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Stability of Synthetic Cathinones in Oral Fluid Samples

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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 µL 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 1-250 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.

Keywords: synthetic cathinones; stability; oral fluid

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 analogue 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’-substitiuted (buphedrone), ring-substituted (mephedrone), N-alkyl-substituted (ethylcathinone), methylenedioxy-substituted (methylone), and pyrrolidinyl-substituted (3’,4’-methylenedioxypyrovalerone (MDPV)). These derivative classes are illustrated in Figure 1.

Synthetic cathinone pharmacological effects may be similar to those of cocaine, amphetamine or (±)-3,4-methylenedioxymethamphetamine (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 (<1mL), 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 anlaysis 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 (> 24h) 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.

Methods and Materials

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).

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-MS 8030 equipped with Dual Ionization Source (DUIS). SPE was performed using a negative pressure manifold from Fisher Scientific. Evaporation under nitrogen was completed using TurboVap LV from Biotage (Charlotte, NC, USA).

Preparation of Standard Solutions

Calibrators' working solutions at 4, 10, 20, 100, 200, 400 and 1000 ng/mL were prepared by appropriate dilution in methanol. Controls' working solutions at 12 ng/mL (low) and 600 ng/mL (high) were prepared in methanol. The combined internal standard solution (MDPV-d_8, mephedrone-d_3, methylone-d_3, and naphyrone-d_5) at 100 ng/mL was prepared in methanol. All solutions were stored in amber vials at -20°C.

Preparation of calibrators and controls samples

The calibrators were prepared spiking the calibrators' working solutions in drug-free OF-buffer mixtures (0.1 mL OF and 0.3 mL Quantisal buffer).
The controls were prepared spiking the controls' working solutions in drug-free OF-buffer mixtures (0.1 mL OF and 0.3 mL Quantisal buffer; 0.1 mL OF and 0.2 mL Oral-Eze buffer) and 0.1 mL neat OF.

Quantisal and Oral-Eze buffer volumes were selected based on the OF/Buffer ratio of the collection devices. Quantisal devices collect 1 mL oral fluid and contain 3 mL of buffer (1/3, OF/Buffer ratio). Oral-Eze devices also collect 1 mL of oral fluid, but contain 2 mL of buffer (1/2, OF/Buffer ratio).

Stability study design

Two pools of stability samples at low (2.5 ng/mL) and high (150 ng/mL) concentrations were prepared in neat OF, OF-Quantisal and OF-Oral-Eze mixtures. Each pool was prepared using a total of 5 mL of OF from 5 different donors (1 mL each). 5 mL of blank OF, 5 mL of OF and 15 mL Quantisal buffer, and 5 mL of OF and 10 mL Oral-Eze buffer were fortified with 12.5 µL of a standards mixture at 1 µg/mL (low concentration) or with 75 µL of a standards mixture at 10 µg/mL (high concentration). The different pools were vortexed for 1 min and split in 18 aliquots (9 conditions by duplicate) in 2 mL polypropylene cryotubes (Fisher Scientific) and stored in the dark at room temperature, 4°C and -20°C for 24 h, one week and one month. A group of aliquots was submitted to 3 freeze and thaw cycles. The pH of each pool was measured by pH indicator strips colorpHast® pH 0-14 from EM Science (Darmstadt, Germany).

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Oral fluid analysis

Two mL of 1M acetic acid and 25 uL of internal standard solution at 100 ng/mL were combined with 0.4 mL OF-Quantisal, 0.3 mL OF-Oral-Eze, or 0.1 mL neat OF, and gently vortexed. The samples were then loaded onto Strata Drug-X B cartridges that were conditioned with 2 mL methanol and 2 mL deionized water. The cartridges were washed first with 2 mL of 1M acetic acid, then with 2 mL methanol. The cartridges were dried under vacuum for 10 min. The elution was done by 2 mL of 2% ammonium hydroxide in dichloromethane:isopropanol (95:5, v/v). Before evaporation, 100 µL of 1% HCl in methanol was added to prevent synthetic cathinones' loss. Evaporation was performed under a stream of nitrogen at 40˚C in a TurboVap LV evaporator. The dried samples were reconstituted with 100 µL of 0.1% formic acid in water and vortexed briefly. The samples were transferred to screw top auto sampler vials, which contained glass inserts.

Liquid Chromatography

Chromatographic separation was accomplished using a Kinetex C18 100x2.1 mm 1.7µm column (Phenomenex, Torrance, CA, USA) and a gradient method. The gradient method employed mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile) at a flow rate of 0.4 mL/min at 35˚C. The initial composition of mobile phase B was 2% and was held for 2 minutes, and then it was increased from 2 to 95% over 8 minutes, it was held at 95% for one minute, and returned to initial composition over 3 minutes.

Mass Spectrometry

The triple quadrupole mass spectrometer (LCMS-8030) was equipped with Dual Ionization Source (DUIS) and operated in the positive ionization mode. The spray voltage was 4.5 kV, corona pin voltage was 4.5 kV, desolvation line (DL)

temperature 250 °C, heat block temperature 400 °C, nebulizing gas flow rate 2 L/min, and dry gas flow rate 15 L/min. Each compound was monitored by 2 transitions in multiple reaction monitoring mode (MRM). These parameters are summarized in Table 1.

Method Validation

The method was validated in OF-Quantisal samples according to SWGTOX guidelines [16]. The studied parameters were linearity, limits of detection (LOD) and quantification (LOQ), imprecision, accuracy, extraction efficiency, process efficiency, matrix effect, carryover, dilution integrity, and autosampler stability. Linearity was determined in five different days by least-squares regression with $1/x^2$ weighting. The acceptable linearity was accomplished when the r^2 (coefficient of determination) was ≥ 0.99 and the residuals were within ±20%. The LOD and LOQ were evaluated with decreasing analyte concentrations in drug fortified preserved OF from 3 different sources. The LOD was the lowest concentration with acceptable chromatography, signal-to-noise ratio > 3, presence of all product ions, correct ion ratio, and a retention time within ±0.2 minutes of the calibrator retention time. The limit of quantification satisfied the LOD criteria and was quantified within ±20% imprecision and 80-120% accuracy.

The imprecision and accuracy was determined at two concentrations, 3 ng/mL (low QC) and 150 ng/mL (high QC) in triplicate on five different days (n=15). Imprecision was determined via coefficient of variation (%CV) of the measured values and expected to be less than 20%. The pooled intra-day was determined by the highest %CV among the five different days (n=15). The total imprecision was determined by the average of the %CV over the five days (n=15). The accuracy was determined via the percentage of the target concentration (n= 15) and was required to be within 80-120%.

For extraction efficiency at each QC concentration (low and high) blank samples were fortified with the appropriate QC solution before and after sample extraction. The extraction efficiency was expressed as a percentage of the mean analyte area of samples fortified before sample extraction (n = 6) divided by mean area of samples fortified after sample extraction (n = 6). To determine matrix effect we compared analyte peak areas in blank extracted samples fortified with the appropriate QC solutions after sample extraction (n=6) to peak areas of neat samples at the same concentrations (n=3). The neat samples were prepared by fortifying QC working solutions into 100 µL 1% HCl in methanol. The neat samples were then evaporated and reconstituted in 100 µL 0.1% formic acid in water. The calculation for determining the matrix effect was (100 × mean peak area of fortified samples after extraction/mean peak area of neat samples) -100. Process efficiency, overall effect of extraction efficiency and matrix effect on quantification of analytes, was expressed as a percentage, which was determined by the mean analyte peak areas of sample fortified before extraction (n=6) divided by the mean peak areas of neat samples of the same concentration (n=3).

To determine carryover, internal standard fortified blank samples (negative calibrator) were injected immediately after samples spiked at 250 ng/mL, the highest calibrator concentration. The carryover was considered negligible if the measured concentration was less than the LOD. The dilution integrity was determined by diluting 500 ng/mL samples to 50 ng/mL, a 1:10 dilution, in duplicate. Dilution was performed with blank OF-Quantisal mixture. The dilution integrity was maintained if the samples quantified within ±20% of 50 ng/mL. Autosampler stability was evaluated reinjecting low and high QC extracts stored 24 h at 10°C in the autosampler. Extracts were stable if quantified within 20% of the QC target value.

Identification Criteria

The identification criteria included retention time in the range of ±0.2 minutes of the calibrator retention time, the presence of two product ions (quantitative and qualitative), and ion ratio between the qualifier and quantifying ion in the range of ±20% from the calibrators average.

Stability Study

The stability aliquots of neat OF, OF-Quantisal and OF_Oral-Eze were stored at room temperature (24 h, 1 week and 1 month), at 4°C (1 week and 1 month), at -20°C (1 week and 1 month), and submitted to 3 freeze-thaw samples. On the day of analysis, internal standard solution was added and samples were analyzed as described above. Each sample was analyzed in duplicate. The % difference was calculated comparing the mean concentration of the fresh QCs (n=2) and the mean concentration of the corresponding QC after a determined storage condition (n=2). Stability was considered acceptable if QC samples quantified within ±20 % of the freshly prepared pool samples on Day 0 (fresh QCs).

Neat OF and OF-Oral-Eze samples were quantified using a OF-Quantisal calibrators and matched-matrix (neat OF or OF-Oral-Eze) low and high QCs. Neat OF and OF-Oral-Eze QCs had to quantified within ±20% of the target value.

Results

Method Validation

The linearity was verified in OF-Quantisal samples from 1 to 250 ng/mL with a $1/x^2$ weighted linear regression in five different days. The determination coefficients (r^2) for the set of five calibration curves were 0.99, except for cathinone, methcathinone, and PVP, which was 0.98. All analytes showed residuals within ±20 %. These results are summarized in Table 2. The LOD was 0.75 ng/mL for cathinone, methcathinone, buphedrone, MEC, and PVP, and 1 ng/mL for methylone, N-ethylcathinone, mephedrone, MDPV, and naphyrone. The LOQ was 1 ng/mL for all analytes (Figure 2).

The imprecision was <20.7% and the accuracy was 84.4–115.3%. The extraction efficiencies were from 87.2 to 114.8%, and process efficiencies from 30.9 to 103.7%. The matrix effects was from -63.8- to -6.2%, with its variation being <15.1% (n=6). These results are summarized in Tables 3 and 4.

No carryover detected after the injection of a sample at 250 ng/mL. The processed samples were stable in the autosampler at 10°C 24 h. The dilution integrity was maintained after 1:10 dilution of 500 ng/mL OF-Quantisal samples.

Stability Study

Synthetic cathinones were not stable in neat oral fluid at room temperature 24 h (% difference up to -60.8% at 2.5 ng/mL, -28.8% at 150 ng/mL), one week (up to -84.3% at 2.5 ng/mL, -85.2% at 150 ng/mL) and one month (up to -100% at 2.5 ng/mL, -99.6% at 150 ng/mL). Only MDPV was stable under these conditions (-3.9 – 18.6% difference). The most unstable cathinones (total loss after one month) were cathinone, methcathinone, N-ethylcathinone and naphyrone. At 4°C, most of synthetic cathinones were stable in neat oral fluid for one week, except cathinone, methcathinone and naphyrone (-31.9 - -21.7% loss); however, most of them were not stable after a month under these conditions (%loss up to -88.2%), except PVP and MDPV (-13.4 – 18.6% difference). All cathinones were stable in neat OF at -20°C and after 3 freeze-thaw cycles, except naphyrone at 2.5 ng/mL (-31.2 - -29.6% loss).

OF samples preserved in Quantisal buffer were stable at room temperature for 24 h. After one week, cathinone, methcathinone and naphyrone experimented losses up to -37.8% at 2.5 ng/mL, and after one month losses from -21.2 to -71.2% were observed for all synthetic cathinones, except for methylone, PVP and MDPV. All synthetic cathinones were stable in Quantisal buffer at 4°C and -20°C up to one month, except N-ethylcathinone, buphedrone and naphyrone at the low concentration 2.5 ng/mL (-34 - -21.6% loss).

In Oral-Eze buffer samples, all synthetic cathinones were stable at room temperature for 24 h, except cathinone at 2.5 ng/mL (-31.3% loss). Cathinone, methcathinone, N-ethylcathinone and naphyrone were not stable after one week (% loss up to -58.3%). Only MPDV was stable after one month stored at this temperature, while the other cathinones % losses were from -29 to -100%. All analytes, except cathinone and naphyrone at low concentration 2.5 ng/mL (-29.7 - -22.2%), were stable in Oral-Eze at -20°C up to one month and after 3 freeze-thaw cycles. These results are summarized in Tables 5-7.

As indicated in the methods and materials section, the % difference was calculated comparing the mean concentration of fresh QCs (n=2) and the mean concentrations of the stored QCs (n=2). Method's inaccuracy was not taken into consideration for these calculations. Stability data were recalculated taking into account the method's analytical inaccuracy (±15%) in the determination of the fresh and stored QC. A maximum and a minimum % difference was calculated. This information is showed in the supplemental Tables 1-3.

Discussion

A validated method was developed for the determination of 10 synthetic cathinones (cathinone, buphedrone, 4- MEC, MDPV, mephedrone, methcathinone, methylone, naphyrone, N-ethylcathinone and PVP) in 0.4 mL preserved OF-Quantisal samples (0.1 mL neat OF) by UHPLC-MSMS achieving 1 ng/mL LOQ. The method was applied to study synthetic cathinone stability in fortified neat and preserved Quantisal and Oral-Eze OF samples stored at room temperature, 4°C and -20°C up to one month, and after 3 freeze/thaw cycles.

Although the popularity of synthetic cathinones is increasing [17], only two OF confirmation methods for synthetic cathinones have been published [1, 9]. Amaratunga et al. [1] developed a method for the determination of 10 synthetic cathinones (butylone, ethylone flephedrone, MDPV, mephedrone, methcathinone, methedrone, methylone, PVP and pyrovalerone) in 0.4 mL of oral fluid-Quantisal buffer mix (0.1 mL neat OF), achieving a LOQ of 1ng/mL, similar to our method. The method was rapid; the SPE procedure did not involved drying steps, and the chromatographic run was 3 min [1]. However, the extraction efficiency of lipophilic cathinones such as MDPV, pyrovalerone and PVP, was low (around 50 %), and lower than in the present method (106.1-114.1%). De Castro et al. [9] developed a method for the determination of 5 synthetic cathinones (flephedrone, mephedrone, methylone, pethedrone and MDPV) in 0.5 mL of neat oral fluid, achieving a LOQ of 0.2 ng/mL. The present method allowed the simultaneous confirmation of 10 synthetic cathinones in 0.4 mL of preserved OF (0.1 mL neat OF) with 1 ng/mL LOQ. Although De Castro et al. [9] achieved a lower LOQ (0.2 ng/mL vs. 1 ng/mL), the amount of sample required was five times the amount employed in our method. In OF analysis, the amount of sample is limited (normally less than 1 mL). Taking into account the necessity of performing different types of test in one sample and that the sample re-analysis may be required, the analytical methods should employed the least amount of sample as possible that allows a sensitive test. With regard to actual concentrations of synthetic cathinones in OF, Amaratunga et al. [1] and De Castro et al. [9] applied their methods to authentic oral fluid specimens. Both authors reported positive cases for MDPV and PVP with concentrations above 20 ng/mL. The sensitivity achieved in the present method (LOQ 1 ng/mL) satisfies the required sensitivity in these authentic cases. During method development, different extraction procedures were evaluated (supported liquid extraction, reverse phase SPE, mixed mode SPE, cation exchange SPE and dilute-and-shoot). Among all of them, cation exchange SPE yielded the best results in terms of extraction efficiency, matrix effect and noise level.

Limited data about NPS stability in biological samples are available. This type of data is critical to perform a correct interpretation of analytical results. Often there is a delay between sample collection and analysis, and the best storage conditions have to be applied until analysis could be performed. Stability of synthetic cathinones in urine [3, 10, 11] and in blood/plasma [10, 12-15] has been reported; however, OF data is scarce [9].

MDPV was reported to be stable in blood at room temperature, 4°C and -20°C for up to 14 days [10]. Mephedrone, 4-MEC, cathinone, methcathinone, ethcathinone and flephedrone showed stability problems in blood samples with losses up to 100% [10, 12, 13, 15]. This stability issues were improved if the samples were preserved under acidic conditions with NaF/citrate buffer at pH 5.9 [13] or with NaF/potassium oxalate and at -20°C [15]. As recently reviewed by Ellefsen et al. [8], other synthetic cathinones were reported to be stable in blood [18, 19], and serum [20] at varying storage conditions (from 24 h to 6 weeks, from room temperature to -20°C). In urine, synthetic cathinones showed stability problems mainly at room temperature for 14 days [10] or in just 24 h [3]. MPDV was reported to be stable under these conditions [3, 10]. Al-Saffar et al. [11] reported losses > 40% at -20°C for 3 months for buphedrone, 3-FMC, 4-FMC, and pentylone. In OF few data are available [9]. De Castro et al. [9] reported that flephedrone, mephedrone, methylone, pethedrone and MDPV were stable in neat OF at 4°C for 24 h, and in neat and preserved Quantisal OF after 3 freeze/thaw cycles. Long term stability (> 24h) and stability in other preserved OF samples (Oral-Eze) is not currently available.

Based on these reported data, synthetic cathinones stability on biological samples depends on the pH of the sample, the storage temperature and the chemical structure of the cathinone. Differences on the benzene ring, nitrogen and phenethylamine backbone [13, 14] may play a role. Tsujika et al. [14] showed that the groups attached at the nitrogen atom affect cathinones stability, reporting that tertiary amines are more stable. With regard to the ring, methylenedioxy-substituted cathinones were more stable probably because did not readily reduce to their corresponding alcohols [21, 22], potentially due to structural affinity differences to reductive enzymes [22].

We investigated the stability of 10 synthetic cathinones with different chemical structures (position-3'-substituted, N-alkyl-substituted, ring-substituted, methylendioxy-substituted and pyrrolidinyl-substituted) in neat OF and preserved OF in 2 different buffers (Quantisal and Oral-Eze) stored at 3 temperatures (room temperature, 4°C and -20°C) for up to one month and after 3 freeze/thaw cycles. In the present study the more stable group was the pyrrolindinyl-substituted (tertiary amines), except naphyrone maybe due to the naphthalene group. The only synthetic cathinone that was stable in all samples under all storage conditions was MPDV, a methylenedioxy and pyrrolindinyl-substittuted derivative. MDPV also showed to be stable in blood [10] and urine [3, 10] under different storage conditions.

Synthetic cathinones showed more stability problems in neat samples, followed by Oral-Eze, and they were more stable in Quantisal at the different storage conditions. The pH of the different pools was tested throughout the experiment. Neat OF pools had a pH of 8, Oral-Eze pH 7 and Quantisal pH 6. Our data agrees with previous observations in other biological samples, that acidic conditions may improve cathinones stability [14].

The main limitation of the study is that no authentic samples could not be analyzed. The most important analyte losses happened at room temperature, being -20°C the most stable temperature for the 3 types of samples. The

cathinones that showed to be more unstable were cathinone and methcathinone, but all the groups were significally affected specially at room temperature for all types of samples, and in neat OF samples at 4°C too. Only MPDV showed good stability under all the storage conditions in any type of samples. These data showed that preservation buffer employed and the storage temperature should be taken into account to guarantee the stability of the synthetic cathinones in oral fluid samples.

Conclusion

We developed a sensitive and selective UHPLC-MSMS method for the simultaneous determination of 10 synthetic cathinones in preserved OF-Quantisal samples. We investigated the stability of these drugs in neat OF and preserved OF (Quantisal and Oral-Eze) samples stored at 3 temperatures (room temperature, 4°C and -20°C) for up to one month. Total losses were observed at room temperature in neat and Oral-Eze samples for some analytes. All compounds were stable in the 3 types of samples at -20°C. These data add important information about NPS stability in OF samples.

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Table 1. LC-MSMS parameters for 10 synthetic cathinones; multiple reaction monitoring (MRM) transitions, retention times, and internal standards. The quantifier transition in underlined.

Analyte	Precursor Ion (*m/z*)	Collision Energy (V)	Retention Time (min)	Internal Standard
Cathinone	<u>150>116.95</u> 150>131.95	-26	2.0	Methylone-d_3
Methcathinone	<u>164>131</u> 164>104.85	-24	2.1	Methylone-d_3
N-ethylcathinone	<u>178>105.05</u> 178>117.25	-22	2.3	Methylone-d_3
Methylone	<u>208>159.95</u> 208>132.05	-19	2.3	Methylone-d_3
Methylone-d_3	<u>211>163.1</u> 211>135.05	-37	2.3	
Buphedrone	<u>178>131.9</u> 178>77.2	-14	2.5	Mephedrone-d_3
Mephedrone	<u>178>145</u> 178>90.95	-23	2.7	Mephedrone-d_3
Mephedrone-d_3	<u>181>163.05</u> 181>118.9	-15	2.7	
4-MEC	<u>192>144.95</u> 192>91.05	-25	2.8	Mephedrone-d_3
PVP	<u>232>90.7</u> 232>77.05	-33	3.2	MDPV-d_8
MDPV	<u>276>175.05</u> 276>14875	-26	3.3	MDPV-d_8
MDPV-d_8	<u>284>174.95</u> 284>135	-19	3.3	
Naphyrone	<u>282>141</u> 282>126.2	-27	4.1	Naphyrone-d_5
Naphyrone-d_5	<u>287>142.25</u> 287>216.2	-35	4.1	

Table 2. Linearity parameters (1-250 ng/ml) for 10 synthetic cathinones in Quantisal-Oral Fluid samples. LOQ was 1 ng/mL for all compounds.

Analyte	Intercept	± SD (n=5)	Slope	± SD (n=5)	R2	± SD (n=5)	LOD (ng/ml)
Cathinone	-0.0005	0.002	0.35	0.04	0.98	0.02	0.75
Methcathinone	-0.0015	0.016	1.51	0.14	0.98	0.02	0.75
Methylone	0.0039	0.007	0.91	0.06	0.99	0.004	1
N-ethylcathinone	0.0029	0.007	0.52	0.03	0.99	0.01	1
Buphedrone	0.0037	0.004	0.49	0.02	0.99	0.005	0.75
Mephedrone	0.0056	0.005	0.89	0.04	0.99	0.003	1
4-MEC	0.0093	0.004	0.77	0.04	0.99	0.01	0.75
PVP	0.0039	0.006	0.62	0.07	0.98	0.02	0.75
MDPV	0.0055	0.009	1.28	0.05	0.99	0.01	1
Naphyrone	0.0176	0.040	4.24	0.31	0.99	0.003	1

Table 3. Pool intra-day imprecision, total imprecision and accuracy for 10 synthetic cathinones in oral fluid-Quantisal samples (low QC at 3 ng/mL, high QC at 150 ng/mL).

Analyte	Pool intra-day Imprecision (CV)		Total Imprecision (CV)		Accuracy (%target)	
	Low QC (n=14)	High QC (n=15)	Low QC (n=14)	High QC (n=15)	Low QC (n=14)	High QC (n=15)
Cathinone	5.4	11.0	10.7	13.0	100.3	100.9
Methcathinone	4.0	10.2	10.9	6.3	95.2	91.4
Methylone	7.8	6.7	12.3	5.8	100.5	98.9
N-ethylcathinone	9.1	6.5	7.9	6.3	97.8	93.5
Buphedrone	9.0	12.5	12.8	8.1	99.1	84.4
Mephedrone	13.2	11.9	11.8	7.1	97.4	98.7
4-MEC	8.5	4.7	11.6	6.5	102.0	92.2
PVP	7.5	7.2	20.7	13.0	115.3	91.2
MDPV	12.1	6.4	12.4	8.9	104.9	98.2
Naphyrone	10.2	7.5	8.7	6.5	102.7	99.5

Table 4. Extraction efficiency, process efficiency, and matrix effects for 10 synthetic cathinones in oral fluid-Quantisal samples (low QC at 3 ng/mL, high QC at 150 ng/mL).

Analyte	Extraction Efficiency (n=6)		Process Efficiency (n=6)		Matrix Effects (CV) (n=6)	
	Low QC	High QC	Low QC	High QC	Low QC	High QC
Cathinone	87.2	97.9	54.6	70.6	-37.4 (15.1)	-28.0 (7.3)
Methcathinone	88.9	105.8	32.2	63.1	-63.8 (11.5)	-40.4 (6.7)
Methylone	99.6	105.8	43.9	66.1	-55.9 (7.1)	-37.5 (5.5)
N-ethylcathinone	102.6	114.8	58.5	76.5	-43.0 (9.2)	-33.4 (3.8)
Buphedrone	100.5	113.1	49.6	81.4	-50.6 (9.2)	-28.1 (4.2)
Mephedrone	101.9	110.5	55.4	75.9	-45.6 (7.8)	-31.3 (4.7)
4-MEC	104.7	112.5	60.0	81.0	-42.7 (8.6)	-28.0 (4.1)
PVP	108.2	109.9	58.0	79.3	-46.4 (12.9)	-27.8 (4.3)
MDPV	106.1	114.1	54.8	73.9	-48.4 (3.7)	-35.2 (5.8)
Naphyrone	101.1	110.6	84.1	103.7	-16.8 (6.5)	-6.2 (3.4)

Table 5. Stability (%difference) and CV (n=2) for 10 synthetic cathinones in neat oral fluid at room temperature (RT) for 24h, one week, and one month, at 4°C and -20°C for one week and one month, and after 3 freeze-thaw cycles (3 F/T) at 2.5 ng/mL (low), and at 150 ng/mL (high).

Analyte Group	Analyte	RT 24 h % difference (CV, n=2)		RT 1 week % difference (CV, n=2)		RT 1 month % difference (CV, n=2)		4°C 1 week % difference (CV, n=2)		4°C 1 month % difference (CV, n=2)		-20°C 1 week % difference (CV, n=2)		-20°C 1 month % difference (CV, n=2)		3 F/T % difference (CV, n=2)	
		Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
	Cathinone	-49.3 (1.3)	-28.5 (1.4)	-81.6 (3.2)	-85.2 (0.7)	-100 (0)	-99.6 (11.7)	-26.4 (8)	-29.2 (10.1)	-87.1 (23.7)	-79.7 (7.3)	-11.8 (14)	1.7 (5.8)	-5.2 (9.8)	2 (5.2)	-8.1 (9.6)	-1.2 (11.3)
	Methcathinone	-16.3 (4.1)	4.2 (0.1)	-69 (8.7)	-84.4 (1)	-100 (0)	-99.2 (3.7)	-21.7 (2.8)	-27.8 (6.2)	-88.2 (10.1)	-79.2 (2.7)	-15.7 (1.3)	2.2 (2.9)	-2.1 (15.2)	-1.6 (1.5)	-12.9 (8.2)	-2.2 (11.7)
Position 3'-substituted	Buphedrone	-15.2 (8.7)	-8.6 (3.6)	-35.9 (4.9)	-45.4 (2.8)	-72.6 (24.5)	-81.5 (1.7)	-8.8 (3.9)	-13.9 (0)	-26.1 (7.2)	-33.1 (2.2)	-2.2 (22.1)	-1.9 (1.6)	4.4 (15.7)	-6.5 (2.6)	-9.5 (12.7)	-6.8 (9.9)
N-alkyl-substituted	N-ethylcathinone	-26.9 (3)	17.2 (1.2)	-67.2 (26.2)	-76.1 (2.6)	-100 (0)	-98.6 (6.7)	-10.6 (0.3)	-24.7 (6.4)	-83.8 (23.3)	-67.6 (1.2)	-19.8 (6.7)	-5.2 (6.1)	6.2 (6)	-5.4 (2.2)	-4.1 (2.7)	-12.8 (8.9)
	4-MEC	-24.7 (9.4)	-16.3 (1.1)	-50.9 (9.1)	-46.1 (0.4)	-86.2 (1.3)	-88.8 (2.3)	-8.6 (12.1)	-10.8 (8.9)	-55.9 (9.2)	-38.5 (2.5)	-0.7 (15.8)	0.4 (0.7)	-1.7 (2.6)	-0.6 (2.6)	9.7 (15.1)	-8.2 (11.5)
Ring-substituted	Mephedrone	-26.9 (0.2)	-15 (1.2)	-48.1 (2)	-59.5 (0.6)	-91.1 (6)	-95 (0.3)	-2.9 (0.2)	-15.7 (6.6)	-61.1 (3.5)	-51.5 (2.5)	-3.9 (1)	-2.6 (1.2)	-2 (2.8)	-5.3 (0.9)	-7.6 (8.2)	-4.9 (11.9)
Methylenedioxy-substituted	Methylone	-8.3 (0.1)	-11.3 (1)	-35.2 (8.3)	-40.2 (0.3)	-73.1 (13.1)	-81.3 (0.3)	8.3 (5.1)	-9.4 (9.9)	-39.6 (1)	-34.9 (0.9)	11.7 (0)	-10.7 (6.4)	10 (11.7)	-1.1 (3.2)	1.5 (7.3)	-9.9 (12.3)
Pyrrolindinyl-substituted	MDPV	0.7 (9.7)	9.7 (4.7)	9.5 (7.5)	9.5 (4)	-3.9 (3)	-7.2 (0.7)	18.6 (5.5)	7.9 (4.9)	7 (6.4)	8.3 (2.3)	1.4 (3.4)	4.6 (6.1)	13.7 (3.6)	7.9 (4.6)	-7.6 (1.9)	-9.9 (7.9)
	Naphyrone	-60.8 (0.2)	-42.2 (2.7)	-84.3 (6.6)	-80.3 (2.1)	-100 (0)	-99.2 (6.6)	-31.9 (5.1)	-23.4 (5.1)	-61.3 (9.5)	-34.8 (3.4)	-29.6 (0.3)	-12.5 (6.2)	-16.4 (3)	1.5 (2.6)	-31.2 (1)	-15.2 (11.6)
	PVP	-17.5 (2.9)	-18.1 (3.9)	-33.3 (20)	-32.8 (5.3)	-52.4 (20.7)	-41.4 (5)	-1.7 (6.2)	-0.1 (8.2)	-3.8 (0.7)	-13.4 (1)	-2.2 (0.6)	-5.6 (1.8)	14.8 (2.1)	7.6 (3.2)	15.3 (6.1)	-1.6 (7.8)

Table 6. Stability (%difference) and CV (n=2) for 10 synthetic cathinones in oral fluid-Quantisal samples at room temperature (RT) for 24h, one week, and one month, at 4°C and -20°C for one week and one month, and after 3 freeze-thaw cycles (3 F/T) at 2.5 ng/mL (low), and at 150 ng/mL (high).

Analyte Group	Analyte	RT 24 h % difference (CV, n=2)		RT 1 week % difference (CV, n=2)		RT 1 month % difference (CV, n=2)		4°C 1 week % difference (CV, n=2)		4°C 1 month % difference (CV, n=2)		-20°C 1 week % difference (CV, n=2)		-20°C 1 month % difference (CV, n=2)		3 F/T % difference (CV, n=2)	
		Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
	Cathinone	-17.4 (2.5)	-5.9 (2.1)	-30.4 (6.3)	-20.6 (1)	-69 (16.2)	-64.1 (0.9)	-11.1 (10.8)	-0.4 (7.4)	-14.6 (0.5)	-14.3 (3.8)	-13.2 (5.2)	0.8 (0.5)	-11.6 (10.7)	4.4 (0.2)	3.4 (1.9)	-4.4 (2.3)
	Methcathinone	0 (0)	0 (0)	-22.4 (1.9)	-19.4 (2.4)	-67.5 (6.1)	-66.7 (1.2)	8.8 (5.4)	8.1 (7.8)	-31.1 (1.9)	-6.7 (2.5)	-8.4 (3.2)	8.1 (1.9)	-1.4 (6.2)	7.4 (0.1)	-6.9 (11.8)	-1 (5.1)
Position 3'-substituted	Buphedrone	-8.2 (12)	-2.9 (1.5)	-7.4 (6.3)	-16.1 (4.3)	-21.1 (2.7)	-26.8 (2.7)	-8.5 (5.4)	-8.6 (4.7)	-21.6 (9.3)	-9.3 (4)	-1.6 (11.3)	1.8 (2.5)	-9.2 (1.5)	-12.5 (0.6)	-26.9 (1.3)	13.1 (4.7)
N-alkyl-substituted	N-ethylcathinone	3.4 (1)	-0.7 (1)	-7.3 (7.4)	-16.7 (4.3)	-42 (14.9)	-48.3 (2.3)	10.6 (10.5)	-2.3 (7.7)	-8.6 (2.8)	-9.5 (4)	-7.8 (2)	0.2 (0.1)	4.7 (2.6)	-0.9 (4.6)	-6.4 (6.9)	-8.6 (5.4)
	4-MEC	-14.5 (3.2)	-7.3 (3.7)	-9.6 (4.9)	-1.9 (3.7)	-28.6 (2.3)	-16.7 (2.1)	-10.5 (6.2)	3.7 (3.2)	-12.3 (1.9)	0.5 (9.1)	-6.2 (15.6)	7.7 (1.4)	0.5 (5.6)	2 (2.2)	-7.6 (0.4)	-9.3 (4)
Ring-substituted	Mephedrone	-11.7 (7.8)	-3.9 (0.2)	-4.4 (11.3)	-11.3 (5.4)	-31.7 (11.4)	-32.8 (2.4)	-2.3 (7.9)	0.7 (5.1)	-14.6 (8.2)	-5.6 (6.5)	-5.2 (4)	2.9 (3.8)	0.5 (4.7)	-3.9 (1.1)	-5.4 (14.3)	-5.7 (2.4)
Methylenedioxy-substituted	Methylone	-4.3 (7.2)	-6.8 (2.9)	6 (7.7)	-5.1 (0.4)	-8.6 (5)	-14.3 (0.8)	-3.1 (6.4)	-1.8 (7.2)	-5.1 (3.3)	-5.9 (4)	10.2 (0)	-7.7 (0.7)	7 (3)	-0.2 (0.2)	-2.9 (3.2)	-6.4 (4.1)
Pyrrolindinyl-substituted	MDPV	-4.7 (5.4)	3.8 (5.5)	-13.5 (2.1)	7.4 (0.5)	-3.3 (4.3)	1.6 (2.4)	10.6 (1.6)	-1 (9.5)	-8.6 (2.4)	11.8 (1.4)	-4.4 (8.3)	4.7 (0.1)	6.6 (0.7)	5.4 (5.1)	-4 (1.2)	-5.9 (2.8)
	Naphyrone	-19.7 (1.4)	-0.8 (0.2)	-37.8 (1.4)	-24.9 (1.5)	-71.2 (6)	-59.5 (1)	-24.2 (3.3)	-4.1 (3.4)	-34 (2)	-10.6 (1.1)	-21.5 (2.2)	-1.9 (0.4)	-3.9 (6.4)	3.2 (6.4)	-23.9 (4.2)	-7.8 (0.2)
	PVP	-0.7 (2.3)	-9.5 (3.9)	-10.4 (7.3)	-3.8 (2.8)	3.1 (15.7)	2.3 (2.8)	-2.4 (8.4)	-3.5 (8.7)	-5.6 (4.4)	-7.2 (3.8)	3.8 (3.8)	-5.4 (9.4)	14.7 (5.3)	13.2 (5.7)	-5.8 (5.7)	-5.5 (0.7)

Table 7. Stability (%difference) and CV (n=2) for 10 synthetic cathinones in oral fluid-Oral-Eze samples at room temperature (RT) for 24h, one week, and one month, at 4°C and -20°C for one week and one month, and after 3 freeze-thaw cycles (3 F/T) (low at 3 ng/mL, high at 150 ng/mL).

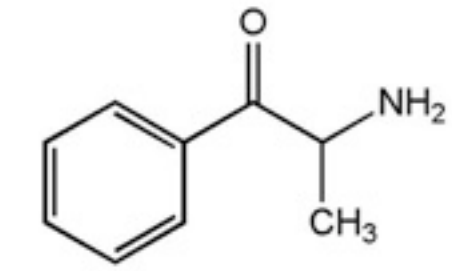
Analyte Group	Analyte	RT 24 h % difference (CV, n=2)		RT 1 week % difference (CV, n=2)		RT 1 month % difference (CV, n=2)		4°C 1 week % difference (CV, n=2)		4°C 1 month % difference (CV, n=2)		-20°C 1 week % difference (CV, n=2)		-20°C 1 month % difference (CV, n=2)		3 F/T % difference (CV, n=2)	
		Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
	Cathinone	-31.3 (2.8)	-15.1 (0.3)	-59.7 (1.1)	-45.8 (2.4)	-88.8 (23.2)	-92.1 (3.1)	-42.4 (5.9)	-19.9 (5.1)	-61.9 (12)	-40.6 (2)	-23.9 (0)	-9 (0.7)	-29.7 (2)	-6.9 (10.8)	-27.2 (5.9)	-10.1 (3.8)
	Methcathinone	0 (0)	0 (0)	0.2 (3.2)	-27.8 (2.7)	-84.8 (4.5)	-83.8 (1.5)	-14 (10.4)	-8.6 (5.3)	-38.9 (13.1)	-23 (1.5)	-11.5 (1.1)	-0.6 (2.2)	-12.9 (1)	3.8 (8)	-18.8 (0.8)	-4.7 (1.7)
Position 3'-substituted	Buphedrone	-8.6 (3)	1.8 (0.5)	7.4 (1.5)	-11.5 (2.2)	-54.1 (5.7)	-41.7 (3.2)	-6.5 (12)	-5.1 (5.8)	7.6 (2.1)	-8.8 (8.2)	8.3 (17.6)	3.3 (1.6)	13.4 (3.7)	-2 (3.7)	-10.3 (6.4)	-6.8 (0.4)
N-alkyl-substituted	N-ethylcathinone	7.3 (6.3)	3 (2.7)	-20.4 (0.1)	-30.4 (3.9)	-83.7 (0.2)	-77 (1.9)	12.1 (5)	-13.7 (4.9)	-35.1 (13.6)	-30.6 (0.3)	-15 (2.1)	-9.4 (1.3)	5.9 (9.3)	-7.6 (12.6)	6.5 (0.4)	-11.4 (0.5)
	4-MEC	-2.2 (5.4)	-6.2 (4.3)	0.7 (12.9)	-7.4 (2.1)	-54.3 (2.1)	-45 (1)	1.5 (11.9)	-1.5 (2.8)	4.8 (4.5)	6.2 (4.1)	11.2 (0.1)	6.3 (0.5)	11.2 (0)	14.6 (2.1)	6.7 (1.3)	-8.2 (1.9)
Ring-substituted	Mephedrone	-2.6 (13)	-4.7 (3.1)	-9.5 (2.7)	-18.9 (0.2)	-62.8 (11.1)	-61.2 (0)	-4.5 (0.8)	-11.9 (5.4)	-2.9 (11.4)	-16.2 (4.5)	-5.1 (2)	-4.8 (0.9)	5.3 (2.3)	-4.1 (6.6)	7.7 (3.9)	-7.6 (0.2)
Methylenedioxy-substituted	Methylone	13 (10.4)	-6.8 (1.7)	2.1 (4.2)	-16.1 (4.1)	-31.5 (3.8)	-40.5 (6.8)	3.6 (2.3)	-12.4 (3.7)	-7.1 (2.4)	-13 (1.1)	19.2 (0)	-12.7 (0.9)	16.5 (2.1)	0.4 (6.6)	-0.6 (5.1)	-13.2 (0.9)
Pyrrolindinyl-substituted	MDPV	4.6 (4.6)	6 (3.9)	-0.5 (9.1)	2.8 (5.2)	-8.3 (0.2)	-2.3 (8.2)	-2.3 (3.7)	-6.5 (1.2)	0.3 (8)	7.7 (0.9)	-3.6 (0.4)	5.2 (0)	14.7 (0.3)	8.7 (2.9)	1.2 (2)	-9.3 (2.8)
	Naphyrone	-19.7 (5.2)	-3.6 (3.6)	-58.3 (0.9)	-50.2 (2.7)	-100 (0)	-96 (1.3)	-25.6 (0.4)	-10 (5.2)	-37 (2.9)	-20.2 (1.4)	-18.2 (0.5)	-2.7 (0.3)	10.3 (7)	12.6 (1.5)	-22.2 (0.4)	-3.2 (0.4)
	PVP	3.9 (0.7)	-9.3 (2.2)	-4.7 (9.5)	-23.3 (2.7)	-29 (8.4)	-18.1 (2.2)	-4.7 (6.1)	-15 (4.8)	17.3 (23.2)	-6.3 (2.7)	6.2 (2)	-8.1 (5.1)	21.6 (0)	20.3 (3.5)	11.8 (7.3)	3 (4.5)

Figure legends

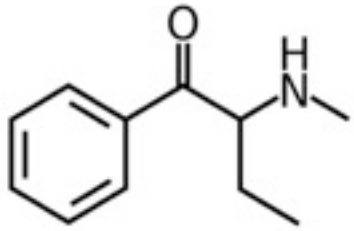
Figure 1. Chemical structure of cathinone and synthetic cathinone derivatives buphedrone, mephedrone, ethylcathinone, methylone and MDPV.

Figure 2. Total ion chromatogram (TIC) of the 10 synthetic cathinones in an oral fluid-Quantisal sample at the limit of quantification (1 ng/mL).

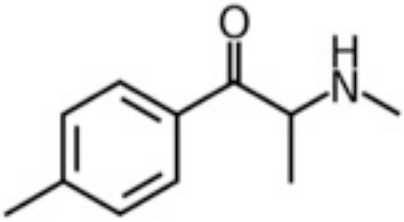
Figure 1.



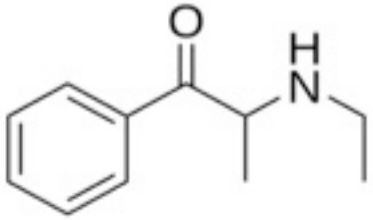
Cathinone



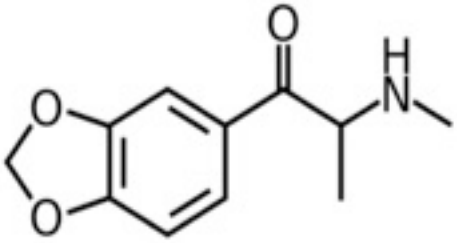
Buphedrone
(Position 3'-substituted)



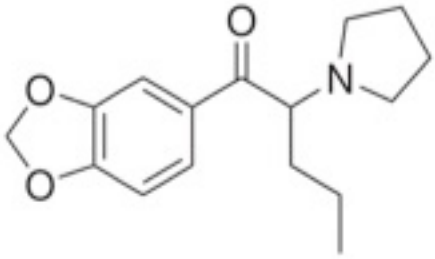
Mephedrone
(Ring-substituted)



Ethylcathinone
(N-alkyl-substituted)



Methylone
(Methylenedioxy-substituted)



MDPV
(Pyrrolidinyl-substituted)

Figure 2.

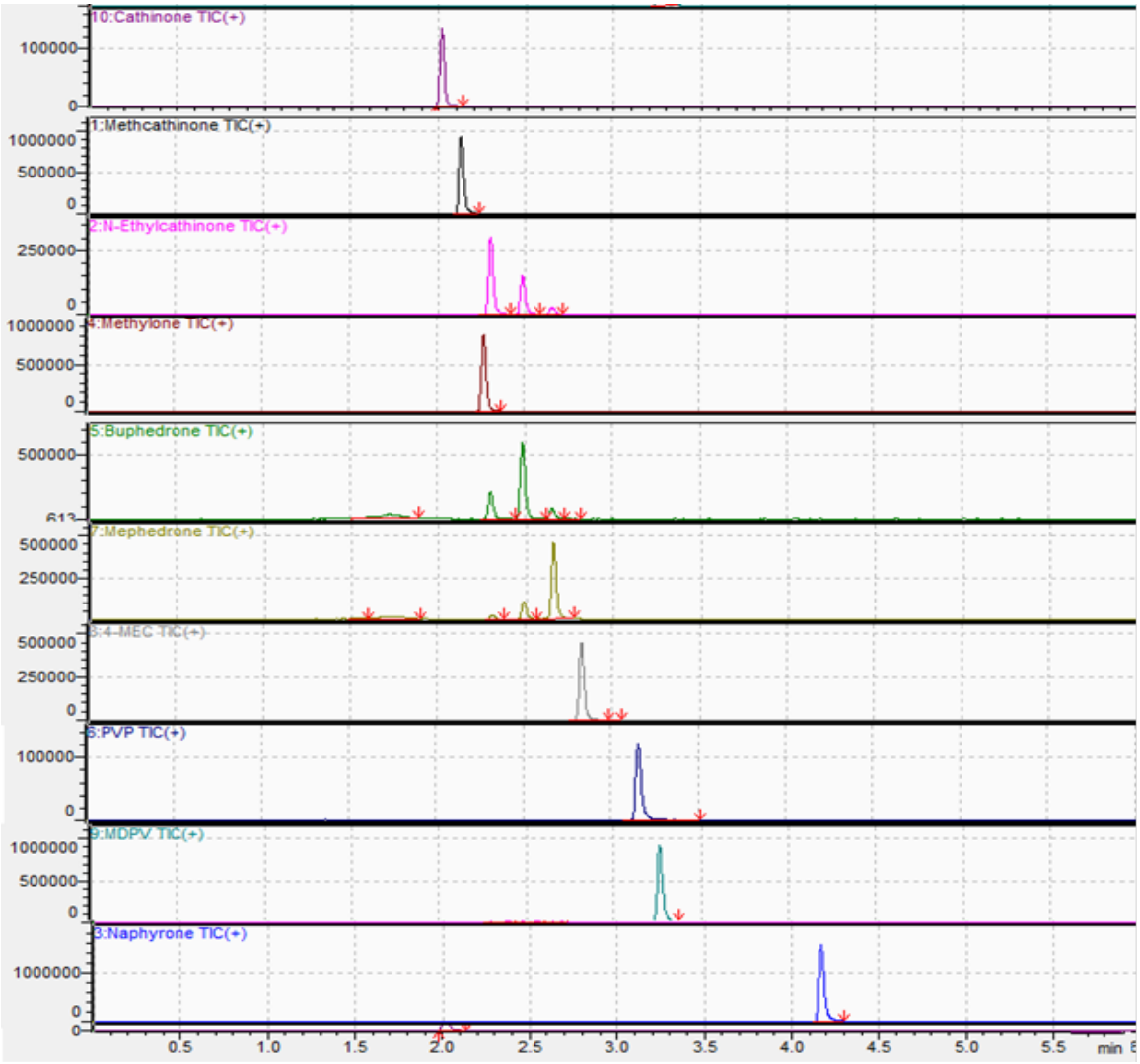

10:Cathinone TIC(+)
100000
0
1:Methcathinone TIC(+)
1000000
500000
0
2:N-Ethylcathinone TIC(+)
250000
0
4:Methylone TIC(+)
1000000
500000
0
5:Buphedrone TIC(+)
500000
7:Mephedrone TIC(+)
500000
250000
3:4-MEC TIC(+)
500000
250000
0
6:PVP TIC(+)
100000
0
9:MDPV TIC(+)
1000000
500000
0
8:Naphyrone TIC(+)
1000000
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
min

Table 1S. Maximum and minimum %difference between fresh and stored sample concentrations (stability), taking into account method's inaccuracy ±15% in the determination of QCs concentrations. These are the results for 10 synthetic cathinones in neat oral fluid samples at 2.5 ng/mL (low) and at 150 ng/mL (high). Storage conditions were room temperature (RT) for 24h, one week, and one month, at 4°C and -20°C for one week and one month, and after 3 freeze-thaw cycles (3 F/T).

Analyte Group	Analyte	RT 24 h max % difference min % difference		RT 1 week max % difference min % difference		RT 1 month max % difference min % difference		4°C 1 week max % difference min % difference		4°C 1 month max % difference min % difference		-20°C 1 week max % difference min % difference		-20°C 1 month max % difference min % difference		3 F/T max % difference min % difference	
		Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
	Cathinone	-62.5 -31.3	-47.2 -3.3	-86.4 -75	-89 -79.9	-100 -100	-99.7 -99.5	-45.6 -0.4	-47.7 -4.2	-90.5 -82.6	-85 -72.6	-34.8 19.4	-24.8 37.6	-29.2 28.3	-24.6 38	-32.1 24.3	-27 33.6
	Methcathinone	-38.5 12.6	-23 41	-77.1 -58.1	-88.5 -78.9	-100 -100	-99.4 -98.9	-42.2 5.9	-46.6 -2.3	-91.3 -84.1	-84.7 -71.9	-37.7 14	-24.5 38.2	-27.7 32.4	-27.3 33.1	-35.7 17.8	-27.7 32.4
Position 3'-substituted	Buphedrone	-37.3 14.8	-32.4 23.7	-52.6 -13.3	-59.6 -26.1	-79.8 -63	-86.3 -75	-32.6 23.4	-36.4 16.5	-45.4 -0.1	-50.6 -9.5	-27.7 32.3	-27.5 32.7	-22.8 41.2	-30.9 26.5	-33.1 22.4	-31.1 26
N-alkyl-substituted	N-ethylcathinone	-45.9 -1	-38.8 12	-75.7 -55.6	-82.3 -67.7	-100 -100	-99 -98.2	-34 20.9	-44.3 1.9	-88 -78.1	-76 -56.1	-40.7 8.5	-29.9 28.3	-21.5 43.6	-30.1 27.9	-29.1 29.8	-35.6 17.9
	4-MEC	-44.3 1.9	-38.1 13.3	-63.7 -33.6	-60.2 -27.1	-89.8 -81.4	-91.7 -84.8	-32.4 23.7	-34.1 20.7	-67.4 -40.3	-54.6 -16.8	-26.6 34.3	-25.8 35.8	-27.4 33	-26.5 34.5	-18.9 48.5	-32.2 24.2
Ring-substituted	Mephedrone	-46 -1.1	-37.1 15.1	-61.6 -29.7	-70.1 -45.2	-93.4 -88	-96.3 -93.3	-28.2 31.4	-37.7 14	-71.3 -47.4	-64.1 -34.4	-29 30.1	-28 31.8	-27.6 32.6	-30 28.1	-31.7 25	-29.7 28.7
Methylenedioxy-substituted	Methylone	-32.2 24.1	-34.4 20	-52.1 -12.3	-55.8 -19.1	-80.1 -63.6	-86.2 -74.7	-20 46.5	-33.1 22.5	-55.4 -18.3	-51.9 -11.9	-17.5 51.1	-34 20.8	-18.7 48.8	-26.9 33.8	-24.9 37.4	-33.4 21.8
Pyrrolindinyl-substituted	MDPV	-25.6 36.3	-18.9 48.4	-19 48.2	-19 48.2	-29 30	-31.4 25.5	-12.3 60.5	-20.2 46	-20.9 44.8	-20 46.5	-25.1 37.2	-22.7 41.5	-16 53.8	-20.2 46	-31.7 25	-33.4 21.8
	Naphyrone	-71 -47	-57.2 -21.7	-88.4 -78.8	-85.4 -73.3	-100 -100	-99.4 -98.9	-49.7 -7.9	-43.4 3.7	-71.4 -47.6	-51.8 -11.8	-48 -4.8	-35.3 18.4	-38.2 13.2	-25 37.3	-49.2 -6.9	-37.3 14.7
	PVP	-39 11.6	-39.5 10.8	-50.7 -9.7	-50.4 -9.1	-64.8 -35.6	-56.7 -20.7	-27.3 33	-26.2 35.1	-28.9 30.1	-36 17.2	-27.7 32.3	-30.2 27.8	-15.1 55.4	-20.5 45.5	-14.8 56	-27.2 33.2

Table 2S. Maximum and minimum %difference between fresh and stored sample concentrations (stability), taking into account method's inaccuracy ±15% in the determination of QCs concentrations. These are the results for 10 synthetic cathinones in oral fluid-Quantisal samples at 2.5 ng/mL (low) and at 150 ng/mL (high). Storage conditions were room temperature (RT) for 24h, one week, and one month, at 4°C and -20°C for one week and one month, and after 3 freeze-thaw cycles (3 F/T).

Analyte Group	Analyte	RT 24 h max % difference min % difference		RT 1 week max % difference min % difference		RT 1 month max % difference min % difference		4°C 1 week max % difference min % difference		4°C 1 month max % difference min % difference		-20°C 1 week max % difference min % difference		-20°C 1 month max % difference min % difference		3 F/T max % difference min % difference	
		Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
	Cathinone	-39 11.7	-30.4 27.3	-48.6 -5.9	-41.3 7.4	-77.1 -58	-73.4 -51.4	-34.3 20.3	-26.4 34.8	-36.8 15.6	-36.7 16	-35.9 17.9	-25.5 36.4	-34.7 19.6	-22.9 41.2	-23.6 39.8	-29.3 29.3
	Methcathinone	-26.1 35.3	-26.1 35.3	-42.7 5	-40.4 9	-76 -56	-75.4 -55	-19.6 47.2	-20.1 46.3	-49.1 -6.8	-31.1 26.2	-32.3 24	-20.1 46.3	-27.2 33.3	-20.6 45.4	-31.2 26	-26.8 33.9
Position 3'-substituted	Buphedrone	-32.1 24.2	-28.2 31.3	-31.5 25.3	-38 13.4	-41.7 6.7	-45.9 -0.9	-32.4 23.8	-32.4 23.7	-42 6.1	-33 22.7	-27.3 33.1	-24.8 37.7	-32.9 22.8	-35.3 18.4	-46 -1.1	-35.8 17.5
N-alkyl-substituted	N-ethylcathinone	-23.6 39.8	-26.6 34.3	-31.5 25.4	-38.4 12.7	-57.2 -21.6	-61.8 -30.1	-18.2 49.7	-27.8 32.2	-32.4 23.7	-33.1 22.5	-31.9 24.7	-26 35.5	-22.6 41.6	-26.7 34.1	-30.8 26.7	-32.4 23.7
	4-MEC	-36.8 15.7	-31.5 25.5	-33.2 22.3	-27.5 32.8	-47.2 -3.4	-38.4 12.7	-33.9 21.1	-23.3 40.3	-35.2 18.6	-25.7 36	-30.7 26.9	-20.4 45.7	-25.7 35.9	-24.6 38	-31.7 25	-33 22.7
Ring-substituted	Mephedrone	-34.7 19.5	-29 30	-29.4 29.3	-34.4 20	-49.5 -7.6	-50.3 -9.1	-27.8 32.2	-25.6 36.3	-36.9 15.6	-30.2 27.7	-29.9 28.3	-23.9 39.3	-25.7 35.9	-29 30	-30.1 27.9	-30.3 27.6
Methylenedioxy-substituted	Methylone	-29.2 29.5	-31.1 26.1	-21.7 43.4	-29.9 28.3	-32.4 23.7	-36.7 16	-28.4 31.1	-27.4 32.9	-29.9 28.4	-30.5 27.3	-18.6 49.1	-31.8 24.9	-20.9 44.8	-26.3 35	-28.2 31.4	-30.8 26.6
Pyrrolindinyl-substituted	MDPV	-29.6 28.9	-23.3 40.4	-36.1 17	-20.6 45.3	-28.5 30.9	-24.9 37.4	-18.2 49.7	-26.8 33.9	-32.4 23.7	-17.3 51.3	-29.4 29.3	-22.6 41.7	-21.2 44.2	-22.1 42.6	-29 29.9	-30.4 27.4
	Naphyrone	-40.6 8.7	-26.7 34.2	-54.1 -15.9	-44.5 1.6	-78.7 -61.1	-70.1 -45.2	-44 2.5	-29.1 29.8	-51.2 -10.6	-33.9 21	-42 6.2	-27.5 32.7	-28.9 30.1	-23.8 39.6	-43.8 3	-31.8 24.8
	PVP	-26.6 34.4	-33.1 22.4	-33.8 21.2	-28.9 30.1	-23.8 39.5	-24.4 38.4	-27.9 32.1	-28.7 30.5	-30.2 27.7	-31.4 25.6	-23.3 40.5	-30.1 28	-15.2 55.2	-16.3 53.2	-30.4 27.5	-30.2 27.8

Table 3S. Maximum and minimum %difference between fresh and stored sample concentrations (stability), taking into account method's inaccuracy ±15% in the determination of QCs concentrations. These are the results for 10 synthetic cathinones in oral fluid-Oral-Eze samples at 2.5 ng/mL (low) and at 150 ng/mL (high). Storage conditions were room temperature (RT) for 24h, one week, and one month, at 4°C and -20°C for one week and one month, and after 3 freeze-thaw cycles (3 F/T).

Analyte Group	Analyte	RT 24 h max % difference min % difference		RT 1 week max % difference min % difference		RT 1 month max % difference min % difference		4°C 1 week max % difference min % difference		4°C 1 month max % difference min % difference		-20°C 1 week max % difference min % difference		-20°C 1 month max % difference min % difference		3 F/T max % difference min % difference	
		Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
	Cathinone	-49.2 -7.1	-37.3 14.8	-70.2 -45.5	-59.9 -26.7	-91.7 -84.4	-94.2 -89.4	-57.4 -22.1	-40.8 8.4	-71.9 -48.5	-56.1 -19.7	-43.7 3	-32.7 23.1	-48 -4.8	-31.2 26	-46.2 -1.5	-33.5 21.6
	Methcathinone	-26.1 35.3	-26.1 35.3	-26 35.5	-46.6 -2.3	-88.8 -79.4	-88 -78.1	-36.4 16.4	-32.5 23.6	-54.9 -17.4	-43.1 4.1	-34.6 19.7	-26.5 34.5	-35.7 17.8	-23.2 40.5	-40 9.9	-29.6 28.9
Position 3'-substituted	Buphedrone	-32.5 23.6	-24.8 37.7	-20.6 45.3	-34.6 19.8	-66.1 -38	-56.9 -21.1	-30.9 26.4	-29.9 28.4	-20.5 45.6	-32.6 23.4	-19.9 46.4	-23.6 39.8	-16.2 53.4	-27.6 32.5	-33.7 21.4	-31.1 26.1
N-alkyl-substituted	N-ethylcathinone	-20.7 45.1	-23.9 39.3	-41.2 7.6	-48.5 -5.8	-88 -78	-83 -68.9	-17.1 51.7	-36.2 16.7	-52 -12.2	-48.7 -6.1	-37.1 15	-33 22.6	-21.7 43.3	-31.7 25	-21.3 44.1	-34.5 19.8
	4-MEC	-27.7 32.3	-30.6 26.9	-25.6 36.2	-31.6 25.3	-66.2 -38.2	-59.3 -25.6	-25 37.3	-27.2 33.3	-22.5 41.8	-21.5 43.7	-17.8 50.4	-21.4 43.9	-17.8 50.5	-15.3 55	-21.2 44.3	-32.1 24.2
Ring-substituted	Mephedrone	-28 31.8	-29.6 28.9	-33.1 22.4	-40 9.7	-72.5 -49.7	-71.3 -47.5	-29.4 29.2	-34.9 19.2	-28.2 31.4	-38.1 13.4	-29.9 28.4	-29.7 28.7	-22.2 42.4	-29.1 29.8	-20.4 45.7	-31.7 25
Methylenedioxy-substituted	Methylone	-16.5 52.9	-31.1 26.2	-24.6 38.1	-38 13.5	-49.4 -7.3	-56 -19.5	-23.4 40.1	-35.2 18.5	-31.3 25.7	-35.7 17.7	-11.9 61.3	-35.5 18.1	-13.9 57.6	-25.8 35.8	-26.6 34.4	-35.9 17.4
Pyrrolindinyl-substituted	MDPV	-22.7 41.6	-21.7 43.4	-26.5 34.6	-24 39.1	-32.2 24	-27.8 32.2	-27.8 32.2	-30.9 26.5	-25.9 35.7	-20.4 45.7	-28.8 30.4	-22.3 42.3	-15.2 55.2	-19.6 47.1	-25.2 36.9	-33 22.7
	Naphyrone	-40.7 8.6	-28.7 30.4	-69.2 -43.5	-63.2 -32.6	-100 -100	-97.1 -94.6	-45 0.7	-33.5 21.7	-53.4 -14.8	-41 8	-39.5 10.7	-28.1 31.6	-18.5 49.2	-16.8 52.3	-42.5 5.3	-28.4 31
	PVP	-23.2 40.6	-33 22.7	-29.6 28.9	-43.3 3.8	-47.5 -3.9	-39.4 10.9	-29.6 28.9	-37.2 15	-13.3 58.7	-30.7 26.8	-21.5 43.7	-32.1 24.3	-10.1 64.5	-11.1 62.8	-17.4 51.2	23.9 39.3