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Estimation of community-wide drugs use via stereoselective profiling of sewage

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ABSTRACT

This paper explores possibilities of applying enantiomeric profiling to solving problems related to estimation of drugs usage in communities via the *sewage epidemiology* approach: for the identification of whether drug residue results from consumption of illicit drug or metabolism of other drugs, verification of potency of used drugs and monitoring of changing patterns of drugs abuse. Due to the very complex nature of wastewater used in sewage epidemiology, which comes from the whole community rather than one individual, verification of the above is challenging but vital in accurate estimations of drugs abuse as well as providing comprehensive information regarding drug abuse trends. The results of this study indicated that amphetamine in raw wastewater was enriched with *R*(−)-enantiomer due to its abuse as racemate. Methamphetamine was found to be racemic or to be enriched with *S*(+)-enantiomer. MDMA was enriched with *R*(−)-MDMA, which was to be expected as MDMA is abused as racemate. MDA was enriched with *S*(+)-enantiomer, which suggests that its presence might be associated with MDMA abuse and not intentional MDA use. Out of the four possible isomers of ephedrine only natural *1R,2S*(−)-ephedrine and *1S,2S*(+)-pseudoephedrine were detected in raw wastewater and their diastereomeric fractions were found to be season dependent with higher contribution from *1S,2S*(+)-pseudoephedrine over winter months and an enrichment with *1R,2S*(−)-ephedrine during the spring and summer months. These findings were accompanied by a decrease of cumulative concentration of ephedrines throughout the sampling campaign between February and August. This is a very important finding indicating that non-enantioselective measurement of ephedrine concentrations cannot be a reliable indicator of actual potency of ephedrines used.

1. Introduction

'*Sewage epidemiology*' is a new and very promising approach for the estimation of drugs consumption in communities via the analysis of sewage. It was first proposed by Daughton (2001), implemented by Zuccato et al. (2005) and followed by others (Banta-Green et al., 2009; Bones et al., 2007; Harman et al., 2011; Huerta-Fontela et al., 2008; Karolak et al., 2010; Kasprzyk-Hordern et al., 2009; Mari et al., 2009; Metcalfe et al., 2010; Postigo et al., 2010; Terzic et al., 2010; van Nuijs et al., 2009). Those wanting to find out more about this new concept are referred to existing reviews (Postigo et al., 2008b; van Nuijs et al., 2010; Zuccato et al., 2008). Significant advances in the development of this new tool were observed in recent years but there are still many uncertainties mainly connected with the not entirely understood fate of drugs and their metabolites in sewers, and the varying and not well understood excretion profiles of drugs, which might be altered by disease, drug interactions, ethnic differences, sex, age and lifestyle. Other sewage epidemiology biases are discussed elsewhere (Lai et al., 2011; van Nuijs et al., 2010). The above uncertainties need to be resolved before this tool is implemented as a routine population screening technique for drugs use. One aspect of drugs properties, namely chirality, has not been previously taken into account in sewage epidemiology, despite its critical importance in drugs' abuse potency and toxicity, as well as their disposition (distribution, metabolism and excretion) in the body.

Most illicit drugs are chiral compounds. Among them are plant-derived substances (e.g. cannabis, cocaine and heroin) and synthetic drugs (e.g. amphetamine, methamphetamine and related designer drugs). A chiral molecule usually has at least one chiral centre (e.g. asymmetric carbon) as a result of which it shows optical activity. It exists in the form of two enantiomers, being the non-superimposable mirror images of each other. Enantiomers of the same drug have similar physico-chemical properties but differ in their biological properties such as distribution, metabolism and excretion, as these processes (due to stereospecific interactions of enantiomers with biological systems) usually favour one enantiomer over the other. Additionally, due to different pharmacological activity, chiral drugs can differ in toxicity. *R,R*(+)-LSD is for example over 20 times more psychoactive than (−)-LSD. Cocaine, similarly to heroin, naturally occurs in the form of *1R,2R,3S,5S*(−)-cocaine. (+)-Cocaine (the unnatural enantiomer) is inactive. Both metabolism and toxicity of (+)- and (−)-cocaine were found to be stereoselective. In cannabinoids, the natural *d*-1-THC and

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d-6-THC have a (*3R,4R*) configuration and a negative rotation. Synthetic (+)-isomers are much less active. For instance (+)-*d*-1-THC is ca. 13 to 230 times less active than the (−)-isomer in cannabimimetic activity (Kasprzyk-Hordern, 2010).

The phenomenon of chirality is often utilised by forensic scientists in for example amphetamine or methamphetamine abuse cases, where distinction between legal and illicit usage has to be made. It can also help with identifying synthetic routes of clandestine manufacture of these drugs. It might be of vital importance in sewage epidemiology in:

- Distinction between legal and illicit use of drugs.
- Verification of the method of synthesis of illicit drugs.
- Identification of whether drug residue results from consumption of illicit drug or metabolism of other (illicit) drug.
- Verification of route of administration.
- Verification of potency of abused drugs.
- Monitoring of changing patterns of drugs abuse.

This paper aims to explore the possibilities of applying enantiomeric profiling to solving problems related to estimation of drugs usage in communities via the *sewage epidemiology* approach. To the authors' knowledge this is the first attempt to undertake such studies in *sewage epidemiology*.

2. Experimental

2.1. Chemicals and materials

All reference standards (*R*(−)-amphetamine, *S*(+)-amphetamine, *R/S*(±)-amphetamine, *R*(−)-methamphetamine, *S*(+)-methamphetamine, *1R,2S*(−)-ephedrine, *1S,2S*(+)-pseudoephedrine, *R/S*(±)-MDA, *R/S*(±)-MDMA) and internal standards (IS): (*R/S*(±)-amphetamine-d11, *R/S*(±)-methamphetamine-d14, *R/S*(±)-MDMA-d5, *R/S*(±)-MDA-d5) (Table 1) were purchased from LGC Standards (Teddington, UK) and Sigma-Aldrich (Gillingham, UK). All solvents used were of LC or LC/MS quality. All internal standards were added to the samples before extraction and were also used for the quantification of the analytes.

Table 1
Selected chiral drugs.

Name	CAS
R/S(±)-Amphetamine $C_9H_{13}N$	R(−): 300-62-9 S(+): 51-64-9 R/S(±): 300-62-9
R/S(±)-Methamphetamine $C_{10}H_{15}N$	R(−): 33817-09-3 S(+): 537-46-2
R/S(±)-MDA $C_{10}H_{13}NO_2$	4764-17-4
R/S(±)-MDMA $C_{11}H_{15}NO_2$	42542-10-9
1R,2S(−)-Ephedrine HCl $C_{10}H_{15}NO \cdot HCl$	50-98-6
1S,2R(+)-Ephedrine HCl $C_{10}H_{15}NO \cdot HCl$	24221-86-1
1S,2S(+)-Pseudoephedrine HCl $C_{10}H_{15}NO \cdot HCl$	345-78-8
1R,2R(−)-Pseudoephedrine $C_{10}H_{15}NO$	90-82-4
R/S(±)-Amphetamine-d11 $C_9H_2D_{11}N$	NA
R/S(±)-Methamphetamine-d14 $C_{10}HD_{14}N$	NA
R/S(±)-MDA-d5 $C_{10}H_8D_5NO_2$	136765-42-9
R/S(±)-MDMA-d5 $C_{11}H_{10}D_5NO_2$	136765-43-0

2.2. Sampling

Grab wastewater samples (2 per each sampling point) were collected from seven WWTPs in England (Table 2) over the period of 8 months (January 2010–August 2010) during 5 sampling campaigns. Samples were collected in 2.5 L silanized amber bottles with Teflon faced phenolic caps. The samples were primarily filtered through GF/D 2.7 μm glass fibre filter (Whatman, UK) and subsequently through 0.7 μm glass fibre filter GF/F (Whatman, UK).

2.3. Sample preparation and analysis

2.3.1. Enantiomeric profiling of chiral drugs with SPE-Chiral LC-MS/MS

Chiral drugs were extracted from wastewater using SPE Gilson ASPEC XL4 (Anachem, UK) and Oasis HLB adsorbents (Waters, UK). A volume of 100 mL of filtered wastewater samples (pH, 7.5 adjusted with NaOH) spiked with 100 ng of internal standards was passed through the cartridge at a rate of 6 mL/min. Analytes were extracted from HLB cartridge with 4 mL of MeOH at a rate of 1 mL/min. The extracts were evaporated to dryness with TurboVap evaporator (Caliper, UK, 40 °C, N_2, 5–15 psi) and finally reconstituted in 0.5 mL of mobile phase. All samples were filtered through 0.2 μm PTFE filters (Whatman, Puradisc, 13 mm) and transferred to maximum recovery deactivated vials with PTFE septa (Waters, UK).

Waters ACQUITY UPLC™ system (Waters, Manchester, UK) consisting of ACQUITY UPLC™ binary solvent manager and ACQUITY UPLC™ sample manager was used for the separation of analytes. Chiral-CBH column, 100×2 mm, 5 μm (Chromtech, Congleton, UK) and Chiral-CBH 10×2.0 mm guard column (Chromtech, Congleton, UK) were used for the separation of enantiomers of chiral drugs. The separation of chiral drugs was undertaken under isocratic conditions with the usage of mobile phase (pH, 5.0) composed of 90% H_2O, 10% 2-propanol and 1 mM ammonium acetate. 20 μL of the sample was injected into the system. The column was kept at 25 °C and the temperature in the sample manager was kept at 4 °C. The flow rate of mobile phase was 0.075 mL/min, which allowed for the introduction of mobile phase from LC into MS without splitting.

A TQD (triple quadrupole) mass spectrometer (Waters, Manchester, UK), equipped with an electrospray ionisation source, was used for drugs of abuse quantification. The analyses were performed in positive mode with a capillary voltage of 3 kV, a source temperature of 150 °C and a desolvation temperature of 200 °C. A cone gas flow of 50 L/h and desolvation gas flow of 450 L/h were used. Nitrogen, used as a nebulising and desolvation gas, was provided by a high purity nitrogen generator (Peak Scientific Instruments Ltd, UK). Argon (99.999%) was used as a collision gas. MassLynx 4.1 (Waters, UK) software was used to collect and analyse the obtained data. Mass spectrometry analyses were performed in the multiple reaction monitoring (MRM) mode, measuring the fragmentation of the protonated pseudo-molecular ions of each chiral drug (Table 3). A dwell time of 50 ms per ion pair was used to maintain high sensitivity of the analysis and required a number of data points across the chromatographic peak. All instrumental and methods validation parameters such as: linearity

Table 2
General information on the studied WWTPs.

WWTP	Parameter		
	Population served (thousands)	Flows [L s^{-1}]	Wastewater (% industrial/% domestic)
WWTP 1	15	89–212	10–15/85–90
WWTP 2	10	32–75	10–15/85–90
WWTP 3	11	40–115	10–15/85–90
WWTP 4	190	366–1300	30/70
WWTP 5	240	603–1231	30/70
WWTP 6	244	476–1378	30/70
WWTP 7	190	395–563	20/80