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Liquid chromatography-tandem mass spectrometry determination of synthetic cathinones and phenethylamines in influent wastewater of eight European cities

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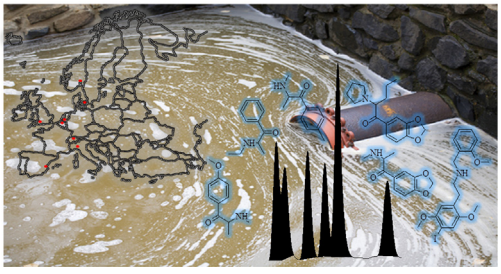
HIGHLIGHTS

- A sensitive UHPLC-MS/MS method for the determination of ten NPS in wastewater.
- In-sample stability was investigated at different pH and temperatures.
- Matrix effects were accounted for using isotopically-labelled internal standards.
- The fully validated methodology was applied to wastewater samples from around Europe.

GRAPHICAL ABSTRACT



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ABSTRACT

The popularity of new psychoactive substances (NPS) has grown in recent years, with certain NPS commonly and preferentially consumed even following the introduction of preventative legislation. With the objective to improve the knowledge on the use of NPS, a rapid and very sensitive method was developed for the determination of ten priority NPS (N-ethylcathinone, methylenedioxypyrovalerone (MDPV), methylone, butylone, methedrone, mephedrone, naphyrone, 25-C-NBOMe, 25-I-NBOMe and 25-B-NBOMe) in influent wastewater. Sample clean-up and pre-concentration was made by off-line solid phase extraction (SPE) with Oasis MCX cartridges. Isotopically labelled internal standards were used to correct for matrix effects and potential SPE losses. Following chromatographic separation on a C_{18}

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column within 6 min, the compounds were measured by tandem mass spectrometry in positive ionization mode. The method was optimised and validated for all compounds. Limits of quantification were evaluated by spiking influent wastewater samples at 1 or 5 ng/L. An investigation into the stability of these compounds in influent wastewater was also performed, showing that, following acidification at pH 2, all compounds were relatively stable for up to 7 days. The method was then applied to influent wastewater samples from eight European countries, in which mephedrone, methylone and MDPV were detected. This work reveals that although NPS use is not as extensive as for classic illicit drugs, the application of a highly sensitive analytical procedure makes their detection in wastewater possible. The developed analytical methodology forms the basis of a subsequent model-based back-calculation of abuse rate in urban areas (*i.e.* wastewater-based epidemiology).

1. Introduction

New psychoactive substances (NPS) are emerging narcotic or psychotropic drugs that are not controlled by legislation, but which may pose a public health threat. It must be noted that here, the term 'new' does not necessarily refer to new inventions but to substances that have recently become available (UNODC, 2014). The use of NPS has grown rapidly over the past decade and there have been increasing reports of the availability and manufacture of such substances, with the number of NPS reported globally more than doubling between 2009 and 2013 (UNODC, 2014). In 2014 alone, 101 NPS were for the first time reported to the EU Early Warning System (EMCDDA, 2015a). Given the nature of the NPS market, with developers limited only by their imagination and ability to sidestep legislation (Reid and Thomas, 2016) there is a sustained need to analyse the extent of the NPS problem.

The analysis of wastewater to estimate (illicit) drug consumption based on biomarkers, has traditionally focussed on the most common illicit drugs - cocaine, cannabis, amphetamine, methamphetamine and 3,4-methylenedioxymethamphetamine (ecstasy, MDMA) (Ort et al., 2014; Thomas et al., 2012), leaving a large information gap on other illicit drugs and NPS. Little research has been made on NPS and their suitable biomarkers, let alone their stability. In the few papers that have been published until now on the determination of NPS in wastewater, the target analytes included are commonly the synthetic cathinones mephedrone, methylone and MDPV (Baz-Lomba et al., 2016; Borova et al., 2015; Castrignanò et al., 2016; Chen et al., 2013; Kankaanpää et al., 2014; Kinyua et al., 2015a; Mwenesongole et al., 2013; Reid et al., 2014; Senta et al., 2015; van Nuijs et al., 2013). Within these studies, the most commonly detected NPS in wastewater are mephedrone and MDPV, generally found at the low ng/L range.

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is the technique of choice for the quantitative determination of illicit drugs in wastewater, due to the low concentrations involved and the high sensitivity of the instrument. In addition to the required validation at realistic concentrations that can be found in the samples, relevant issues must be considered, such as the way to correct/minimize matrix effects, and the proper identification of the compound detected. The use of isotopically-labelled internal standards (ILIS) is one of the most efficient tools to correct for matrix effects as well as potential losses from solid phase extraction (SPE). When utilising LC-MS/MS instruments in selected reaction monitoring (SRM) mode, at least two transitions should be incorporated in the method (one for quantification and the other(s) for confirmation). However, the specificity of the transitions should be taken into account, as non-specific transitions (such as the loss of water) can lead to false negatives due to the non-compliance of ion ratios (Pozo et al., 2006).

The purpose of this study was to develop and validate a sensitive LC-MS/MS method for the quantitative determination of a number of NPS of the synthetic cathinone and phenethylamine families: butylone, ethylone, methylone, naphyrone, methedrone, methylenedioxypyrovalerone (MDPV), mephedrone, 25-I-NBOMe, 25-C-NBOMe and 25-B-NBOMe. These compounds were selected on the basis of their frequent detection in analytical, forensic and toxicological studies (Borova et al., 2015; Chen et al., 2013; Elliott and Evans, 2014; Ibáñez et al., 2014; Kankaanpää et al., 2014; Kinyua et al., 2015a; Mwenesongole et al., 2013; Reid et al., 2014; Senta et al., 2015; Uralets et al., 2014) as well as reports from the EMCDDA (EMCDDA, 2015b) and UNODC (UNODC, 2014). The developed method, using Oasis MCX SPE cartridges for sample pre-treatment, followed by UHPLC-MS/MS measurement, has been applied to influent wastewater samples from around Europe, with an additional study on stability being made. Special emphasis is placed on the reliable confirmation of the NPS detected in water, with up to three SRM transitions being acquired, which, together with ion ratios, allowed simultaneous detection, quantification and confirmation of positive samples.

2. Experimental

2.1. Chemicals and materials

See Supporting Information for this section as well as the structures of all compounds (Fig. S1).

2.2. Samples

A number of different influent wastewater (IWW) samples were utilised in the development and validation of the present method: from Zurich, Switzerland; Copenhagen, Denmark and Castellon, Spain. The developed method was applied to IWW samples. The 24-h composite samples were taken in March 2015 from Zurich, Switzerland; Copenhagen, Denmark; Oslo, Norway; Castellon, Spain; Milan, Italy; Brussels, Belgium, Utrecht, The Netherlands and Bristol, United Kingdom. All samples were collected in high density polyethylene bottles, transported to Castellon and stored in the dark at −20 °C until pre-treatment.

2.3. Instrumentation

A Waters Acquity UHPLC system (Milford, MA, USA) was interfaced to a triple quadrupole mass spectrometer (Xevo TQS, Waters Micromass, Manchester, UK) equipped with Z-Wave devices and an electrospray ionization interface (ESI) operated in positive-ion mode. The chromatographic separation was performed using an Acquity UPLC BEH C_{18} column, 1.7 μm, 50 mm × 2.1 mm (i.d.) (Waters) at a flow rate of 0.3 mL min^{-1}. The mobile phases used were water with 5 mM ammonium acetate and 0.01% formic acid