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Estimation of drug abuse in 9 Polish cities by wastewater analysis

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ABSTRACT

The aim of this work was to measure illicit drug residues in raw sewage samples collected from nine Polish cities in order to determine trends in illicit drug use in these urban populations. This is the first study involving an analysis of samples from several sewage treatment plants in Poland and covering such a large population. Concentration of illicit drugs was determined using a high performance liquid chromatography–tandem mass spectrometry method. The samples were subjected to a multistep preparation procedure with a solid phase extraction as a main pre-treatment step. Among the selected drugs investigated in the study, amphetamine was found in the greatest amounts in all sewage samples and consequently was the most prevalent drug of abuse. Higher loads of illicit drug residues were found during weekends compared to the weekdays, especially for 3,4-methylenedioxymethamphetamine (ecstasy) and benzoylecgonine, main metabolite of cocaine.

1. Introduction

In recent years, an increased interest in the measurement of illicit drugs in urban sewage has been observed. Raw sewage can be considered a diluted urine sample collected from the population served by a given sewage treatment plant (STP). This approach, called sewage-based epidemiology (SBE), was proposed by Daughton and Ternes [1] in 1999 and applied for the first time by Zuccato et al. [2] in 2005 to estimate cocaine use in medium-size Italian cities. These authors also showed temporal changes in drug use in communities with the highest amounts of illicit drug residues occurring on weekends [3]. Due to encouraging results consistent with official epidemiological data and advances in analytical techniques allowing for detection of excreted drugs at very low concentration, the use of SBE approach for mapping drug consumption in urban populations has become increasingly popular. Over the past few years, a number of research groups have used this methodology to analyze sewage samples collected in various European countries, e.g. Spain [4], Belgium [5], Switzerland [6], United Kingdom [7], Croatia [8], France [9], Slovakia [10], Finland [11], Sweden [12], as well as North America [13] and Australia [14]. Recently, SBE-based estimates of drug use from Asian locations have been reported [15–17].

Since illicit drug consumption is a hidden and stigmatized activity, it is difficult to obtain real figures of the prevalence of drug abuse. An estimation of illicit drug usage in Poland is currently established using indirect methods, such as population surveys, medical data and police statistics [18]. Collected data concerning drugs and drug addiction are submitted to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) under yearly reporting obligation [18,19]. Despite various information sources, the knowledge on drug abuse in Polish population is still incomplete and SBE has a considerable potential as a new source of information on drug use. The most relevant advantages of the SBE include providing objective data covering the entire communities, and the ability to monitor near-real time trends in drug consumption [20].

Although SBE has been widely applied in many European populations, only a few studies concerned the countries located in Central Europe, including Poland. Wastewater analyses proved that amphetamine was the most commonly used synthetic drug in the northern regions of Europe, whereas the highest consumption rates of methamphetamine in Europe occurred in the cities located in the south of Poland [10,21]. To date, limited information regarding methamphetamine use in Poland has been obtained. According to the latest National Report, amphetamine produced in Polish clan labs is a main stimulant on the Polish drug market. However, the data on drug seizure revealed an arrival of methamphetamine into Poland [19]. The information from self-report surveys can be misleading because consumers may be dishonest or unaware of the exact chemical composition of the

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drugs they use. Since drug seizures are subject to randomness and population surveys can provide subjective data, an additional source of information on drug abuse in Polish society is required. A combination of various drug-related information sources may help in painting a more accurate picture of the illicit drug use in the Polish population.

The aim of this work was to measure illicit drug residues in raw sewage samples collected from nine Polish cities in order to determine trends in illicit drug use in these urban populations. To the best of our knowledge, this is the first study that involves an analysis of samples from several sewage treatment plants in Poland and covers such a large population. So far, the data on illicit drug content in sewage samples were known from only one Polish city [22]. This study compared the profiles of illicit drug consumption between various cities of western Poland (the Greater Poland). Moreover, an attempt was made to compare the profiles of drug abuse between Polish and other European cities. Although similar analyses have been already carried out for many European countries, Poland is the furthest to the east among them.

2. Materials and methods

2.1. Illicit drugs of interest

Illicit drugs selected for the study involved a group of amphetamine-like stimulants: amphetamine (AMP), methamphetamine (METH), 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), 3,4-methylenedioxyethylamphetamine (MDEA), and cocaine (COC). Estimation of illicit drug use was based on determination of drug target residues (DTRs, also called urinary biomarkers) in wastewater samples. Metabolism of a particular drug of abuse in human organism and its stability in wastewater were taken into account in the selection of its DTR [3,23]. For amphetamine-like stimulants, the compounds used as DTRs were the parent drugs, whereas for cocaine its primary metabolite benzoylecgonine (BE) was used. Additionally, the presence of cocaine in wastewater samples was monitored. The list of determined compounds is included in Table S1 in Supplementary material.

2.2. Reagents and materials

Reference standards of all analyzed illicit drugs and their metabolites: AMP, METH, MDMA, MDEA, COC and BE, as well as their deuterated analogues used as internal standards (ISs): AMP-D6, METH-D5, MDMA-D5, MDEA-D5, COC-D3, and BE-D3 were acquired from LGC Standards (Teddington, UK). All standards were purchased as 1.0 mg/mL solutions in methanol and stored in the dark at −20 °C until analysis. Formic acid and ammonium formate from Sigma–Aldrich (St. Louis, MO, USA) were used. Deionized water was acquired from Millipore Simplicity UV purification system (Waters, Milford, MA, USA). All other reagents were obtained from J.T. Baker (Griesheim, Germany). For solid phase extraction (SPE) procedure Oasis MCX (Waters, Milford, MA, USA) cartridges were used.

2.3. Sample collection

The study investigated samples from nine sewage treatment plants (STPs), representing nine cities in Poland. The project included the following cities: Poznań (central STP of the city of Poznań in Koziegłowy), Piła, Szamotuły, Ostrów Wielkopolski, Leszno, Gniezno, Słupca, Grodzisk Wielkopolski, and Kościan. All the cities are located in the Greater Poland Voivodship (Fig. 1). The cities vary in terms of population, the smallest are Słupca, Grodzisk Wielkopolski, and Szamotuły (population below 20,000 inhabitants) and the biggest one is Poznań (nearly 550,000 inhabitants), which is the capital of the Greater Poland. The STPs included in the project serve the majority of inhabitants living in these cities and cover a total population of 1,033,511 inhabitants. Since the total population of the Greater Poland in 2014 was 3,472,579, the study investigated nearly one third (29.76%) of the voivodship population. The total population of Poland in 2014 was 38,478,602, meaning the studied population constituted 2.69% of the population of Poland [24]. The data on each STP are presented in Table 1.

Three 24-h composite samples of untreated wastewater were collected into polyethylene bottles from each STP during 2013 and 2014. The sample collection took place in a time-, volume- or flow-proportional mode depending on the STP (Table 1). The pooled wastewater samples were obtained over a 24-hour period starting at 8:00 a.m. or 7:00 a.m. Sampling frequency for each STP is presented in Table 1. The samples were collected during one weekend day and two weekdays from each STP to assess variations in drug abuse during a week.

2.4. Sample preparation

Wastewater sample preparation and analysis followed the methodology proposed by Castiglioni et al. [23]. The method was tested and used by many research groups for an analysis of illicit drugs in waste and surface water [3,8,25]. Briefly, wastewater samples were filtered through glass microfiber filters GF/A (Whatman, Kent, UK), acidified to pH = 2.0 with 37% HCl and spiked with 10 ng of each IS, except for AMP-D6 (50 ng). Stable isotope-labeled internal standards are recommended to compensate for potential errors arising from the loss of the analytes during sample preparation process, the matrix effects and the variation in an instrument response. Therefore, corresponding deuterated molecules for each target analyte were chosen as internal standards (Table S1). For conditioning of SPE cartridges, 6 mL of methanol, 3 mL of deionized water and 3 mL of acidified water (pH = 2.0) were used. After loading a sample onto the conditioned cartridge and drying off the sorbent, illicit drugs and their metabolites were eluted with 3 mL of methanol and 3 mL of 2% ammonia solution in methanol. The extract was then evaporated to dryness in a vacuum concentrator (miVac Duo, GeneVac, UK), redissolved in 1 mL of eluent A of mobile phase and filtered through 0.45 μm Mini-UniPrep syringeless filters (Whatman, Kent, UK). Each collected wastewater sample yielded three independently prepared samples for LC–MS/MS analysis: two 500 mL samples and one 100 mL sample.

2.5. Sample analysis

Quantification of the selected illicit drugs and their metabolites was carried out using liquid chromatography–tandem mass spectrometry (LC–MS/MS) system composed of a high performance liquid chromatograph 1260 Infinity (Agilent Technologies, Santa Clara, CA, USA) and a triple quadrupole mass spectrometer 4000 QTRAP (AB Sciex, Framingham, MA, USA) equipped with an electrospray ion source. LC–MS/MS is a reference analytical method for sewage analysis because it provides necessary selectivity and sensitivity [26]. For chromatographic separation, XTerra C18 column (100 mm × 2.1 mm, 3.5 μm) (Waters, Milford, MA, USA) and a gradient elution were used. The column was maintained at 35 °C and the injection volume was 20 μL. The analyses were performed using 5 mM ammonium formate solution with 0.1% formic acid as an eluent A and eluent B consisting of acetonitrile with 0.1% formic acid and 10% solvent A. The gradient program was 0–5 min 2% B, 5–14 min linear from 2% to 90% B, 14–16 min 90%, 16–18 min linear from 90% to 2% B,