Published, Feb 26, 2018.
RA02I001.001

Method of Test for Synthetic Cathinones in Urine (1)

1. Scope

This method is applicable to the determination of 24 synthetic cathinones and related metabolites (*N*-benzylmethcathinone etc. listed as the attached table) in urine.

2. Method

After extraction-purification and derivatization, 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, isopropanol and ethyl acetate, HPLC grade;

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

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

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

N-benzylmethcathinone etc. listed in the attached tables, reference standards;

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

2.3. Apparatus

2.3.1. Volumetric flask: 10 mL and 1000 mL

2.3.2. Solid phase extraction cartridge: Bond Elut SPEC DAU cartridge

15 mg, 3 mL, or an equivalent product

2.3.3. Micro-reaction vial: 3 mL

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 10 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 24 reference standards accurately weighed into each 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, collect the eluent and transfer to the micro-reaction vial. Evaporate the eluent to dryness by gently flushing with a steam of nitrogen at 40℃. Dissolve the residue with 50 μL of HFBA and 50 μL of ethyl acetate. Cap the micro-reaction vial, vortex the residue solution to homogeneous, and react at 90℃ for 15 min. Allow the solution to cool to room temperature and evaporate with nitrogen gas at 40℃ until dry. Dissolve the residue with 100 μL of ethyl acetate 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: 140℃, 1 min;
temperature ramp 1: 2℃/min;
hold 1 temperature: 150℃, 10 min;
temperature ramp 2: 5℃/min;
hold 2 temperature: 180℃, 7 min;
temperature ramp 3: 20℃/min;
final temperature 200℃.

Inlet temperature: 260℃

Injection volume: 2 μL

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

Interface temperature: 280℃

Ion source: 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:

The amount of each synthetic cathinone in the sample (ng/mL)

$$= \frac{C \times V \times 1000}{M}$$

Where:

C: the concentration of each synthetic 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. The 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

Hong, W. Y., Ko, Y. C., Lin, M. C., Wang, P. Y., Chen, Y. P., Chiueh, L. C.,

Shih, D. Y., Chou, H. K. and Cheng, H. F. 2016. Determination of synthetic cathinones in urine using gas chromatography-mass spectrometry techniques. J. Anal. Toxicol. 40:12-16.

Table. SIM fragmentation patterns of 24 synthetic cathinones and 10 isotope-labeled internal standards

NO.	Analyte	Monitored ions (*m/z*)	Corresponding internal standard
1	*N*-Benzylmethcathinone	171*, 143, 431	4-Methylmethcathinone-d_3
2	4-Bromomethcathinone	254*, 183, 210	4-Methylmethcathinone-d_3
3	Butylone	268*, 210, 269	Butylone-d_3
4	4-Chloromethcathinone	254*, 139, 210	4-Methylmethcathinone-d_3
5	2,5-Dimethoxy-4-methylmethcathinone	179*,136, 254	4-Methylmethcathinone-d_3
6	3,4-Dimethylmethcathinone	133*,105, 254	4-Methylmethcathinone-d_3
7	3,4-Dimethylmethcathinone norephedrine	331*, 240, 358	3,4-Dimethylmethcathinone norephedrine-d_4
8	Ethcathinone	268*, 240, 105	Methcathinone-d_3
9	*N*-Ethylcathinone ephedrine	268*, 240, 358	*N*-Ethylcathinone ephedrine-d_5
10	4-Ethylethcathinone	133*, 268, 240	4-Methylmethcathinone-d_3
11	4-Ethylmethcathinone	133*, 254, 105	4-Methylmethcathinone-d_3
12	Ethylone	268*, 240, 269	Ethylone-d_5
13	4-Fluoroethcathinone	268*, 240, 123	Methcathinone-d_3
14	4-Fluoroephedrine	254*, 210, 362	4-Fluoroephedrine-d_3
15	4-Fluoromethcathinone	254*, 210, 213	Methcathinone-d_3
16	Methcathinone	254*, 210, 105	Methcathinone-d_3
17	4-Methoxyethcathinone	135*, 268, 240	4-Methylmethcathinone-d_3
18	4-Methoxymethcathinone	135*, 254, 210	4-Methylmethcathinone-d_3
19	4-Methylephedrine	254*, 210, 358	4-Methylephedrine-d_3
20	4-Methylethcathinone	119*, 268, 240	4-Methylmethcathinone-d_3
21	4-Methyl-*N*-ethyl-norephedrine	268*, 240, 372	4-Methyl-*N*-ethyl-norephedrine-d_5
22	4-Methylmethcathinone	254*, 210, 255	4-Methylmethcathinone-d_3
23	Methylone	254*, 210, 255	Methylone-d_3
24	Pentylone	149*, 282, 240	Ethylone-d_5
25	Butylone-d_3 (I.S.)	271*	-
26	3,4-Dimethylmethcathinone norephedrine-d_4 (I.S.)	243*	-
27	*N*-Ethylcathinone ephedrine-d_5 (I.S.)	273*	-
28	Ethylone-d_5 (I.S.)	273*	-
29	4-Fluoroephedrine-d_3 (I.S.)	257*	-
30	Methcathinone-d_3 (I.S.)	257*	-
31	4-Methylephedrine-d_3 (I.S.)	257*	-
32	4-Methyl-*N*-ethyl-norephedrine-d_5 (I.S.)	273*	-
33	4-Methylmethcathinone-d_3 (I.S.)	257*	-
34	Methylone-d_3 (I.S.)	257*	-

*Quantitative ion