SN

中华人民共和国出入境检验检疫行业标准

SN/T 1985—2007

进出口动物源性食品中吩噻嗪类药物残留量检测方法　液相色谱-质谱/质谱法

Determination of phenothiazines residues 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：中华人民共和国深圳出入境检验检疫局、中国检验检疫科学研究院、中华人民共和国辽宁出入境检验检疫局。

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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吩噻嗪类药物残留量检测方法　液相色谱-质谱/质谱法

1　范围

本标准规定了动物源性食品中甲苯噻嗪、异丙嗪、氯丙嗪、乙酰丙嗪、丙酰丙嗪等五种吩噻嗪类药物残留量的制样、液相色谱-质谱/质谱测定和确证方法。

本标准适用于肌肉、内脏、水产品和牛奶等动物源性食品中甲苯噻嗪、异丙嗪、氯丙嗪、乙酰丙嗪、丙酰丙嗪等五种吩噻嗪类药物残留的测定。

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研究是否可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试验方法

3　样品制备与保存

3.1　制样要求

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。

3.2　动物肌肉、肝脏、肾脏和水产品

从所取全部样品中取出有代表性样品可食部分约500 g，用组织捣碎机充分捣碎均匀，均分成两份，分别装入洁净容器中，密封，并标明标记，于－18℃以下冷冻存放。

3.3　牛奶样品

从所取全部样品中取出有代表性样品约500 g，充分混匀，均分成两份，分别装入洁净容器中，密封，并标明标记，于－18℃以下冷冻存放。

4　方法提要

试样中的吩噻嗪类药物残留用碱性乙腈溶液提取，经固相萃取柱净化，浓缩、定容后，用液相色谱-质谱/质谱仪测定，外标法定量。

5　试剂和材料

除另有说明外，所用试剂均为分析纯，水为GB/T 6682规定的一级水。

5.1　乙腈，色谱纯。

5.2　25%氨水。

5.3　乙酸铵，色谱纯。

5.4　乙酸，色谱纯。

5.5　硫酸。

5.6　氯化钠。

5.7　1%氨水的乙腈溶液：准确吸取10 mL 25%的氨水，转移入1 000 mL容量瓶，用乙腈定容至刻度，混合均匀。

5.8 乙酸铵溶液(50 mmol/L,pH 4.5):准确称取 3.854 g 乙酸铵,溶于 900 mL 水中,用乙酸调节 pH 至 4.5,转移到 1 000 mL 容量瓶中,水定容至刻度,混合均匀。

5.9 10%氯化钠溶液:称取 100.0 g 氯化钠,用水溶解并定容到 1 000 mL 容量瓶中,混合均匀。

5.10 硫酸溶液(10 mmol/L):准确吸取 0.54 mL 浓硫酸,小心沿烧杯壁滴入 500 mL 水中,搅匀后转移入 1 000 mL 容量瓶中,用水定容至刻度,混合均匀。

5.11 标准物质:甲苯噻嗪,纯度≥99%;异丙嗪,纯度≥99%;氯丙嗪,纯度≥99%;乙酰丙嗪,纯度≥99%;丙酰丙嗪,纯度≥99%。

5.12 标准溶液

5.12.1 标准储备溶液:分别称量经折算相当于 5 mg(精确到 0.1 mg)的甲苯噻嗪、异丙嗪、氯丙嗪、乙酰丙嗪、丙酰丙嗪,用甲醇溶解并定容至 10 mL 棕色容量瓶中,得质量浓度分别为 500 mg/L 的标准储备溶液。此储备液可在−20℃以下避光存放 12 个月。

5.12.2 混合标准中间溶液:准确吸取各种吩噻嗪类标准储备溶液 0.2 mL 于 10 mL 棕色容量瓶中,用甲醇定容至刻度,各种吩噻嗪类药物的浓度为 10 mg/L,此中间溶液可在−20℃以下避光存放 3 个月。

5.12.3 混合标准工作溶液:标准工作溶液根据需要使用前用空白样品基质溶液配制。

5.13 Oasis HLB 固相萃取(SPE)柱(60 mg,3 mL)或相同材料的其他型号。

5.14 氮气:纯度≥99.99%。

6 仪器与设备

6.1 液相色谱串联四极杆质谱仪或相当者,配电喷雾离子源。

6.2 分析天平:感量 0.1 mg,0.01 g。

6.3 旋涡混合器。

6.4 离心机,5 000 r/min。

6.5 pH 计。

6.6 固相萃取真空装置。

6.7 机械真空泵。

6.8 组织捣碎机。

6.9 均质器。

6.10 超声波仪。

6.11 吹氮浓缩仪。

7 测定步骤

7.1 样品提取

7.1.1 肌肉、水产品、肝脏和肾脏

称取均质试样 6 g(精确到 0.01 g),置于 50 mL 具螺旋盖聚丙烯离心管中,加入 15 mL 1%氨水的乙腈溶液。肌肉和水产品样品均质提取 2 min;肝脏和肾脏样品漩涡混合 1 min,超声提取 10 min。3 000 r/min以上离心 5 min,收集上清液于一具刻度离心管中。离心后的残渣用约 15 mL 1%氨水的乙腈溶液重复上述提取步骤 1 次,合并上清液,定容到 30 mL,混匀。取 10 mL 提取液于 45℃下吹氮浓缩至 2 mL 以下。用 10%氯化钠溶液定容到 10 mL,混匀。

7.1.2 牛奶

称取混匀牛奶样品 6 g(精确到 0.01 g),置于 50 mL 具刻度螺旋盖聚丙烯离心管中,用 1%氨水的乙腈溶液定容至 30 mL,漩涡混合 1 min,超声提取 10 min,3 000 r/min 以上离心 5 min。取 10 mL 上清液,之后与 7.1.1 同。

7.2 固相萃取净化

将固相萃取真空装置与真空泵连接好，装上 HLB 固相萃取柱，依次以 5 mL 甲醇和 5 mL 水预处理。将 10 mL 提取液过柱，流速控制在约 1 滴/s。依次用 2 mL 超纯水和 1 mL 10 mmol/L 的硫酸溶液淋洗柱子，淋洗液完全通过小柱后，抽真空 5 min 以上。以 3 mL 乙腈洗脱，洗脱液用干净的 15 mL 具刻度试管收集。在 45℃下氮气吹至约 0.5 mL，用水定容至 1 mL，混匀。溶液以 0.45 μm 滤膜过滤，滤液可直接供高效液相色谱/串联质谱仪测定。

7.3 测定

7.3.1 液相色谱条件

a) 色谱柱：YMC-Pack Pro C_{18}，3 μm，100 mm×2.0 mm(内径)或同等性能的其他色谱柱；

b) 流动相：乙腈+50 mmol/L pH4.5 乙酸铵溶液，梯度洗脱(梯度时间表见表 1)；

c) 流速：300 μL/min；

d) 柱温：30℃；

e) 进样量：15 μL。

表 1 液相色谱的梯度洗脱条件

时间/min	流速/(μL/min)	乙腈/%	50 mmol/L pH4.5 乙酸铵溶液/%
0.00	300	5.0	95.0
0.50	300	5.0	95.0
1.00	300	65.0	35.0
7.00	300	95.0	5.0
8.00	300	95.0	5.0
8.50	300	5.0	95.0
15.00	300	5.0	95.0

7.3.2 质谱条件

离子化模式：电喷雾电离正离子模式(ESI+)；质谱扫描方式：多反应监测(MRM)；分辨率：单位分辨率；其他参考质谱条件参见附录 A。

7.3.3 液相色谱-质谱/质谱仪测定

根据样液中被测吩噻嗪类药物残留的含量情况，选定峰高相近的标准工作溶液。标准工作溶液和样液中吩噻嗪类药物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。各种吩噻嗪类药物的参考保留时间为：(1)甲苯噻嗪 4.9 min，(2)异丙嗪 5.3 min，(3)氯丙嗪 5.6 min，(4)乙酰丙嗪 5.2 min，(5)丙酰丙嗪 5.5 min；标准品色谱图参见附录 B 中图 B.1。

8 结果计算与表述

8.1 定性标准

8.1.1 保留时间

待测样品中化合物色谱峰的保留时间与标准溶液相比变化范围应在±2.5%之内。

8.1.2 信噪比

待测化合物的定性离子的重构离子色谱峰的信噪比应大于等于 3(S/N≥3)，定量离子的重构离子色谱峰的信噪比应大于等于 10(S/N≥10)。

8.1.3 定量离子、定性离子及子离子丰度比

每种化合物的质谱定性离子必须出现，至少应包括一个母离子和两个子离子，而且同一检测批次，对同一化合物，样品中目标化合物的两个子离子的相对丰度比与浓度相当的标准溶液相比，其允许偏差不超过表 2 规定的范围。

表 2　定性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

8.2　结果计算与表述

采用外标法定量，按式(1)计算吩噻嗪类药物残留量。

$$X = \frac{A_X \times c_S \times V}{A_S \times m} \quad \cdots\cdots(1)$$

式中：

X——样品中待测组分的含量，单位为毫克每千克(mg/kg)；

A_X——测定液中待测组分的峰面积；

A_S——标准液中待测组分的峰面积；

m——最终样液所代表的样品质量，单位为克(g)；

c_S——标准液中待测组分的含量，单位为毫克每升(mg/L)；

V——定容体积，单位为毫升(mL)。

9　测定低限与回收率

9.1　测定低限

肌肉/肾脏/肝脏/牛奶：甲苯噻嗪 0.01 mg/kg，异丙嗪、氯丙嗪、乙酰丙嗪、丙酰丙嗪均为 0.001 mg/kg；鱼肉：甲苯噻嗪、异丙嗪、氯丙嗪、乙酰丙嗪、丙酰丙嗪均为 0.001 mg/kg。

9.2　回收率

回收率见表 3。

表 3　吩噻嗪类药物的残留回收率

药物名称	添加浓度/(mg/kg)	回收率/%				
		猪肉	猪肾	猪肝	牛奶	鱼肉
甲苯噻嗪	0.01(鱼肉为 0.001)	70.8～98.2	70.2～102	70.5～99.5	70.0～105	60.2～98.5
	0.02(鱼肉为 0.002)	71.5～95.0	72.5～104	80.0～97.0	73.5～110	67.0～95.0
	0.04(鱼肉为 0.004)	71.0～98.0	70.8～102	73.5～94.3	71.8～100	71.5～97.5
异丙嗪	0.001	71.3～106	83.5～110	77.3～109	67.3～102	61.8～99.5
	0.002	73.0～105	74.5～107	90.5～109	73.5～109	65.0～99.5
	0.004	79.0～103	87.5～108	95.0～110	77.5～109	76.5～104
氯丙嗪	0.001	65.7～102	69.7～91.7	66.1～97.8	62.1～96.5	61.3～96.5
	0.002	74.0～98.0	70.5～99.0	73.0～101	69.0～93.5	70.0～98.5
	0.004	75.0～102	70.5～99.0	77.0～95.5	64.5～88.3	69.0～99.8
乙酰丙嗪	0.001	77.2～105	65.8～102	70.2～108	70.0～109	62.1～90.5
	0.002	75.5～105	84.5～110	87.0～107	69.5～105	66.5～96.0
	0.004	75.5～103	83.3～110	75.3～105	68.5～94.5	74.0～98.5
丙酰丙嗪	0.001	72.1～110	63.8～89.7	66.1～99.5	66.9～93.4	61.8～94.5
	0.002	83.5～104	61.0～92.5	71.0～99.0	65.5～99.0	70.0～97.5
	0.004	72.8～105	75.3～97.3	76.8～101	73.0～93.8	70.3～92.0

附 录 A
（资料性附录）
参考质谱条件[1)]

参考质谱条件：

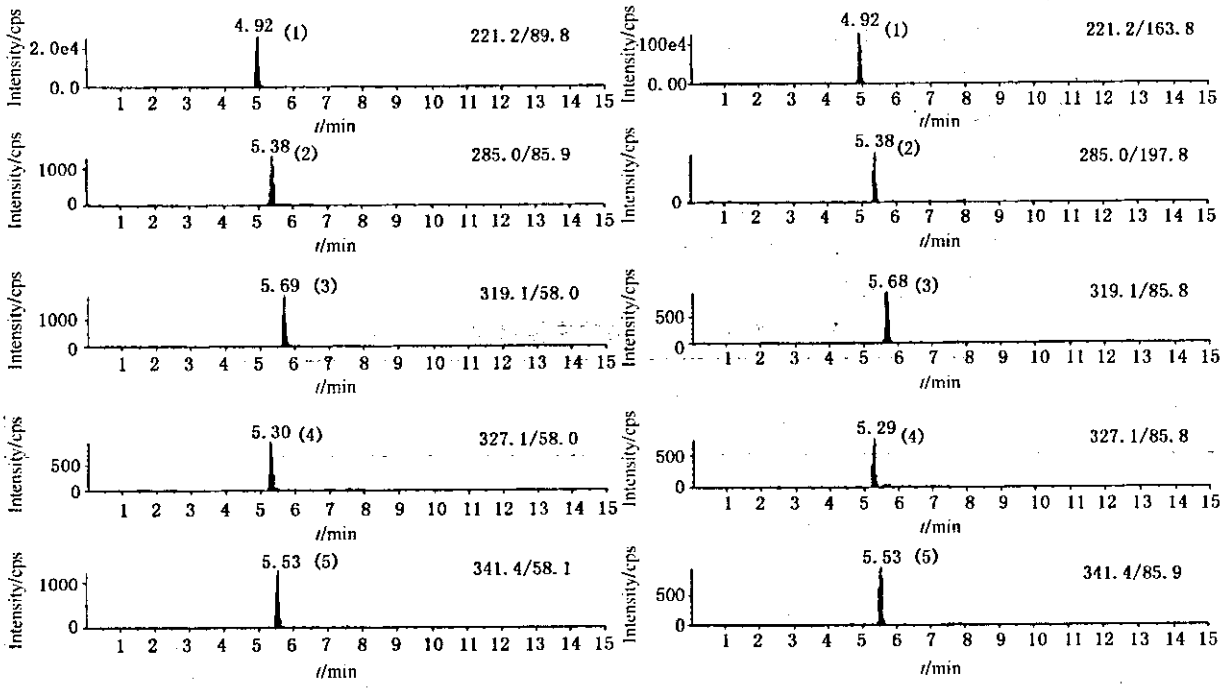
a) 雾化气(NEB)：7.00 L/min(氮气)；
b) 气帘气(CUR)：9.00 L/min(氮气)；
c) 喷雾电压(IS)：2 500 V；
d) 去溶剂温度(TEM)：500℃；
e) 去溶剂气流 7.00 L/min(氮气)；
f) 碰撞气(CAD)：6.00 mL/min(氮气)；
g) 其他质谱参数见表 A.1。

表 A.1 十八种噻嗪类药物的主要参考质谱参数

化合物	母离子 (m/z)	子离子 (m/z)	驻留时间/ ms	DP	FP	CE/ eV	CXP
甲苯噻嗪	221.2	89.8[a] 163.8	100 100	36 36	170 170	39 39	4 12
异丙嗪	285.0	85.9[a] 197.8	50 50	41 41	150 150	31 33	14 18
氯丙嗪	319.1	58.0[a] 85.8	50 50	36 36	150 150	61 29	4 6
乙酰丙嗪	327.1	58.0[a] 85.8	50 50	51 51	180 180	65 31	10 8
丙酰丙嗪	341.4	58.1[a] 85.9	50 50	51 51	210 210	71 27	4 6
注：对于不同质谱仪器，仪器参数可能存在差异，测定前应将质谱参数优化到最佳。							
[a] 离子为定量离子。							

1) 非商业性声明：附录 A 所列参考质谱条件是在 API 3000 型液质联用仪上完成的，此处列出试验用仪器型号仅为提供参考，并不涉及商业目的，鼓励标准使用者尝试不同厂家或型号的仪器。

附 录 B
（资料性附录）
标准品的多反应监测(MRM)色谱图



(1)——甲苯噻嗪,0.02 mg/L;

(2)——异丙嗪,0.002 mg/L;

(3)——氯丙嗪,0.002 mg/L;

(4)——乙酰丙嗪,0.002 mg/L;

(5)——丙酰丙嗪,0.002 mg/L。

图 B.1 五种吩噻嗪类药物残留的多反应监测(MRM)色谱图

Foreword

Annex A and annex B of this standard are informative annexes.

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

This standard was drafted by Shenzhen Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Chinese Academy of Inspection and Quarantine, and Liaoning Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

This standard was mainly drafted by Yue Zhenfeng, Xie Liqi, Tang Shaobing, Zhao Fengjuan, Peng Tao, Hua Honghui, Han Ruiyang, Wang Hongwei, Hou Lexi.

This standard was 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 only for reference.

Determination of phenothiazines residues in foodstuffs of animal origin for import and export—LC-MS/MS Method

1 Scope

This standard specifies the methods of qualified and quantified determination by liquid chromatography-mass spectrometry of 5 phenothiazines residues of xylazine, promethazine, chloropromazine, acepromazine and propionylpromazine in foodstuffs of animal origin.

This standard is applicable to determination of 5 phenothiazines residues of xylazine, promethazine, chloropromazine, acepromazine and propionylpromazine in animal muscle, animal viscera, aquatic product and milk.

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 Sample preparation and storage

3.1 Requirement of sample preparation

Certain measures should be taken to prevent contamination of the samples or decomposition of the residues during the sample preparation procedure.

3.2 Animal muscle, liver, kidney and aquatic product

About 500 g representative edible samples should be taken from all samples, then grinded and blended by a tissue blender to produce homogenous samples and put in suitable clean containers. After being sealed and labeled, the samples should be stored at below −18℃.

3.3 Milk

About 500 g representative samples should be taken from all samples, then mixed to produce homogenous samples, and put in suitable clean containers. After being sealed and labeled, the samples should be stored at below −18℃.

4 Principle

The residues of phenothiazines drugs in the test sample are extracted with alkaline acetonitrile. After being cleaned up with SPE Column, concentrated and reconstituted, the residues are determined by liquid chromatography-mass spectrometry, quantified by external standard method.

5 Reagents and materials

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

5.1 Acetonitrile: HPLC Grade.

5.2 25% $NH_3 \cdot H_2O$.

5.3 Ammonium acetate: HPLC Grade.

5.4 Acetic acid: HPLC Grade.

5.5 Sulfuric acid.

5.6 Sodium chloride.

5.7 1% ammonium in acetonitrile: accurately draw 10 mL 25% $NH_3 \cdot H_2O$ into a 1 000 mL volumetric flask, dilute to volume with acetonitrile, and mix to homogeneity.

5.8 50 mmol/L ammonium acetate solution (pH 4.5): Accurately weigh 3.854 g ammonium acetate, dissolve the material in 900 mL water, adjust pH value to 4.5 by acetic acid, transfer to a 1 000 mL volumetric flask, dilute to volume with water, and mix to homogeneity.

5.9 10% sodium chloride solution: Accurately weigh 100.0 g sodium chloride, dissolve and dilute to 1 000 mL with water, and mix to homogeneity.

5.10 10 mmol/L sulfuric acid solution: accurately draw 0.54 mL concentrate sulfuric acid, slowly and carefully drop into 500 mL water, then transfer to a 1 000 mL volumetric flask, dilute to volume

with water, and mix to homogeneity.

5.11 Standard chemicals: xylazine, purity≥99%; promethazine, purity≥99%; chloropromazine, purity≥99%; acepromazine, purity≥99%; proplonylpromazine, purity≥99%.

5.12 Standard solution:

5.12.1 Standard stock solution: Accurately weigh 5 mg (accurate to 0.1 mg) standard chemical of xylazine, promethazine, chloropromazine, acepromazine and propionylpromazine respectively, dissolve in 10 mL methanol, and mix to homogeneity. The concentration of the solution is 500 mg/L. The solution can be stored at the temperature below −20℃ for more than 12 months.

5.12.2 Mixed standard solution: accurately measure 0.2 mL standard stock solution of each phenothiazine to a same 10 mL black volumetric flask, dilute with methanol to 10 mL, and mix to homogeneity. The concentration of the mixed standard solution is 10 mg/L. The solution can be stored at the temperature below −20℃ for more than three months.

5.12.3 Standard working solution: according to the requirement, accurately measure an adequate volume of mixed standard solution, dilute with blank matrix extract solution just before use.

5.13 Oasis HLB column (60 mg, 3 mL) or equivalent.

5.14 Nitrogen: purity≥99.99%.

6 Apparatus and equipment

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

6.2 Analytical balance, sensitivity: 0.1 mg, 0.01 g.

6.3 Mechanical Shaker.

6.4 Centrifuge with cooler, 5 000 r/min.

6.5 pH meter.

6.6 SPE vacuum container.

6.7 Mechanical vacuum pump.

6.8 Tissue blender.

6.9 Homogenizer.

6.10 Ultrasonic extractor.

6.11 Evaporator with nitrogen flow.

7 Analytical procedure

7.1 Extraction procedure

7.1.1 Muscle, aquatic product, liver and kidney

Accurately weigh 6 g of the test sample (accurate to 0.01 g) into a 50 mL polypropylene centrifuge tube with cap, add 15 mL 1% ammonium in acetonitrile. To muscle and aquatic product, homogenize for 2 min; to liver and kidney, vortex for 1 min, ultrasonicate for 10 min. After centrifugation at 3 000 r/min for 5 min, the supernatant is collected in a graduated polypropylene centrifuge tube. The residues are re-extracted once with about 15 mL 1% ammonium in acetonitrile as the above procedure, combine the supernatants, supplement to 30 mL with acetonitrile, mix to homogeneity. An aliquot of 10 mL extract solution is evaporated to less than 2 mL at 45℃ under nitrogen flow. Dilute to 10 mL with 10% sodium chloride solution, mix to homogeneity.

7.1.2 Milk

Accurately weigh 6 g of the test sample (accurate to 0.01 g) into a 50 mL graduated polypropylene centrifuge tube with cap, dilute the sample to 30 mL with 1% ammonium in acetonitrile, vortex for 1 min, ultrasonicate for 10 min. After centrifugation at 3 000 r/min for 5 min, an aliquot of 10 mL supernatant is evaporated to less than 2 mL at 45℃ under nitrogen flow. Dilute to 10 mL with 10% sodium chloride solution, mix to homogeneity.

7.2 Cleanup procedure

Connect the SPE vacuum container with a mechanical vacuum pump, fit on the HLB column, and pretreat the column with 5 mL methanol and 5 mL water. Load the 10 mL extract solution to the HLB column, the flow rate is adjusted to 1 drop/s. Rinse the column with 2 mL water and 1 mL 10 mmol/L sulfuric acid solution, then dry the column with vacuum pump for at least 5 min. The phenothiazines residues are eluted with 3 mL acetonitrile. The eluted solution is evaporated to about 0.5 mL at 45℃ under nitrogen flow, then dilute to 1 mL with water, vortex to homogeneity. After being filtrated with a 0.45 μm filter, the final solution is ready for analysis by LC-MS/MS.

7.3 Determination

7.3.1 LC operating conditions

a) Column: YMC-Pack Pro C_{18}, 3 μm, 100 mm×2.0 mm i.d. or equivalent;

b) Mobile phase: acetonitrile +50 mmol/L ammonium acetate solution (pH 4.5), the elution gradient is listed in table 1;

c) Flow rate: 300 μL/min;

d) Column temperature: 30℃;

e) Injection volume: 15 μL.

Table 1—Elution gradient of LC

Time/min	Flow/(μL/min)	rate acetonitrile/%	50 mmol/L ammonium acetate solution(pH4.5)/%
0.00	300	5.0	95.0
0.50	300	5.0	95.0
1.00	300	65.0	35.0
7.00	300	95.0	5.0
8.00	300	95.0	5.0
8.50	300	5.0	95.0
15.00	300	5.0	95.0

7.3.2 MS operating conditions

Ionization mode: ESI+; scan mode: MRM; resolution: unit resolution; other reference mass operating conditions are listed in Annex A.

7.3.3 LC-MS/MS determination

According to the approximate concentration of phenothiazines residues in the test sample solution, the standard working solution is selected with similar response to that of sample solution. The response of phenothiazines residues in the standard working solution and sample solution should be in the linear range of the instrumental detection. The standard working solution should be injected randomly in between the injections of sample solution of equal volume. Under the above chromatograph conditions, the reference retention time of xylazine, promethazine, chloropromazine, acepromazine and propionylpromazine is about 4.9 min, 5.3 min, 5.6 min, 5.2 min and 5.5 min respectively. The multiple reaction monitoring (MRM) chromatograms of standard working solution are showed in figure B.1 of annex B.

8 Calculation and expression of result

8.1 Qualification criteria

8.1.1 Retention time

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 ±2.5%.

8.1.2 Signal to noise ratio

The signal to noise ratio of qualified reconstituted ion chromatogram peak for each analyte should be no less than 3(S/N≥3), and the signal to noise ratio of quantified multiple reaction monitoring (MRM) chromatogram peak for each analyte should be no less than 10(S/N≥10).

8.1.3 Quantification ion, qualification ion and ion ratio

The qualification ions for every compound should be found, and at least include one precursor ion and two daughter ions. 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.

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

8.2 Calculation and expression of result

The calculation of phenothiazine residue concentration in the sample is according to the following formula(1)

$$X = \frac{A_x \times c_s \times V}{A_s \times m} \quad \cdots\cdots (1)$$

where

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

A_x Peak area of phenothiazine in the sample solution;

A_s Peak area of phenothiazine in the standard working solution;

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

c_s the concentration of phenothiazine in the standard working solution, mg/L;

V the final volume of sample solution, mL.

9 Limit of quantification and recovery

9.1 Limit of quantification

For the muscle, kidney, liver and milk, the limit of quantification for xylazine is 0.01 mg/kg, and for promethazine, chloropromazine, acepromazine and propionylpromazine is 0.001 mg/kg respectively; for fish muscle, the limit of quantification for all phenothiazines residues is 0.001 mg/kg.

9.2 Recovery

Recovery see table 3.

Table 3—Recoveries of phenothiazines residues

Compound	Spiked level/(mg/kg)	Recoveries/%				
		Swine muscle	Swine kidney	Swine liver	Milk	Fish muscle
xylazine	0.01(for fish muscle 0.001)	70.8~98.2	70.2~102	70.9~99.5	70.0~105	60.2~98.5
	0.02(for fish muscle 0.002)	71.5~99.9	72.5~104	80.0~97.0	73.5~110	67.0~95.0
	0.04(for fish muscle 0.004)	71.0~93.0	70.8~102	73.5~94.3	71.8~100	71.5~97.5
promethazine	0.001	71.3~106	83.5~110	77.3~109	67.3~102	61.8~99.5
	0.002	73.0~105	74.5~107	90.5~109	73.5~109	65.0~99.5
	0.004	79.0~103	87.5~108	95.0~110	77.5~109	76.5~104
chloropromazine	0.001	65.7~102	69.7~91.7	66.1~97.8	62.1~96.5	61.3~96.5
	0.002	74.0~98.0	70.5~99.0	73.0~101	69.0~93.5	70.0~98.5
	0.004	75.0~102	70.5~99.0	77.0~95.5	64.5~88.3	69.0~99.8
acepromazine	0.001	77.2~105	65.8~102	70.2~108	70.0~109	62.1~90.5
	0.002	75.5~105	84.5~110	87.0~107	69.5~105	66.5~96.0
	0.004	75.5~103	83.3~110	75.3~105	68.5~94.5	74.0~98.5
propionylpromazine	0.001	72.1~110	63.8~89.7	66.1~99.5	66.9~93.4	61.8~94.5
	0.002	83.5~104	61.0~92.5	71.0~99.0	65.5~99.0	70.0~97.5
	0.004	72.8~105	75.3~97.3	76.8~101	73.0~93.8	70.3~92.0

Annex A
(Informative)
Reference mass conditions[1)]

Reference mass conditions:

a) Nebulizer gas: 7.00 L/min(Nitrogen);

b) curtain gas: 9.00 L/min(Nitrogen);

c) ion spray voltage: 2 500 V;

d) heat temperature: 500℃;

e) heat gas: 7.00 L/min(Nitrogen);

f) collision gas: 6.00 mL/min (Nitrogen).

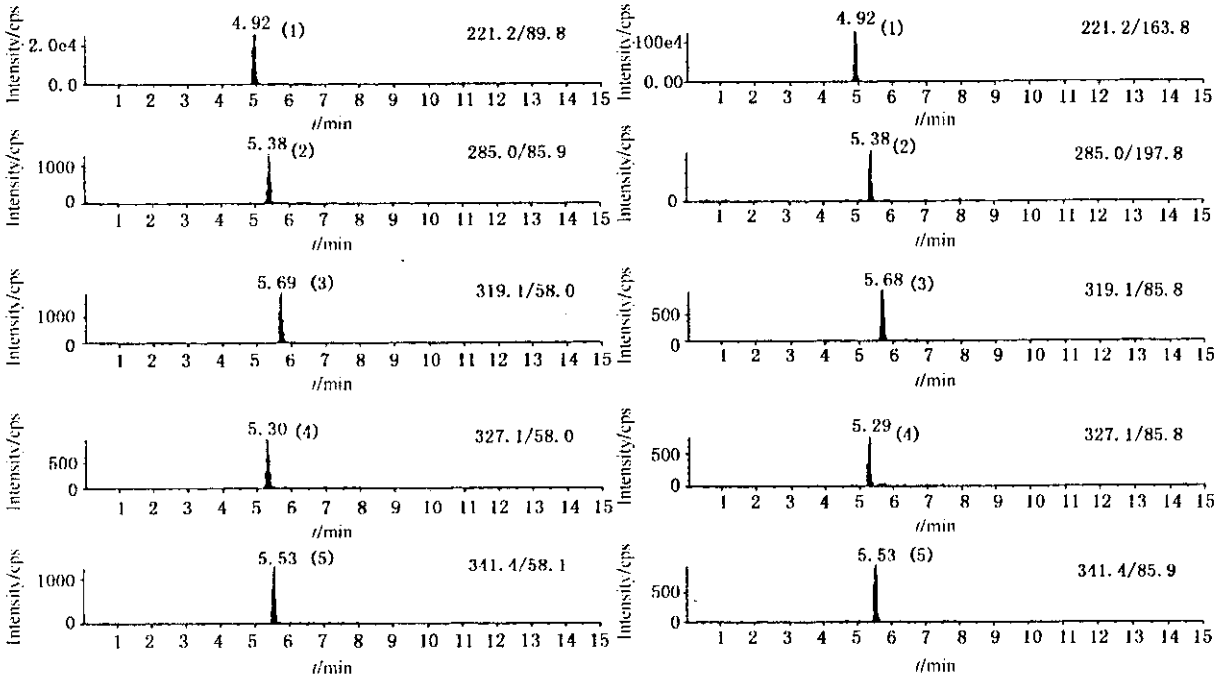
g) Other mass operating conditions are listed in table A.1.

Table A.1—Main MS parameters of phenothiazines

Compound	Precursor (m/z)	Daughter ion (m/z)	Dwell time/ ms	DP	FP	CE/ eV	CXP
xyzaline	221.2	89.8[a]	100	36	170	39	4
		163.8	100	36	170	39	12
promethazine	285.0	85.9[a]	50	41	150	31	14
		197.8	50	41	150	33	18
chloropromazine	319.1	58.0[a]	50	36	150	61	4
		85.8	50	36	150	29	6
acepromazine	327.1	58.0[a]	50	51	180	65	10
		85.8	50	51	180	31	8
propionylpromazine	341.4	58.1[a]	50	51	210	71	4
		85.9	50	51	210	27	6
Note: For the different MS equipment, the parameters may be different, and the MS parameters should be optimized to the best before analysis.							
[a] mark is the quantification ion.							

1) Non-commercial statement: the reference mass parameters in Annex A are accomplished by API 3 000 LC-MS/MS, the equipment and its type involved in the standard method is only for reference and not related to any commercial aim, and the analysts are encouraged to use equipments of different corporation or different type.

Annex B
(Informative)
Multiple reaction monitoring(MRM)chromatogram of standard solution



(1) Xyzaline.0.02 mg/L;

(2) Promethazine.0.002 mg/L;

(3) Chloropromazine.0.002 mg/L;

(4) Acepromazine.0.002 mg/L;

(5) propionylpromazine.0.002 mg/L.

Figure B.1—Multiple reaction monitoring(MRM)chromatogram of 5 phenothiazines residues of standard working solution

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