Journal of Chromatography A, xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Multiresidual analysis of emerging amphetamine-like psychoactive substances in wastewater and river water

Ivan Senta, Ivona Krizman, Marijan Ahel, Senka Terzic*

Division for Marine and Environmental Research, Rudjer Boskovic Institute, Bijenicka 54, Zagreb 10000, Croatia

ARTICLE INFO

Article history:
Received 14 September 2015
Received in revised form
10 November 2015
Accepted 10 November 2015
Available online xxx

Keywords:
Novel psychoactive substances
Designer drugs
Illicit drugs
Amphetamine
Wastewater
Liquid chromatography-tandem mass spectrometry

ABSTRACT

Besides the common illicit drugs, such as cocaine, heroin, and marijuana, there is a growing concern about the use of modern "designer drugs" that have emerged in large numbers over the past few years. In this work, a sensitive and selective method for simultaneous determination of 25 synthetic amphetamine-like psychoactive compounds, including amphetamine, sympathomimetic substituted amphetamines, synthetic cathinones and ketamine, in raw wastewater (RW), secondary effluent (SE) and river water was developed. Samples were enriched by solid-phase extraction (SPE) on mixed-mode reversed-phase/strong cation-exchange sorbent (Oasis MCX) and analysed by reversed-phase liquid chromatography coupled to electrospray ionisation tandem mass spectrometry (LC-MS/MS). The target compounds were separated on a Synergi Polar column and detected using multiple reaction monitoring (MRM) in positive ionisation mode. Accurate quantification was achieved by using several deuterated analogues as surrogate standards. Careful optimisation and validation of the procedure resulted in a reliable determination of all target analytes in low ng/L range for all matrices, which makes the method suitable for the application in wastewater-based epidemiology. The method was applied for assessment of selected compounds in municipal wastewater and river water from Croatia. It was shown that most of the wastewater samples contained detectable levels of the well-known synthetic illicit drugs, amphetamine and 3,4-methylenedioxy-methamphetamine (MDMA) (concentrations up to 545 ng/L and 55 ng/L in RW, respectively), as well as ephedrine (up to 108 ng/L) and pseudoephedrine (up to 698 ng/L), which are used as ingredients of popular over-the counter cough and cold medications. Other target amphetamine-like psychoactive substances, recently reported for their potential abuse, were detected only occasionally and in low concentrations (<10 ng/L).

1. Introduction

Numerous substances, known as legal highs, designer drugs or novel psychoactive substances (NPS) have been released on the semi-legal drug market during the last decade [1]. Although "traditional" illicit drugs (e.g. cannabis, cocaine, heroin, ecstasy) still dominate the market, the pronounced increase in number and amount of versatile NPS, having largely unknown toxicological effects, is sufficient reason for alert.

Presently, a large proportion of all NPS belongs to different amphetamine-like substances, including numerous synthetic cathinones and sympathomimetic amines, which were designed to mimic the pharmacological effects of the original drugs [2]. Synthetic cathinones are derivatives of cathinone, an alkaloid naturally present in the leaves of khat plant (*Catha edulis*) [3]. Structurally, they are closely related to amphetamines (α-methylphenethylamines), with the only difference being the presence of a keto group on β-carbon [4]. They are produced by substitutions at three locations of the cathinone molecule: phenyl ring, amino group and propanone terminus. Many functional groups can be introduced to the molecule, forming a large family of structurally closely-related compounds [5], which, due to their strong amphetamine-like sympathomimetic effects, represent good candidates for new designer drugs. Some synthetic cathinones such as mephedrone, pentedrone and 2,4-methylenedioxypyrovalerone (MDPV) have been regularly reported on the illicit drug market in some European countries [1]. Moreover, it seems that a segment of traditional drug market, especially of *traditional* amphetamine type drugs, might be partially replaced with new designer drugs, as observed for MDMA and mephedrone [6]. Besides cathinones, some other substituted phenylalkylamines, including substituted amphetamines

* Corresponding author. Tel.: +385 1 4560 940; fax: +385 1 4680 242.
E-mail address: terzic@irb.hr (S. Terzic).

http://dx.doi.org/10.1016/j.chroma.2015.11.043
0021-9673/

and 2,5-dimethoxy phenethylamines, have also emerged [7]. Moreover, certain licit drugs, such as ketamine, have also been widely abused during recent years [2]. Obviously, there is an urgent need for improved analytical methodologies to systematically assess increasing importance of NPS as potential drugs of abuse.

The classical information sources on the drug abuse, which traditionally include medical records, population surveys and crime statistics, have been complemented in the past decade by an innovative approach based on the analysis of selected urinary drug biomarkers in the municipal wastewater (wastewater-based epidemiology–WBE), which was proven to be very useful in providing valuable information on current drug consumption at national [8–12] and international level [13,14]. However, until recently, WBE was focused mainly on several most frequently used illicit drugs (e.g. cocaine, cannabis, amphetamine, MDMA, heroin, metamphetamine), whose metabolic patterns in humans can be found in literature [15]. In contrast, the selection of the most suitable urinary biomarkers of different NPS, as well as a study of their stability in the sewage, still warrants further research.

The newest data on the metabolic patterns of selected phenethylamine-based designer drugs indicated rather slow metabolism rate of the studied compounds, with parent drugs being the key biomarkers of the drug consumption [16], which is in accordance with the metabolic patterns of classical amphetamine-type drugs (e.g. MDMA, amphetamine) [15]. Very recently, a couple of reports addressed the determination of new drugs of abuse in municipal wastewaters, but these included only a smaller number of representatives of synthetic cannabinoids and amphetamine-like drugs [17–19]. The fully validated methods suitable for the determination of a larger number of NPS in municipal wastewater are still missing.

Therefore, the aim of this work was to develop and validate a multiresidual method for the determination of trace levels of 25 synthetic drugs, mostly belonging to the groups of synthetic cathinones and other substituted phenylalkylamines, in aqueous samples, in order to provide a tool for the early reconaissance of the advent of these compounds in urban communities as a part of drug abuse mitigation activities, as well as to study their occurrence and fate in the aquatic environment. The stability of target analytes during sample collection, storage and preparation was also investigated and the method was applied for determination of target analytes in municipal wastewater and river water samples from Croatia, in order to assess their relative abundance, compared to some common illegal amphetamine drugs.

2. Experimental

2.1. Chemicals and materials

Reference standard solutions of all analytes (see Table 1 for the list and abbreviations and Table S1 in Supplementary material for structures, nomenclature and properties), including their deuterated analogues (used as surrogate standards) were purchased from Lipomed (Arlesheim, Switzerland) and Sigma–Aldrich (Germany), at concentration of 1 mg/mL (analytes) and 0.1 mg/mL (deuterated analogues), respectively. Working standard solutions, containing all analytes at 10, 1 and 0.1 μg/mL, were prepared by diluting the reference standard solutions with methanol. The mixture of deuterated surrogates (2 μg/mL) was also prepared in methanol. All solutions were kept in dark at −20 °C. LC-MS grade formic and acetic acid, ammonium formate, ammonium acetate, 85% phosphoric acid and 7N ammonia solution in methanol were purchased from Sigma–Aldrich (Germany). 25% ammonia solution in water was supplied by Merck (Darmstadt, Germany). HPLC grade methanol was purchased from VWR International (Vienna, Austria). Ultrapure water was produced using Elix-Milli-Q system (Millipore, Bedford, MA).

Solid-phase extraction (SPE) cartridges Oasis HLB (200 mg/6 mL) and Oasis MCX (150 mg/6 mL) were purchased from Waters (Milford, MA, USA). Glass-fiber filters (GF/D) were delivered by Whatman (USA).

2.2. Sample collection and preparation

All wastewater samples, including raw wastewater (RW) and secondary effluent (SE), for the method development and validation were collected at the central wastewater treatment plant (WWTP) of the city of Zagreb. Additional wastewater samples were collected at the WWTPs of the cities of Vinkovci and Velika Gorica. River water samples were collected at two locations along the Sava river, downstream of the main wastewater outfalls of the cities of Zagreb and Velika Gorica (Sava A and Sava B, respectively). Samples from WWTPs of the cities of Zagreb and Vinkovci were 24-h composite samples, while samples from WWTP of Velika Gorica and Sava river were grab samples. Sample volumes were 125 mL, 200 mL and 250 mL for RW, SE and river water, respectively. Before the filtration, surrogate standards (15 ng of each) were added and samples were equilibrated for at least 15 min. Samples were then filtered through glass-fiber filters to remove the suspended matter and pH was adjusted if necessary. Two types of SPE cartridges were tested during the method development: Oasis HLB and Oasis MCX. In the final procedure, samples were acidified to pH 2 using 85% phosporic acid and percolated (≤5 mL/min) through MCX cartridges pre-conditioned with 6 mL of methanol, 6 mL of ultrapure water and 6 mL of water acidified to pH 2. Before the elution, cartridges were washed with 6 mL of ultrapure water and 6 mL of methanol. If the samples were not analysed on the same day, cartridges were then wrapped up in aluminum foil and stored at −20 °C until analysis. Adsorbed analytes were eluted from the cartridges using 6 mL of 0.5% ammonia solution in methanol. The extracts, collected in silanized glass tubes, were then evaporated to approximately 100 μL under a nitrogen stream and dilluted to 500 μL with 0.1% formic acid in water.

2.3. LC-MS/MS analysis

Instrumental analysis was performed on a Thermo Electron TSQ AM (San Jose, CA, USA) LC-MS/MS system, consisted of HPLC pump and autosampler (Surveyor) interfaced to a triple-quadrupole mass spectrometer equipped with an electrospray ionisation source. Different HPLC columns, eluents and gradient programs were tested during the method development. Four chromatographic columns supplied by Phenomenex (Torrance, CA, USA) were tested, including Luna 2.5 μm C18(2)-HST column (50 mm × 2 mm) and three different types of Synergi 4 μm RP 80 Å columns, Polar, Fusion and Hydro (150 mm × 3 mm). In the final method, the target compounds were separated on the column Synergi Polar, using water and methanol, both acidified with 0.1% (*v*/*v*) of formic acid as eluent A and B, respectively. The following gradient program was applied: 0–20 min from 10% to 35% of eluent B, 20–30 min from 35% to 85% of B, 30–32 min from 85% to 95% of B, 32–34 min 95% of B (isocratic hold), 34–35 min from 95% to 5% of B, 35–45 min 10% of B (reconditioning to initial conditions). The injection volume was 15 μL.

The target compounds were ionised using electrospray ionisation in positive polarity. The MS source settings were as follows: capillary voltage 3500 V, capillary temperature 330 °C, desolvation gas (N_2) 10 arbitrary units, auxiliary gas (N_2) 40 arbitrary units. Identification and quantification of target compounds was done in multiple reaction monitoring (MRM) mode, using argon as a collision gas. Two most abundant precursor/product ion transitions

Please cite this article in press as: I. Senta, et al., Multiresidual analysis of emerging amphetamine-like psychoactive substances in wastewater and river water, J. Chromatogr. A (2015), http://dx.doi.org/10.1016/j.chroma.2015.11.043