Forensic Science International 274 (2017) 13–21

Contents lists available at ScienceDirect

Forensic Science International

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

Stability of synthetic cathinones in oral fluid samples

Briana Miller, Jiyoung Kim, Marta Concheiro*

Department of Sciences, John Jay College of Criminal Justice, City University of New York, New York, NY, USA

ARTICLE INFO

Article history:
Available online 1 December 2016

Keywords:
Synthetic cathinones
Stability
Oral fluid

ABSTRACT

Synthetic cathinones are new stimulant drugs derived from cathinone that have been sold as "legal highs" worldwide. These compounds can elicit powerful effects such as delusions, hallucinations as well as other potentially dangerous behavior. New analogs with varying effects and potencies are constantly introduced in the market to evade legislation, and they are not detected by routine screening and confirmation methods. Oral fluid is an alternative matrix of increasing interest in forensic toxicology. Its collection is non-invasive and easily supervised, and positive drug findings typically reflect recent drug exposure. The focus of this research was to develop a method for the determination of 10 synthetic cathinones (cathinone, methcathinone, buphedrone, mephedrone, 4-methylethcathinone, 3,4-methylenedioxypyrovalerone (MDPV), methylone, naphyrone, alpha-pyrrolidinovalerophenone (PVP) and *N*-ethylcathinone) in preserved oral fluid (Quantisal™), as well as evaluate their stability in preserved (Quantisal and Oral-Eze™) and neat oral fluid samples stored under different conditions, using ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC–MSMS). Four-hundred microliters oral fluid-Quantisal buffer mixture (100 μL oral fluid and 300 μL buffer) were subjected to cation exchange solid phase extraction. The chromatographic reverse-phase separation was achieved with a gradient mobile phase of 0.1% formic acid in water and in acetonitrile in 5 min. We used a Shimadzu triple quadrupole mass spectrometer in multiple reaction monitoring (MRM) mode. The assay was linear from 1 to 250 ng/mL, with the limits of detection of 0.75–1 ng/mL. Imprecision (n = 15) was <20.7% and accuracy (n = 15) was 84–115.3%. Extraction efficiency was 87.2–116.8% (n = 6), process efficiency was 30.9–103.7% (n = 6), and matrix effect was −65.1 to −6.2% (CV 2.5–15.1%, n = 6). The stability was performed for neat oral fluid, oral fluid in Quantisal buffer, and oral fluid in Oral-Eze buffer samples stored up to one month at room temperature, 4 °C and −20 °C, and after 3 freeze–thaw cycles. Losses up to −71.2 to −100% were observed in neat and preserved samples stored at room temperature up to one month. At 4 °C, losses up to −88.2% occurred in neat OF and Oral-Eze samples, while Quantisal samples showed losses up to −34%. All types samples were stable if stored at −20 °C and after 3 freeze–thaw cycles.

1. Introduction

Synthetic cathinones are novel psychoactive substances (NPS) that can elicit powerful effects such as delusions, hallucinations and potentially dangerous behavior [1]. Since the mid-2000s, synthetic cathinones gained popularity in the recreational drug market worldwide because of their unregulated status, low cost and ready accessibility via the Internet and head shops [2]. They are advertised as "legal highs" and sold as "bath salts" or "plant food", and are labeled as "not for human consumption" to avoid drug abuse legislation [3]. Constantly new synthetic cathinones are synthesized to circumvent existing laws on controlled substances, and/or to enhance pharmacological activity.

Synthetic cathinones are derivatives of cathinone, a naturally occurring beta-ketone amphetamine analog found in the leaves of the *Catha edulis* plant. Synthetic cathinones are phenylalkylamines derivatives, and are often termed "bk-amphetamines" for the beta-ketone component [4]. The main cathinone derivative classes are position 3′-substituted (buphedrone), ring-substituted (mephedrone), *N*-alkyl-substituted (ethylcathinone), methylenedioxy-substituted (methylone), and pyrrolidinyl-substituted (3′,4′-methylenedioxypyrovalerone (MDPV)). These derivative classes are illustrated in Fig. 1.

Synthetic cathinone pharmacological effects may be similar to those of cocaine, amphetamine or (±)-3,4-methylenedioxy

* Corresponding author at: Department of Sciences, John Jay College of Criminal Justice, City University of New York, 524 West 59th Street, Rm 5.66.02, New York, NY 10019, USA.
E-mail address: mconcheiro-guisan@jjay.cuny.edu (M. Concheiro).

http://dx.doi.org/10.1016/j.forsciint.2016.11.034
0379-0738/

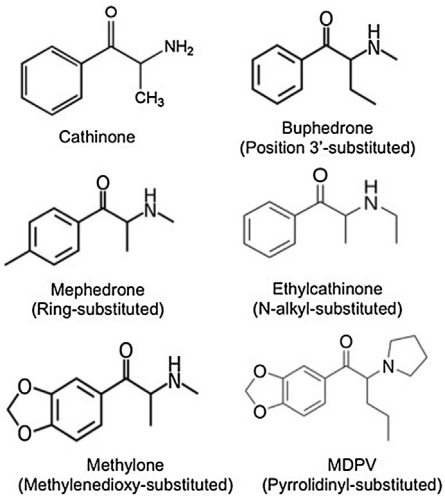

Fig. 1. Chemical structure of cathinone and synthetic cathinone derivatives buphedrone, mephedrone, ethylcathinone, methylone and MDPV.

methamphetamine (MDMA), depending upon the class [3]. Desired effects reported by users of synthetic cathinones were increased energy, empathy, openness, and increased libido. Cardiac, psychiatric, and neurological signs and symptoms are the most common adverse effects reported in synthetic cathinone users who require medical care [4].

Currently, bupropion is the only cathinone derivative that carries a medical indication in the US and Europe [4]. It is prescribed for the treatment of depression and as a smoking-cessation aid. Only cathinone and methcathinone were listed as Schedule I drugs, with diethylcathinone and pyrovalerone as Schedule IV of the United Nations 1971 Convention on Psychotropic Substances. As a consequence of synthetic cathinones' abuse potential, mephedrone, MDPV and methylone were permanently controlled as Schedule I drugs in the United States Controlled Substances Act in 2013 [3]. Ten additional cathinones were temporarily scheduled as class I drugs in 2014, 4-methylethcathinone (4-MEC), 4-methyl-α-pyrrolidinopropiophenone (4-MPPP), α-pyrrolidinopentiophenone (α-PVP), butylone, pentedrone, pentylone, 4-fluoromethcathinone (4-FMC), 3-fluoromethcathinone (3-FMC), naphyrone, and α-pyrrolidinobutiophenone (α-PBP) in 2014 [5], and extended for another year in 2016 [6]. Although many other cathinone derivatives are not yet under international control, restrictive legislation has been introduced in multiple countries.

Oral fluid is an alternative matrix that has increasing interest in forensic and clinical toxicology. Its collection is non-invasive and easily supervised, and its window of detection may be similar to blood indicating recent drug exposure [7]. However, the use of oral fluid may pose analytical challenges because the sample volume is low (<1 mL), drug concentrations are much lower (low ng/mL) than in urine (ng/mL and μg/mL) and salivation may be reduced after the intake of drugs with sympathomimetic properties [7].

There are different devices available for oral fluid collection. The general procedure consists of a swab or pad that is inserted into the mouth to draw the oral fluid. The swab or pad is then placed into a vial that contains a buffer to preserve the sample [1]. Examples of the most common commercially available oral fluid devices are Quantisal™ (Immunalysis Corp., Pomona, CA, USA) and Oral-Eze® (Capitol Vial, Inc., Auburn, AL, USA). These devices employ different buffers to improve the stability of the compounds in oral fluid samples and to avoid bacterial growth.

Several articles described analytical methods for the determination of synthetic cathinones in urine and blood/plasma; [8] however, only two confirmation methods have been published in oral fluid [1,9]. Amaratunga et al. [1] developed a method for the determination of 10 synthetic cathinones in 400 μL of oral fluid-Quantisal buffer mix, achieving a limit of quantification of 1 ng/mL. De Castro et al. [9] developed a method for the determination of 5 synthetic cathinones in 500 μL of neat oral fluid, achieving a limit of quantification of 0.2 ng/mL. Both methods were developed by liquid chromatography tandem mass spectrometry (LC–MSMS).

Information about stability of drugs in biological samples is critical for accurate interpretation of analytical results. Many times biological specimens cannot be assayed immediately after collection due to laboratory workload, instrumentation downtime, shipment delay, or if a second analysis or a counter-test is requested after some time. This delay in the analysis can be problematic if the analytes are not stable in the biological samples. Although synthetic cathinones stability is compromised in blood and urine [3,10–15], few data are available in oral fluid [9]. De Castro et al. [9] showed that cathinones were stable in neat oral fluid and in Quantisal buffer samples at 4 °C for 24 h and after 3 freeze–thaw cycles. Long-term information (>24 h) or stability data in other collection buffers is not currently available.

We developed a method for the determination of 10 synthetic cathinones in preserved oral fluid (Quantisal) by ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC–MSMS) to evaluate synthetic cathinones' stability in preserved (Quantisal and Oral-Eze) and in neat oral fluid fortified samples stored under different conditions (room temperature, 4 °C and −20 °C) from 24 h to one month and after 3 freeze–thaw cycles.

2. Methods and materials

2.1. Chemicals and materials

Cathinone, methcathinone, methylone, *N*-ethylcathinone, buphedrone, mephedrone, 4-methylethcathinone, α-pyrolidinovarophenone (PVP), MDPV, and naphyrone (1 mg/mL), and internal standards MDPV-d_8, mephedrone-d_3, methylone-d_3, and naphyrone-d_5 (100 μg/mL) were obtained from Cerilliant (Round Rock, TX, USA). Solid phase extraction (SPE) cation exchange cartridges Strata Drug-X B 60 mg/3 mL were from Phenomenex (Torrance, CA, USA). Glacial acetic acid, acetonitrile, ammonium hydroxide, and formic acid were acquired from Pharmco-Aaper (Shelbyville, KY, USA). Methanol, dichloromethane, and isopropanol were acquired from Fisher Scientific (Pittsburgh, PA, USA). All solvents used in the extraction were high performance liquid chromatography (HPLC) grade and in the chromatographic instrument were liquid chromatography–mass spectrometry (LC–MS) grade. Quantisal buffer was obtained from Immunalysis Corp. (Pomona, CA, USA) and Oral-Eze buffer from Capitol Vial, Inc. (Auburn, AL, USA). Neat drug-free OF was obtained from healthy volunteers by spitting into a Corning® polypropylene 50 mL tube (Fisher Scientific).

2.2. Instrumentation

Ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC–MSMS) instrument was from Shimadzu (Columbia, MD, USA). The Nexera UHPLC system consisted of a binary LC-20ADXR HPLC pump, Nexera LC-30AD micro mixer, online degassing unit DGU-20A3R and cooled autosampler SIL-20SCHT UFLC. The mass spectrometer was a triple quadrupole LC–