-8.8	-8.7	-9.8
O-H-LSD	-2.7	0.9	12.1	-17.1	-6.0	2.7	Out of Linearity range[b]		
Human indicators									
Caffeine	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	-4.0	15.1	7.0
1,7-dimethylxanthine	<MQL	<MQL	<MQL	2.9	11.0	3.7	-11.1	2.5	-2.4
Nicotine	<MQL	<MQL	<MQL	0.8	1.1	2.7	-5.7	-2.8	-3.8
Continine	<MQL	<MQL	<MQL	-14.0	-14.1	-11.9	Out of Linearity range[b]		
Opiods, morphine derivatives and their metabolites									
Heroin	<MQL	<MQL	<MQL	7.7	3.2	2.4	-10.6	-11.0	-12.6
6-acetylmorphine	1.1	-0.6	-2.3	-6.2	-14.8	-16.6	1.9	-2.7	-2.1
Codeine	0.8	10.3	10.3	-2.5	-4.2	-3.2	0.1	-3.5	-3.0
Norcodeine	20.8	23.0	16.0	-18.7	-19.4	-19.5	5.3	-2.6	-2.6
Oxycocodone	-5.6	21.6	-2.2	2.5	5.6	2.6	Out of Linearity range[b]		
Oxymorphone	-7.3	8.5	9.1	17.0	16.1	17.3	Out of Linearity range[b]		
Morphine	0.0	0.0	0.0	8.9	16.2	2.0	7.7	8.2	5.2
Normorphine	<MQL	<MQL	<MQL	7.7	2.7	-2.1	-5.0	-3.3	-6.2
Dihydrocodeine	8.5	5.6	3.6	2.9	4.9	3.8	-0.4	0.4	-0.2
Buprenorphine	<MQL	<MQL	<MQL	0.2	-5.2	-0.4	0.6	2.4	2.1
Norbuprenorphine	<MQL	<MQL	<MQL	-10.6	-8.0	-4.9	Out of Linearity range[b]		
Methadone	21.9	22.7	18.3	9.9	11.9	9.6	Out of Linearity range[b]		
EDDP	8.2	1.6	-4.7	-16.8	-19.4	-24.1	-1.3	-5.9	-2.6
EMDP	-5.6	-11.7	-1.5	-14.9	-9.3	-1.0	Out of Linearity range[b]		
Fentanyl	17.5	21.0	21.9	14.0	17.5	13.1	Out of Linearity range[b]		
Norfentanyl	-28.1	-24.9	-22.9	-2.5	-2.6	-3.8	Out of Linearity range[b]		
Propoxyphene	-24.6	-18.6	-25.0	1.2	3.2	2.1	-5.6	-8.3	-9.5
Norpropoxyphene	<MQL	<MQL	<MQL	-6.2	14.8	23.9	-22.7	-22.7	-18.2
Tramadol	11.2	11.9	12.5	11.4	10.5	13.7	-5.3	-11.8	-10.6
Nortramadol	<MQL	<MQL	<MQL	-14.7	-28.2	-33.1	Out of Linearity range[b]		
Benzodiazpines and their metabolites									
Temazepam	<MQL	<MQL	<MQL	4.5	1.0	-2.4	Out of Linearity range[b]		
Diazepam	10.7	7.9	5.5	9.6	7.7	6.2	-1.7	-3.2	-2.6
Nordiazepam	10.2	-9.1	-22.5	-5.4	-19.2	-27.3	-3.6	-11.6	-18.7
Nitrazepam	11.4	9.5	-9.9	-11.3	-26.7	-20.5	Out of Linearity range[b]		
7-aminonitrazepam	<MQL	<MQL	<MQL	-26.4	-24.1	-28.3	Out of Linearity range[b]		
Oxazepam	-1.7	-0.1	9.2	6.1	0.8	1.8	-2.8	-6.2	-7.0
Chlordiazepoxide	-6.9	-4.5	-5.9	1.1	-5.8	-6.6	-8.6	-7.1	-8.1
Antidepressants and their metabolites									
Dosulepin	21.1	21.5	2.2	18.5	27.3	12.6	Out of Linearity range[b]		

Amitriptyline	19.5	23.9	4.7	26.0	17.2	13.4	Out of Linearity range[b]		
Nortriptyline	29.3	29.2	12.7	30.1	21.8	13.5	Out of Linearity range[b]		
Fluoxetine	<MQL	<MQL	<MQL	-2.9	1.1	-2.3	-1.6	-4.4	-4.2
Norfluoxetine	<MQL	<MQL	<MQL	-0.7	-4.4	-6.9	Out of Linearity range[b]		
Venlafaxine	0.6	-5.3	10.7	-8.3	-8.5	-11.6	Out of Linearity range[b]		
Dissociative anesthetics and their metabolites									
Phencyclidine	3.1	8.9	3.0	3.9	-3.5	-5.0	-10.4	-11.0	-13.6
Ketamine	14.3	19.7	10.3	2.4	3.2	3.0	0.6	-1.4	0.7
Norketamine	-4.9	5.3	7.3	-1.7	-0.4	-3.5	-3.3	-1.0	1.4
Other									
Methaqualone	7.3	4.5	2.0	-3.9	-5.8	-10.0	Out of Linearity range[b]		
Sildenafil (viagra)	<MQL	<MQL	<MQL	15.2	8.6	15.3	Out of Linearity range[b]		
Drug precursors									
Ephedrine/Pseudoephedrine	<MQL	<MQL	<MQL	4.1	2.1	-7.3	1.5	0.6	3.5
Norepehedrine	<MQL	<MQL	<MQL	-11.2	-19.5	-22.4	Out of Linearity range[b]		

[a] Surface water spiked with compounds before extraction

[b] Out of linearity range: Concentration higher than calibration range, see table 1