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中华人民共和国出入境检验检疫行业标准

SN/T 2579—2010

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进出口蜂王浆中 10 种硝基咪唑类  
药物残留量的测定  
液相色谱-质谱/质谱法

Determination of ten nitroimidazoles residues  
in royal jelly for import and export—  
LC-MS/MS

2010-05-27 发布

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中华人民共和国  
国家质量监督检验检疫总局 发布

## 前 言

本标准按照 GB/T 1.1—2009 给出的规则起草。

本标准由国家认证认可监督管理委员会提出并归口。

本标准由中华人民共和国浙江出入境检验检疫局负责起草。

本标准主要起草人：谢文、陈笑梅、丁慧瑛、奚君阳、张慧敏。

# 进出口蜂王浆中 10 种硝基咪唑类 药物残留量的测定 液相色谱-质谱/质谱法

## 1 范围

本标准规定了进出口蜂王浆中硝基咪唑及其代谢物残留测定的抽样和液相色谱-质谱/质谱测定方法。

本标准适用于蜂王浆中羟基甲硝唑、2-甲硝咪唑、羟基二甲硝咪唑、甲硝唑、二甲硝咪唑、洛硝哒唑、氯甲硝咪唑、苯硝咪唑、羟基异丙硝唑、异丙硝唑残留量的检测。

## 2 规范性引用文件

下列文件对于本文件的应用是必不可少的。凡是注日期的引用文件，仅注日期的版本适用于本文件，凡是不注日期的引用文件，其最新版本（包括所有的修改单）适用于本文件。

GB/T 6682 分析实验室用水规格和试验方法

## 3 方法提要

用甲醇沉淀样品中蛋白质，上清液加磷酸盐缓冲溶液后，乙酸乙酯提取，再用 Oasis(HLB)和 C<sub>18</sub> 固相萃取小柱净化，液相色谱质谱/质谱测定，外标法或内标法定量。

## 4 试剂和材料

除另有规定外，所有试剂均为分析纯，水为 GB/T 6682 规定的一级水。

- 4.1 乙腈：高效液相色谱级。
- 4.2 甲醇：高效液相色谱级。
- 4.3 乙酸乙酯：高效液相色谱级。
- 4.4 甲酸：高效液相色谱级。
- 4.5 氯化钠。
- 4.6 磷酸二氢钠。
- 4.7 氢氧化钠。
- 4.8 0.1 mol/L 氢氧化钠溶液：称取 4 g 氢氧化钠，并用水稀释至 1 L。
- 4.9 磷酸盐缓冲溶液：溶解 13.8 g 磷酸二氢钠于 950 mL 水中，用 0.1 mol/L 氢氧化钠溶液调节溶液 pH 值到 8.0，最后用水稀释至 1 L。
- 4.10 羟基甲硝唑、2-甲硝咪唑、羟基二甲硝咪唑、甲硝唑、二甲硝咪唑、洛硝哒唑、氯甲硝咪唑、苯硝咪唑、羟基异丙硝唑、异丙硝唑标准品：纯度大于等于 98%，见附录 A 表 A.1。
- 4.11 羟基二甲硝咪唑-D3、二甲硝咪唑-D3、洛硝哒唑-D3、羟基异丙硝唑-D3、异丙硝唑-D3 同位素标准品：纯度大于等于 98%。

- 4.12 标准储备溶液:分别称取经折算适量标准品(4.10)(精确至 0.1 mg),分别用甲醇溶解并定容至 100 mL,溶液浓度为 100  $\mu\text{g}/\text{mL}$ ,1  $^{\circ}\text{C}$ ~4  $^{\circ}\text{C}$  冰箱保存。有效期 3 个月。
- 4.13 同位素内标标准储备溶液:分别称取适量标准品(4.11)(精确至 0.1 mg),分别用甲醇溶解定容至 100 mL,溶液浓度为 100  $\mu\text{g}/\text{mL}$ ,1  $^{\circ}\text{C}$ ~4  $^{\circ}\text{C}$  冰箱保存。
- 4.14 空白样品提取液:用不含 10 种硝基咪唑及其代谢物的蜂王浆样品,按照第 7 章制备空白样品溶液。
- 4.15 标准工作溶液:根据需要用空白样品提取液(4.14)将标准储备液稀释成浓度为 5 ng/mL、10 ng/mL、20 ng/mL、50 ng/mL、60 ng/mL 的混合标准工作溶液,相当于样品中含有 5  $\mu\text{g}/\text{kg}$ 、10  $\mu\text{g}/\text{kg}$ 、20  $\mu\text{g}/\text{kg}$ 、50  $\mu\text{g}/\text{kg}$ 、60  $\mu\text{g}/\text{kg}$  硝基咪唑类药物。二甲硝咪唑-D3、洛硝哒唑-D3 溶液浓度为 20 ng/mL,羟基二甲硝咪唑-D3、羟基异丙硝唑-D3、异丙硝唑-D3 溶液浓度 10 ng/mL。
- 4.16 Oasis(HLB)固相萃取小柱,500 mg,5 mL 或相当者。使用前依次用 5 mL 甲醇,5 mL 水预洗。
- 4.17  $\text{C}_{18}$  固相萃取小柱,500 mg,3 mL 或相当者。使用前依次用 5 mL 甲醇预洗。
- 4.18 微孔滤膜:0.45  $\mu\text{m}$ ,有机相。

## 5 仪器和设备

- 5.1 高效液相色谱-质谱/质谱仪:配有电喷雾离子源。
- 5.2 天平:感量为 0.000 1 g 和 0.01 g。
- 5.3 固相萃取装置。
- 5.4 离心机:大于等于 6 000 r/min。
- 5.5 pH 计:测量精度 $\pm$ 0.02 pH 单位。
- 5.6 旋涡混合器。
- 5.7 减压浓缩仪。
- 5.8 具塞离心管:聚四氟乙烯,50 mL。

## 6 试样制备与保存

- 6.1 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  $^{\circ}\text{C}$  保存。
- 6.2 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。

## 7 测定步骤

### 7.1 提取

称取 2 g 试样(精确到 0.01 g)置于 50 mL 具塞离心管中,加 0.2 mL 二甲硝咪唑-D3、洛硝哒唑-D3 同位素内标溶液(200 ng/mL),羟基二甲硝咪唑-D3、羟基异丙硝唑-D3、异丙硝唑-D3 同位素内标溶液(100 ng/mL),加 10 mL 水,混匀,静置 2 min,再加甲醇至 20 mL,于旋涡混合器上以 2 000 r/min 混匀 1 min,以 6 000 r/min 离心 10 min,移取 10.0 mL 上清液,加 10 mL 磷酸盐缓冲溶液(4.9)稀释,混匀,加 2 g 氯化钠和 20 mL 乙酸乙酯提取,于旋涡混合器上以 2 000 r/min,混匀 1 min,以 6 000 r/min 离心 5 min,将上层乙酸乙酯收集于浓缩瓶中,样品残渣再加 20 mL 乙酸乙酯,重复上述操作,合并乙酸乙酯提取溶液,在 45  $^{\circ}\text{C}$  以下水浴减压浓缩至近干。

## 7.2 净化

加 15 mL 水分次溶解残渣并将溶液转移至 HLB 固相萃取小柱中(4.16),弃去流出液,抽干,用 6 mL 甲醇洗脱,控制流速 1 mL/min~2 mL/min,收集全部洗脱液并将溶液转移至 C<sub>18</sub> 固相萃取小柱中(4.17),再加 9 mL 甲醇洗脱,控制流速 1 mL/min~2 mL/min,收集全部流出液,洗脱液在 50 °C 以下水浴中减压浓缩至近干,用 1.0 mL 甲醇-0.15% 甲酸溶液(1+9, 体积比)溶解残渣,混匀,溶液过 0.45 μm, 滤膜,供液相色谱-质谱/质谱仪测定。

## 7.3 测定

### 7.3.1 液相色谱-串联质谱条件

- a) 色谱柱:C<sub>8</sub> 柱,150 mm×4.6 mm(内径),5 μm 或相当者;
- b) 流动相梯度洗脱程序见表 1;

表 1 梯度洗脱程序

时间/min	甲醇/%	0.15%甲酸水溶液/%
0	13	87
8.0	13	87
8.1	40	60
15.5	80	20
19.0	100	0
19.1	13	87
25.0	13	87

- c) 流速:0.4 mL/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;
- h) 雾化气压力(GS1)、气帘气压力(CUR)、辅助气流速(GS2)均为高纯氮气或其他合适气体;使用前应调节各气体流量以及离子源温度(TEM)使质谱灵敏度达到检测要求,详细参考条件及监测离子对(m/z)参见附录 B。

### 7.3.2 高效液相色谱-串联质谱测定

根据试样中被测样液的含量,选定浓度相近的混合基质标准溶液,待测物的响应值应在仪器检测的线性范围内。对混合基质标准溶液及样液等体积参插进样测定。在上述色谱条件下羟基甲硝唑、2-甲硝咪唑、羟基二甲硝咪唑、甲硝唑、二甲硝咪唑、洛硝咪唑、氯甲硝咪唑、苯硝咪唑、羟基异丙硝唑、异丙硝唑的参考保留时间分别约为 8.9 min、9.4 min、11.4 min、11.9 min、13.8 min、14.3 min、15.8 min、16.3 min、17.5 min、18.5 min,标准溶液的选择性离子流图参见附录 C 中图 C.1。

### 7.3.3 定性测定

按照上述条件测定样品和混合基质标准工作液,如果检测的质量色谱峰保留时间与混合基质标准

工作液一致,允许偏差小于±2.5%。定性离子对的相对丰度与浓度相当混合基质标准工作液的相对丰度一致,相对丰度偏差不超过表2的规定,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	>20~50	>10~20	≤10
允许的相对偏差/%	±20	±25	±30	±50

7.3.4 空白试验

除不加试样外,均按上述操作步骤进行。

8 结果计算和表述

用色谱数据处理机或按式(1)计算试样中硝基咪唑类药物的残留含量,计算结果需扣除空白值:

$$X_i = \frac{c_i V}{m} \dots\dots\dots(1)$$

式中:

- $X_i$  —— 试样中硝基咪唑类药物残留量,单位为微克每千克( $\mu\text{g}/\text{kg}$ );
- $c_i$  —— 基质标准溶液中硝基咪唑类药物的浓度,单位为纳克每毫升( $\text{ng}/\text{mL}$ );
- $V$  —— 样液最终定容体积,单位为毫升( $\text{mL}$ );
- $m$  —— 最终样液代表的试样质量,单位为克( $\text{g}$ );

9 方法的测定低限(LOQ)和回收率

9.1 测定低限

硝基咪唑类药物残留量测定低限为  $10 \mu\text{g}/\text{kg}$ 。

9.2 回收率

回收率的实验数据(在不同添加浓度范围内)见表3。

表3 10种硝基咪唑及其代谢物添加回收率范围( $n=6$ )

化合物	添加浓度/ $(\mu\text{g}/\text{kg})$	回收率/%
羟基甲硝唑	10	74.3~90.1
	20	72.5~99.5
	50	80.0~92.4
2-甲硝咪唑	10	70.7~79.3
	20	74.5~81.5
	50	70.2~83.6
羟基二甲硝咪唑	10	95.0~104.0
	20	95.5~101.0
	50	98.0~100.0

表 3 10 种硝基咪唑及其代谢物添加回收率范围( $n=6$ )(续)

化合物	添加浓度/ $(\mu\text{g}/\text{kg})$	回收率/%
甲硝唑	10	90.3~96.4
	20	91.5~100.0
	50	90.8~97.8
二甲硝咪唑	10	94.6~103.0
	20	88.0~97.0
	50	91.2~100.0
洛硝哒唑	10	94.9~105.0
	20	88.5~96.5
	50	93.2~99.0
氯甲硝咪唑	10	95.3~101.0
	20	88.0~94.5
	50	84.6~89.4
苯硝咪唑	10	75.3~90.1
	20	74.0~83.5
	50	76.4~80.0
羟基异丙硝唑	10	93.4~99.0
	20	95.5~103.0
	50	97.2~100.6
异丙硝唑	10	88.7%~96.6
	20	89.5~98.0
	50	93.6~98.8

附 录 A  
(规范性附录)  
硝基咪唑标准品信息

表 A.1 硝基咪唑类药物标准品信息

中文名称	英文名称	CAS 编号	分子式	相对分子质量
羟基甲硝唑	1-(2-hydroxyethyl)-2-hydroxy-methyl-5-nitroimidazol(MNZOH)	4812-40-2	C <sub>8</sub> H <sub>9</sub> N <sub>3</sub> O <sub>4</sub>	187.15
2-甲硝咪唑	2-methyl-5-nitroimidazole	88054-22-2	C <sub>4</sub> H <sub>5</sub> N <sub>3</sub> O <sub>2</sub>	127.10
羟基二甲硝咪唑	2-hydroxymethyl-1-methyl-5-nitroimidazole(DMZOH、HMMNI)	936-05-0	C <sub>5</sub> H <sub>7</sub> N <sub>3</sub> O <sub>3</sub>	157.13
甲硝唑	metronidazole(MNZ)	443-48-1	C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub>	171.15
二甲硝咪唑	dimetridazole(DMZ)	551-92-8	C <sub>8</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub>	141.13
洛硝哒唑	ronidazole(RNZ)	7681-76-7	C <sub>6</sub> H <sub>8</sub> N <sub>4</sub> O <sub>4</sub>	200.15
氯甲硝咪唑	5-chloro-1-methyl-4-nitroimidazole	4897-25-0	C <sub>4</sub> H <sub>4</sub> ClN <sub>3</sub> O <sub>2</sub>	161.55
苯硝咪唑	5-nitrobenzimidazole	94-52-0	C <sub>7</sub> H <sub>5</sub> N <sub>3</sub> O <sub>2</sub>	163.14
羟基异丙硝唑	ipronidazole-OH(IPZOH)	35175-14-5	C <sub>7</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub>	185.18
异丙硝唑	ipronidazole(IPZ)	14885-29-1	C <sub>7</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub>	169.18
羟基二甲硝咪唑-D3	DMZOH-D3	—	—	160.2
二甲硝咪唑-D3	DMZ-D3	—	—	144.2
洛硝哒唑-D3	RNZ-D3	—	—	203.2
羟基异丙硝唑-D3	IPZOH-D3	—	—	188.2
异丙硝唑-D3	IPZ-D3	—	—	172.2



**附录 B**  
(资料性附录)

**API 4000 LC-MS/MS 系统电喷雾离子源参考条件<sup>1)</sup>**

API 4000 LC-MS/MS 系统电喷雾离子源参考条件:

- a) 电喷雾电压(IS):4 800 V;
- b) 雾化气压力(GS1):289.59 kPa(42 psi);
- c) 气帘气压力(CUR):186.165 kPa(27 psi);
- d) 辅助气流速(GS2):310.275 kPa(45 psi);
- e) 离子源温度(TEM):540 °C;
- f) 碰撞气(CAD):5;
- g) 定性离子对、定量离子对、去簇电压(DP)、碰撞气能量(CE)及碰撞室出口电压(CXP)见表 B.1。

**表 B.1 定性离子对、定量离子对、去簇电压(DP)、碰撞气能量和碰撞室出口电压**

名称	离子对 m/z	去簇电压 (DP)/V	碰撞气能量 (CE)/V	碰撞室出口电压 (CXP)/V	内标化 合物名称
羟基甲硝唑	188.1/123.0 <sup>a</sup> 188.1/126.0	53	19 26	10	羟基二甲硝唑-D3
2-甲硝唑	128.0/82.0 <sup>a</sup> 128.0/42.0	60	26 51	10	—
羟基二甲硝唑	158.0/140.1 <sup>a</sup> 158.0/55.0	48	16 31	10	羟基二甲硝唑-D3
甲硝唑	172.0/128.2 <sup>a</sup> 172.0/82.1	62	21 35	11 14	羟基二甲硝唑-D3
二甲硝唑	142.0/96.0 <sup>a</sup> 142.0/81.0	62	23 36	8 14	二甲硝唑-D3
洛硝唑	201.1/140.2 <sup>a</sup> 201.1/110.2	37	17 25	8	洛硝唑-D3
氯甲硝唑	162.0/116.0 <sup>a</sup> 162.0/145.0	68	26 24	10	—
苯硝唑	164.0/118.0 <sup>a</sup> 164.0/91.0	64	32 52	10	—
羟基异丙硝唑	186.3/168.1 <sup>a</sup> 186.3/122.3	45	19 29	9 5	羟基异丙硝唑-D3

1) 非商业性声明:附录表 B 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。

表 B.1 定性离子对、定量离子对、去簇电压(DP)、碰撞气能量和碰撞室出口电压(续)

名 称	离子对 m/z	去簇电压 (DP)/V	碰撞气能量 (CE)/V	碰撞室出口电压 (CXP)/V	内标化 合物名称
异丙硝唑	170.2/109.0 <sup>a</sup> 170.2/124.1	63	37 26	10	异丙硝唑-D3
羟基二甲硝咪唑-D3	161.2/143.1	51	18		6
二甲硝咪唑-D3	145.2/99.0	85	25		10
洛硝哒唑-D3	204.2/143.3	50	17		10
羟基异丙硝唑-D3	189.2/171.2	60	20		9
异丙硝唑-D3	173.1/127.2	67	28		6

<sup>a</sup> 该离子对为定量离子对。

附录 C  
(资料性附录)

硝基咪唑及其代谢物标准品选择性离子流图

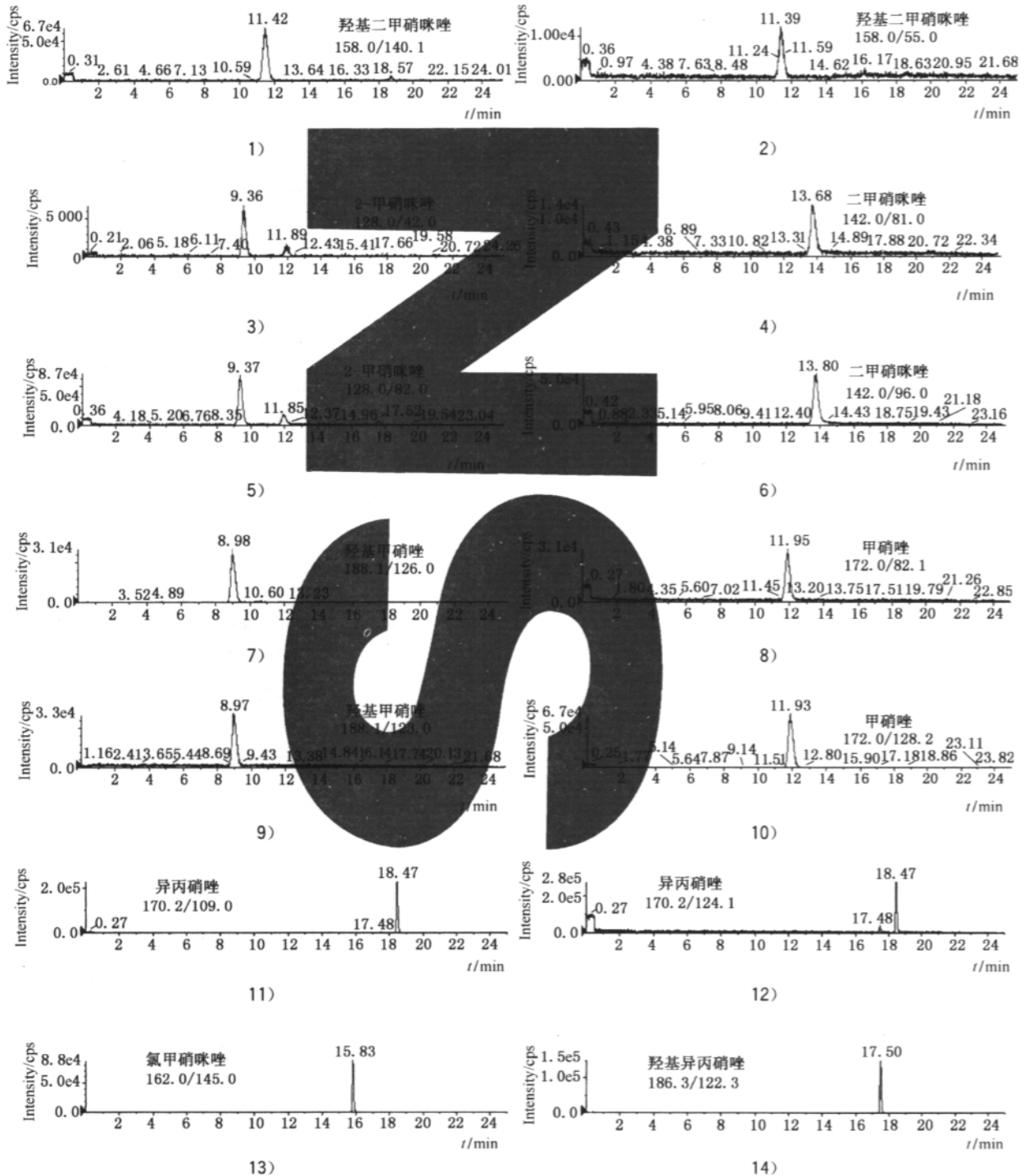


图 C.1 硝基咪唑及其代谢物(10 ng/mL)的标准品的选择性离子流图

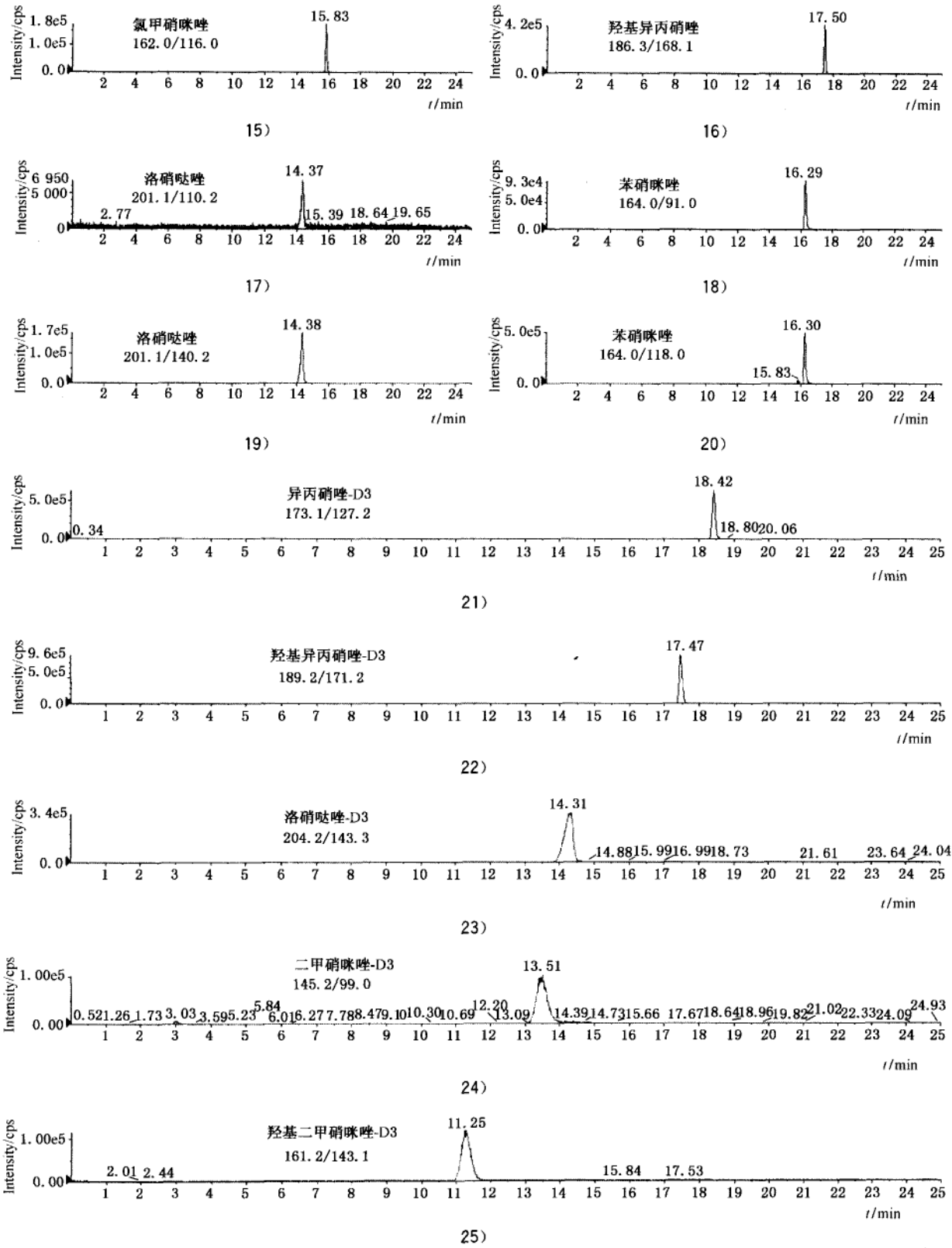


图 C.1 硝基咪唑及其代谢物(10 ng/mL)的标准品的选择性离子流图(续)

## Foreword

This standard was proposed by and is under the charged 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 People's Republic of China.

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# Determination of ten nitroimidazoles residues in royal jelly for import and export— LC-MS/MS

## 1 Scope

This standard specifies the method of sample preparation and determination of ten nitroimidazoles residues in royal jelly for import and export by LC-MS/MS.

This standard is applicable to the determination of residues of 1-(2-hydroxyethyl)-2-hydroxy-methyl-5-nitroimidazol, 2-methyl-5-nitroimidazole, 2-hydroxymethyl-1-methyl-5-nitroimidazole, metronidazole, dimetridazole, ronidazole, 5-chloro-1-methyl-4-nitroimidazole, 5-nitrobenzimidazole, ipronidazole-OH, ipronidazole in royal jelly.

## 2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this Professional Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based in this Professional Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies.

GB/T 6682 Water for analytical laboratory use—Specification and test methods.

## 3 Principle

Methanol is used to precipitate protein. The supernatant solution is diluted with phosphate buffer solution, extracted with ethyl acetate. After Oasis (HLB) and C<sub>18</sub> solid phase extraction cartridge purification, the residues are determined by LC-MS/MS, and quantified by external standard method or internal standard method.

## 4 Reagents and materials

Unless otherwise specified, all reagents used should be analytical grade, “water” is the first grade water prescribed by GB/T 6682.

- 4.1 Acetonitrile:HPLC grade.
- 4.2 Methanol:HPLC grade.
- 4.3 Ethyl acetate:HPLC grade.
- 4.4 Formic acid:HPLC grade.
- 4.5 Sodium chloride.
- 4.6 Sodium dihydrogen phosphate
- 4.7 Sodium hydroxide.
- 4.8 0.1 mol/L sodium hydroxide solution: Weigh 4 g sodium hydroxide into 1 L volumetric flask, then dilute to 1 L with water.
- 4.9 Phosphate buffer solution: Dissolve 13.8 g sodium dihydrogen phosphate in 950 mL water, adjust to pH 8.0 with 0.1 mol/L sodium hydroxide solution and dilute to 1 L with water.
- 4.10 1-(2-hydroxyethyl)-2-hydroxy-methyl-5-nitroimidazole, 2-methyl-5-nitroimidazole, 2-hydroxymethyl-1-methyl-5-nitroimidazole, metronidazole, dimetridazole, ronidazole, 5-chloro-1-methyl-4-nitroimidazole, 5-nitrobenzimidazole, ipronidazole-OH, ipronidazole. Standards: Purity  $\geq 98\%$ . Information see annex A table A. 1.
- 4.11 DMZ-D3, RNZ-D3, DMZOH-D3, IPZOH-D3, IPZ-D3 standards. Purity  $\geq 98\%$ .
- 4.12 Standard stock solution: Accurately weigh an adequate amount of each standard (4.10) (accurate to 0.1 mg), dissolve in methanol and prepare a solution of 100  $\mu\text{g}/\text{mL}$  as the standard stock solution respectively, stored at 1  $^{\circ}\text{C} \sim 4^{\circ}\text{C}$ . They are stable for three months.
- 4.13 Isotope internal standard stock solution: Weigh an adequate amount of each isotope internal standard(4.11) (accurate to 0.1 mg), dissolve with methanol respectively, the concentration of solution is 100  $\mu\text{g}/\text{mL}$  as the standard stock solution, stored at 1  $^{\circ}\text{C} \sim 4^{\circ}\text{C}$ .
- 4.14 Blank sample solution: According to section 7, blank solution is prepared with royal jelly without nitroimidazoles.
- 4.15 Calibration curve standard working solution: According to the requirement, dilute the standard stock solution to 5 ng/mL, 10 ng/mL, 20 ng/mL, 50 ng/mL, and 60 ng/mL, mix standard working solution with blank sample solution(4.13) before using. It is same as 5  $\mu\text{g}/\text{kg}$ , 10  $\mu\text{g}/\text{kg}$ , 20  $\mu\text{g}/\text{kg}$ , 50  $\mu\text{g}/\text{kg}$ , and 60  $\mu\text{g}/\text{kg}$  nitroimidazoles in sample. The concentrations of DMZ-D3 and RNZ-D3 are 20 ng/mL, DMZOH-D3, IPZOH-D3 and IPZ-D3 are 10 ng/mL.

4.16 Oasis (HLB) solid-phase extraction (SPE) cartridge: 500 mg, 5 mL or equivalent. It should be conditioned with 5 mL methanol followed by 5 mL water before use.

4.17 C<sub>18</sub> solid-phase extraction (SPE) cartridge: 500 mg, 3 mL or equivalent. It should be conditioned with 5 mL methanol before use.

4.18 Membrane filter: 0.45 μm, organic type.

## 5 Apparatus and equipment

5.1 Liquid chromatography-tandem mass spectrometry, equipped with electrospray ion source.

5.2 Analytical balance, accuracy: 0.000 1 g and 0.01 g.

5.3 Solid phase extraction vacuum container.

5.4 Centrifuge: ≥6 000 r/min.

5.5 pH meter, accuracy ± 0.02 pH.

5.6 Vortex mixer.

5.7 Rotary vacuum evaporator.

5.8 Centrifuge tube: Polytetrafluoroethylene, 50 mL.

## 6 Sample preparation and storage

6.1 Royal jelly is about 500 g. The sample is melted under room temperature. Keep the prepared sample into two sample bottles, seal and label. The test sample is stored at room temperature. The rest sample is stored in -18 °C refrigerator.

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

## 7 Procedure

### 7.1 Extraction

Weigh ca 2 g test sample (accurate to 0.01 g) into a 50 mL centrifuge tube. Add 0.2 mL DMZ-D3,



RNZ-D3(200 ng/mL), DMZOH-D3, IPZOH-D3, IPZ-D3(100 ng/mL), and 10 mL water, mix it. Wait for 2 min. Add to 20 mL with methanol. Vortex for 1 min under 2 000 r/min, centrifuge for 10 min under 6 000 r/min. Transfer 10.0 mL supernatant layer into a container. Add 10 mL phosphate buffer solution(4.8), and mix it. Add 2 g sodium chloride and 20 mL ethyl acetate, vortex for 1 min under 2 000 r/min, centrifuge for 5 min under 6 000 r/min. The supernatant layer was transferred into flask. Repeat the extraction in the same way with 20 mL ethyl acetate and combined the solution. Ethyl acetate is evaporated to nearly dryness in a water bath below 45 °C.

## 7.2 Clean up

The residues are redissolved with 15 mL water. Transfer the solution into HLB cartridge (4.15), discard the eluate. The cartridge is dried under vacuum to "dryness". Elute the cartridge with 6 mL methanol, flow rate is 1 mL/min~2 mL/min. Transfer the solution into C<sub>18</sub> cartridge(4.16). Elute the cartridge with 9 mL methanol, flow rate is 1 mL/min~2 mL/min, the eluate is evaporated to nearly dryness in a water bath below 50 °C. The residues are reconstituted in 1.0 mL methanol—0.15% formic acid(1+9, V/V), mix it. The solution is passed through a 0.45 μm filter. The filtrate is ready for LC-MS/MS determination.

## 7.3 Determination

### 7.3.1 LC-MS/MS operating conditions

- a) LC column: C<sub>8</sub> column, 150 mm × 4.6 mm(i. d), 5 μm, or equivalent;
- b) Mobile phase: See table 1;

Table 1—Gradient of mobile phase

Time/min	Methanol/%	0.15% Formic acid/%
0	13	87
8.0	13	87
8.1	40	60
15.5	80	20
19.0	100	0
19.1	13	87
25.0	13	87

- c) Flow rate: 0.4 mL/min;
- d) Injection volume: 30 μL;
- e) Ion source: electrospray ionization;

- f) Polarity: positive modes;
- g) Monitoring model: Multiple reaction monitor (MRM);
- h) Nebulizer gas(GS1), curtain gas(CUR), auxiliary heater gas(GS2) are all high purity nitrogen(N<sub>2</sub>) or equivalent. Optimize the flow rate of each gas and ion source temperature to reach the requirement of the sensitivity of mass spectrometry. Detailed parameters, qualifier and quantifier MRM are listed in annex B.

### 7.3.2 LC-MS/MS determination

According to the concentrations of nitroimidazoles in sample solution, select the standard working solution of similar concentration to that of sample solution. The responses of nitroimidazoles in the sample solution should be within the linear range of the calibration curve. The standard working solution should be injected randomly in between the injections of the sample solution of equal volume. Under the above LC-MS/MS operating condition, the retention time of 1-(2-hydroxyethyl)-2-hydroxy-methyl-5-nitroimidazole, 2-methyl-5-nitroimidazole, 2-hydroxymethyl-1-methyl-5-nitroimidazole, metronidazole, dimetridazole, ronidazole, 5-chloro-1-methyl-4-nitroimidazole, 5-nitrobenzimidazole, ipronidazole-OH, ipronidazole is 8.9 min, 9.4 min, 11.4 min, 11.9 min, 13.8 min, 14.3 min, 15.8 min, 16.3 min, 17.5 min, 18.5 min. Selected ion chromatograms of the standards are shown in Figure C. 1 of annex C.

### 7.3.3 Confirmation

Under above determination condition, the variation range of the retention time for the peak of analyte in unknown sample and in the standard working solution can not be out of range of  $\pm 0.25\%$ . For the same analysis batch and the same compound, the variation range of the ion ratio between the two daughter ions for the unknown sample and the standard working solution at the similar concentration can not be out of range of table 2, and then the corresponding analyte must be present in the 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

### 7.3.4 Blank test

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

## 8 Calculation and expression of result

Calculate the residue content of nitroimidazoles residues in the sample by LC-MS/MS data processor

or according to the following formula (1), the blank value should be subtracted from the about result of calculation.

$$X_i = \frac{c_i \times V}{m} \dots\dots\dots(1)$$

Where

$X_i$  —the residue content of nitroimidazoles residues in the sample,  $\mu\text{g}/\text{kg}$ ;

$c_i$  —the concentration of nitroimidazoles residues in the matrix standard working solution,  $\text{ng}/\text{mL}$ ;

$V$  —the final volume of sample solution,  $\text{mL}$ ;

$m$  —mass of test sample of final sample solution,  $\text{g}$ .

## 9 Limit of quantification(LOQ)and recovery

### 9.1 Limit of quantification

The limit of quantifications of nitroimidazoles residues are  $10 \mu\text{g}/\text{kg}$ .

### 9.2 Recovery

According to the experimental data, the corresponding recoveries of fortifying concentrations are shown in table 3.

Table 3—Recovery of nitroimidazoles( $n = 6$ )

Compound	Spiked level/ $(\mu\text{g}/\text{kg})$	Recovery/%
MNZOH	10	74.3~90.1
	20	72.5~99.5
	50	80.0~92.4
2-methyl-5-nitroimidazole	10	70.7~79.3
	20	74.5~81.5
	50	70.2~83.6
DMZOH	10	95.0~104.0
	20	95.5~101.0
	50	98.0~100.0
metronidazole	10	90.3~96.4
	20	91.5~100.0
	50	90.8~97.8

Table 3—Recovery of nitroimidazoles( $n = 6$ ) (continued)

Compound	Spiked level/( $\mu$ g/kg)	Recovery/%
dimetridazole	10	94.6~103.0
	20	88.0~97.0
	50	91.2~100.0
ronidazole	10	94.9~105.0
	20	88.5~96.5
	50	93.2~99.0
5-chloro-1-methyl-4-nitroimidazole	10	95.3~101.0
	20	88.0~94.5
	50	84.6~89.4
5-nitrobenzimidazole	10	75.3~90.1
	20	74.0~83.5
	50	76.4~80.0
ipronidazole-OH	10	93.4~99.0
	20	95.5~103.0
	50	97.2~100.6
ipronidazole	10	88.7~96.6
	20	89.5~98.0
	50	93.6~98.8

**Annex A**  
**(Normative annex)**  
**Standard information**

**Table A. 1—Standard information of nitroimidazoles standards**

Compound	CASNo	Molecular formula	Molecular weight
1-(2-hydroxyethyl)-2-hydroxy-methyl-5-nitroimidazol(MNZOH)	4812-40-2	C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>4</sub>	187. 15
2-methyl-5-nitroimidazole	88054-22-2	C <sub>4</sub> H <sub>5</sub> N <sub>3</sub> O <sub>2</sub>	127. 10
2-hydroxymethyl-1-methyl-5-nitroimidazole(DMZOH, HMMNI)	936-05-0	C <sub>5</sub> H <sub>7</sub> N <sub>3</sub> O <sub>3</sub>	157. 13
metronidazole(MNZ)	443-48-1	C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub>	171. 15
dimetridazole(DMZ)	551-92-8	C <sub>5</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub>	141. 13
ronidazole(RNZ)	7681-76-7	C <sub>6</sub> H <sub>8</sub> N <sub>4</sub> O <sub>4</sub>	200. 15
5-chloro-1-methyl-4-nitroimidazole	4897-25-0	C <sub>4</sub> H <sub>4</sub> ClN <sub>3</sub> O <sub>2</sub>	161. 55
5-nitrobenzimidazole	94-52-0	C <sub>7</sub> H <sub>5</sub> N <sub>3</sub> O <sub>2</sub>	163. 14
ipronidazole-OH(IPZOH)	35175-14-5	C <sub>7</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub>	185. 18
ipronidazole(IPZ)	14885-29-1	C <sub>7</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub>	169. 18
DMZOH-D3	—	—	160. 2
DMZ-D3	—	—	144. 2
RNZ-D3	—	—	203. 2
IPZOH-D3	—	—	188. 2
IPZ-D3	—	—	172. 2

**Annex B**  
(Informative annex)  
**API 4000 LC-MS/MS conditions<sup>1)</sup>**

API 4000 LC-MS/MS conditions:

- a) Electrospray capillary voltage: 4 800 V;
- b) GS1: 289.59 kPa(42 psi);
- c) CUR: 186.165 kPa(27 psi);
- d) GS2: 310.275 kPa(45 psi);
- e) Ion source temperature: 540 °C;
- f) Collision gas(CAD): 5;
- g) Qualifier and quantifier MRM, Decustering potential (DP), Collision energy (CE), Collision cell exit potential(CXP) are shown in table B. 1.

Table B. 1—Transitions, DP, CE, CXP

Compound	Transitions m/z	DP/V	CE/V	CXP/V	Internal compound
MNZOH	188.1/123.0 <sup>a</sup> 188.1/126.0	53	19 26	10	DMZOH-D3
2-methyl-5-nitroimidazole	128.0/82.0 <sup>a</sup> 128.0/42.0	60	26 51	10	—
DMZOH	158.0/140.1 <sup>a</sup> 158.0/55.0	48	16 31	10	DMZOH-D3
metronidazole	172.0/128.2 <sup>a</sup> 172.0/82.1	62	21 35	11 14	DMZOH-D3
dimetridazole	142.0/96.0 <sup>a</sup> 142.0/81.0	62	23 36	8 14	DMZ-D3
ronidazole	201.1/140.2 <sup>a</sup> 201.1/110.2	37	17 25	8	RNZ-D3

1) Non-commercial statement: Parameters listed in Annex B are accomplished by API 4000 LC-MS/MS. The equipment and its type involved in the standard method is only for reference and not related to commercial aims, and the analysts are encouraged to use equipments of different corporation or different type.

Table B. 1—Transitions, DP, CE, CXP (continued)

Compound	Transitions m/z	DP/V	CE/V	CXP/V	Internal compound
5-chloro-1-methyl- 4-nitroimidazole	162.0/116.0 <sup>a</sup> 162.0/145.0	68	26 24	10	—
5-nitrobenzimidazole	164.0/118.0 <sup>a</sup> 164.0/91.0	64	32 52	10	—
ipronidazole-OH	186.3/168.1 <sup>a</sup> 186.3/122.3	45	19 29	9 5	IPZOH-D3
ipronidazole	170.2/109.0 <sup>a</sup> 170.2/124.1	63	37 26	10	IPZ-D3
DMZOH-D3	161.2/143.1	51	18		6
DMZ-D3	145.2/99.0	85	25		10
RNZ-D3	204.2/143.3	50	17		10
IPZOH-D3	189.2/171.2	60	20		9
IPZ-D3	173.1/127.2	67	28		6
<sup>a</sup> product ion is used for quantification					

Annex C  
(Informative annex)

Selected ion chromatograms of nitroimidazoles standards.

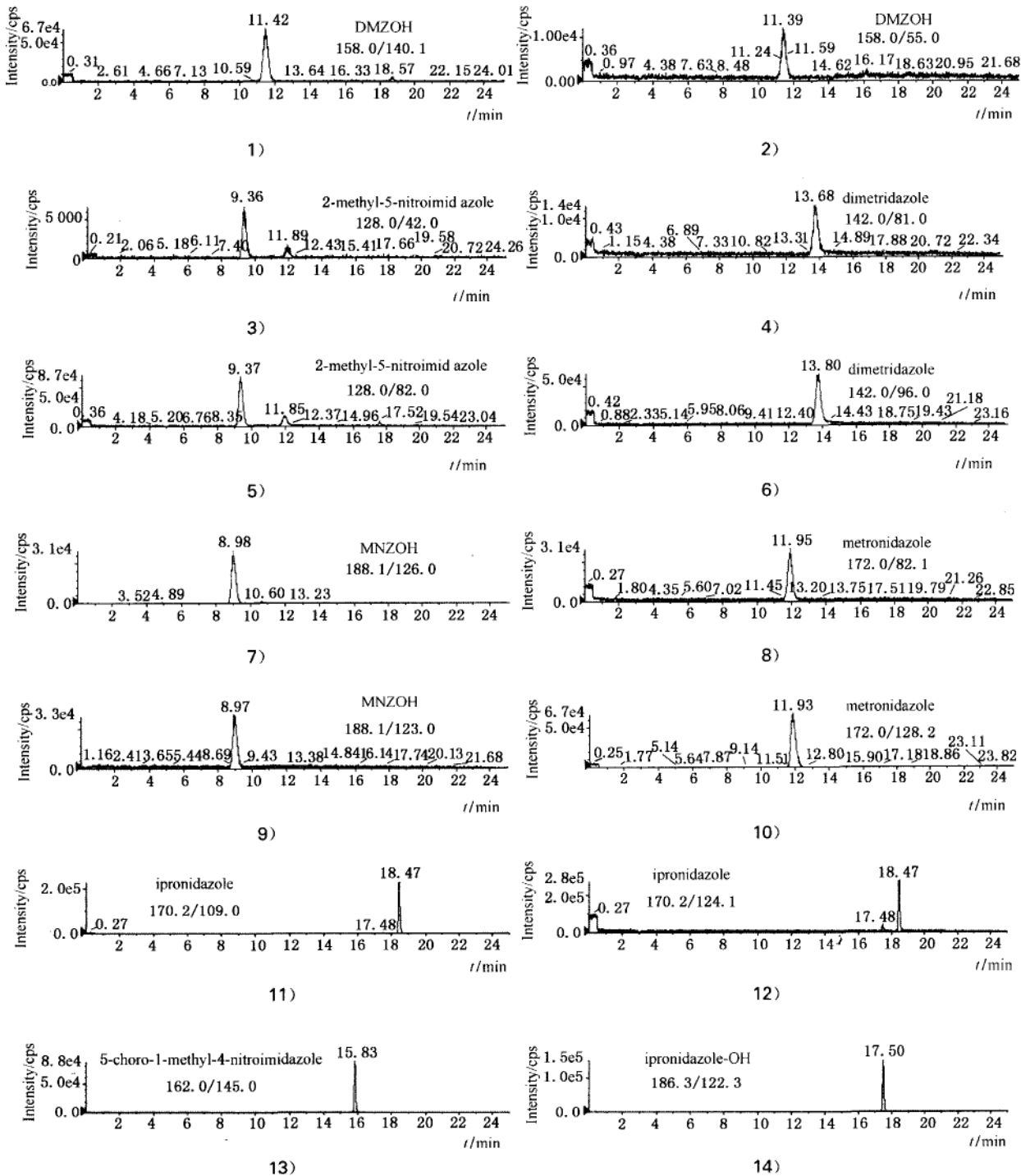


Figure C.1—Selected ion chromatograms of nitroimidazoles standards(10 ng/mL)



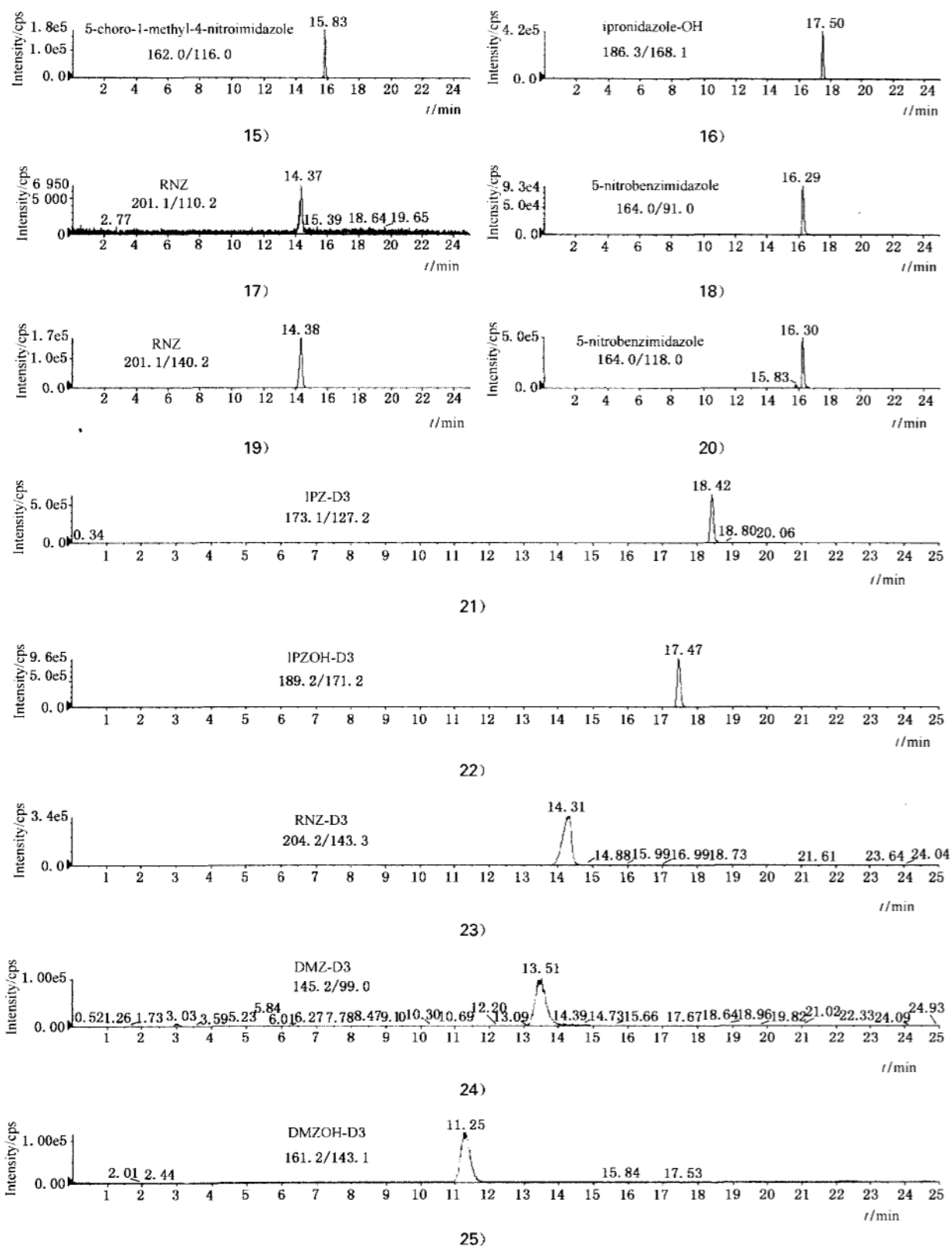


Figure C. 1—Selected ion chromatograms of nitroimidazoles standards(10 ng/mL) (continued)