SN

中华人民共和国出入境检验检疫行业标准

SN/T 2061—2008

进出口蜂王浆中硝基呋喃类代谢物残留量的测定 液相色谱-质谱/质谱法

Determination of nitrofuran metabolites residues in royal jelly for import and export—HPLC-MS/MS

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前　言

本标准的附录A、附录B为资料性附录。

本标准由国家认证认可监督管理委员会提出并归口。

本标准起草单位：中华人民共和国浙江出入境检验检疫局。

本标准主要起草人：陈笑梅、刘海山、谢文、单慧君、俞春燕、杨磊、钱艳、吴娟。

本标准系首次发布的出入境检验检疫行业标准。

进出口蜂王浆中硝基呋喃类代谢物残留量的测定 液相色谱-质谱/质谱法

1 范围

本标准规定了蜂王浆中呋喃唑酮代谢物 3-氨基-2-噁唑烷酮(3-Amino-oxazolidin-2-one,AOZ)、呋喃它酮代谢物 3-氨基-5-吗啉代甲基-2-噁唑烷酮(3-Amino-5-morpholinomethyl-oxazolidin-2-one,AMOZ)、呋喃西林代谢物氨基脲(semicarbazide,SEM)和呋喃妥因代谢物 1-氨基乙内酰脲(1-aminohydantoin,AHD)质谱/质谱测定方法。

本标准适用于蜂王浆中 AOZ、AMOZ、SEM 和 AHD 残留的定性确证和定量测定。

2 方法提要

样品经稀盐酸水解蛋白结合态中的硝基呋喃代谢物,用 2-硝基苯甲醛(2-NBA)衍生化,三氯乙酸沉淀去除蛋白质,调 pH 值 7.5 后,清液过 HLB 小柱净化,分析物采用液相色谱-串联质谱测定,内标法定量。

3 试剂和材料

除另有规定外,试剂均为分析纯,水为超纯水。

3.1 甲醇:色谱纯。

3.2 乙腈:色谱纯。

3.3 乙酸乙酯:色谱纯。

3.4 邻硝基苯甲醛(2-NBA)。

3.5 二甲亚砜:色谱纯。

3.6 乙酸铵:色谱纯。

3.7 5 mmol/L 乙酸铵水溶液:称取 0.19 g 乙酸铵溶于适量水中,再定容到 500 mL。

3.8 50%三氯乙酸:称 50 g 三氯乙酸,加 50 g 水溶解。

3.9 1 mol/L 氢氧化钠溶液:称取 40 g 氢氧化钠,用水溶解定容至 1 000 mL。

3.10 2-NBA 衍生剂(50 mmol/L):称取 0.037 8 g 邻硝基苯甲醛到烧杯中,加入 5 mL 二甲亚砜溶解,该溶液的浓度为 50 mmol/L,现配。

3.11 0.1 mol/L 磷酸氢二钾:称取 8.71 g 无水磷酸氢二钾,加入 500 mL 水溶解。

3.12 0.2 mol/L 盐酸溶液:量取 17 mL 浓盐酸用水定容至 1 000 mL。

3.13 0.2%乙酸-乙腈(7+3,体积比):0.2%乙酸 70 mL 与 30 mL 乙腈混匀。

3.14 标准溶液配制

3.14.1 硝基呋喃类代谢物标准品:AOZ、SEM·HCl、AHD·HCl、AMOZ 纯度均大于 99%。

3.14.2 硝基呋喃类代谢物同位素标准品:AOZ-D_4 纯度大于 98%,SCA-$^{15}N_2$-^{13}C·HCl 纯度大于 99%,AHD-$^{13}C_3$ 纯度大于 98%,AMOZ-D_5 纯度大于 98%。

3.14.3 标准储备液:分别称取适量 AOZ、AMOZ、SEM·HCl 和 AHD·HCl 硝基呋喃代谢物标准,用甲醇溶解并定容 1.0 mg/mL,0℃～4℃保存,有效期 6 个月。

3.14.4 混合标准溶液:分别准确移取适量 1.0 mg/mL AOZ、SEM、AHD、AMOZ 标准储备液(3.16),

用甲醇稀释定容至 10 ng/mL，0℃～4℃保存，有效期 7 d。

3.14.5 同位素内标储备液：分别称取适量 AOZ-D_4、AHD-$^{13}C_3$、AMOZ-D_5 和 SCA-$^{15}N_2$-^{13}C · HCl 硝基呋喃代谢物同位素标准品，用甲醇溶解并定容到 25 mL 棕色容量瓶中，该溶液浓度为 1.0 mg/mL，0℃～4℃保存。

3.14.6 混合同位素内标溶液：分别准确移取适量 1 mg/mL AOZ-D_4、SCA-$^{15}N_2$-^{13}C、AHD-$^{13}C_3$、AMOZ-D_5 储备液(3.14.3)，用甲醇稀释定容为 10 ng/mL，0℃～4℃保存。

3.14.7 混合标准工作溶液：分别取混合标准溶液(3.14.4)0 μL，60 μL，100 μL，200 μL，400 μL，1 000 μL。加入混合同位素内标溶液(3.14.6)200 μL，3 mL 0.2 mol/L 稀盐酸和 100 μL 2-NBA 衍生剂，混匀后 37℃放置过夜。用 1 mol/L 氢氧化钠溶液调节 pH 7.5±0.2，5 mL 乙酸乙酯液液萃取，将有机相在 40℃氮吹至干后，用 1.0 mL 0.2%乙酸-乙腈(7+3，体积比)(3.13)溶解残渣，该混合标准工作溶液相当于样品中含有 0 μg/kg，0.3 μg/kg，0.5 μg/kg，1.0 μg/kg，2.0 μg/kg，5.0 μg/kg 的硝基呋喃代谢物残留。

3.15 HLB 小柱：OASIS HLB 小柱，60 mg，3 mL 或相当者。使用前用 5 mL 甲醇和 5 mL 水预淋洗。

4 仪器和设备

4.1 液相色谱-质谱/质谱联用仪：配有电喷雾源(ESI)。

4.2 旋转浓缩器。

4.3 混匀器。

4.4 真空固相萃取装置。

4.5 pH 计：测量精度±0.02。

4.6 浓缩瓶：100 mL。

4.7 具盖塑料离心管：50 mL。

4.8 离心机：4 000 r/min。

4.9 烘箱。

5 试样制备与保存

取 500 g 代表性蜂王浆样品，在室温下解冻，等样品全部融化后搅匀，将试样均分成两份，分别装入样品瓶中，密封，并标明标记。一份作为试验样，另一份在－18℃保存。

在制样的操作过程中，应防止样品污染或发生残留物含量的变化。

6 测定步骤

6.1 提取和净化

称取混匀的蜂王浆样品 2 g(精确至 0.01 g)于 50 mL 具盖塑料离心管中，分别加入 200 μL 混合同位素内标溶液(3.14.6)，25 mL 0.2 mol/L 盐酸溶液和 100 μL 2-NBA 衍生剂(3.10)，混匀后放入 37℃±2℃烘箱内，避光放置过夜。加 1.0 mL 50%三氯乙酸，轻轻混匀后，4 000 r/min 离心 5 min，将上清液过滤。滤液用 1 mol/L 氢氧化钠溶液调节 pH 7.5±0.2，过经预淋洗的 HLB 小柱(3.15)，再加 10 mL 水清洗 HLB 小柱，用 10 mL 乙酸乙酯洗脱，收集洗脱液，40℃以下真空浓缩至干后，用 1.0 mL 0.2%乙酸-乙腈(7+3，体积比)(3.13)溶解残渣，过 0.45 μm 的滤膜，滤液供液相色谱串联质谱测定。

6.2 测定

6.2.1 液相色谱串联质谱条件

a) 色谱柱：C_8 150 mm×5 mm，5 μm 或相当者；

b) 流动相梯度洗脱条件见表 1；

c) 流速 300 μL/min；

d) 进样量:30 μL;
e) 离子源:电喷雾离子源;
f) 扫描方式:正离子扫描;
g) 检测方式:多反应监测(MRM);
h) 分辨率:单位分辨;
i) 其他参考条件参见附录 A。

表 1 梯度洗脱条件

时间/min	乙腈/%	5 mmol/L 醋酸铵水溶液/%
0.0	30	70
7	90	10
12	90	10
12.1	30	70
17	30	70

6.2.2 液相色谱/串联质谱测定

根据试样中被测样液的含量情况,选取响应值相近的标准工作液进行色谱分析。标准工作液和样液中待测物的响应值均应在校正曲线线性响应范围内,如果超出,应稀释。在上述色谱条件下 SEM、AHD、AMOZ 和 AZO 的参考保留时间分别约为 9.8 min、10.1 min、10.7 min 和 11.2 min,四种硝基呋喃代谢物标准品多反应监测(MRM)色谱图参见附录 B 中图 B.1。

6.2.3 定性测定

按照上述条件测定样品和标准工作溶液,如果样品的质量色谱峰保留时间与标准品一致,允许偏差小于±2.5%;定性离子对的相对丰度与浓度相当的标准工作溶液的相对丰度一致,相对丰度允许偏差不超过表 2 规定的范围,则可判断样品中存在相应的被测物。

表 2 定性确证时相对离子丰度的最大允许偏差

相对离子丰度/%	>50	≥50~20	≥20~10	≤10
允许的相对偏差/%	±20	±25	±30	±50

6.2.4 定量测定

按照内标法进行定量计算。

6.2.5 空白试验

除不加试样外,均按上述操作步骤进行。

7 结果计算和表述

用色谱数据处理机或用式(1)计算试样中硝基呋喃代谢物残留含量:

$$X=\frac{c_0'\times A_i\times c_{is}\times A_0\times V}{A_0'\times c_0\times A_{is}\times m_i}\times 100\% \qquad \cdots\cdots(1)$$

式中:

X——试样中被测组分的残留量;
c_0'——样品溶液中内标物的浓度;
A_i——样品溶液中待测组分的峰面积;
A_0'——样品溶液中内标物峰面积;
c_{is}——标准溶液的浓度;
c_0——标准溶液中内标物的浓度;
A_{is}——标准溶液峰面积;
A_0——标准溶液中内标物峰面积;

V——样品溶液最终定容的体积；

m_i——样品溶液所代表的试样质量，单位为克(g)。

8 测定低限、回收率

8.1 测定低限

硝基呋喃类代谢物残留测定低限均为 0.5 μg/kg。

8.2 回收率

在蜂王浆样品中添加硝基呋喃类代谢物浓度在 0.5 μg/kg、1.0 μg/kg、2.0 μg/kg 时，回收率见表 3。

表 3 蜂王浆中硝基呋喃类代谢物测定回收率范围

添加浓度	0.5 μg/kg	1.0 μg/kg	2.0 μg/kg
SEM	87.6%～104.0%	89.1%～106.0%	91.0%～106.0%
AHD	87.2%～100.4%	87.6%～102.0%	84.0%～97.0%
AMOZ	91.6%～103.2%	90.7%～104.0%	87.5%～104.0%
AOZ	91.6%～104.2%	89.6%～105.0%	88.0%～101.0%

附 录 A
（资料性附录）
API 4000 LC-MS/MS 系统电喷雾离子源参考条件[1)]

监测离子对及电压、气压参数：

a) 气帘气压力(CUR)：172.375 kPa(25 Psi)；
b) 雾化气压力(GS1)：289.59 kPa(42 Psi)；
c) 辅助气压力(GS2)：310.275 kPa(45 Psi)；
d) 电喷雾电压(IS)：4 800 V；
e) 离子源温度(TEM)TEM：540℃；
f) 碰撞气压力(CAD)：34.475 kPa(5 Psi)；
g) EP 电压：9 V；
h) 离子对、DP、CE 和 CXP 见表 A.1。

表 A.1 离子对、DP、CE 和 CXP

名 称	定性离子对 m/z	保留时间 /min	去簇电压 (DP)/V	碰撞气能量 (CE)/V	碰撞室出口电压(CXP)/V
SEM NBA	209.2/166.2[a]	9.8	60	15	11
	209.2/192.2	9.8	60	17	11
SCA $^{15}N_2$ ^{13}C NBA	212.2/168.2	9.8	60	15	11
AHD NBA	249.2/134.1[a]	10.1	66	18	11
	249.2/104.1	10.1	66	32	11
AHD $^{13}C_3$ NBA	252.2/134.2	10.1	66	18	11
AMOZ NBA	335.2/262.3[a]	10.7	66	25	18
	335.2/291.3	10.7	66	17.7	18
AMOZ D_5 NBA	340.3/296.4	10.7	66	18	11
AOZ NBA	236.2/134.2[a]	11.2	66	18.3	12
	236.2/104.1	11.2	66	32	18
AOZ D_4 NBA	240.2/134.2	11.2	66	18	11
[a] 为定量离子对。					

1) 非商业性声明：附录 A 所列参数是在 API 4000 质谱仪完成的，此处列出试验用仪器型号仅是为了提供参考，并不涉及商业目的，鼓励标准使用者尝试不同厂家和型号的仪器。

附 录 B
（资料性附录）
硝基呋喃类代谢物标准品多反应监测(MRM)色谱图

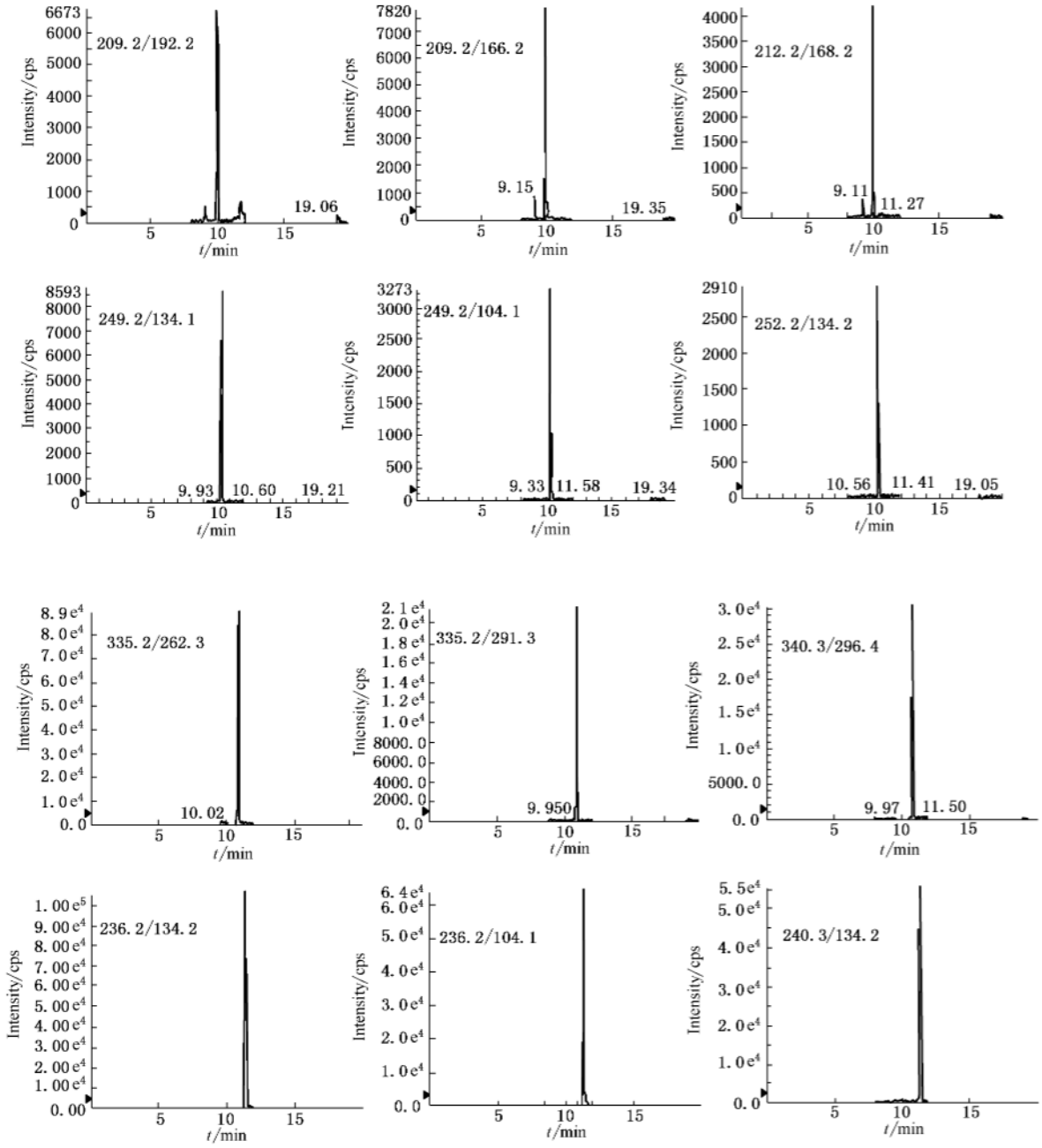

图 B.1 硝基呋喃类代谢物标准品(2.0 μg/kg)和同位素内标(1.0 μg/kg)多反应监测(MRM)色谱图

Foreword

Annex A、annex B of this standard are both informative annexes.

This standard is proposed by and is under the charge of Certification and Accreditation Administration of the People's Republic of China.

This standard is drafted by Zhejiang Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Chen Xiaomei, Liu Haishan, Xie Wen, Shan Huijun, Yu Chunyan, Yang Lei, Qian Yan, Wu Juan.

This standard is a professional standard for entry-exit inspection and quarantine promulgated for the first time.

Note: This English Version, a translation from the Chinese text, is solely for guidance.

Determination of nitrofuran metabolites residues in royal jelly for import and export —HPLC-MS/MS

1 Scope

This standard specifies the method of determination of furazolidone metabolite 3-Amino-oxazolidin-2-one(AOZ), furaltadone metabolite 3-Amino-5-morpholinomethyl-oxazolidin-2-one(AMOZ), nitrofurazone metabolite semicarbazide(SEM), and nitrofurantoin metabolite 1-aminohydantoin(AHD) residues in royal jelly by high-performance liquid chromatography-tandem mass spectrometry.

This standard is applicable to the quantify and confirm of AOZ, AMOZ, AHD, SEM residues in royal jelly.

2 Principle

Protein in sample need to be precipitated by trichloroacetic acid solution after combined hydrolysis of the protein-bound nitrofuran metabolites and derivatisation of the resulting metabolites with 2-nitrobenzaldehyde (NBA) during an overnight incubation. Adjust the solution to pH 7.5 and clean up by HLB cartridge. It was determined and confirmed by high-performance liquid chromatography-tandem mass spectrometry. Internal standard method was used to quantify.

3 Reagents and materials

Unless specified notes, all reagents should be analytical grade, "water" is redistilled or deionized.

3.1 Methanol: HPLC grade.

3.2 Acetonitrile: HPLC grade.

3.3 Ethyl acetate: HPLC grade.

3.4 2-Nitrobenzaldehyde(2-NBA).

3.5 Dimethyl sulfoxide: HPLC grade.

3.6 Ammonium acetate: HPLC grade.

3.7 5 mmol/L Ammonium acetate solution: Dissolve 0.19 g Ammonium acetate in 500 mL water.

3.8 50% Trichloroacetic acid solution: Dissolve 50 g trichloroaceticacid in 50 mL water.

3.9 1 mol/L Sodium hydroxide solution: Dissolve 40 g sodium hydroxide in 1 000 mL water.

3.10 2-NBA solution(50 mmol/L). Dissolve 0.037 8 g 2-nitrobenzaldehyde in 5 mL dimethyl sulfoxide, prepared freshly.

3.11 0.1 mol/L Dipotassium hydrogen phosphate solution: Dissolve 8.71 g dipotassium hydrogen phosphate anhydrous in 500 mL water.

3.12 Hydrochloric acid(0.2 mol/L): Dilute 17 mL hydrochloric acid to 1 000 mL with water.

3.13 0.2% Acetic acid-acetonitrile(7+3, *V*/*V*): Mix 70 mL 0.2% acetic acid with 30 mL acetonitrile.

3.14 Standard solution Preparation:

3.14.1 Nitrofuran metabolites standard: AOZ, SEM · HCl, AHD · HCl, AMOZ, purity>99%.

3.14.2 Nitrofuran metabolites isotope standard: AOZ-D_4 purity>98%, AHD-$^{13}C_3$ purity>98%, AMOZ-D_5 purity>98%. SCA-$^{15}N_2$-^{13}C · HCl purity>99%.

3.14.3 Nitrofuran metabolites standard stock solution: Accurately weigh an appropriate amount of AOZ, AMOZ, SEM · HCl and AHD · HCl, and dissolve with methanol to prepare a standard stock solution of 10 ng/mL. This standard stock solution should be stored at 0℃ ~ 4℃ and stable for 6 months.

3.14.4 Nitrofuran metabolites mix standard solution: According to the requirement, pipette adequate amount of standard stock solution(3.14.3) and dilute with methanol to prepare mix standard interim solution of 10 ng/mL. This standard stock solution should be stored at 0℃ ~4℃ and stable for 7 d.

3.14.5 Nitrofuran metabolites isotope standard stock solution: Accurately weigh an appropriate amount of AOZ-D_4, SCA-$^{15}N_2$-^{13}C · HCl, AHD-$^{13}C_3$ and AMOZ-D_5, and dissolve with methanol to prepare an isotope standard stock solution of 1.0 mg/mL. This isotope standard stock solution should be stored at 0℃ ~4℃.

3.14.6 Nitrofuran metabolites mix isotope standard solution: According to the requirement, pipette adequate amount of isotope standard stock solution(3.14.5) and dilute with methanol to prepare mix isotope standard interim solution of 10 ng/mL. This isotope standard stock solution should be stored at 0℃ ~4℃.

3.14.7 Nitrofuran metabolites mix standard working solution: Pipette 0, 60, 100, 200, 400, 1 000 μL nitrofuran metabolites mix standard solution (3.14.4) in six centrifuge tubes. Add 200 μL 10 ng/mL nitrofuran metabolites mix isotope standard solution (3.14.6), 3 mL 0.2 mol/L HCl(3.11) and 100 μL 2-NBA solution (3.4) in each tube, shake the centrifuge tube with vortex mixer and place it in an oven at 37℃, and keep away from light overnight. Adjust the pH of the solution to 7.5±0.2 with 1 mol/L sodium hydroxide solution. Extract the solution with 5 mL ethyl acetate, evaporate the organic phase by nitrogen evaporator at 40℃. Dissolve the residue with 1.0 mL 0.2% acetic

acid-acetonitrile(7+3, *V*/*V*)(3.13). The six standard solutions correspond to samples containing 0, 0.3, 0.5, 1.0, 2.0, 5.0 μg/kg nitrofuran metabolites.

3.15 HLB cartridge: OASIS HLB Extraction cartridge, 60 mg 3 mL, wash the cartridge with 5 mL methanol followed by 5 mL water.

4 Apparatus and equipment

4.1 High-performance liquid chromatography-tandem mass spectrometer. equipped with electrospray ion source.

4.2 Rotary evaporator.

4.3 Vortex mixer.

4.4 Solid phase extraction equipment.

4.5 pH meter: Accurate to 0.02.

4.6 Concentratiom flask: 100 mL.

4.7 Plastic centrifuge tube: 50 mL.

4.8 Centrifuge: 4 000 r/min.

4.9 Oven.

5 Preparation and storage of test sample

Around 500 g of royal jelly sample is prepared. Melt it at room temperature and mix thoroughly after the sample melted. Keep the prepared sample into two sample bottles, seal and label. One is used as test sample and the other is stored at below −18℃ temperature.

In the course of sample preparation, precautions must be taken to avoid contamination or any factors that may cause the change of residue content.

6 Determination procedure

6.1 Extraction and cleanup

Weigh 2 g royal jelly (accurate to 0.01 g) into 50 mL centrifuge tube. Add 200 μL 10 ng/mL nitrofuran metabolites mix isotope standard solution (3.14.6). Add 25 mL 0.2 mol/L HCl and 100 μL 2-NBA solution. Vortex the centrifuge tube and place it in an oven at 37℃ ± 2℃. Keep it away from light overnight. Add 1.0 mL 50% trichloroacetic acid (3.7), shake the centrifuge tube lightly. Centrifuge for 5 min at 4 000 r/min, filter the supernatant layer. Adjust pH of the filtrate to 7.5 ± 0.2 with

1 mol/L sodium hydroxide solution. Transfer the filtrate to the HLB cartridge, then wash the cartridge with 10 mL water, and elute the column with 10 mL ethyl acetate. Evaporate the solution to dryness in a water bath below 40℃, dissolve the residue with 1.0 mL 0.2% acetic acid-acetonitrile (7+3, *V*/*V*)(3.13). Filter the solution with 0.45 μm filter. The solution is used for analysis by LC-MS/MS.

6.2 Determination

6.2.1 LC-MS/MS operating conditions

a) Column: C_8 5 μm, 150 mm×5 mm(i.d.),5 μm or equivalent;

b) Mobile phase: see table 1;

Table 1—Gradient of mobile phase

Time/min	Acetonitrile/%	5 mmol/L Ammonium acetate solution/%
0	30	70
7.0	90	10
12.0	90	10
12.1	30	70
17	30	70

c) Flow rate: 300 μL/min;

d) Injection volume: 30 μL;

e) Source: ESI;

f) Polarity: Positive;

g) Mode: Multiple reaction monitoring(MRM);

h) Resolution:unit;

i) other instrumental settings may be adjusted, if necessary, to optimize performance. See Annex A.

6.2.2 LC-MS/MS determination

According to the approximate concentration of analyte in sample solution, select the standard working solution with similar responses to that of sample solution. The responses of the analyte in the standard working solution and the sample solution shall be within the linear range of the instrument detection. If it is out of the range, the sample solution must be diluted. Under the above LC-MS/MS operating condition, the retention times of SEM-NBA, AHD-NBA, AMOZ-NBA, AOZ-NBA are about

9.8 min, 10.1 min, 10.7 min and 11.2 min. For MRM chromatograms of four nitrofuran metabolites standards are shown in Figure B.1 of annex B.

6.2.3 Qualitative determination

Under LC-MS/MS conditions, the working solution and sample solution were injected. If the retention times of sample chromatogram peaks are consistent with that of standard solution, the permitted tolerance is less than ±2.5%. The relative intensities of sample transitions shall correspond to those of standard solution transitions for confirmation. The concentration of standard solution should be same with those of sample solution. The permitted tolerances listed in table 2, then the corresponding analyte must be present in sample.

Table 2—Maximum permitted tolerances for relative ion intensities while confirmation

Relative intensity/%	>50	>20~50	>10~20	≤10
Permitted tolerances/%	±20	±25	±30	±50

6.2.4 Quantitative determination

Internal standard method is used to quantify.

6.2.5 Blank test

The operation of the blank test is the same as the method described in the determination procedure, but with the omission of sample addition.

7 Calculation and expression of result

Calculate the contents of nitrofuran metabolites residues in the test sample by LC-MS/MS data process or accord to the formula (1):

$$X = \frac{c_0' \times A_i \times c_{is} \times A_0 \times V}{A_0' \times c_0 \times A_{is} \times m_i} \times 100\% \quad \cdots\cdots (1)$$

where

X——the residue content of nitrofuran metabolites in sample;

c_0'——the concentration of inter standard in sample;

A_i——area of nitrofuran metabolites in sample solution;

A_0'——area of inter standard in sample solution;

c_{is}——concentration of standard solution;

c_0——concentraton of inter standard in standard solution;

A_{is}——area of standard solution;

A_0——area of inter standard solution in standard solution;

V——the final volume of sample solution, mL;

m_i——the corresponding mass of the test sample in the final sample solution, g.

8 Limit of quantification (LOQ) and recovery

8.1 Limit of quantification

The limits of quantification of nitrofuran metabolites are 0.5 μg/kg.

8.2 Recovery

According to the experiment data, the fortifying concentrations of nitrofuran metabolites and corresponding recoveries are listed in table 3:

Table 3—The fortifying concentrations of nitrofuran metabolites and corresponding recoveries

Spike level	0.5 μg/kg	1.0 μg/kg	2.0 μg/kg
SEM	87.6%～104.0%	89.1%～106.0%	91.0%～106.0%
AHD	87.2%～100.4%	87.6%～102.0%	84.0%～97.0%
AMOZ	91.6%～103.2%	90.7%～104.0%	87.5%～104.0%
AOZ	91.6%～104.2%	89.6%～105.0%	88.0%～101.0%

Annex A
(informative annex)
LC-MS/MS operating conditions[1)]

Optimal LC-MS/MS operating conditions:

a) CUR:172.375 kPa(25 Psi);

b) GS1:289.59 kPa(42 Psi);

c) GS2:310.275 kPa(45 Psi);

d) IS:4 800 V;

e) TEM:540℃;

f) CAD:34.475 kPa(5 Psi);

g) EP:9 V;

h) MRM transitions,DP、CE and CXP are listed in Table A.1.

Table A.1—MRM transitions,DP、CE and CXP

Compound	MRM Transitions m/z	Rententi on time /min	DP/V	CE/V	CXP/V
SEM-NBA	209.2/166.2[a]	9.8	60	15	11
	209.2/192.2	9.8	60	17	11
SCA-$^{15}N_2$-^{13}C-NBA	212.2/168.2	9.8	60	15	11
AHD-NBA	249.2/134.1[a]	10.1	66	18	11
	249.2/104.1	10.1	66	32	11
AHD-$^{13}C_3$-NBA	252.2/134.2	10.1	66	18	11
AMOZ-NBA	335.2/262.3[a]	10.7	66	25	18
	335.2/291.3	10.7	66	17.7	18
AHOZ-D_5-NBA	340.3/296.4	10.7	66	18	11
AOZ-NBA	236.2/134.2[a]	11.2	66	18.3	12
	236.2/104.1	11.2	66	32	18
AOZ-D_4-NBA	240.2/134.2	11.2	66	18	11
[a] represents quatitative ion pair.					

1) Non-commercial statement:the equipments and their models involved in the standardard method are not related to commercial motive. The analysts are encouraged to use different equipments and models.

Annex B

(informative annex)

MRM chromatograms of four nitrofuran metabolites standards

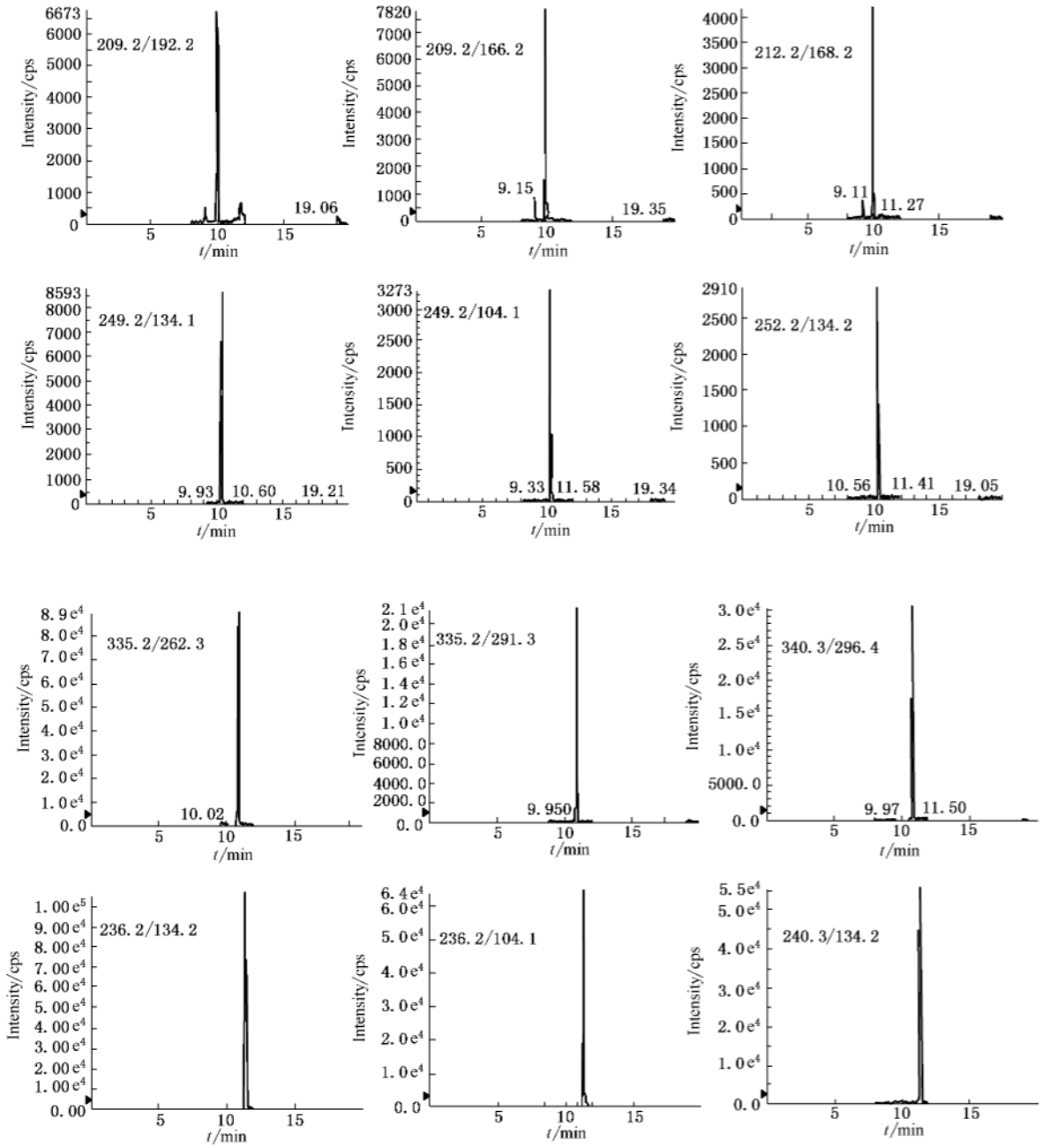

Figure B.1 —MRM chromatograms of four nitrofuran metabolites standards (2.0 μg/kg) and isotope standard(1.0 μg/kg)