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# Method of Test for Synthetic Cathinones in Urine (2)

## 1. Scope

This method is applicable to the determination of 49 synthetic cathinones and related metabolites (benzedrone etc. listed as the attached table) in urine.

## 2. Method

After extraction-purification, analytes are determined by gas chromatography/mass spectrometry (GC/MS).

**2.1.** Equipment

**2.1.1.** Gas chromatograph/mass spectrometer

**2.1.1.1.** Ion source: electron ionization (EI)

**2.1.1.2.** Column: HP-5MS, 0.25 μm, 0.25 mm i.d. × 30 m, or an equivalent product

**2.1.2.** Vortex mixer

**2.1.3.** pH meter

**2.1.4.** Heater

**2.1.5.** Nitrogen evaporator

**2.1.6.** Solid phase vacuum extraction manifold

**2.2.** Chemicals

Methanol and isopropanol, HPLC grade;

Acetic acid, dichloromethane, ammonium hydroxide (32%), potassium hydrogen phosphate anhydrous ($K_2HPO_4$), potassium dihydrogen phosphate anhydrous ($KH_2PO_4$), and sodium hydroxide, reagent grade;

Artificial urine (UTAK 88121-CDF (L) or an equivalent product), reagent grade;

Deionized water, resistivity ≥ 18 MΩ·cm (at 25℃);

Benzedrone etc. listed in the attached tables, reference standards;

Butylone-$d_3$ etc. and other isotope-labeled internal standards (listed in the attached table).

**2.3.** Apparatus

**2.3.1.** Volumetric flask: 10 mL

**2.3.2.** Solid phase extraction cartridge: Bond Elut SPEC DAU cartridge, 15 mg, 3 mL, or an equivalent product

**2.4.** Reagent solution preparation

**2.4.1.** 5 M sodium hydroxide solution

Dissolve and dilute 200 g of sodium hydroxide with deionized water to

1000 mL.

**2.4.2.** 0.1 M phosphate buffer solution

Dissolve 1.7 g of potassium hydrogen phosphate anhydrous and 12.14 g of potassium dihydrogen phosphate anhydrous with 900 mL of deionized water, adjust with 5 M sodium hydroxide solution to pH 6.0 and dilute with deionized water to 1000 mL.

**2.4.3.** 0.1 M acetic acid solution

Dissolve and dilute 6 g of acetic acid with deionized water to 1000 mL.

**2.4.4.** Eluting solution

Mix 80 mL of dichloromethane, 20 mL of isopropanol and 2 mL of ammonium hydroxide.

**2.5.** Internal standard solution preparation

Transfer 1 mg of the 7 isotope-labeled internal standards accurately weighed into each 10-mL volumetric flask, dissolve and dilute with methanol to volume as the internal standard stock solutions. Store at -20℃ in the dark. Prior to use, mix adequate volume of the internal standard stock solutions, and dilute with methanol to 10 μg/mL as the internal standard solution.

**2.6.** Standard solution preparation

Transfer 1 mg of the 49 reference standards accurately weighed into a 10-mL volumetric flask, dissolve and dilute with methanol to volume as the standard stock solutions. Store at -20℃ in the dark. Prior to use, mix adequate volume of the standard stock solutions, and dilute with methanol to 10 μg/mL as the standard solution.

**2.7.** Sample solution preparation

Mix accurate 2 mL of the homogenized sample, 20 μL of the internal standard solution and 1 mL of 0.1 M phosphate buffer solution. Transfer the sample solution into the solid phase extraction cartridge pre-rinsed with 1 mL of methanol and 1mL of deionized water, and discard the eluent. Wash the cartridge 1 mL of 0.1 M acetic acid solution and 1 mL methanol and discard the eluent. Evaporate the cartridge with nitrogen gas for 4 min until dry. Add 2 mL of eluting solution to the cartridge, and collect the eluent. Evaporate the eluent to dryness by gently flushing with a steam of nitrogen at 40℃. Dissolve the residue with 100 μL of methanol as the sample solution.

**2.8.** Calibration curve

Use the artificial urine as the blank sample. Separately take 10-400 μL of the standard solution and dilute with artificial urine to volume of 2 mL. Add 20 μL of the internal standard solution and 1 mL of 0.1 M phosphate buffer solution to the above solution. Prepare the calibration solutions following the procedure described in section 2.7. Operate GC/MS according to the following conditions. Establish the calibration curve of each cathinone by the ratios of the peak area of each cathinone to that of the respective internal standard vs. the added concentrations (1-40 μg/mL).

GC/MS operating conditions(1):

Column: HP-5MS, 0.25 μm, 0.25 mm i.d. × 30 m

Temperature program: initial temperature 180℃, 2 min;
temperature ramp 1: 5℃/min;
hold temperature: 195℃, 10 min;
temperature ramp 2: 15℃/min;
final temperature 240℃, 5 min.

Inlet temperature: 260℃

Injection volume: 2 μL

Flow rate of carrier gas: helium, 0.8 mL/min

Interface temperature: 280℃

Ionization mode: EI, 70 eV

Ion source temperature: 230℃

Injection mode: splitless

Detection mode: selected ion monitoring (SIM). Monitored ions are shown in the attached table.

Note 1: All the parameters can be adjusted depending on the equipment used if the above conditions are not applicable.

**2.9.** Identification and quantification

Accurately inject 2 μL of the sample solution and the standard solutions into GC/MS separately. Operate according to the conditions in section 2.8. Identify each cathinone based on the retention time and the relative ion intensities(2). Calculate the amount (ng/mL) of each cathinone in the sample using the following formula:

$$\text{The amount of each cathinone in the sample (ng/mL)} = \frac{\mathrm{C} \times \mathrm{V}}{\mathrm{M}} \times 10^3$$

Where:

C: the concentration of each cathinone in the sample solution calculated by the calibration curve (μg/mL)

V: the final make-up volume of the sample (0.1 mL)

M: the volume of the sample (mL)

Note 2: Relative ion intensities are calculated by peak areas of quantitative ions divided by peak areas of qualitative ions. Maximum permitted tolerances of relative ion intensities are as the following:

| Relative ion intensity (%) | Tolerance (%) |
|---|---|
| > 50 | ± 10 |
| > 20-50 | ± 15 |
| > 10-20 | ± 20 |
| ≤ 10 | ± 50 |

**Remark**

1. Limit of quantification (LOQ) for each cathinone is 50 ng/mL.
2. Further validation should be performed when interference compounds appear in the samples.

**Reference**

Abiedalla, Y. F., Abdel-Hay, K., DeRuiter, J. and Clark, C. R. 2017. GC–MS, MS/MS and GC–IR analysis of a series of methylenedioxyphenyl-aminoketones: precursors, ring regioisomers and side-chain homologs of 3,4-methylenedioxypyrovalerone. J. Chromatogr. Sci. 55: 99-108.

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## Reference chromatogram

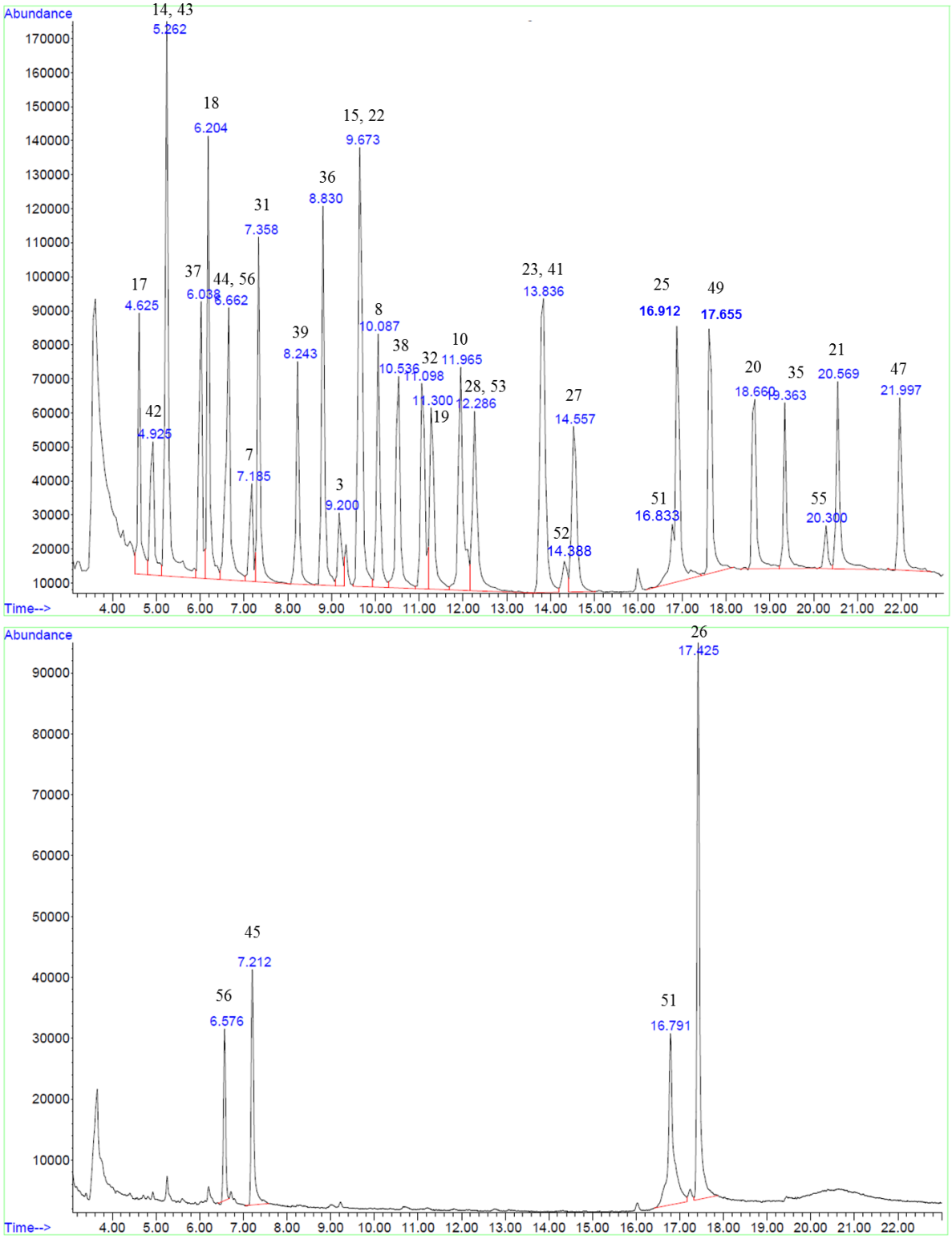


Figure. The SIM chromatogram of 49 synthetic cathinones and 7 isotope-labeled internal standards in urine analyzed by GC/MS.

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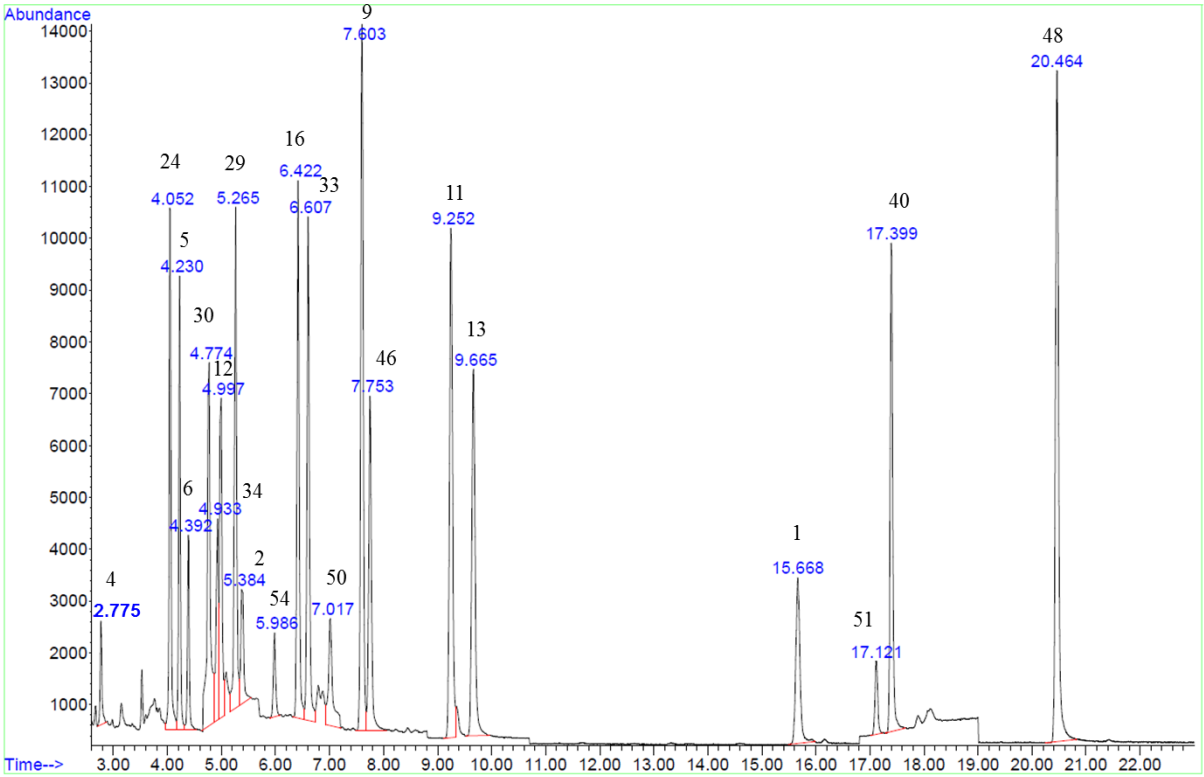


Figure. The SIM chromatogram of 49 synthetic cathinones and 7 isotope-labeled internal standards in urine analyzed by GC/MS (continued).

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Table. SIM fragmentation patterns of 49 synthetic cathinones and 7 isotope-labeled internal standards

| NO. | Analyte | Monitored ions (*m/z*) | Corresponding internal standard |
|---|---|---|---|
| 1 | Benzedrone | 91*, 134, 65 | 3,4-Methylenedioxypyrovalerone-$d_8$ |
| 2 | 4-Bromoethcathinone | 72*, 76, 155 | Methylone-$d_3$ |
| 3 | 4-Bromo-α-pyrrolidinopropiophenone | 98*, 56, 69 | 3,4-Methylenedioxy-α-pyrrolidinopropiophenone-$d_8$ |
| 4 | Cathinone | 77*, 105, 51 | Methylone-$d_3$ |
| 5 | 4-Chlorodimethylcathinone | 72*, 111, 75 | Methylone-$d_3$ |
| 6 | 4-Chloroethcathinone | 72*, 111, 75 | Methylone-$d_3$ |
| 7 | 4-Chloro-α-pyrrolidinopropiophenone | 98*, 56, 112 | α-Pyrrolidinovalerophenone-$d_8$ |
| 8 | 4-Chloro-α-pyrrolidinovalerophenone | 126*, 55, 111 | 3,4-Methylenedioxy-α-yrrolidinopropiophenone-$d_8$ |
| 9 | Dibutylone | 86*, 71, 87 | Butylone-$d_3$ |
| 10 | 3,4-Dimethyl-α-pyrrolidinovalerophenone | 126*, 105, 55 | 3,4-Methylenedioxy-α-pyrrolidinopropiophenone-$d_8$ |
| 11 | N,N-Dimethylpentylone | 100*, 58, 101 | Butylone-$d_3$ |
| 12 | α-Ethylaminohexanophenone | 114*, 58, 77 | Methylone-$d_3$ |
| 13 | N-Ethylpentylone | 100*, 58, 101 | Butylone-$d_3$ |
| 14 | 4-Fluoro-α-pyrrolidinobutiophenone | 112*, 70, 95 | α-Pyrrolidinovalerophenone-$d_8$ |
| 15 | 4-Fluoro-α-pyrrolidinoheptiophenone | 154*, 95, 123 | 3,4-Methylenedioxy-α-pyrrolidinopropiophenone-$d_8$ |
| 16 | 4-Fluoro-α-pyrrolidinohexanophenone | 126*, 127, 95 | Methylone-$d_3$ |
| 17 | 4-Fluoro-α-pyrrolidinopropiophenone | 98*, 56, 123 | α-Pyrrolidinovalerophenone-$d_8$ |
| 18 | 4-Fluoro-α-pyrrolidinovalerophenone | 126*, 55, 95 | α-Pyrrolidinovalerophenone-$d_8$ |
| 19 | 4-Methoxy-α-pyrrolidinobutiophenone | 112*, 55, 70 | 3,4-Methylenedioxy-α-pyrrolidinopropiophenone-$d_8$ |
| 20 | 4-Methoxy-α-pyrrolidinoheptiophenone | 154*, 96, 77 | 3,4-Methylenedioxypyrovalerone-$d_8$ |
| 21 | 4-Methoxy-α-pyrrolidinooctanophenone | 168*, 96, 69 | Naphyrone-$d_5$ |
| 22 | 4-Methoxy-α-pyrrolidinopropiophenone | 98*, 69, 56 | 3,4-Methylenedioxy-α-pyrrolidinopropiophenone-$d_8$ |
| 23 | 4-Methoxy-α-pyrrolidinovalerophenone | 126*, 77, 55 | 3,4-Methylenedioxy-α-pyrrolidinobutiophenone-$d_8$ |
| 24 | 4-Methylbuphedrone | 72*, 91, 119 | Methylone-$d_3$ |
| 25 | 3,4-Methylenedioxypyrovalerone | 126*, 149, 127 | 3,4-Methylenedioxypyrovalerone-$d_8$ |
| 26 | 3,4-Methylenedioxypyrovalerone metabolite | 126*, 55, 151 | 3,4-Methylenedioxypyrovalerone -$d_8$ |
| 27 | 3,4-Methylenedioxy-α-pyrrolidinobutiophenone | 112*, 149, 70 | 3,4-Methylenedioxy-α-pyrrolidinobutiophenone-$d_8$ |
| 28 | 3,4-Methylenedioxy-α-pyrrolidinopropiophenone | 98*, 56, 149 | 3,4-Methylenedioxy-α-pyrrolidinopropiophenone-$d_8$ |
| 29 | 4-Methyl-α-ethylaminopentiophenone | 100*, 101, 58 | Methylone-$d_3$ |
| 30 | 4-Methylpentedrone | 86*, 91, 87 | Methylone-$d_3$ |
| 31 | 4-Methyl-α-pyrrolidinobutiophenone | 112*, 91, 70 | α-Pyrrolidinovalerophenone-$d_8$ |
| 32 | 4-Methyl-α-pyrrolidinohexanophenone | 140*, 91, 119 | 3,4-Methylenedioxy-α-pyrrolidinopropiophenone-$d_8$ |
| 33 | 4-Methyl-α-pyrrolidinopropiophenone | 98*, 56, 99 | Methylone-$d_3$ |
| 34 | Mexedrone | 88*, 91, 119 | Methylone-$d_3$ |
| 35 | Naphyrone | 126*, 155, 127 | Naphyrone-$d_5$ |
| 36 | Pyrovalerone | 126*, 91, 119 | 3,4-Methylenedioxy-α-pyrrolidinopropiophenone-$d_8$ |
| 37 | α-Pyrrolidinobutiothiophenone | 112*, 55, 70 | α-Pyrrolidinovalerophenone-$d_8$ |
| 38 | α-Pyrrolidinoheptiophenone | 154*, 77, 105 | 3,4-Methylenedioxy-α-pyrrolidinopropiophenone-$d_8$ |
| 39 | α-Pyrrolidinohexanophenone | 140*, 77, 105 | 3,4-Methylenedioxy-α-pyrrolidinopropiophenone-$d_8$ |
| 40 | α-Pyrrolidinononanophenone | 182*, 183, 110 | 3,4-Methylenedioxypyrovalerone-$d_8$ |
| 41 | α-Pyrrolidinooctanophenone | 168*, 105, 169 | 3,4-Methylenedioxy-α-pyrrolidinobutiophenone-$d_8$ |
| 42 | α-Pyrrolidinopropiophenone | 98*, 77, 56 | α-Pyrrolidinovalerophenone-$d_8$ |

| | | | |
|---|---|---|---|
| 43 | α-Pyrrolidinopropiothiophenone | 98*, 111, 56 | α-Pyrrolidinovalerophenone-$d_8$ |
| 44 | α-Pyrrolidinovalerophenone | 126*, 77, 105 | α-Pyrrolidinovalerophenone-$d_8$ |
| 45 | α-Pyrrolidinovalerophenone metabolite | 126*, 110, 55 | α-Pyrrolidinovalerophenone-$d_8$ |
| 46 | Tertylone | 100*, 149, 57 | Butylone-$d_3$ |
| 47 | 3,4-Tetramethylene-α-pyrrolidinohexanophenone | 140*, 55, 69 | Naphyrone-$d_5$ |
| 48 | 3,4-Tetramethylene-α-pyrrolidinovalerophenone | 126*, 127, 91 | 3,4-Methylenedioxypyrovalerone-$d_8$ |
| 49 | 3,4-Trimethylene-α-pyrrolidinovalerophenone | 126*, 145, 55 | 3,4-Methylenedioxypyrovalerone-$d_8$ |
| 50 | Butylone-$d_3$ (I.S.) | 75* | - |
| 51 | 3,4-Methylenedioxypyrovalerone-$d_8$ (I.S.) | 134* | - |
| 52 | 3,4-Methylenedioxy-α-pyrrolidinobutiophenone-$d_8$ (I.S.) | 120* | - |
| 53 | 3,4-Methylenedioxy-α-pyrrolidinopropiophenone-$d_8$ (I.S.) | 106* | - |
| 54 | Methylone-$d_3$ (I.S.) | 61* | - |
| 55 | Naphyrone-$d_5$ (I.S.) | 131* | - |
| 56 | α-Pyrrolidinovalerophenone-$d_8$ (I.S.) | 134* | - |

*Quantitative ion