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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.

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 5–10% [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 uncer-

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tainty 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 h [23].