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Method of Test for Synthetic Phenethylamines in Urine (2)

1. Scope

This method is applicable to the determination of 20 synthetic phenethylamines (5-(2-Aminopropyl)-2,3-dihydrobenzofuran (5-APDB) 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: temperature ≥ 90℃

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℃);

5-APDB etc. listed in the attached tables, reference standards;

3,4-methylenedioxyamphetamine-d_5 (MDA-d_5) 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

2.3.3. 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 2 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 20 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 with 1 mL of 0.1 M acetic acid solution and 1 mL of 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 10-200 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 phenethylamine by the ratios of the peak area of each phenethylamine to that of the respective internal standard vs. the added concentrations (1-20 μg/mL).

GC/MS operating conditions(1):

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

Temperature program: initial temperature 120℃, 0.5 min;
temperature ramp 1: 15℃/min;
hold 1 temperature: 180℃;
temperature ramp 2: 3℃/min;
hold 2 temperature: 200℃;
temperature ramp 3: 30℃/min;
final temperature 285℃, 3 min.

Inlet temperature: 260℃

Injection volume: 2 μL

Flow rate of carrier gas: helium, 0.9 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 phenethylamine based on the retention time and the relative ion intensities(2). Calculate the amount (ng/mL) of each

phenethylamine in the sample using the following formula:

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

Where:

C: the concentration of each phenethylamine 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 phenethylamine is 50 ng/mL.
2. Further validation should be performed when interference compounds appear in the samples.

Reference

Nisbet, L. A., Wylie, F. M., Logan, B. K. and Scott, K. S. 2019. Gas chromatography-mass spectrometry method for the quantitative identification of 23 new psychoactive substances in blood and urine. J. Anal. Toxicol. 43: 346-352

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Reference chromatogram

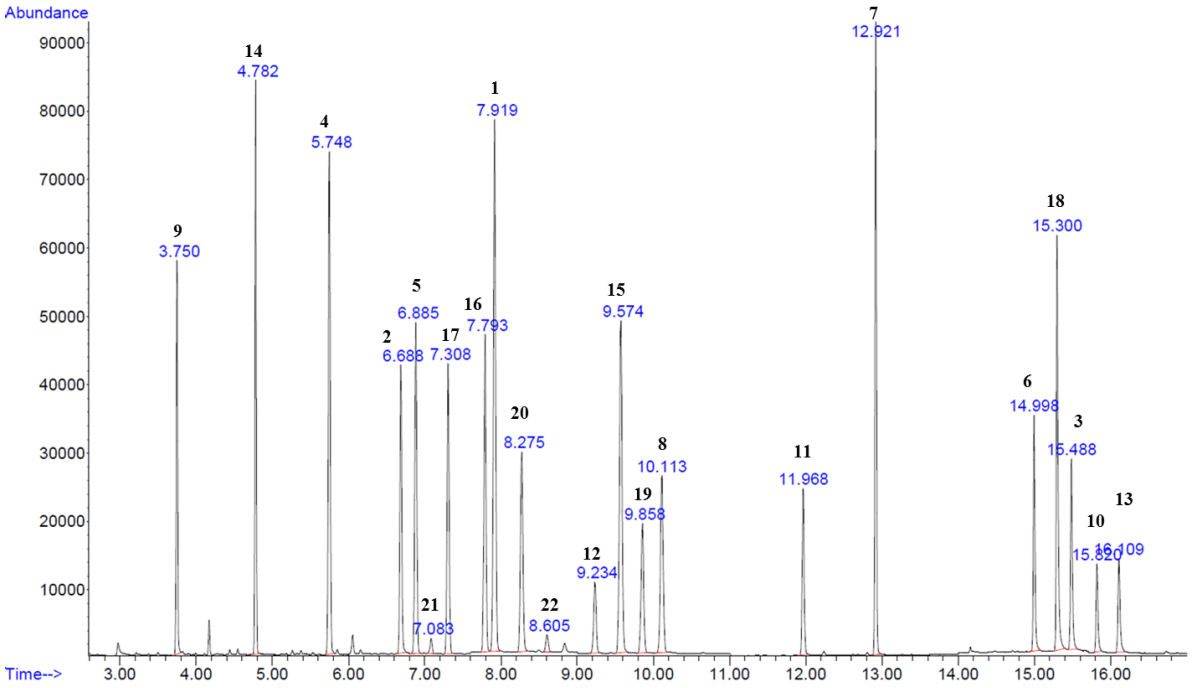

Figure. SIM chromatogram of 20 synthetic phenethylamines and 2 isotope-labeled internal standards in urine analyzed by GC/MS.

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Table. SIM fragmentation patterns of 20 synthetic phenethylamines and 2 isotope-labeled internal standards

NO.	Analyte	Monitored ions (*m/z*)	Corresponding internal standard
1	5-(2-Aminopropyl)-2,3-dihydrobenzofuran (5-APDB)	133*, 160, 134	MDA-d_5
2	1-(4-Bromophenyl)propan-2-amine (4-BA)	240*, 196, 198	MDA-d_5
3	4-Bromo-*N*-[(2-fluorophenyl)methyl]-2,5-dimethoxybenzeneethanamine (25B-NBF)	109*, 242,244	MDMA-d_5
4	1-(4-Chlorophenyl)propan-2-amine (4-CA)	240*, 152,125	MDA-d_5
5	1-(4-Chlorophenyl)-*N*-methylpropan-2-amine (4-CMA)	254*, 152,210	MDA-d_5
6	4-Chloro-*N*-[(2-fluorophenyl)methyl]-2,5-dimethoxybenzeneethanamine (25C-NBF)	109*, 198,185	MDMA-d_5
7	*N*-[(2-Chlorophenyl)methyl]-1-phenylpropan-2-amine (Clobenzorex)	125*, 127,118	MDMA-d_5
8	1-(4-Chloro-2,5-dimethoxyphenyl)propan-2-amine (DOC)	185*, 212,155	MDMA-d_5
9	*N*,*N*-Dimethyl-1-phenylpropan-2-amine (*N*,*N*-DMA)	72*, 91,56	MDA-d_5
10	2,5-Dimethoxy-*N*-[(2-methoxyphenyl)methyl]-3,4-dimethyl-benzeneethanamine (25G-NBOMe)	192*, 121,525	MDMA-d_5
11	2-(3,5-Dimethoxy-4-propoxyphenyl)ethanamine (Proscaline)	167*, 180,435	MDMA-d_5
12	2-(4-Ethyl-2,5-dimethoxyphenyl)ethanamine (2C-E)	179*, 192,405	MDA-d_5
13	*N*-(2-Fluorobenzyl)-4-iodo-2,5-dimethoxyphenethylamine (25I-NBF)	109*, 290,277	MDMA-d_5
14	1-(4-Methylphenyl)propan-2-amine (4-MA)	132*, 105,240	MDA-d_5
15	5-(2-Methylaminopropyl)-2,3-dihydrobenzofuran (5-MAPDB)	133*, 160,254	MDA-d_5
16	*N*-(2-Methoxyethyl)-1-phenylpropan-2-amine (PMEA)	148*, 268,121	MDA-d_5
17	1-(4-Methoxyphenyl)-*N*-methylpropan-2-amine (PMMA)	148*, 254,210	MDA-d_5

18	*N*-(*o*-Methoxybenzyl)-3,4-dimethoxyamphetamine (3,4-DMA-NBOMe)	121*, 178,151	MDMA-d_5
19	1-(7-Methoxy-1,3-benzodioxol-5-yl)propan-2-amine (MMDA)	165*,192,405	MDA-d_5
20	3-(1-Phenylpropan-2-ylamino)propanenitrile (Fenproporex)	293*,118,56	MDA-d_5
21	3,4-Methylenedioxyamphetamine-d_5 (MDA-d_5) (I.S.)	167*	-
22	3,4-Methylenedioxymethamphetamine-d_5 (MDMA-d_5) (I.S.)	258*	-

*Quantitative ion