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Towards finding a population biomarker for wastewater epidemiology studies

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HIGHLIGHTS

- We suggested five criteria for choosing a population biomarker in wastewater.
- We evaluated seven potential population biomarkers.
- 5-Hydroxyindoleacetic acid (5-HIAA) and cotinine satisfy the criteria.

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ABSTRACT

Wastewater analysis has the potential to provide objective information on community drug use. Introducing a population biomarker (PB) in the sample analysis may significantly reduce errors in the back-calculation associated with population estimation and wastewater volume measurement. A number of potential PBs have been suggested but no systematic evaluation has been conducted so far. This study evaluated the eligibility of the previously suggested PB candidates (creatinine, cholesterol, coprostanol and cotinine) as well as three new ones (cortisol, androstenedione and 5-hydroxyindoleacetic acid (5-HIAA)) using five criteria. We assessed the quantification method, affinity to particulate matter and stability of candidates in wastewater, as well as the constancy of inter-day excretion and correlation between excretion and census population. All PB candidates were quantifiable in wastewater. Cholesterol and coprostanol were eliminated from further consideration due to affinity to particulate matters in the wastewater. Creatinine, cortisol and androstenedione were disqualified for stability reasons. On a population scale, both cotinine and 5-HIAA were excreted (RSD = 8.01 ± 1.13% and 10.20 ± 0.89%, respectively) at a constant rate and concentrations of each correlated well with the census population ($r = 0.9809$ and 0.9442, respectively). Overall, both cotinine and 5-HIAA are eligible PBs, but the neurotransmitter metabolite 5-HIAA may be more suitable for international comparisons.

1. Introduction

With the development of modern analytical chemistry, trace substances can be measured in wastewater samples as a means to gain information on the contributing population. One current application is the analysis of illicit drugs or their metabolites in the municipal wastewater as a direct measurement of drug use in the sampling area (Daughton, 2001). Initially, procedures of this approach were described by Zuccato et al. (2008), Eq. (1).

$$\text{Drug consumption per capita} = \frac{\text{DTR concentration} \times \text{Wastewater volume}}{\text{DTR excretion factor} \times \text{Population}}$$

Eq. (1). Calculation of drug prevalence using flow rate and population data, derived from Zuccato et al. (2008). DTR: drug target residue.

Since all parameters used in this approach are objective data, this method has the potential to provide reliable information on population drug use. Validation work has been conducted to optimise drug target residue (DTR) concentrations (e.g. Baker and Kasprzyk-Hordern, 2011a) and DTR excretion factors (e.g. Khan and Nicell, 2011; Khan and Nicell, 2012). However, little has been done so far in regards to the wastewater volume and population data. These data are potential sources of error in the current method (Lai et al., 2011), and the reliability of the final estimation can potentially be significantly improved if these two errors are reduced, or, if the use of these two parameters can be avoided.

A promising solution is the introduction of a population biomarker (PB), which is either an endogenous compound (Chiaia et al., 2008; Daughton, 2012) or an exogenous substance excreted by a large proportion of the population (Baker and Kasprzyk-Hordern, 2011b; Bisceglia,

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2010). By using a PB, drug use can be calculated in a way which does not require an accurate estimate of the population. DTR concentrations are normalised against the PB concentration in wastewater samples according to Eq. (2).

$$\text{Drug consumption per capita} = \frac{\text{DTR concentration}}{\text{PB concentration}} \times \frac{\text{PB excretion rate}}{\text{DTR excretion factor}}$$

Eq. (2). Proposed calculation of drug prevalence using a population biomarker. PB: population biomarker.

Based on our understanding, a PB should meet at least five criteria, namely: (1) be quantifiable; (2) have little affinity to particulate matter in wastewater or to filter paper; (3) be stable in wastewater; (4) be constantly excreted and (5) the total excretion should correlate with census population, meaning there should be no contribution other than human metabolism.

The first criterion indicates that the concentration of the PB in wastewater should be above the limit of quantification (LOQ) of the most appropriate analytical method currently available. The following factors serve to estimate the required concentration range of a potential PB in urine: (1) the LOQs of most analytes in recent reported wastewater studies are at or above the ng/L level (Castiglioni et al., 2008); (2) daily urine production is approximately 1 L; and (3) daily water use is normally several hundreds of litres per capita (SA Water, 2008). Hence, the minimum concentration of a PB in human urine should at least be in or above the μg/L range to ensure its quantification in wastewater.

Wastewater contains particulate matter which requires the filtration of samples prior to analysis. If a PB adsorbs to these particulates or to filter paper, the analytical recovery and accuracy of the method will be significantly reduced (Deo and Halden, 2010; Heidler and Halden, 2008). This could also impact on the total amount of a PB within a collected sample as the proportion of suspended particulate matter may be difficult to keep constant.

Stability is another important criterion when choosing a PB. For example, for a metropolitan wastewater treatment plant (WWTP) serving 500,000 people, it may take as long as 7 h for the wastewater to reach the sampling point (Reid et al., 2011). This lag time may increase to15 h for smaller WWTPs (Castiglioni et al., 2013). Additionally, the collected samples typically stay in the autosamplers at WWTPs for a maximum of 24 h if collected by a composite sampler. Hence, even if analyte concentration can be stabilised after collection, analyte loss in the sewage pipelines and the autosamplers at WWTPs is difficult to estimate if appreciable decomposition occurs within hours.

Since a PB should reflect the contributing population, its excretion from the population should theoretically be relatively constant over days when there is no population change. The total excretion should also correlate with census population, especially when a PB may infiltrate wastewater systems from agricultural activities (e.g. urea) or surface water contamination.

Chiaia et al. (2008) proposed creatinine as a potential PB. This compound is a breakdown substance of muscle tissues and is commonly used clinically to normalise concentrations of disease biomarkers and drugs (Biradar et al., 2011; Pesce et al., 2011). However, stability issues have been raised in relation to creatinine (Bisceglia, 2010), and these need to be resolved.

Daughton (2012) evaluated creatinine, cholesterol and coprostanol as potential PBs and suggested that coprostanol was the best choice. However, it was not fully assessed against the criteria put forward in this study and hence it is not known whether it is a suitable PB.

Some researchers have also suggested the metabolites of caffeine and nicotine as potential PBs (Baker and Kasprzyk-Hordern, 2011b; Bisceglia, 2010). Since nicotine is largely excreted as its metabolite cotinine, we chose cotinine as an example of an exogenously derived compound and evaluated its eligibility.

In this study we developed analytical methods for the previously suggested PB candidates creatinine, cholesterol, coprostanol and cotinine as well as three new ones, namely the stress hormone cortisol (Chang et al., 2007), the sex hormone precursor androstenedione (Chang et al., 2011) and the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) (Fig. 1). The five criteria set above were applied consecutively to each candidate with the aim of finding at least one PB that meets all of them. Substances failing to meet any of the criteria were eliminated from the candidate list and were not evaluated using subsequent criteria.

2. Methods

2.1. Chemicals and reagents

Creatinine, cholesterol, coprostanol, cortisol and 5-HIAA were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Creatinine-d_3 (methyl-d_3), 5-hydroxyindole-4,6,7-d_3-3-acetic-d_2 acid (5-HIAA-d_5), and 4-androsten-17α-ol-3-one-2,2,4,6,6-d_5 (androstenolone-d_5) were purchased from C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada). The above substances were received in powder form and were dissolved in appropriate solvents prior to use. Creatinine and its deuterated analogue were dissolved in 1:1 (v/v) water:ethanol. 5-HIAA and 5-HIAA-d_5 were dissolved in acetonitrile as it was found to be unstable in ethanol and methanol (data not shown). Cholesterol, coprostanol, cortisol and androstenolone-d_5 were dissolved in ethanol. Androstenedione was kindly provided as an ethanol solution by Dame Roma Mitchell Cancer Research Laboratories at the University of Adelaide. Cotinine and cotinine-d_3 were purchased from Cerilliant Corp. (Round Rock, TX) as certified methanol solutions at concentrations of 1 mg/mL and 0.1 mg/mL, respectively.

Methanol and formic acid from Merck Pty. Ltd. (Kilsyth, VIC, Australia), and distilled water prepared by a water still (Labglass Pty. Ltd., Brisbane, QLD, Australia) were used as the mobile phase for liquid chromatography/tandem mass spectrometry analysis (LC–MS/MS). Sodium metabisulphite ($Na_2S_2O_5$) and sodium chloride (NaCl) were of food grade. All other reagents were of analytical grade from Chem-Supply Pty. Ltd. (Gillman, SA, Australia).

2.2. Sample preparation

2.2.1. Large-volume injection (for creatinine)

Creatinine was analysed by large-volume injection (LVI) using the method reported previously with some modifications due to its hydrophilicity and high concentration in wastewater (Chiaia et al., 2008). Generally, wastewater samples filtered by glass microfibre filters (GF/A 1.6 μm, Whatman™, Kent, U.K.) were spiked with creatinine-d_3 to give a final concentration of 1 μg/L and further filtered with 0.2 μm 25 mm nylon syringe filters (Thermo Fisher, Waltham, MA) prior to LC–MS/MS analysis.

2.2.2. Liquid–liquid extraction (for cholesterol, coprostanol and 5-HIAA)

For the analysis of cholesterol and coprostanol, wastewater samples were first filtered using glass microfibre filters. The pH was then adjusted to 4.5 using 2.5% acetic acid. A 10 mL 9:1 (v:v) mixture of hexane:ethyl acetate was used in the extraction step for a 35 mL sample, and a 5 mL portion of the organic solvent was transferred and evaporated to dryness. The residue was reconstituted in 200 μL hexane and analysed by gas chromatography–mass spectrometry (GC–MS).

In the case of 5-HIAA, the 35 mL aliquot was spiked with 20 μg/L of 5-HIAA-d_5 prior to extraction. The pH was adjusted to 2 using hydrochloric acid and the solution was extracted once with 10 mL of ethyl acetate. The residue was reconstituted in 20 μL of acetonitrile followed by mixing with 180 μL of water prior to LC–MS/MS analysis.