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中华人民共和国出入境检验检疫行业标准

SN/T 2226—2008

进出口动物源性食品中乌洛托品残留量的检测方法　液相色谱-质谱/质谱法

Determination of urotropine residue in foodstuffs of animal origin for import and export—LC-MS/MS method

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前　言

本标准的附录A和附录B均为资料性附录。

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：中华人民共和国黑龙江出入境检验检疫局、中华人民共和国重庆出入境检验检疫局、中华人民共和国辽宁出入境检验检疫局。

本标准主要起草人：刘永、杨长志、王国民、康庆贺、林维宣、李贤良、吴岩、程阳、王冰、郭伟。

本标准系首次发布的出入境检验检疫行业标准。

进出口动物源性食品中乌洛托品残留量的检测方法　液相色谱-质谱/质谱法

1　范围

本标准规定了进出口动物源性食品中乌洛托品残留量的液相色谱-质谱/质谱检测方法。

本标准适用于进出口鸡肉、鸡肝脏、鸡肾脏和猪肉中乌洛托品残留量的检测和确证。

2　规范性引用文件

下列文件中的条款通过本标准的引用而成为本标准的条款。凡是注明日期的引用文件，其随后所有的修改单（不包括勘误的内容）或修订版均不适用于本标准，然而，鼓励根据本标准达成协议的各方面研究使用这些文件的最新版本。凡是不注明日期的引用文件，其最新版本适用于本标准。

GB/T 6682　分析实验室用水规格和实验方法（GB/T 6682—2008，ISO 3696：1987，MOD）

3　方法提要

用乙腈提取试样中的乌洛托品残留，正己烷除去脂溶性杂质，阳离子交换固相萃取柱净化，洗脱液浓缩后残渣用碱性乙腈定溶，液相色谱-质谱/质谱仪测定，外标法定量。

4　试剂和材料

所有试剂除特殊注明外，所用试剂均为分析纯，水为GB/T 6682规定的一级水。

4.1　乙腈：色谱纯。

4.2　正己烷：色谱纯。

4.3　甲醇：色谱纯。

4.4　乙酸（99%）。

4.5　氨水（25%）。

4.6　盐酸（36%）。

4.7　乙酸铵。

4.8　0.1%乙酸溶液：量取0.5 mL乙酸（4.4），用水稀释至500 mL。

4.9　0.1 mol/L盐酸溶液：量取4.2 mL盐酸（4.6），用水稀释至500 mL。

4.10　20 mmol/L乙酸铵缓冲液（pH＝4）：准确称取0.77 g乙酸铵溶于500 mL水中，用乙酸溶液（4.8）调pH值至4。

4.11　5%氨水-甲醇（5＋95，体积比）溶液：量取5 mL氨水（4.5），用甲醇稀释至100 mL。

4.12　0.1%乙酸-乙腈（2＋8，体积比）溶液：量取200 mL乙酸溶液（4.8），用乙腈稀释至1 000 mL。

4.13　乙腈饱和的正己烷溶液：20 mL乙腈中加入100 mL正己烷，充分振荡后，静置分层，取上层正己烷层备用。

4.14　无水硫酸钠：经650 ℃灼烧4 h，冷却后在干燥器中储存备用。

4.15　乌洛托品标准物质（Urotropine，分子式：$C_6H_{12}N_4$）：纯度大于99.0%。

4.16　标准储备溶液：准确称取按其纯度折算为100%质量的乌洛托品标准品0.010 g，用乙腈溶解并定容至100 mL，浓度相当于100 μg/mL。储备液在0 ℃～4 ℃冰箱避光保存，可使用6个月。

4.17　标准中间溶液：准确量取标准储备溶液（4.16）1.0 mL，用乙酸-乙腈溶液（4.12）溶解并定容至

100 mL，浓度相当于1.0 μg/mL，在0 ℃～4 ℃冰箱避光保存，使用前配制。

4.18　标准工作溶液：根据需要用1.0 mL适当浓度的标准工作溶液溶解空白样品浓缩残渣，配制成适当浓度的标准工作溶液，使用前配制。

4.19　固相萃取柱（OasisMCX）：60 mg，3 mL，或相当者。OasisMCX固相萃取柱使用前分别用3 mL甲醇、3 mL水、3 mL乙酸铵溶液预处理，并保持柱体湿润。

4.20　微孔滤膜：0.22 μm，有机相。

5　仪器和设备

5.1　高效液相色谱-质谱/质谱仪：配电喷雾离子源（ESI）或相当者。

5.2　分析天平：感量为0.1 mg，0.01 g。

5.3　固相萃取装置。

5.4　pH计：测量精度±0.02。

5.5　旋转蒸发器。

5.6　氮吹仪。

5.7　旋涡混匀器。

5.8　组织捣碎机。

5.9　均质器（10 000 r/min）。

5.10　离心机（4 000 r/min）。

5.11　50 mL玻璃离心管。

6　试样的制备与保存

取样品中有代表性的约200 g，用组织捣碎机捣碎，混匀，均分成两份作为试样，分别装入洁净容器，密封并做好标识，于−18 ℃冰箱内保存。

在抽样和制样操作过程中，应防止样品受到污染或发生残留物含量的变化。

7　测定步骤

7.1　提取

准确称取5 g试样（精确到0.01 g）于50 mL离心管中，加入2 g无水硫酸钠、20 mL乙腈，用均质器以10 000 r/min均质1 min，以4 000 r/min离心5 min，上清液转移至50 mL具塞离心管中，样品再用10 mL乙腈重复上述提取、离心操作，合并两次提取的上清液，加入经乙腈饱和的正己烷溶液（4.13）10 mL，用漩涡振荡器混匀2 min后，以4 000 r/min离心3 min，弃去上清液，乙腈相过无水硫酸钠，转移至浓缩瓶，浓缩近干后待净化。

7.2　净化

用1.0 mL 20 mmol/L乙酸铵缓冲液（4.10）溶解浓缩瓶中残渣，以1 mL/min～2 mL/min的速度过固相萃取柱（4.19），再用2.0 mL 20 mmol/L乙酸铵溶液分两次洗涤浓缩瓶，过固相萃取柱，用1.0 mL水、1.0 mL甲醇依次淋洗固相萃取柱，弃去流出液，在15 mmHg以下减压抽1 min使柱体干涸；用3.0 mL 5%氨水-甲醇溶液（4.11）洗脱，洗脱液在45 ℃下用氮吹仪浓缩至近干，用1.0 mL 0.1%乙酸-乙腈溶液（4.12）溶解残渣，经0.22 μm滤膜过滤，供液相色谱-质谱/质谱测定。

7.3　测定

7.3.1　液相色谱条件

a）　色谱柱：WatersACQUITY UPLC® HILIC 2.1 mm（内径）×50 mm，粒径1.7 μm，或相当者；

b）　流动相：0.1%乙酸-乙腈（2+8，体积比）；

c）　流速：0.25 mL/min或根据仪器条件优化；

d） 柱温：30 ℃；

e） 进样量：5 μL。

7.3.2 质谱条件

a） 离子源：电喷雾离子源；

b） 扫描方式：正离子；

c） 检测方式：多反应监测(MRM)；

d） 其他质谱/质谱参考条件参见附录 A 中表 A.1。

7.3.3 定量测定

根据样液中乌洛托品残留量浓度大小，选定峰高相近的标准工作溶液，标准工作溶液和样液中乌洛托品残留的响应值均应在仪器的检测线性范围内。对标准工作溶液和样液等体积参差进样测定，在上述色谱条件下，乌洛托品的参考保留时间约为 3.06 min，标准溶液的多反应监测色谱图参见附录 B 中图 B.1。

7.3.4 定性测定

在相同的实验条件下，样品与标准工作液中待测物质的质量色谱峰相对保留时间在 2.5%以内，并且在扣除背景后的样品质量色谱图中，所选择的离子对均出现，同时与标准品的相对丰度允许偏差不超过表 1 规定的范围，则可判断样品中存在对应的被测物。

表 1 使用定性液相色谱-质谱/质谱时相对离子丰度最大容许误差

相对离子丰度/%	>50	>20～50	>10～20	≤10
最大允许偏差/%	±20	±25	±30	±50

7.4 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

用数据处理软件中的外标法，或绘制标准曲线，按照式(1)计算样品中乌洛托品残留量。

$$X = \frac{c \times V}{m} \qquad \cdots\cdots (1)$$

式中：

X——试样中待测组分含量，单位为微克每千克(μg/kg)；

c——由标准曲线而得的样液中待测组分的浓度，单位为纳克每毫升(ng/mL)；

V——样液最终定容体积，单位为毫升(mL)；

m——最终样液所代表的试样质量，单位为克(g)。

9 测定低限、回收率

9.1 测定低限

本方法乌洛托品测定低限为 5 μg/kg。

9.2 回收率

回收率试验数据见表 2。

表 2 乌洛托品添加回收率($n=10$)

食品名称	添加浓度/(μg/kg)	平均测定值/(μg/kg)	回收率/%	相对标准偏差/%
鸡肉	5	3.83	73.2～86.7	5.5
	10	8.38	73.8～91.5	5.1
	20	16.70	76.7～93.6	6.3

表 2（续）

食品名称	添加浓度/(μg/kg)	平均测定值/(μg/kg)	回收率/%	相对标准偏差/%
鸡肝	5	3.89	70.1～91.8	8.3
	10	8.12	72.2～93.2	7.7
	20	16.71	75.7～92.6	6.5
鸡肾	5	3.81	70.0～86.8	8.1
	10	7.38	69.8～85.8	6.5
	20	16.14	75.4～92.9	7.2
猪肉	5	3.91	71.4～84.7	7.4
	10	8.70	77.3～97.4	6.4
	20	17.20	75.9～101.1	6.6

附录A

（资料性附录）

串联质谱条件1)

串联质谱条件：

a） 电离方式：ESI$^+$；

b） 毛细管电压：3.0 kV；

c） 源温度：110 ℃；

d） 去溶剂温度：350 ℃；

e） 锥孔气流：50 L/h；

f） 去溶剂气流：550 L/h；

g） 碰撞气：氩气，碰撞气压 3.30×10^{-3} Pa；

h） 监测模式：多反应监测条件见表A.1。

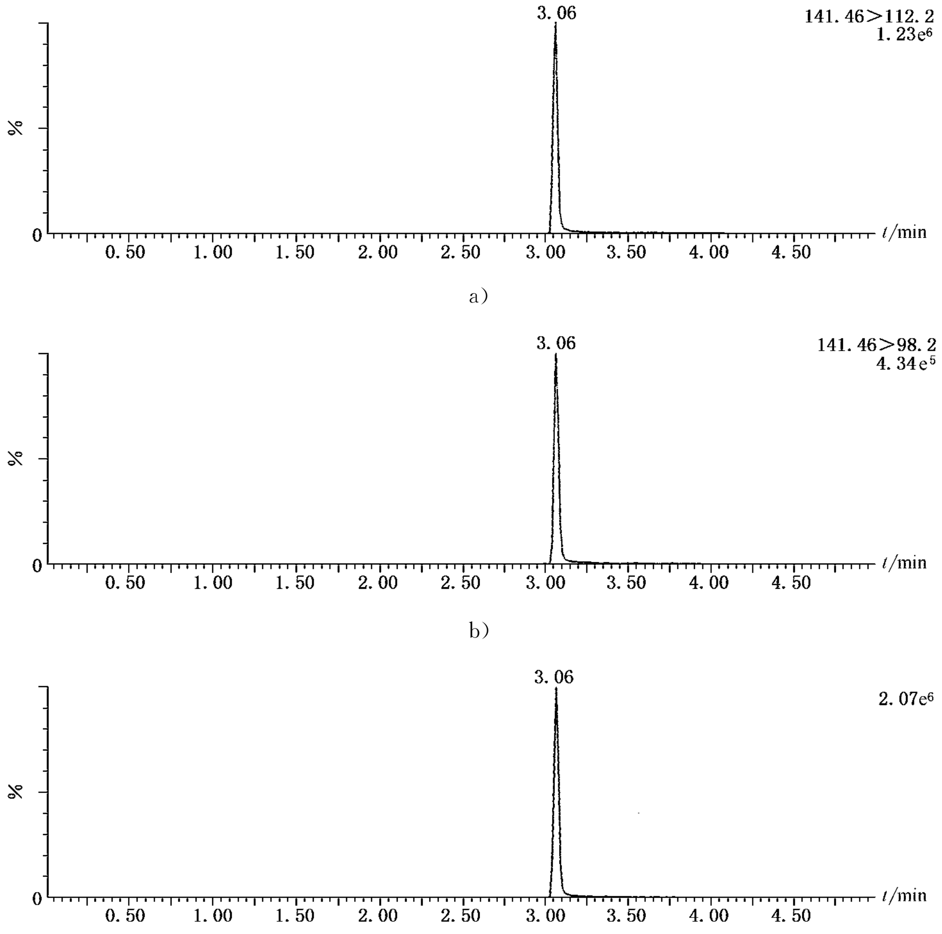
表A.1 多反应监测条件

化合物	母离子	子离子	驻留时间/s	锥孔电压/V	碰撞能量
乌洛托品	141.1	112.2[a]	0.2	25	13
		98.2	0.2	25	13

[a] 离子用于定量。

1） 非商业性声明：附录表A所列参数是在Waters Quattro Premier质谱仪上完成的，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采用不同厂家或型号的仪器。

附 录 B
（资料性附录）
标准样品色谱图



a）

b）

c）

图 B.1 乌洛托品高效液相色谱-质谱/质色谱图

Foreword

Annex A and Annex B of this standard is informative annexes.

This standard was proposed by and is under the charge of China National Regulatory Commission for Certification and Accreditation.

The standard was drafted by Hei longjiang Entry-Exit Inspection and Quarantine Bureau of the People’s Republic of China，Chongqing Entry-Exit Inspection and Quarantine Bureau of the People’s Republic of China，Liaoning Entry-Exit Inspection and Quarantine Bureau of the People’s Republic of China.

The main drafters of this standard are Liu Yong，Yang Changzhi，Wang Guomin，Kang Qinghe ，Lin Weixuan，Li Xianliang，Wu Yan，Cheng Yang，Wang Bing，Guo Wei.

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 urotropine residue In foodstuffs of animal origin for import and export —LC-MS/MS method

1 Scope

This standard specifies the methods of sample preparation and determination by liquid chromatography mass spectrometry of Urotropine residues in foodstuffs of animal origin.

This standard is applicable to the determination and qualification of Urotropine residue in animal-derived foods including pork,poultry and their liver,kidney.

2 Normative references

The following normative documents contain provisions which,through reference in this text,constitute provisions of this national standard. For dated references,subsequent amendments to,or revisions of,any of these publications do not apply. However,parties to agreements based in this national 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(GB/T 6682—2008, ISO 3696:1987,MOD)

3 Principles

The residues of Urotropine residues in the test sample are extracted with acetonitrile. n-hexane is used to remove lipid soluble matters. The test sample are cleaned-up with MCX solid-phase extraction cartridges. After being concentrated,the residues are dissolved volume with acetonitrile-alkaline,and determined by liquid chromatography mass spectrometry with electrospray ionization and triquadruple mass spectrometer,quantified by external standard method.

4 Reagents and materials

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

4.1 Acetonitrile: HPLC grade.

4.2 n-hexane: HPLC grade.

4.3 Methanol: HPLC grade.

4.4 Acetic acid (99%).

4.5 Ammonium hydroxide (25%).

4.6 Hydrochloric acid(36%).

4.7 Ammonia-AC.

4.8 0.1% Acetic acid solution: 0.5 mL acetic acid diluted to 500 mL with water.

4.9 0.1 mol/L hydrochloric acid solution: 4.2 mL hydrochloric acid diluted to 500 mL with water.

4.10 20 mmol/L ammonia-AC(pH=4)solution: Dissolve 0.77 g ammonia-AC diluted to 500 mL with water. Adjusted pH=4 with acetic acid solution(4.8).

4.11 5% Ammonium hydroxide-methanol solution: Mix 5 mL Ammonium hydroxide (5.5) diluted to 100 mL with methanol.

4.12 0.1% Ammonium hydroxide-acetonitrile (2+8)solution: Mix 200 mL acetic acid solution (4.8) diluted to 1 000 mL with acetonitrile.

4.13 n-hexane saturated with acetonitrile: Add 20 mL n-hexane into 100 mL acetonitrile, mix adequately, then wait for delamination separation, use the substrate layer.

4.14 Dehydrate sodium sulphate: Ignite for 4 h at 650 ℃, cool to room temperature in desiccators and keep in a tightly closed container.

4.15 Standards: Urotropine: Purity should be no less than 99.0%.

4.16 Stock standard solution: Dissolve 0.010 g Urotropine (5.15) with dilution solution(5.12) in 100 volumetric flask and the concentration of standard is 100 μg/mL. The solution should be stored at the temperature 0 ℃～4 ℃ for more than six months.

4.17 Intermediate standard solution: Add 1 mL stock standard solution(5.16) to a 100 mL volumetric flask and dilute to volume with dilution solution(5.12). The concentration of intermediate standard solution is 1 μg/mL. The solution should be stored at the temperature 0 ℃～4 ℃ for more than one month.

4.18 Working standard solution: According to the requirement, dilute 1.0 mL intermediate standards solution (5.17) with blank matrix extraction solution to proper concentration. The solution should be prepared just before using.

4.19 Oasis MCX SPE cartridges (3 mL, 60 mg) or equivalent: Pretreat the cartridge with 3 mL methanol, 3 mL water, 3 mL ammonia-AC solution before using, and keep the cartridge wet.

4.20 0.22 μm membrane filter.

5 Apparatus and equipment

5.1 Liquid chromatography-mass spectrometry, equipped with electrospray ion source and triquadruple mass spectrometer or equivalent.

5.2 Analytical balance: sensitivity at 0.1 mg, 0.01 g.

5.3 SPE vacuum container.

5.4 pH meter: accurate to ±0.02.

5.5 Rotatory evaporator.

5.6 Nitrogen flow appearance.

5.7 Vortex mixer.

5.8 Tissue blender.

5.9 Homogenizer (10 000 r/min).

5.10 Centrifuge (4 000 r/min).

5.11 50 mL centrifuge tube.

6 The preparation and storing of the sample

About 200 g representative samples should be taken from all samples, then grinded and blended by a tissue blender to produce homogenous samples, put in suitable clean container. After being sealed and labeled, the samples should be stored at below −18 ℃ in refrigerator.

While sampling and sample preparation, precaution should be taken to avoid contamination or any factors that may cause the change of residue content.

7 Procedure

7.1 Extraction

An aliquot of 5.0 g of the test sample (accurate to 0.01 g) was weighed in a 50 mL centrifuge tube. Add 2 g sodium sulfate and 20.0 mL acetonitrile. For extraction, homogen at 10 000 r/min for 1 min, then centrifuge at 4 000 r/min for 5 min. Extract again with 10 mL acetonitrile. Combine the supernatants and mix the extraction with Acetonitrile-n-hexane saturated solution (4.13) 10 mL.

Vortexes for 1 min, then centrifuge at 4 000 r/min for 3 min. Abandon the supernatants, and the acetonitrile phase permeated the dehydrate sodium sulphate. Concentrate with rotator evaporator, then it is ready for cleaned-up.

7.2 Clean-up

The residue is to dissolve with 1.0 mL 20 mmol/L pH = 4 Ammonia-AC Solution (4.10). Load the solution to MCX SPE cartridge (4.19) at the flow rate of 1 mL/min～2 mL/min, then wash the heart-bottle twice with 1.0 mL 20 mmol/L pH = 4 Ammonia-AC Solution and permeate the MCX SPE cartridge. Rinse the columns with 1 mL water and 1 mL methanol, and abandon the effluent.

Dry the columns with vacuum pump at less than 15 mmHg for at least 1 min. Elute with 3.0 mL 5% Ammonium hydroxide- methanol solution (4.11), then dry the eluant with nitrogen flow appearance (at less than 45 ℃), then dissolved the residue to 1 mL with 0.1% acetic acid —Acetonitrile (2+8) solution (4.12). After filtered with 0.22 μm filter, the final solution is ready for LC-MS/MS determination.

7.3 Determination

7.3.1 HPLC operating condition

a) LC column: Waters ACQUITY UPLC® HILIC 1.7 μm, 2.1 (i. d.) × 50 mm, or equivalent;

b) Mobile phase: 0.1% acetic acid ∶ acetonitrile = 20 ∶ 80;

c) Flow rate: 0.25 mL/min;

d) Column temperature: 30 ℃;

e) Injection volume: 5 μL.

7.3.2 MS operating condition

a) Ion source: ESI;

b) Scanning model: positive ion;

c) Determination model: multiple reaction monitor(MRM);
d) Other parameters are listed annex A.

7.3.3 Quantification test

According to the method, detect the residues of Urotropine in the test sample solution, the standard working solution, The response of Urotropine should be in the linear range of the instrumental detection. If the response out of the linear range, dilute with the extract of the blank sample to suitable concertration. Reconstituted ion chromatogram of standard working solution is listed in figure B.1 of annex B.

7.3.4 Qualification test

Under the same conditions of experiment, the retention time of the unknow sample is the same as the stand working solution, the qualification ions for every compound must be found. 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 1.

Table 1—Maximum permitted tolerances for relative ion intensities while confirmation

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

7.4 Blank tests

The operation of blank test is the same as the procedure that prescribed above, but omission of sample.

8 Calculation and expression of result

Calculate the content of Urotropine residue concentration in the sample is carried out by LC/MS/MS data processor or according to the formula (1):

$$X = \frac{c \times V}{m} \qquad \cdots\cdots (1)$$

Where:

X—the residue content of Urotropine in the test sample, mg/kg;

c—the concentration of Urotropine in the test sample calculated by calibration curve, ng/mL;

V—the final volume of sample solution, mL;

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

9 Limit of quantification and recovery

9.1 Limit of quantification

The limit of quantification for Urotropine is 5 μg/kg.

9.2 Recovery

The results of recoveries were showed on table 2.

Table 2—The data of recovery（$n=10$）

Samples	Added concentration/(μg/kg)	Mean of results/(μg/kg)	Recovery/%	RSD/%
Pork	5	3.9	71.4～84.7	7.4
	10	8.7	77.3～97.4	6.4
	20	17.2	75.9～101.1	6.6
Chicken	5	3.8	73.2～86.7	5.5
	10	8.4	73.8～91.5	5.1
	20	16.7	76.7～93.6	6.3
Liver	5	3.5	70.1～79.3	8.3
	10	8.1	72.2～80.4	7.7
	20	16.7	75.7～79.9	6.5
Kidney	5	3.6	71.8～80.1	8.1
	10	7.4	73.2～79.4	6.5
	20	16.1	80.1～90.3	7.2

Annex A

(Informative)

LC-MS/MS condition[1)]

LC-MS/MS condition

a） Ion source：ESI^+；

b） Capillary voltage：3.0 kV；

c） Source temperature：110 ℃；

d） Desolvation temperature：350 ℃；

e） Cone gas flow：Nitrogen，50 L/h；

f） Desolvation gas flow：Nitrogen，600 L/h；

g） Collision gas pressure：Argon，3.30×10^{-3} Pa；

h） Monitoring model：multiple reaction monitor（MRM）see table A.1.

Table A.1—MRM condition

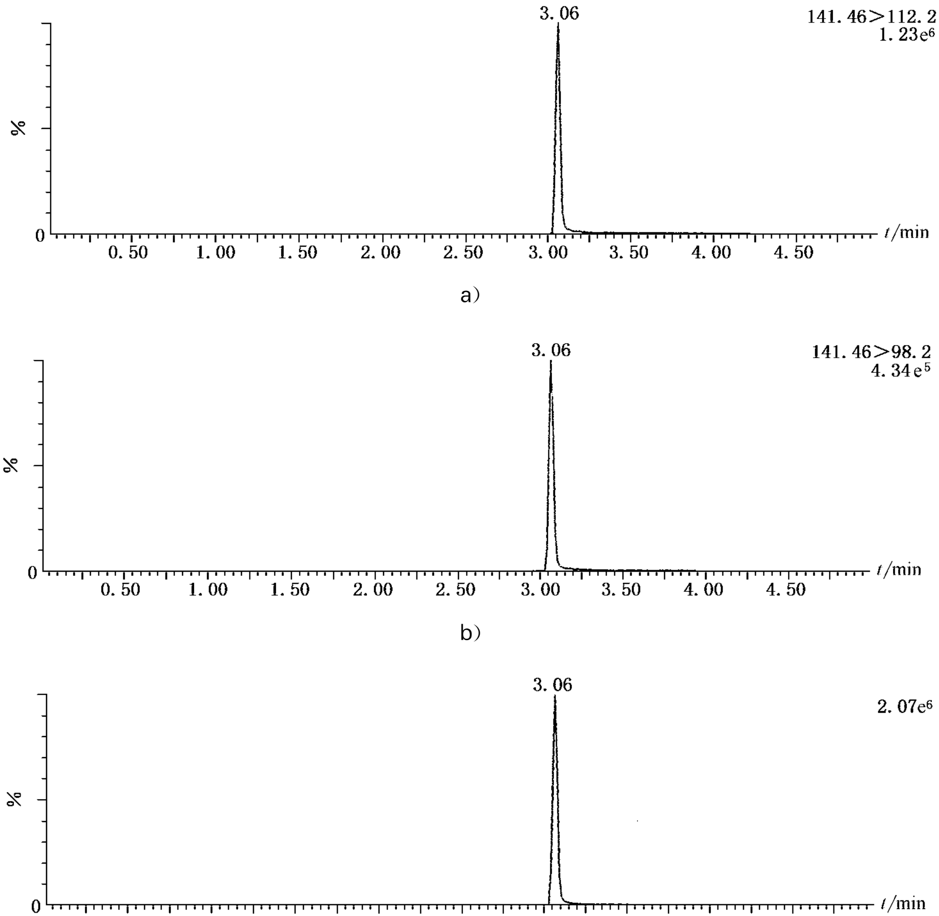
Compound	Precursor ion	Product ion	Dwell time/s	Cone voltage/V	Collision energy
Urotropine	141.1	112.2[a]	0.2	25	13
		98.2	0.2	25	13
[a] is used for quantification.					

1） Non-commercial statement：the equipments and their types Waters Quattro Premier involved in the standard method are not related to commercial aims，and the analysts are encouraged to equipments of different corporation or different type.

Annex B

(Informative)

MRM chromatogram of the standard



a)

b)

c)

Figure B.1—The MRM chromatogram of Urotropine standard

SN/T 2226—2008

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