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A high-throughput solid-phase microextraction and post-loop mixing large volume injection method for water samples.

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Abstract:

This article presents a novel approach for the analysis of 13 drugs in wastewater for use in wastewater-based epidemiology (WBE) studies. Sample preparation remains one of the principal bottlenecks in modern high-throughput analysis by ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The proposed methodology is based on the micro-extraction of small volumes (1 mL) of wastewater using a HLB 96-well microplate and both large volume injection (LVI) and post-loop mixing injection (PLM). With this configuration, the limits of quantification (LOQ) were below the reported environmental concentrations of the target compounds in wastewater. Furthermore, both the complexity of collecting, transporting and storing the wastewater sample, sample preparation time, cost and amount of solvent used are all diminished, enhancing the suitability of this methodology for future WBE studies. A new workflow is also proposed in order to create a virtual specimen library bank for WBE by using high-resolution mass spectrometry (HRMS). The method was validated and the limits of quantification were between 0.2 and 6.3 ng L^{-1}. The relative standard deviations (RSD) for a standard mixture at 200 ng L^{-1} (n=6) was between 3.4 and 14.4% while the recoveries for the 13 drug target residues (DTR) were between 92 and 110%. The developed and validated method was finally successfully applied to 10 wastewater samples collected from Oslo, Norway.

Keywords

Wastewater-based epidemiology, Liquid chromatography-mass spectrometry, High-throughput analysis, 96-well plate microextraction, Post-loop mixing, Large volume injection

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1. Introduction

Wastewater-based epidemiology (WBE) has been established as a complementary tool to estimate drug use at the population level by the quantitative measurement of endogenous and exogenous biomarkers excreted by humans in wastewater [1]. Recently WBE has also been shown to be an effective approach for estimating population level human exposure to a wide range of pollutants [2, 3]. WBE has the potential to provide real-time data on geographical and temporal trends in illicit drug use [4]. Traditional methods used for this purpose are usually based on population surveys, sales data, clinical cases, seizures or mortality rates related to use, but these approaches lack representativeness, are time consuming and expensive [5].

The WBE procedure consists of several steps involving sample collection, chemical analysis and the drug target residue (DTR) back-calculation, which are subject to a certain number of sources of uncertainty that have been described and progressively diminished by using a harmonized approach [6]. The appropriate collection of representative composite wastewater samples to compensate for the flow fluctuations during the sampling has been described by Ort and colleagues [7], presenting an acceptable uncertainty when estimating the population weighted loads of around 5 to 10% [6]. Furthermore, wastewater data has been shown to present low temporal representativeness when assessing annual averages [8]. Consequently, the annual estimates for a certain substance based on WBE studies must consist of several stratified random samples (typically 56 samples per year for an acceptable level of sample size related uncertainty < 10% [9]) rather than only one consecutive week as most of the WBE studies, such as the European-wide monitoring for the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) [10]. However, increasing the sampling frequency to decrease the annual estimate uncertainty may therefore imply a greater activity from the wastewater treatment plant (WWTP) operators in order to collect the samples to be analyzed. Therefore, there is a need to develop more suitable and cost-effective alternatives to classic methods for the long-term monitoring of exposure and substance use at community level through WBE [8].

Sample analysis is critical to achieve reliable concentration of the DTR. The uncertainty related to the analytical variability is estimated to be up to 26% [6]. Most of the DTR are found in wastewater in the ng L^{-1} range and therefore a pre-concentration step is usually required [11]. Solid phase extraction (SPE) is the most common procedure for this purpose and large volumes of wastewater are necessary in order to reach the required limits of detection for determining environmental concentrations (between 50 and 1000 mL) [12]. However, the majority of the above procedures are tedious and time-consuming. Miniaturization of the sample preparation has become an alternative in modern high-throughput methods. Solid phase microextraction (SPME) differs from SPE in the ratio sorbent *versus* sample volume. Therefore, all the different SPME configurations are an equilibrium extraction technique since only a small portion of the analyte is extracted from the sample whereas SPE techniques are based on the complete extraction of all the analytes from the sample. Micro-SPE (μSPE) is a miniaturized version of SPE with the same concept of extracting all the analytes but in this case, with a smaller sample volume and a reduced amount of packed sorbent [13].

Large volume injection (LVI) methods are another alternative that provide the advantage of reducing sample preparation steps, improving the reproducibility and minimizing potential contamination of the sample. Furthermore, LVI increases sample throughput at minimal cost [14] and the water sample can be injected in the initial aqueous mobile phase without causing serious peak broadening. However, to date, LVI methods have normally presented low sensitivity with respect to the environmental levels [15], and require modern and very sensitive instruments that are not always available in the analytical laboratories [16].

Ultra-high-performance liquid chromatography (UHPLC) has recently emerged providing higher sensitivity, better separations and improved throughput [5]. UHPLC columns are packed with much smaller particles and support greater pressures that increases the efficiency and decreases the run time. However, UHPLC columns become a problem when using LVI due to lower sample capacity leading to chromatographic distortions such as peak broadening or volume over-load issues [17]. The post-loop mixing (PLM) approach efficiently avoids the above problems by completely diluting the sample into organic mobile phase before the sample reaches the mixer and is diluted and carried to the column by the aqueous mobile phase. The initial elution solvent rate is such that the sample is retained at the head of the column in a narrow band (i.e. A:water 97%; B:methanol 3%). In this case, rather than injecting the wastewater sample directly, the sample is extracted by µSPE and then a larger volume of the eluent is injected into the system directly in organic solvent without reconstitution in water.

At present the main development focus within the WBE field is based on the development of analytical methods for new markers [18-20] and reduction of the uncertainty related to both the in-sewer transformation [21] and the estimation of the population of the WWTP catchment areas [22]. However, due to the relatively low uncertainty and the inter-laboratory exercises for external quality control assurance, the analytical methods have remained unaltered, tedious and inefficient. Therefore, the combination of µSPE with PLM together with LVI provides a perfect compromise between sample throughput, cost, sensitivity and chromatographic separation.

The aim of this study was to develop, validate and apply a novel high-throughput WBE procedure for the analysis of 13 DTR by off-line µSPE-PLM-LVI-UHPLC coupled to tandem mass spectrometry (MS/MS). The selected compounds were amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), benzoylecgonine, cocaine, cocaethylene, atenolol, citalopram, carbamazepine, fexofenadine, methylphenidate, metoprolol and lidocaine. Thus, this procedure will potentially improve the technical and environmental WBE feasibility by: i) reducing sample preparation and analysis time; ii) reducing costs; iii) reducing the amount of solvents needed; iv) improving the whole method efficiency, v) making the sample collection and storage easier for the WWTP operator (from 1L to 5 mL or from one big bottle to one small glass vial) and vi) enabling the creation of a virtual specimen library bank for WBE by archiving and retrospectively analyzing the data acquired in HRMS mode. Finally, to demonstrate the feasibility of this approach, µSPE-PLM-LVI-UHPLC-MS/MS was applied to the analysis of 10 wastewater samples.

2. Experimental

2.1 Reagents and materials.

Reference standards for 13 drugs and/or their main metabolites chosen for the analysis were the following: amphetamine, methamphetamine, MDMA, cocaine, benzoylecgonine, cocaethylene, atenolol, citalopram, carbamazepine, fexofenadine, methylphenidate, metoprolol, and lidocaine dissolved in methanol (MeOH) or acetonitrile (ACN) at concentrations of 1 mg mL^{-1} or 100 µg mL^{-1}. Standard solutions of each compound were made in methanol at 100 µg mL^{-1} and then diluted into final mix solutions to a concentration of 10 and 1 ng mL^{-1}. Corresponding isotope-labeled internal standards (ILIS) were amphetamine-d8, methamphetamine-d11, MDMA-d5, cocaine-d3, benzoylecgonine-d3, cocaethylene-d3, atenolol-d7, fexofenadine-d6, metoprolol-d7 and lidocaine-d6 dissolved in MeOH or ACN at concentrations of 100 µg mL^{-1}. The ILIS solutions were made in methanol at 10 µg mL^{-1} and then diluted to a mix working solution at 10 ng mL^{-1}. All reference standards and ILIS were purchased from Cerilliant (Round Rock, TX, USA). The standards and working solutions were stored at -20 °C.

HPLC-grade MeOH was purchased from Rathburn Chemicals Ltd. (Walkerburn, SCT, UK). HPLC-grade ACN was acquired from VWR Chemicals (Oslo, Norway). Ammonium hydroxide (NH_4OH) solution ≥ 25% in water was obtained from Fluka - Sigma-Aldrich (Oslo, Norway) and formic acid (FA) 98-100% (for analysis) was purchased from Merck - Millipore (Oslo, Norway).

2.2 Wastewater samples

Influent wastewater samples were collected from Vestfjorden Avløpselskap (VEAS), the Oslo wastewater treatment plant (WWTP) in June 2016. A total of 10 flow proportional samples were collected with an EFCON® Wall Mounted Vacuum sampler from the VEAS raw inlets between the 17th and the 30th of June. The sampler was operated at 4 °C and the wastewater samples were firstly collected in high-density polyethylene (HDPE) bottles and then homogenized, poured into the 7 mL glass vials and stored at -20 °C immediately following collection.

Weekend composite samples consisted of a three-day composite sample from Friday (08:00) to Monday (08:00) while weekdays were twenty-four-hour composite samples. VEAS treats sewage for a *de jour* population of approximately 600,000 people of which the city contributes about 70.5% and the adjoining areas representing the other 29.5%. The total length of the sewer line is 42.3 km and the mean residence time in the sewer system is 5 hours [23].

2.3 Sample preparation and µ-SPE

Sample preparation is a crucial step to remove any matrix components that may compete with the target analytes in the ionization process during the UHPLC-MS/MS analysis. Prior to extraction, 5 mL of influent wastewater were spiked with 50 µL of the ILIS working solution to reach a concentration of 100 ng L^{-1}. Following vortex stirring, 1 ml of sample was centrifuged at 16,200 ×*g* for 5 min at 4°C in a Heraeus Fresco Biofuge (Thermo Scientific, Waltham, MA, USA) and the supernatant was used for analysis. µSPE was performed using Waters Oasis HLB µElution plates, 30 µm (Milford, MA, USA). The plate was conditioned by washing and rinsing with 1 ml of MeOH and 1 ml of ultrapure water under suction. The wastewater samples were loaded onto the plate under suction and washed with 1 ml of ultrapure water. The plate was vacuum dried for 15 min. Analytes were eluted into a 96 well plate using 50 µl of 1% NH_4OH in MeOH, 100 µl of MeOH and 50 µl of 1% FA in MeOH.

The final 200 µl extract was divided in two LC vials for separate analysis for both target and retrospective purposes (Figure 1). No solvent evaporation or residue re-dissolution were needed before injection and therefore, the eluent consisted only of methanol. Analysis was performed by injecting 37 µl into the PLM-LVI-UHPLC-MS/MS.

2.4 LC–MS/MS analysis

Wastewater analysis was carried out with a Waters Acquity UPLC system (Milford, MA, USA) equipped with a binary solvent manager and a sample manager. The UHPLC was coupled to a Waters Quattro Premier XE Micromass triple quadrupole mass spectrometer (Milford, MA, USA) with a T-wave collision cell and electrospray ionization interface (ESI), operated in positive ionization mode. Selected parent and product ions together with ionization and collision energy parameters are presented in Table 1. Mass spectrometer parameters were tuned with a direct infusion of standard solutions. Information about the HRMS acquisition parameters and other information can be found in Baz-Lomba et al. 2016 [24].

Chromatographic separation was carried out using a Waters Acquity UPLC BEH C8 column, 1.7 µm, 2.1 x 100 mm (Milford, MA, USA). The column temperature was kept at 50°C and the temperature of the sample manager was 4°C. A constant flow rate of 0.4 ml min^{-1} was used with a mobile phase consisting of 0.1% ammonium hydroxide (solvent A) and acetonitrile (solvent B). The elution gradient changed as follows: 0 min (3% B); 4.9 min (3% B); 5.1 min (40% B); 8.5 min (60% B); 9 min (95% B); 10 min (95% B); 10.5 min (3% B); 11 min (3% B). The sample injection volume was 37 µL.

The cone and desolvation gas used was nitrogen with flow rates of 50 L h^{-1} and 800 L h^{-1}, respectively. The collision gas used was argon with a flow rate of 0.15 mL min^{-1}. Other operational parameters were capillary voltage, 3.2 kV; source temperature, 100 °C and desolvation temperature, 450 °C. The loop and needle volumes were 50 and 250 µL respectively and the injection mode was partial loop with needle overfill mode (PLNO). The PLNO mode provides the best partial loop accuracy, precision, and linearity and only sample and mobile phase were injected onto the column avoiding air gaps or weak wash solvent.

Data acquisition was performed working in multiple reaction-monitoring mode (MRM). Infusion solutions of individual standards were prepared to optimize MS conditions and to select MS/MS transitions for both target analytes and ILIS. The best results in terms of

sensitivity were those using ESI operating in positive ionization mode, using the protonated molecule $[M+H]^+$ as precursor ion. The most abundant product ion of each target analyte was typically used for quantification and one additional product ion was used for confirmation. Furthermore, the retention times were also compared with those from reference standards (± 0.2 minutes). Each DTR was quantified using its ILIS as a surrogate internal standard, except citalopram, carbamazepine and methylphenidate for which the ILIS with the most similar retention time and chemical structures were selected. All data were acquired and processed using MassLynx v4.1 (Milford, MA, USA).

2.5 Method validation

Method validation was performed in terms of linearity, method quantification limits (LOQ), relative and absolute recoveries (trueness), repeatability and matrix effects. The performance of the method was evaluated following EU guidelines with minor modifications [25]. The linearity of the method was studied by analyzing standard solutions in methanol in triplicate at eight concentrations, in the range of 0.025 to 10 ng mL^{-1}, together with the ILIS at 0.5 ng mL^{-1}. Satisfactory linearity was considered when the correlation coefficient (R^2) was higher than 0.99, based on relative responses (analyte peak area/ILIS peak area). The LOQs were calculated in wastewater samples with known concentrations (all compounds were present in sample) as the concentrations giving a signal-to-noise ratio (S/N) of ≥10.

Relative and absolute recoveries were tested in triplicate in wastewater samples spiked at 100 ng L^{-1}. Adequate blank samples were not found since the target compounds were present in all the wastewater samples. Therefore, an additional set of three wastewater samples were analyzed by spiking only the ILIS before extraction to account for the analyte background. Relative recoveries were calculated by spiking the ILIS before the µSPE while for the absolute recoveries, meant for the assessment of the µSPE efficiency, were spiked after the extraction, right before the injection in the LC-MS/MS system. Calibration standards in solvent were used for quantification and the relative recoveries between 80% and 120% were considered satisfactory. Precision (expressed as repeatability) was assessed as the relative standard deviation (RSD) of six wastewater samples spiked at 200 ng L^{-1}. The matrix effects that occurred during the ionization (ESI) were assessed by spiking three wastewater extracts at 1 ng mL^{-1} together with the respective ILIS right before analysis and comparing its responses with that for those spiked at the same concentration in mobile phase. A non-spiked wastewater sample (only with ILIS) was analyzed simultaneously to subtract its response from the spiked sample:

$$Matrix\ effect\ (\%) = \frac{Response\ in\ ww\ extract - Response\ ww\ blank}{Response\ in\ mobile\ phase}\ x\ 100$$

3. Results and discussion

3.1 Large volume injection and post-loop mixing injection

The SPE extract is commonly evaporated under a current of nitrogen and reconstituted into the initial mobile phase to improve the chromatographic separation and avoid the sample to significantly penetrate the column without an optimal retention [24]. The PLM configuration, described in Figure 2, avoids the eluent reconstitution following µSPE and chromatographic peak distortion when using LVI with UHPLC. In the PLM-LVI configuration, the position of the mixer and line A (aqueous phase) are changed in such a way that line B (organic phase, acetonitrile) goes directly to the loop in the autosampler, drags the sample and meets the aqueous phase in the mixer located right after the autosampler and before the HPLC column. At this stage, the sample is diluted in the mixer and stacked at the head of the column. Furthermore, the PLM-LVI configuration mitigates one of the main issues when using LVI with UHPLC columns related to the lower sample capacity leading into chromatographic distortions such as peak broadening or volume over-load issues. By using a high initial water ratio (i.e 97%), the sample is completely diluted in water right before the UHPLC column and retained in a narrow band at the head of the column.

The ratio of the organic phase versus aqueous phase will depend on the characteristics of the target compounds and becomes a critical feature in the development of the method. Optimization of the percentages of organic phase (acetonitrile) in water was achieved by comparing the peak shapes of the early-eluting compounds. The initial gradient was tested at 1,2,3,4,5 and 10% of acetonitrile. If the initial ratio of acetonitrile was too high, the polar analytes could not be retained at the column head due to the strong elution strength and therefore, the peak width of the analytes increased significantly. Both the loop and tubing (from autosampler to mixer) volumes were taken into account to estimate the time to fill the loop and drag the sample into the column (approximately 60 µL). The best compromise between peak shape and total run time was found to be 3% acetonitrile in water. Using a flow rate of 0.4 mL min^{-1}, the initial gradient was held for 5 minutes at 3% acetonitrile and once the analytes were retained at the head of the column the % acetonitrile was increased.

3.2 Method validation

The principal aim of this study was to prove the concept and applicability of a µSPE- PLM-LVI -UHPLC-MS/MS configuration for WBE. Therefore, neither the µSPE nor the UHPLC conditions were optimized. However, all the conditions and parameters used in this study were previously developed “in-house” for validated and published methods [24, 26]. Furthermore, the analytical method used in this study has been validated through an external inter-laboratory exercise with other 27 international laboratories for some of the studied compounds (cocaine, benzoylecgonine, amphetamine, methamphetamine and MDMA), successfully meeting all the external quality control requirements [27].

The mean correlation coefficients (R^2) of the calibration curves, which are higher than 0.99 (Table 2) show good linearity of the method in the range of 0.025 to10 ng mL^{-1}. The method LOQs were below 10 ng L^{-1} for all the compounds, ranging from 0.2 ng L^{-1} for carbamazepine to 6.3 ng L^{-1} for MDMA, being better than achieved with and SPE-UHPLC-MS/MS method on the same 16-year old MS system [26] and were below the reported environmental concentrations of the target compounds in wastewater.

The absolute recoveries for the μSPE performed with Waters Oasis HLB were satisfactory with values higher than 79% for all the compounds except for amphetamine with only a 36% recovery. Satisfactory relative recoveries were found for all the compounds, ranging from 92% for citalopram to 110% for cocaine. Precision (n=6) for spiked wastewater samples at 200 ng L^{-1} was satisfactory in all cases with RSD values ranged from 3.4 to 14.4%.

3.3 Matrix effects

Ion suppression or enhancement is commonly observed in complex environmental matrices such as wastewater as a consequence of the matrix effect, which affects sensitivity, accuracy and the evaluation of method recovery. The matrix effect observed for the target compounds dissolved in wastewater is presented in Table 2. Little or no signal suppression was observed for MDMA, citalopram, carbamazepine and metoprolol. Atenolol and fexofenadine, both co-eluting at the beginning of the chromatographic run, showed a high ion suppression while the rest of the compounds showed a moderate ions suppression/enhancement (± 20%). The matrix suppression and recoveries were acceptable for the compounds for which no corresponding isotope-labelled internal standards were available.

3.4 Analysis of wastewater samples

The developed method was applied to the analysis of ten 24-hour flow proportional influent samples (72-hour for the weekend samples). Standard calibration curves were used to calculate the concentrations of the target compounds and injected in duplicate at the beginning and at the end of the run. Fortified "blank" samples were injected as internal quality control during the sequence.

The target compounds were found in all the inlet wastewater samples with changing concentrations (Table 3). Carbamazepine showed the highest concentrations with an average (n=10) of 1200 ng L^{-1} while cocaethylene and metoprolol showed the lowest concentrations with an average of 9 and 7 ng L^{-1} respectively. Amphetamine and methamphetamine concentrations show similar levels ranging from 200 to 600 ng L^{-1} respectively. MDMA was the compound with the highest coefficient of variance among the 10 samples (61%) when comparing week days with the weekend due to its recreational use during the weekend in agreement with previous works [28]. Cocaine and its main metabolite, benzoylecgonine, ranged from 100 to 700 ng L^{-1} and show a benzoylecgonine/cocaine ratio of approximately 2~3, in agreement with previous publications [12]. For the rest of the pharmaceuticals, concentrations ranged from 25 to 48 ng L^{-1} for atenolol, from 35 to 71 ng L^{-1} for citalopram, from 117 to 205 ng L^{-1} for fexofenadine, from 140 to 263 ng L^{-1} for methylphenidate and from 55 to 108 ng L^{-1} for lidocaine.

3.5 Environmental feasibilities and implications for the future

In summary, the 96-well plate for μSPE provides the highest throughput for the analysis of wastewater samples to date. The main advantages are the reduction of the time invested per sample, the final cost per sample is lower (only the cartridges are approximately 25% less expensive and the amount of ILIS used compared with classic methods is approximately 100 times less), slightly decrease of matrix effects due to the reduction of the volume extracted and from the environmental point of view, is more feasible due to the reduction of the solvents used for the extraction, by approximately a 90%.

Furthermore, the HLB sorbent, with a hydrophilic-lipophilic-balanced sorbent, offers the possibility to extract a wide range of compounds with different psychochemical characteristics enabling the simultaneous analysis of a wide range of drugs and pharmaceuticals in one single extraction. The use of this generic extraction methodology also is very suitable for HRMS and retrospective analysis, which have been proposed as a good alternative for data storage and environmental repository without the need of additional sample analyses [29]. Furthermore, this workflow does not imply additional extractions and both analysis are performed using the same extract. Therefore, the approach proposed in Figure 1 will allow the performance of different tasks such as pre- and post-target analysis, potential elucidation of metabolites and transformation products, retrospective analysis and non-target analysis only with one extraction and two analysis.

The PLM-LVI configuration complements the µSPE reducing even more the sample preparation time by avoiding the reconstitution of the eluent. Furthermore, this configuration also improves the efficiency of the method by injecting larger volumes. Most of the published analytical methods for the analysis of wastewater samples reconstitute the eluent in 250-1000 µL for a final injection of a few µL (normally between 2-5 µL) [24, 30]. In this study, we elute 200 µL that are split in two for target and retrospective analysis, and 37 µL out of 100 µL are injected into the system. This configuration would also allow the introduction of robots or automated µSPE that would simplify and improve substantially the method in the future.

The reduction of the time invested for collecting, extracting and analyzing the sample together with substantial reduction of the cost, increase the possibilities for the laboratories to perform real-time monitoring. The fact that the staff at the WWTP move from collecting 500-1000 ml in big plastic bottles to 5 mL in small glass vials could potentially increase the number of collaborations between laboratories and treatment plants.

4. Conclusions

A novel analytical methodology based on the use of µSPE-PLM-LVI-UHPLC-MS/MS has been developed for the simultaneous quantification and confirmation of 13 widely consumed drugs in urban wastewater and applied to 10 influent wastewater samples from Oslo, Norway. A high throughput analytical procedure has been fully validated, obtaining satisfactory accuracy and precision and high sensitivity. The method LOQs are comparable with previous studies and below the environmental concentrations found is Oslo during the last years.

The combination of µSPE with PLM-LVI has been demonstrated to be a promising compromise to reduce the sample preparation time and still reach the required detection levels for environmental samples. Furthermore, reducing total cost and amounts of solvents, increasing the method efficiency and improving the collection and handling of the samples, have upgraded the technical and environmental feasibility of classic WBE methods. These results highlight the potential of µSPE-PLM-LVI-UHPLC-MS/MS for WBE studies in the future.

In addition, a 200 µL µSPE extract is enough for both the quantitative and HRMS analysis, which will enable the creation of a virtual specimen library bank for WBE. This additional workflow will archive all the data for retrospective analysis, functioning as a backup for cases when old samples are not available or degraded.

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456 *Figure 1. Illustrative workflow of the analytical procedure using Waters Oasis HLB µElution plate (5 mg,*
457 *30 µm particle size) for the sample extraction (n=3) and an UPLC-MS/MS for target quantification and*
458 *UPLC-HRMS for virtual library bank acquisition.*

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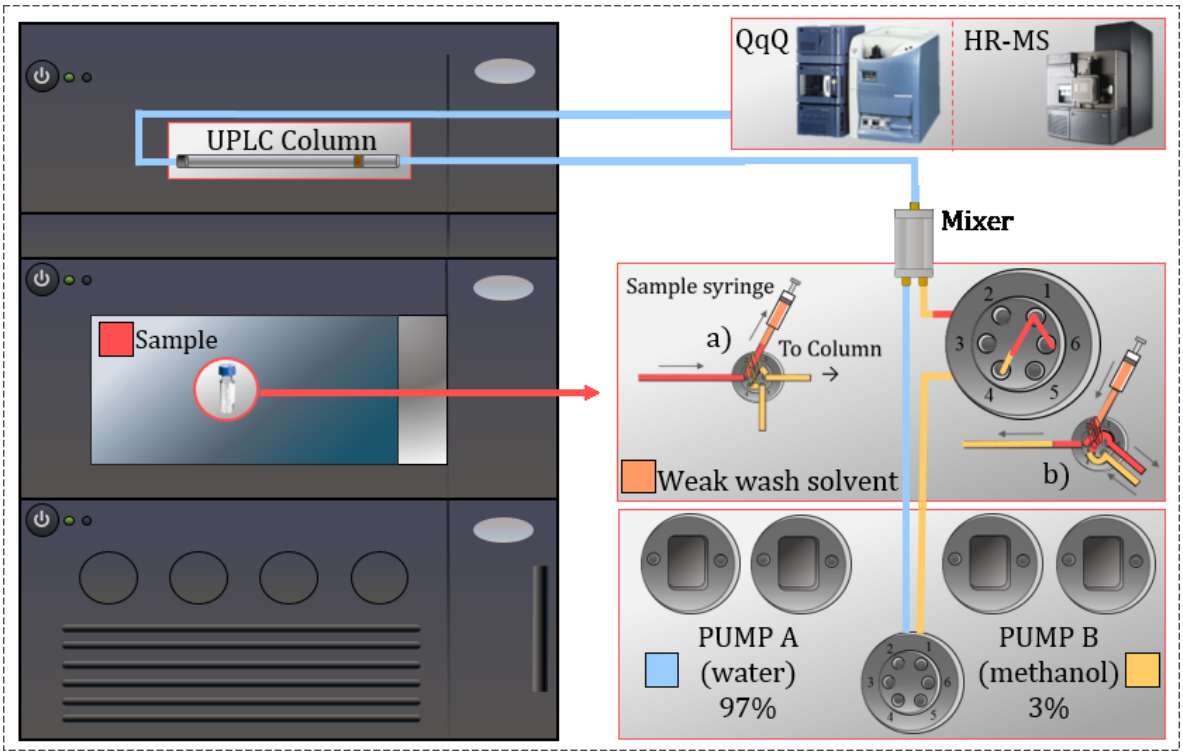

Figure 2. Schematic representation of post-loop mixing process. Initial mobile phase rate (flow 0.5 mL min^{-1}) is set at 97% A:3% B during the first 5 minutes in order to retain the sample at the head of the UHPLC column

465 *Table 1. MS/MS optimized conditions for selected compounds.*

			Quantitation			Confirmation
Compound	ESI	Retention time	MRM (Q1 > Q3)	Cone (V)	Collision (V)	MRM (Q1 > Q3)
Amphetamine	+	6.6	136.1 > 91.1	20	15	136.1 > 119.1
Amphetamine-d8	+	6.6	144.1 > 97.1	20	15	-
Methamphetamine	+	7.0	150.1 > 91.1	20	15	150.1 > 119.1
Methamphetamine-d11	+	7.0	161.2 > 127.1	20	15	-
MDMA	+	6.9	194.2 > 163.2	20	15	194.2 > 105.1
MDMA-d5	+	6.9	199.2 > 165.2	20	15	-
Cocaine	+	7.4	304.2 > 182.2	30	20	304.2 > 105
Cocaine-d3	+	7.4	307.2 > 185.2	30	22	-
Benzoylecgonine	+	5.9	290.2 > 168.2	30	20	290.2 > 105
Benzoylecgonine-d3	+	5.9	293.2 > 171.2	30	20	-
Cocaethylene	+	8.0	318.2 > 196.2	30	20	318.2 > 82.1
Cocaethylene-d3	+	8.0	321.2 > 199.1	30	20	-
Atenolol	+	6.0	267.2 > 190	25	20	267.2 > 145
Atenolol-d7	+	6.0	274 > 145	30	20	-
Citalopram	+	8.1	325.2 > 262.2	30	22	325.2 > 109.2
Carbamazepine	+	6.5	237.1 > 194.1	25	20	237.1 > 192.1
Fexofenadine	+	5.9	502.3 > 466.3	20	30	502.3 > 171.1
Fexofenadine-d6	+	5.9	508.3 > 472.5	30	30	-
Methylphenidate	+	7.2	243.3 > 84	20	20	243.3 > 174.1
Metoprolol	+	6.7	268.2 > 116	25	20	268.2 > 191
Metoprolol-d7	+	6.7	275.2 > 123.1	28	20	-
Lidocaine	+	7.7	235.3 > 86	25	20	235.3 > 58.1
Lidocaine-d6	+	7.7	241.3 > 86	25	15	-

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Table 2. Method performance parameters: linearity, recoveries, repeatability, matrix effect and method limits of quantification.

	MeOH	Wastewater					ILIS used for correction
	Linearity (R^2)	Relative recovery (RSD)	Absolute recovery (RSD)	Repeatability (RSD)	Matrix Effects	LOQ	
	ng mL^{-1}	Both in %	Both in %	%	%	ng L^{-1}	
	n=3	[100 ng L^{-1}] n=3	[100 ng L^{-1}] n=3	[200 ng L^{-1}] n=6	n=3		
Amphetamine	0.025 - 10 (0.99931)	105 (14)	36 (18)	14.4	80	3.5	Amphetamine-d8
Methamphetamine	0.025 - 10 (0.99941)	94 (10)	95 (3)	9.3	117	1.1	Methamphetamine-d11
MDMA	0.025 - 10 (0.99973)	99 (3)	86 (5)	3.5	104	6.3	MDMA-d5
Cocaine	0.025 - 10 (0.99991)	110 (8)	79 (1)	6.8	117	4.3	Cocaine-d3
Benzoylecgonine	0.025 - 10 (0.99979)	103 (5)	86 (14)	4.3	87	2.9	Benzoylecgonine-d3
Cocaethylene	0.025 - 10 (0.99997)	98 (3)	86 (1)	3.4	118	1.0	Cocaethylene-d3
Atenolol	0.025 - 10 (0.99871)	104 (12)	87 (3)	11.1	55	4.4	Atenolol-d7
Citalopram	0.025 - 10 (0.99984)	92 (10)	87 (7)	11.3	96	1.1	Cocaethylene-d3
Carbamazepine	0.025 - 10 (0.99937)	104 (9)	93 (15)	11.3	102	0.2	Metoprolol-d7
Fexofenadine	0.025 - 10 (0.99980)	96 (8)	90 (12)	8.8	21	5.6	Fexofenadine-d6
Methylphenidate	0.025 - 10 (0.99979)	105 (7)	91 (11)	4.9	78	1.9	Cocaine-d3
Metoprolol	0.025 - 10 (0.99954)	109 (16)	94 (3)	12.6	104	2.1	Metoprolol-d7
Lidocaine	0.025 - 10 (0.99989)	100 (3)	92 (5)	3.6	113	0.3	Lidocaine-d6

472 *Table 3. Concentrations of the target compounds quantified in 10 wastewater samples from Oslo in*
473 *2016 (ng L^{-1}).*

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	Wastewater Concentration (ng/L)									
Compound \ Date	17-19/06/16 Weekend	20/06/16 Monday	21/06/16 Tuesday	22/06/16 Wednesday	23/06/16 Thursday	24-26/06/16 Weekend	27/06/16 Monday	28/06/16 Tuesday	29/06/16 Wednesday	30/06/16 Thursday
Amphetamine	459	282	227	372	426	594	393	372	402	349
Methamphetamine	447	300	250	375	398	480	386	353	395	354
MDMA	117	61	28	45	44	145	78	44	38	35
Benzoylecgonine	644	340	236	405	535	718	495	420	456	371
Cocaine	300	119	108	195	257	306	187	194	195	176
Cocaethylene	16	6	4	7	10	16	9	8	9	8
Atenolol	42	30	25	39	44	38	33	43	48	32
Citalopram	65	58	35	60	66	59	58	66	71	55
Carbamazepine	1379	1241	888	1315	1433	1277	1168	1200	1389	1091
Fexofenadine	205	165	117	167	178	182	142	166	165	165
Methylphenidate	185	167	140	205	263	204	215	232	232	177
Metoprolol	6	5	3	7	10	9	7	7	7	5
Lidocaine	89	87	55	71	85	83	72	77	108	78

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