Quantitative Measurement of Synthetic Cathinones in Oral Fluid

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Synthetic cathinones have recently emerged as a substitute for common drugs of abuse. Synthetic cathinones can elicit powerful adverse effects such as delusions, hallucinations and potentially dangerous behavior. To develop a method to analyze 10 synthetic cathinones in oral fluid, we implemented a combined approach of solid-phase extraction and ultra-high-performance liquid chromatography–tandem mass spectrometry. The developed analytical procedure was a sensitive, precise and selective method suited for high-throughput toxicological screening of synthetic cathinones. The method was validated using standard parameters including accuracy, precision, linearity, sensitivity, matrix effect and recovery. Human subject samples were analyzed using the developed method to demonstrate the applicability of the method.

Introduction
Cathinone ([S]-2-amino-1-phenyl-1-propanone) is a beta-ketone amphetamine found in the leaves of Catha edulis (Khat). This plant is commonly found in East Africa and in the Middle East and people chew the leaves of this plant for their stimulant effect (1). Synthetic cathinones are chemically synthesized derivatives of cathinones that encompass a beta-ketone moiety. Synthetic cathinones have recently emerged as a substitute for common drugs of abuse. These synthetic products are sold under misleading names in order to evade current legislation that covers common drugs of abuse. Synthetic cathinones can elicit powerful adverse effects such as delusions, hallucinations and potentially dangerous behavior (2). However, there is limited information available on the pharmacological and toxicological effects of the synthetic cathinones (3). Law enforcement authorities and poison control centers have reported increasing incidents of overdose and deaths resulting from the use of this new class of abuse drugs. Considering the increased use of synthetic cathinones and their adverse effects, it is essential to develop analytical techniques that identify and quantitate them accurately. Analytical techniques that identify synthetic cathinones are scientific tools for law enforcement authorities and treatment centers to aid prevention and treatments.

Because these drugs are new, precise identification of these drugs using conventional screening techniques is unlikely. Immunoassay is a conventional screening technique used for the detection of drugs of abuse; however, it is difficult to develop an immunoassay method that detects an entire class of synthetic cathinones because of the variations found among the different synthetic cathinones created by the modifications of functional groups. In addition, the low specificity of the immunoassay methods is a substantial drawback to the technique: drug metabolites and other structurally similar molecules to the drug of interest might also react with the antibody, thus producing a false-positive signal.

Development and implementation of selective and sensitive confirmation techniques can enable the detection of new designer drugs. In the past, gas chromatography–mass spectrometry (GC–MS) was used as a confirmation technique. However, there are a couple of limitations in GC–MS: extensive sample preparation and its inability to detect polar and thermally labile compounds. Conversely, ultra-high-performance liquid chromatography (UHPLC)–tandem mass spectrometry (MS–MS) is the current method of choice for confirmation of drugs, because it does not suffer from the drawbacks of GC–MS. UHPLC can separate a large number of compounds with high resolution within a short period. The MS–MS detector exhibits sensitivity and specificity superior to those of conventional mass spectrometric techniques because it involves an ion fragmentation step and multiple mass filtration steps.

Body fluids, such as blood and urine, have been routinely used for drugs of abuse testing and therapeutic drug monitoring. Oral fluid has become a popular choice as a matrix for drug testing due to the inherent advantages it has over other matrices (4–6). The noninvasive and observed collection procedure of oral fluid sampling makes adulteration almost impossible. The drug concentration in oral fluid is correlated with drug concentration in blood. According to previous reports, drugs with a molecular structure similar to that of synthetic cathinones have shown higher drug concentrations in oral fluid compared with blood. For example, the oral fluid-to-blood concentration ratio has been reported as 2.0 (7) and 2.9 (8) for methamphetamine in previous studies. Similarly, if the oral fluid-to-blood concentration ratio is determined for synthetic cathinones, the oral fluid concentration can be used to estimate the concentrations of synthetic cathinones in blood. To date, there are a limited number of validated methods available for the detection of synthetic cathinones in biological matrices such as urine, blood or oral fluid.

One of the essential components of a drug testing method is the sample cleanup process that separates interfering matrix compounds out of the sample before quantitation or identification of target analytes: extraction and centrifugation are common cleanup methods. However, most of the available methods that detect synthetic cathinones are dilute-and-shoot techniques in which the sample is directly injected into a separation and quantitation instrument after dilution. With these methods, the matrix components are injected into the instrument, thus producing possible interferences with the accurate quantification of target analytes. Therefore, dilute-and-shoot techniques are not suitable for the toxicological screening process in a production laboratory setting.

To develop a method for analyzing synthetic cathinones in oral fluid, we implemented a combined approach of solid-phase extraction and UHPLC–MS–MS. This work details a validation of a rapid, sensitive and robust method to analyze 10 synthetic cathinones in oral fluid. In addition, human subject samples were analyzed to provide the implications of the method.
Experimental

Supplies and reagents
Reference standards of 4-methylmethcathinone (mephedrone), methylenedioxyxypyrovalerone (MDPV), 4-fluoromethcathinone (lephedrone), 4-methoxymethcathinone (methedrone), 3,4-methylenedioxy-N-methylcathinone (methylone), β-keto-N-methylbenzodioxolylbutanamine (butylone), 3,4-methylenedioxy-N-ethylcathinone (ethylone), α-pyrrolidinopentiophenone (PVP), α-methymalino-propiophenone (methcathinone), 4-methyl-β-ketone-prolantane (pyrovalerone), 4-methylenmethcathinone-d3 (mephedrone-d3), MDPV-d8 and 3,4-methylenedioxy-N-ethylcathinone-d5 (ethylone-d5) were purchased from Cerilliant (Round Rock, TX, USA). Solid-phase extraction cartridges were purchased from Cerilliant (Round Rock, TX, USA). LC–MS-grade methanol, LC–MS-grade acetonitrile, formic acid, phosphoric acid and ammonia were purchased from VWR (Radnor, PA, USA). Negative calibrator oral fluid, extraction buffer and Quantisal™ devices (for the collection of oral fluid) were purchased from Immunalysis (Pomona, CA, USA). Solid-phase extraction cartridges were purchased from Waters (Milford, MA, USA).

Sample collection
Oral fluid samples were collected with a Quantisal™ collection device. The Quantisal™ collection device consists of a cotton pad, which absorbs oral fluid from the oral cavity, an extraction buffer, which preserves oral fluid during transportation and a tube, which contains the buffer and the pad. Under the routine collection procedure, the cotton pad absorbs 1 mL of oral fluid and there is 3 mL of extraction buffer in the tube. Therefore, the dilution of neat oral fluid in samples is 1:3. For this reason, standards and quality control (QC) samples were prepared in oral fluid–extraction buffer mixture (1:3 v/v). Because original oral fluid was diluted with extraction buffer during the collection, detected drug concentrations were adjusted accordingly.

Prior to initiation of the research study, all human subject samples were de-identified and any demographic data or other means of patient identification were permanently deleted from the sample records.

Standard preparation
Purchased reference standards of the analytes were individually diluted in methanol to make 10,000 ng/mL working solutions. Spiking solutions were prepared from working solutions as mixtures of the 10 synthetic cathinones in methanol at concentrations eight times higher than that of the corresponding calibration standard. Oral fluid–extraction buffer (1:3, v/v) mixtures were spiked with spiking solutions to prepare calibration standards at 1.0, 2.0, 50.0, 100.0, 250.0 and 500.0 ng/mL concentrations. QC standards, which consisted of all the 10 analytes, were independently prepared in oral fluid–extraction buffer (1:3, v/v) mixture at four different concentrations as follows: near lower limit of quantitation at 1.0 (QCLOQ), 2.0 (QCLow), 250.0 (QCMid) and 500.0 ng/mL (QCHigh). A spiking solution of deuterated standards (mephedrone-d3, ethylone-d5 and MDPV-d8) was prepared as a mixture in methanol at a concentration of 2000 ng/mL each analyte (IS Solution).

Extraction procedure
Human subject samples, calibration standards and QC standards were purified by solid-phase extraction prior to UHPLC–MS–MS analysis. Solid-phase extraction was performed in a vacuum manifold (Waters) using a µ-elution MCX 96-well cartridge (Waters). Ten microliters of IS Solution and 300 µL of 4% phosphoric acid were added to 400 µL of calibration standard, QC standard or human subject samples followed by mixing in a rotary shaker (VWR). The sample size of 400 µL of human subject sample was equivalent to 100 µL of neat oral fluid. Prepared samples were loaded into the solid-phase extraction wells. There is no preconditioning of the extraction cartridge involved in this method. The wells were washed with 200 µL of 2% formic acid in water followed by 200 µL of deionized (DI) water/methanol (50:50, v/v). The compounds were eluted with two portions of 25 µL of 5% NH₃ in water: acetonitrile (60:40, v/v). Fifty microliters of 2% formic was added to the eluent before it was injected into the UHPLC–MS–MS system.

UHPLC–MS–MS analysis
Chromatographic separation was performed on an Acquity UHPLC system (Waters) equipped with an Acuity BEH (C-18, 100 × 2.1 mm, 1.7 µm) analytical column (Waters). Mobile phase A was 0.1% formic acid in water: acetonitrile (95:5, v/v), and mobile phase B was 0.1% formic acid in acetonitrile. The flow rate was kept at 0.7 mL/min, and the column temperature was kept at 50°C throughout the analysis. The total run time was 3.0 min. The gradient conditions were as follows: from time 0 to 0.5 min, the mobile phase ratio was 95% A: 5% B; from time 0.5 to 1.8 min, the mobile phase ratio was increased from 95% A: 5% B to 64% A: 36% B; from time 1.81 to 2.8 min, the mobile phase ratio was 100% A: 0% B; from time 2.81 to 3.0 min, the mobile phase ratio was 95% A: 5% B. Injection volume was 7.0 µL. The needle was washed with 600 µL of DI water: acetonitrile (90 : 10, v/v) and 200 µL of water: acetonitrile (50 : 50, v/v) in between injections.

Electrospray ionization (ESI) mass spectrometry was performed on a TQD instrument (Waters). Analysis was performed in positive ionization (ESI+) and multiple reaction monitoring (MRM) mode. The source temperature was 150°C, and the desolvation temperature was 450°C. The flow rates of desolvation gas (nitrogen) and cone gas (nitrogen) were set at 850 and 50 mL/min, respectively. Collision gas (argon) flow was set at 0.18 mL/min. Collision cell pressure was 3.59e-3 mbar of argon. The MRM conditions were developed and optimized using the IntellStart software (Waters); however, the settings were critically reviewed to ensure that the precursor ions and the product ions could be explained with respect to the molecular structure of each drug. Water and carbon dioxide fragments were excluded during the method development to prevent the uncertainties that arise from these fragmentations during the quantification. Dwell time was 0.005 s for all the transitions.

The mass spectrometer method is summarized in Table I. Two MRM transitions, a qualifier and a quantifier, were monitored for the standards; only one MRM transition was monitored for internal standards. The ratio of the peak area between the quantifier ion and the qualifier ion (ion transition ratio) was calculated by replicate analysis (n = 6) of 50 ng/mL calibration standard, and the average ion transition ratio was utilized as an acceptance criterion for the method validation. Deuterated internal standards were assigned as follows: MDPV-d8 was used as the internal standard for MDPV, PVP and pyrovalerone; ethylone-d5 was
used as the internal standard for ethylone, methylene and butylone; mephedrone-d3 was used as the internal standard for mephedrone, flephedrone, methedrone and methcathinone.

**Method validation**

**Linearity and carryover**

Linearity of the assay was assessed by analyzing six replicates of the above-listed extracted calibration standards (deuterated standards were added to each calibration standard prior to extraction). A blank sample (matrix sample extracted without the internal standard) and a zero sample (matrix sample extracted without the internal standards) were added to each calibration standard prior to extraction. A zero sample was analyzed after the highest calibration standard of each curve to evaluate the carryover of the target analytes. The acceptance criterion was that no interfering substance be present in the blank samples.

**Selectivity**

Blank matrix samples \((n = 6)\) were spiked with a 4000 ng/mL concentration of the following list of drugs, including common over-the-counter drugs, drugs of abuse and some prescribed drugs: amphetamine, methamphetamine, ephedrine, cocaine, hydrocodone, codeine, 6-monoacetyl morphine, morphine, hydromorphone, methadone, phencyclidine, dextromethorphan, oxycodone, propoxyphene, fentanyl, buprenorphine, acetaminophen, carisoprodol, tramadol, phenetermine, bupropion, methylphenidate, zolpidem, lamotrigine, fluoxetine, sertraline, aripiprazole, clomipramine, duloxetine, mirtazapine, gabapentin, escitalopram, quetiapine, trazodone, buspirone, hydroxyzine, paroxetine, clonidine, risperidone, amitriptyline, nortriptyline, venlafaxine, oxymethyleneflaxine, alprazolam, lorazepam, clonazepam, cyclobenzaprine, tapentadol, pregabalin, haloperidol, ziprasidone, diazepam, 3,4-methylendioxymphetamine and 3,4-methylendioxy-N-methylamphetamine. The acceptance criterion was that no false-positive signal for target analytes be present in the spiked samples.

Blank samples \((n = 10)\), which were collected from 10 drug-free volunteers, were tested without the addition of the internal standard in order to evaluate the presence of interference from the endogenous compounds. The acceptance criterion was that no interfering substance be present in the blank samples.

QCHigh samples \((n = 3)\) were analyzed without internal standards in order to evaluate the interference coming from the target analytes to the selectivity of the internal standards. Similarly, internal standards were added to blank samples \((n = 3)\) at a concentration of 250 ng/mL and analyzed in order to evaluate the interference from the deuterated standard to the selectivity of the target analytes.

**Matrix effect and recovery**

Matrix effect (ME) and recovery were quantitatively assessed according to the method described in Matuszewski et al. (9).

Three sets of samples in two different concentrations (50.0 and 500 ng/mL) were prepared as follows: Set A consisted of neat standards \((n = 6)\); Set B consisted of spiked blank extracts \((n = 6)\) and Set C consisted of spiked and extracted samples \((n = 6)\). Samples of Sets B and C were prepared by using six oral fluid samples obtained from drug-free healthy volunteers. Each set was analyzed by UHPLC–MS–MS. The matrix effect was calculated by comparing the peak areas of the analytes of Set A with those of the corresponding samples of Set B and reported in percentage. The recovery was calculated by comparing the peak areas of the analytes of Set B with those of the corresponding samples of Set C and reported in percentage. In the matrix effect calculations, the values < 100% represent ion suppression and the values > 100% represent ion enhancement.

### Table I

**Summary of the UHPLC–MS–MS method**

<table>
<thead>
<tr>
<th>Target analyte</th>
<th>Retention time (min)</th>
<th>Precursor ion (m/z)</th>
<th>Product ions (m/z)</th>
<th>Collision energy (V)</th>
<th>Core voltage (V)</th>
<th>Ion transition ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDPV</td>
<td>1.51</td>
<td>276.1</td>
<td>126</td>
<td>28</td>
<td>38</td>
<td>3.1</td>
</tr>
<tr>
<td>Mephedrone</td>
<td>1.01</td>
<td>178.1</td>
<td>119</td>
<td>22</td>
<td>20</td>
<td>6.6</td>
</tr>
<tr>
<td>Flephedrone</td>
<td>0.67</td>
<td>182</td>
<td>123</td>
<td>24</td>
<td>26</td>
<td>4.3</td>
</tr>
<tr>
<td>Methedrone</td>
<td>0.74</td>
<td>194</td>
<td>145.8</td>
<td>32</td>
<td>24</td>
<td>2.4</td>
</tr>
<tr>
<td>Ethylone</td>
<td>0.72</td>
<td>222.1</td>
<td>146.1</td>
<td>28</td>
<td>30</td>
<td>2.1</td>
</tr>
<tr>
<td>Butylone</td>
<td>0.87</td>
<td>222.1</td>
<td>174</td>
<td>22</td>
<td>30</td>
<td>3.9</td>
</tr>
<tr>
<td>Methcathinone</td>
<td>0.57</td>
<td>164.1</td>
<td>104.9</td>
<td>26</td>
<td>24</td>
<td>4.9</td>
</tr>
<tr>
<td>Methyline</td>
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<td>29</td>
<td>2.6</td>
</tr>
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<td>PVP</td>
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<td>190.9</td>
<td>20</td>
<td>32</td>
<td>1.2</td>
</tr>
<tr>
<td>Pyrovalerone</td>
<td>1.81</td>
<td>246.2</td>
<td>161</td>
<td>18</td>
<td>38</td>
<td>2.0</td>
</tr>
<tr>
<td>Ethylone-d5</td>
<td>0.72</td>
<td>227.1</td>
<td>179.1</td>
<td>24</td>
<td>30</td>
<td>N/A</td>
</tr>
<tr>
<td>Mephedrone-d3</td>
<td>1.01</td>
<td>181</td>
<td>148.2</td>
<td>20</td>
<td>27</td>
<td>N/A</td>
</tr>
<tr>
<td>MDPV-d8</td>
<td>1.51</td>
<td>284.2</td>
<td>175</td>
<td>20</td>
<td>35</td>
<td>N/A</td>
</tr>
</tbody>
</table>

### Accuracy and precision and sensitivity

Intraday accuracy and intraday precision were assessed by analyzing a batch of samples that contained a calibration curve and replicates \((n = 6)\) of QCLOQ, QCLow, QCMid and QCHigh. This experiment was repeated for 5 days to assess interday accuracy and interday precision. Precision was calculated by using the coefficient of variation (%CV). Accuracy was calculated as the absolute percent relative error (%RE) = \((|E - T| / T) 	imes 100\), where \(E\) is the experimentally determined concentration and \(T\) is the theoretical concentration. The acceptance criterion for precision was that the %CV should be \(\leq 15\%\) for QC samples \((\leq 20\%\ CV\) at lower limit of quantification (LLOQ)). The acceptance criterion for accuracy was that %RE should be \(\leq 15\%\) for QC samples \((\leq 20\%\ at\ LLOQ)\).

An LLOQ was defined as the lowest point of the calibration curve that had a signal-to-noise ratio of \(\geq 10\) and that passed the data acceptance criteria for accuracy and precision.

In addition, the ion transition ratios of all the analytes were compared \((\pm 20\%)\) with the average ion transition ratios of the 50 ng/mL calibration standard to deem all the QC samples to be acceptable.
matrix effect between the six samples from different sources was evaluated by calculating %CV (%CV of ≤15% was considered acceptable).

Processed samples stability
Processed sample stability was evaluated by allowing the QCLow (n = 6) and QCHigh (n = 6) samples to remain in the autosampler at 15°C and reanalyzing them at 1 hr intervals for 6 hrs. Absolute peak areas of the analytes were plotted against time to evaluate stability. The acceptance criterion for the instability of the processed samples was indicated by a negative slope, significantly different from zero (P ≤ 0.05) (10).

Results and discussion
To quantitatively identify 10 synthetic cathinones in oral fluid, an analytical procedure was developed: a combined approach of a solid-phase extraction method and a UHPLC–MS–MS method. The developed analytical procedure was a sensitive, precise and selective method suited for high-throughput toxicological screening of synthetic cathinones. The solid-phase extraction method did not involve preconditioning or drying steps, so that the samples could rapidly be separated from their matrix components and directly be injected into the UHPLC–MS–MS system after the extraction. The UHPLC–MS–MS method was able to quantitatively identify the synthetic cathinones within 3.0 min. All the synthetic cathinones had an LLOQ of 1.0 ng/mL.

Sample preparation
Previous methods that have been developed to detect individual synthetic cathinones or synthetic cathinones panels mainly focused on detecting them in powder-form (11), urine (12), hair (13) or blood (14). Strano-Rossi et al. (15) reported a technique that can quantify synthetic cathinones and synthetic cannabinoids in oral fluid. In comparison with the present study, they used a different collection device (DCD5000) that did not contain a preservative buffer. When the collected oral fluid is not preserved during the transportation and storage, degradation of the drugs and the metabolites may occur. Even though their UHPLC–MS–MS method was able to detect the synthetic cathinones with an limit of detection (LOD) of 1 ng/mL, the run time was 15 min to test the entire drug profile.

Generally, oral fluid drug concentrations and the specimen volumes are relatively low compared with urine. Therefore, it was essential to develop analytical methods that allowed accurate analysis of lower drug concentration in a limited volume of oral fluid. Our method used only 400 μL of the sample for the analysis, and it was able to quantify drug concentrations as low as 1.0 ng/mL for all the synthetic cathinones tested.

The main purpose of using a solid-phase extraction procedure is to remove the interfering matrix components present in a biological matrix because matrix compounds may either enhance or reduce the MS–MS signals of the target analytes. In addition, the matrix compounds reduce the longevity of the UHPLC column. Most of the previous studies that were designed to identify synthetic cathinones involved dilute-and-shoot techniques that do not involve sample preparation steps (9, 14–17). Although these approaches are ideal for a high-throughput environment, the inherent column clogging and high back pressure in long-term sample processing are substantial drawbacks. The previously described method by Strano-Rossi et al. used the dilute-and-shoot technique. According to their results, the analysis of synthetic cathinones suffered from significant ion suppression. For example, the matrix effect for mephedrone was in the range of 0.1–0.5%. This may be due to matrix compounds that co-eluted with mephedrone.

For sample preparation of synthetic cathinones, certain laboratories follow liquid–liquid extraction (13, 18). This is an extraction technique with low separation power and also a time-consuming application. The solid-phase extraction method described here involved two washing steps that removed matrix interferences of different polarities. As a result, most of the target analytes suffered from only minor ion enhancement or ion suppression effects (Table II). After analyzing samples for an extended period of time using this method, column clogging, change in retention times of the peaks and deformation of the peak shapes were not observed.

UHPLC–MS–MS method
One of the reasons why synthetic cathinones have become an emerging threat is that neither conventional immunoassay methods nor chromatographic methods can accurately identify them. Implementation of a versatile UHPLC–MS–MS technique enables an analyst to detect the target analytes with an increased sensitivity and selectivity. In the current method, the synthetic cathinones were identified by using different MRM transitions (Table I). Figure 1 shows the chromatograms for quantifying ions of the synthetic cathinones at a concentration of 2 ng/mL. Although unique precursor ion–product ion combinations were assigned for each target analyte, it was found that some cathinones exhibited common fragments (ethylone and butylone). To address this issue, gradient conditions were optimized to obtain baseline separation between ethylone and butylone; ethylone eluted at 0.72 min and butylone eluted at 0.87 min. However, the gradient chromatographic method was unable to separate some of the analytes with a baseline separation, but the MS–MS was able to identify the unique fragments of those analytes.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>QC sample</th>
<th>Matrix effect (%)</th>
<th>%CV (matrix effect)</th>
<th>Recovery (%)</th>
<th>%CV (recovery)</th>
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<tr>
<td>MDPV</td>
<td>50 ng/mL</td>
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<td>2.9</td>
<td>47.2</td>
<td>7.7</td>
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<td>100.4</td>
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<td>4.6</td>
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</table>
Method validation

The described method was validated in general accordance with the ‘Guidance for Industry: Bioanalytical Method Validation’ (19). Calibration curves for all of the synthetic cathinones were linear over the range of 1.0–500.0 ng/mL. Average correlation coefficient was calculated using the analyzed six replicate curves. The average correlation coefficients of MDPV, mephedrone, flephedrone, methedrone, ethylone, butylone, methcathinone, methylone, PVP and pyrovalerone were 0.998, 0.999, 0.995, 0.997, 0.999, 0.992, 0.998, 0.998 and 0.995, respectively. The blank samples analyzed after the highest standard of the calibration curve showed no peaks for the target analytes. This supports that the method is free from carryover issues.

The accuracy of a UHPLC–MS-MS method is defined as the closeness of the results, which were obtained by the analysis, to the true value of the analyte. Precision of a UHPLC–MS-MS method is defined as the closeness of the individual measurements of the analysis, when the method is performed multiple times with the same homogeneous samples. Table III summarizes the accuracy and precision (interday and intraday) values calculated according to the method described in the preceding text. Intraday precision and interday precision were ≤15% for all the analytes (≤20% at LLOQ). Similarly, intraday accuracy and interday accuracy were ≤15% for all the analytes (≤20% at LLOQ). The results indicate that the UHPLC–MS-MS method is capable of analyzing all the 10 analytes within the acceptable limits of precision and accuracy.

In a UHPLC–MS-MS method, quantification data are acceptable above the LLOQ. The LLOQ was determined using the acceptance criteria described previously. The LLOQ for all the synthetic cathinones was 1.0 ng/mL.

Selectivity of the UHPLC–MS-MS method is defined as its ability to differentiate among and quantify the target analytes in the presence of other interfering compounds in the sample. These interfering compounds could be commonly found exogenous compounds in the matrix or they could be endogenous compounds in the biological matrix. Blank oral fluid samples spiked with the previously described list of drugs were tested with the method. All the samples tested negative for the target drugs, which supports that the method is free from exogenous interference. Ten oral fluid samples collected from drug-free individuals tested negative for the target analytes, indicating that the method can exclude the interference from the endogenous materials.

The UHPLC–MS-MS method should be able to distinguish between the deuterated standard and the target analyte. The three QC samples analyzed without addition of the internal standards did not show any peaks relevant to internal standards, indicating that the assigned MRM transitions for the target analytes were free from interferences from the MRM transitions of internal standards. The three blank samples spiked with deuterated standards tested negative for the target analytes. No interfering peaks were detected at target analytes’ retention times. This result implies that the ions of the deuterated standards do not interfere with the quantification of the target analytes.

The extent of the matrix effect was quantified using the method described previously. Table II presents the mean value of matrix effect of synthetic cathinones at two different concentrations and corresponding %CV over six different oral fluid samples. Most of the analytes exhibited relatively lower ion enhancement or ion suppression effects. However, pyrovalerone shows significant ion suppression (%ME is 50.0 at 50.0 ng/mL and 77.9 at 500 ng/mL). This may be due to co-eluting lipophilic substances that interfere with ionization of pyrovalerone. Observed %CV for matrix effect for the analytes was < 15%, indicating the lack of significant variation of matrix effect between different samples.

Figure 1. Chromatograms corresponding to quantifying transition ions of synthetic cathinones at a concentration of 2 ng/mL.
samples obtained from different individuals. We implemented internal standards, which were structurally similar to the target analytes. The use of internal standards compensated for the matrix effect.

The method showed satisfactory recoveries for most of the analytes. Table II summarizes the mean values of recovery and corresponding %CV over six different oral fluid samples. The recoveries for the analytes varied between 34.5 and 90.3%. MDPV, pyrovalerone and PVP exhibited relatively lower recoveries. Low recovery of these drugs may occur due to their lipophilic nature. However, they were quantitatively identified in low concentrations because of the sensitivity UHPLC–MS–MS method: LLOQ for MDPV, pyrovalerone and PVP is 1.0 ng/mL.

The stability experiments showed that the processed samples are stable over a period of 6 h.

**Analysis of human subject samples**

Human subject samples were analyzed using the developed method to demonstrate the applicability of the method. Five of the de-identified human subject samples were found to be positive for synthetic cathinones. Among those five samples, three samples were found to be positive for both MDPV and PVP. The drug concentrations found in different samples were as follows: 450.6 ng/mL of MDPV, 935.0 ng/mL of PVP, 468.0 ng/mL of MDPV, 285.1 ng/mL of PVP and 831.7 ng/mL of MDPV, 81.9 ng/mL of PVP. Two samples were found to be positive only for MDPV, and the concentrations were as follows: 20.5 and 24.5 ng/mL. The samples with high synthetic cathinone concentrations were diluted to reduce the concentrations of the synthetic cathinone, so that the concentrations would fit within the linear range of the calibration curve.

**Conclusion**

The use of synthetic cathinones is an evolving threat to public safety because of the availability of these drugs and the lack of analytical methods to detect them. Modern forensic toxicology laboratories must be able to detect these compounds, necessitating development and implementation of versatile analytical techniques. Ability to test for these drugs in oral fluid has been poorly studied. Our experiments summarize the validation of an integrated method of solid-phase extraction and UHPLC–MS–MS that can quantitatively identify 10 synthetic cathinones in oral fluid. This is a rapid and robust method that can be used in high-throughput toxicological screening applications.

**References**


