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Enantiomeric profiling of amphetamine and methamphetamine in wastewater: A 7-year study in regional and urban Queensland, Australia

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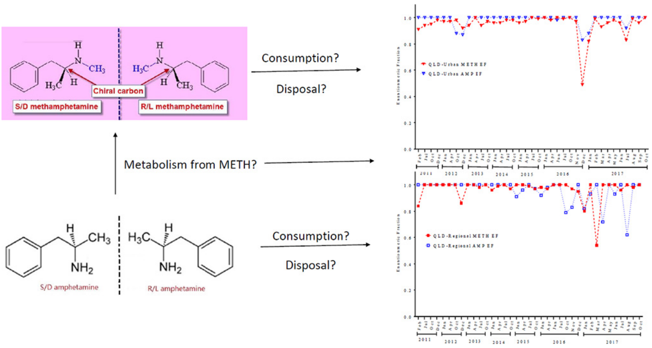
HIGHLIGHTS

- Seven years monitoring of enantiomeric amphetamine (AMP) and methamphetamine (METH)
- Chiral and metabolite ratios suggested AMP comes predominantly from METH consumption
- Amphetamine may have been used occasionally in Queensland recently.
- Comparable estimates of AMP consumption acquired using two different methods

GRAPHICAL ABSTRACT



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ABSTRACT

Enantiomeric profiling was used in this study to investigate the consumption of amphetamine and methamphetamine in regional and urban Southeast Queensland, Australia over a period of seven years. S(+) methamphetamine was predominantly consumed in both urban and regional areas, showing a two and three fold increase in urban and regional catchments respectively between 2011 and 2017. The ratio of amphetamine to methamphetamine (AMP/METH) in wastewater reflected the expected excretion profile of methamphetamine consumption indicating the presence of amphetamine in this study was primarily the result of methamphetamine metabolism. However, the occasional occurrence of R(−) amphetamine in samples containing higher AMP/METH ratios, suggested the consumption of racemic amphetamine. The R(−) methamphetamine enantiomer was also identified in several samples, possibly indicative that the phenyl-2-propanone (P2P) synthesis process rather than the more typical reduction of ephedrines was also being used to manufacture methamphetamine. Furthermore, we identified two samples with a significantly different enantiomer ratio for the METH and AMP as well as a much lower AMP/METH concentration ratio suggesting contribution from direct disposal of methamphetamine into the sewer. This study demonstrated that enantiomeric profiling in wastewater-based epidemiology can provide valuable information for evaluating the origin of amphetamine in wastewater as either a metabolite of methamphetamine consumption or amphetamine itself.

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1. Introduction

Amphetamine-type substance abuse is a major concern in society due to its negative impact on population health and social wellbeing (Degenhardt et al., 2017; NCAN, 2017). In Australia, methamphetamine use has been increasing dramatically over the past several years, as indicated by the Australian national survey (AIHW, A.I.o.H.a.W, 2011, 2014, 2017). Increasing consumption is also reflected by the elevated number of detections and total mass of methamphetamine seized (AFP, A.F.P, 2011, 2013, 2017). In addition, wastewater-based epidemiology (WBE) results have objectively confirmed such increases in specific wastewater catchments by measuring the methamphetamine load in the influent (ACIC, A.C.I.C, 2017; Lai et al., 2016a; Lai et al., 2011; Tscharke et al., 2016). Such information is useful for public health and law enforcement authorities to better focus the use of resources to targeted locations.

To monitor amphetamine consumption through WBE, the parent drug is often used as the target biomarker. This may bias the result as amphetamine is a drug of abuse that is excreted 30% as the unchanged parent, yet is also a metabolite of methamphetamine (Thomas et al., 2012; van Nuijs et al., 2011). Following the administration of methamphetamine, approximately 40% methamphetamine and 4–7% amphetamine is excreted (Fig. S1) (Cody, 2002). Enantiomeric profiling can help to resolve such bias as recent studies have shown that the additional resolution afforded by enantiomeric profiling of chiral drug residues in wastewater can provide greater insights into the drug market and allow the potential designation of the amphetamine source (Archer et al., 2018; Castrignanò et al., 2018; Xu et al., 2017). Both amphetamine and methamphetamine have one chiral centre, resulting in two enantiomers, S(+) and R(−). The illicit use of amphetamine and methamphetamine follows a different enantiomeric pattern; S(+) methamphetamine is the dominant abused enantiomer due to its stronger stimulant effects over R(−) enantiomer. Meanwhile, illicit amphetamine is usually racemic due to the most common clandestine synthesis of amphetamine, the Leuckart method, which produces racemic amphetamine. After metabolism, the enantiomeric profile of the drug remains the same, and hence metabolism of S(+) methamphetamine will only produce S(+) amphetamine, and usually, the S(+) enantiomer will metabolise faster than the R(−) enantiomer if administrated in racemic form (Cody and Schwarzhoff, 1993). Consequently, the enantiomeric profiling of urine samples has been used to differentiate between the licit and illicit use of amphetamine, depending if a racemic or pure S(+) amphetamine was detected (George and Braithwaite, 2000). Examples of enantiomeric profiling in WBE include: comparison of licit and illicit drug consumption (Kasprzyk-Hordern and Baker, 2012b); differentiating between the consumption and direct disposal of unused drugs (Emke et al., 2014; Petrie et al., 2016); understanding chiral drug consumption patterns (Vazquez-Roig et al., 2014); and investigating the geographical consumption behaviour of chiral drugs (Xu et al., 2017). Therefore, for amphetamine it is possible to distinguish between amphetamine residues in wastewater that are present due to the consumption of amphetamine, or due to the metabolism of methamphetamine (Xu et al., 2017).

The main objective of this study was to use enantiomeric profiling as a tool to examine if illicit amphetamine was consumed in urban and regional areas in South East Queensland, Australia over a seven-year period (2011–2017). Subsequent aims were to investigate the possibility of using enantiomer profiling and amphetamine/methamphetamine ratio to differentiate between the consumption and direct disposal of unused drugs; evaluate temporal chiral drug consumption patterns in two catchments with different urbanization level. In addition, factors that can contribute to the enantiomeric profile of amphetamine and methamphetamine in wastewater are discussed in detail.

2. Materials and methods

2.1. Chemicals and reagents

Racemic amphetamine, methamphetamine, amphetamine-d6, methamphetamine-d9 standards were purchased from Cerilliant (Texas, US). LC grade methanol was purchased from Merck (Germany). Acetic acid was purchased from Sigma-Aldrich (Germany). Ammonium hydroxide solution (28.0–30.0% NH_3 basis) was purchased from Sigma-Aldrich (USA). Analytical grade hydrochloric acid (32%) was purchased from Univar (Ingleburn, Australia). Deionized water was produced by a MilliQ system (Millipore, 0.22 μm filter, 18.2 mΩ.cm^1).

2.2. Sampling, sample preparation and chemical analysis

Twenty-four hour time-proportional (every 15 min) composite raw influent wastewater samples were collected using auto-sampler at two wastewater treatment plants (WWTPs) in Southeast Queensland, Australia; one in an urban (QLD-urban) and one in a regional (QLD-regional) area. From the years 2011 to 2015, one random sample per season were chosen from each site ($n = 20$), and from 2016 to 2017 one random sample from each month was selected ($n = 17$), for 37 samples from each WWTP. During sample collection, wastewater was refrigerated at 4 °C. After a 24-h cycle, the wastewater was aliquot into 500-mL HDPE bottles and adjusted to pH 2 with 2.0 M hydrochloric acid. The samples were frozen and transported to the laboratory to be stored at −20 °C until analysis. Both amphetamine and methamphetamine were observed to be stable in such storage condition over a period of 5 years as presented in Fig. S2. .

Samples were defrosted at room temperature and a 40 mL aliquot of each wastewater sample was filtered using 0.22 μm regenerated cellulose filters (Agilent). Then, 10 μL of a 5 mg/L deuterium-labelled internal standard mix was spiked into the samples and mixed before solid phase extraction. The Solid Phase Extraction (SPE) procedure followed validated methods described in Xu et al. (2017). Briefly, an Oasis HLB cartridge (60 mg, 3 mL, Waters, UK) was pre-conditioned with 2 mL methanol followed by 2 mL MilliQ water at pH 7.5 (adjusted using ammonium hydroxide). The wastewater samples were loaded onto the cartridges at a flow rate of 1–2 mL/min. Each cartridge was rinsed with 5:95 methanol/water and dried under vacuum. The cartridges were then eluted with 5 mL of methanol into solvent washed centrifuge tubes. The eluate was reduced under a gentle nitrogen stream at 40 °C and transferred to a 2 mL amber glass vial. Eluates were further reduced to dryness under a gentle stream of nitrogen. Samples were reconstituted into 200 μL of methanol and vortex mixed prior to analysis by LC-MS/MS.

Sample analysis was conducted using a liquid chromatography system (Prominence, Shimadzu, Japan) coupled to a tandem mass spectrometer (6500$^+$ QTrap, Sciex). A 5 μL injection volume was separated by a Chirobiotic V2 column (250 mm * 2.1 mm, 5 μm) (Sigma-Aldrich, UK) at 25 °C. The flow rate was set at 0.25 mL/min under isocratic conditions, where the mobile phase consisted of methanol, acetic acid and ammonium hydroxide (100:0.1:0.025, v:v:v). Details of the instrumental parameters are provided in Table S1. The method performance data is presented in Table S2. An example chromatogram of calibration standards and wastewater samples is provided in Fig. S3.

2.3. Consumption estimation and enantiomeric fraction

As both amphetamine and methamphetamine has low LogKow value (1.76 and 2.22), sorption to the solids is considered negligible. The consumption of drugs was back-calculated using the previously published method outlined in (Lai et al., 2011). Briefly, the concentration in the wastewater samples calculated by internal standard method ($C_{i,}$ mg/L) was multiplied by the daily flow (F, L) to get the daily mass