SN

中华人民共和国出入境检验检疫行业标准

SN/T 0193.1—2015
代替 SN/T 0193.1—1993

出口皮革及皮革制品中五氯酚残留量检测方法　第1部分：液相色谱-质谱/质谱法

Determination of pentachlorophenol residue in leather and leather products for export—Part 1: HPLC-MS/MS method

2015-02-09 发布　　2015-09-01 实施

中华人民共和国
国家质量监督检验检疫总局　发布

前　言

SN/T 0193《出口皮革及皮革制品中五氯酚残留量检测方法》共分为2个部分：

——第1部分：液相色谱-质谱/质谱法

——第2部分：气相色谱法

本部分为SN/T 0193的第1部分。

本部分按照GB/T 1.1—2009给出的规则起草。

本部分代替SN/T 0193.1—1993《进出口皮革及皮革制品中五氯酚残留量检验方法　第1部分：乙酰化气相色谱法》。

本部分与SN/T 0193.1—1993主要技术差异如下：

——对前处理方法和检测仪器进行改进，降低了方法检出限。

——略去了抽样步骤。

本部分由国家认证认可监督管理委员会提出并归口。

本部分起草单位：中华人民共和国上海出入境检验检疫局、中华人民共和国新疆出入境检验检疫局、嘉兴市质量技术监督局。

本部分主要起草人：赵善贞、韩丽、曲栗、伊雄海、周瑶、曹晨、张新、张旖、姜苏杰。

本部分所代替标准的历次版本发布情况为：

——SN/T 0193.1—1993。

出口皮革及皮革制品中五氯酚残留量检测 方法 第1部分:液相色谱-质谱/质谱法

1 范围

SN/T 0193的本部分规定了出口鞣质猪皮、牛皮、羊皮及其制品中五氯酚残留量的液相色谱-质谱/质谱测定方法。

本部分适用于鞣质猪皮、牛皮、羊皮中五氯酚残留量定性确证和定量测定。

2 方法提要

试样中的五氯酚用1%氢氧化钠溶液提取,HLB小柱净化,液相色谱-质谱/质谱检测和确证,外标法定量。

3 试剂材料

除另有说明外,所用试剂均为分析纯,水为去离子水。

3.1 乙腈:高效液相色谱级。

3.2 甲醇:高效液相色谱级。

3.3 甲酸:高效液相色谱级。

3.4 乙酸铵:高效液相色谱级。

3.5 氢氧化钠。

3.6 HLB固相萃取小柱(60 mg,3 mL),或相当者。

3.7 1%的NaOH溶液:称取1.0 g氢氧化钠(精确至0.01 g)(3.5),水溶解并定容至100 mL。

3.8 5 mmol/L乙酸铵溶液,含0.1%的甲酸:精确称取0.385 g乙酸铵(精确至0.01 g)(3.4),加入1 mL甲酸,水稀释定容至1 L。

3.9 含0.1%甲酸的乙腈:移取1 mL甲酸(3.3)于1 L乙腈(3.1)中。

3.10 水-甲醇(95+5,V_1+V_2):量取95 mL水,加入5 mL甲醇(3.2),混匀。使用前配制。

3.11 水-乙腈(90+10,V_1+V_2):量取90 mL水,加入10 mL乙腈(3.1),混匀。使用前配制。

3.12 标准品:五氯酚(pentachlorophenol CAS号:87-86-5),纯度≥95%。

3.13 标准储备溶液的配制:称取0.01 g五氯酚标准品(精确至0.000 1 g)(3.12),用甲醇溶解定容至终浓度为1.0 mg/mL,−18 ℃避光冷冻保存,有效期为12个月。

3.14 标准中间溶液的配制:移取100 μL五氯酚标准储备液(3.13),用甲醇稀释至终浓度约为1.0 μg/mL,4 ℃避光冷藏保存,有效期为6个月。

3.15 基质标准工作液的配制:根据需要,临用时吸取一定量的标准中间溶液(3.14),用基质空白溶液配制成适当浓度的基质标准工作溶液(参考线性浓度范围为1.0 ng/mL～100.0 ng/mL),临用前现配。

3.16 0.22 μm有机相滤膜。

4 仪器和设备

4.1 液相色谱-质谱/质谱仪:配有电喷雾离子源(ESI)。

4.2 分析天平:感量 0.000 1 g 和 0.01 g。

4.3 旋涡混匀器。

4.4 离心机:10 000 r/min。

4.5 固相萃取装置。

4.6 氮吹浓缩装置。

5 试样制备与保存

取样品中有代表性的部分约 500 g,用剪刀绞碎成约为 1 cm^2 的小块,均分成两份,分别装入洁净容器作为试样,密封,并标明标记。将试样置于−18 ℃避光保存。

注: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

称取 0.5 g 样品(精确至 0.01 g)于 50 mL 塑料离心管中,加入 25 mL 氢氧化钠溶液(3.7),涡旋混合 5 min,8 000 r/min 离心 5 min。取上清液,待过固相萃取小柱。

6.2 净化

固相萃取小柱依次用 3 mL 甲醇、3 mL 水活化,转移提取液(6.1)至固相萃取柱的顶部,以 0.5 mL/min 流速过柱。用 3 mL 的水-甲醇(3.10)溶液淋洗柱子;用 6 mL 甲醇溶液洗脱小柱,收集洗脱液,于 40 ℃±5 ℃的温度下蒸发洗脱液至干,用 1.0 mL 水-乙腈溶液(3.11)振荡溶解残渣后,过 0.22 μm 有机相滤膜(3.16),滤液供液相色谱-质谱测定。

7 测定

7.1 参考高效液相色谱条件

参考高效液相色谱条件如下:

a) 色谱柱:C_{18}柱,50 mm×2.1 mm,粒径 1.8 μm 或相当者;

b) 流动相:A:5 mmol/L 乙酸铵水溶液,含 0.1%甲酸,B:含 0.1%甲酸的乙腈;

c) 流速:0.4 mL/min,梯度洗脱程序见表 1;

d) 柱温:常温;

e) 进样量:10 μL。

表 1 梯度洗脱程序表

梯度时间/min	流动相 A/%	流动相 B/%
0	95	5
3	80	20
4	80	20
4.5	95	5
9	95	5

7.2 参考质谱条件

参考质谱条件如下：

a） 离子源：电喷雾 ESI，负离子模式；

b） 扫描方式：多反应监测 MRM；

c） 其他参考质谱条件：参见附录 A。

7.3 定量测定

根据试样中被测物的含量情况，选取响应值适宜的标准工作液进行色谱分析，标准曲线工作液应有5个浓度水平。基质标准工作液和待测液中五氯酚的响应值均应在仪器线性响应范围内，如果含量超过线性范围，应用基质空白溶液稀释到合适浓度后分析。在上述色谱条件下的五氯酚的参考保留时间为 5.68 min。标准溶液中待测物的多反应监测色谱图参见附录 B 中图 B.1。

7.4 定性测定

按照液相色谱-质谱/质谱条件测定样品和标准工作溶液，如果检测的质量色谱峰保留时间与标准品一致，定性离子对的相对丰度用相对于最强离子丰度的强度百分比表示，应当与浓度相当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对应的被测物。

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%

7.5 空白试验

除不加试样外，均按上述操作步骤进行。

7.6 结果计算和表述

用色谱数据处理机或按式(1)计算试样中五氯酚的残留含量，计算结果需扣除空白值：

$$X_i = \frac{\rho_i \times V}{m} \times \frac{1\,000}{1\,000} \qquad \cdots\cdots(1)$$

式中：

X_i ——试样中五氯酚残留量，单位为微克每千克(μg/kg)；

ρ_i ——从标准曲线上得到的五氯酚残留量的溶液质量浓度，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(LOQ)

本方法中五氯酚的测定低限为 50 μg/kg。

由于测试结果取决于所使用仪器，因此不可能给出液相色谱-质谱/质谱分析的通用参数。设定的参数应保证色谱测定时被测组分与其他组分能够得到有效的分离，下列给出的参数证明是可行的。

8.2 回收率

采用本方法对鞣质猪皮、羊皮、牛皮等3种基质进行添加回收试验，五氯酚在3种基质中的回收率资料参见附录C中表C.1。

附 录 A
（资料性附录）
API 4000 四级杆质谱仪参数[1)]

A.1 电喷雾电压(IS)：5 500 V。

A.2 雾化气压力(GS1)：380 kPa(50 psi)。

A.3 气帘气压力(CUR)：172 kPa(25 psi)。

A.4 辅助气流速(GS2)：310 kPa(45 psi)。

A.5 离子源温度(TEM)：500 ℃。

A.6 监测离子对、碰撞电压(CE)、去簇电压(DP)如表 A.1 所示。

表 A.1 五氯酚的定性、定量离子对以及 CE、DP 参考值

化合物	母离子 (Q_1)	子离子 (Q_3)	CE/V	DP/V
五氯酚	262.7*	262.7*	−5	−75
	264.7	264.7	−5	−72
	266.7	266.7	−5	−68
	262.7	199.6	−44	−34
其中：* 为定量离子对。				

1) 非商业性声明：附录 A 所列参考质谱条件是在 Agilent 1200 液相色谱仪、ABAPI4000 质谱仪上完成的，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）
五氯酚标准的多反应监测（MRM）色谱图

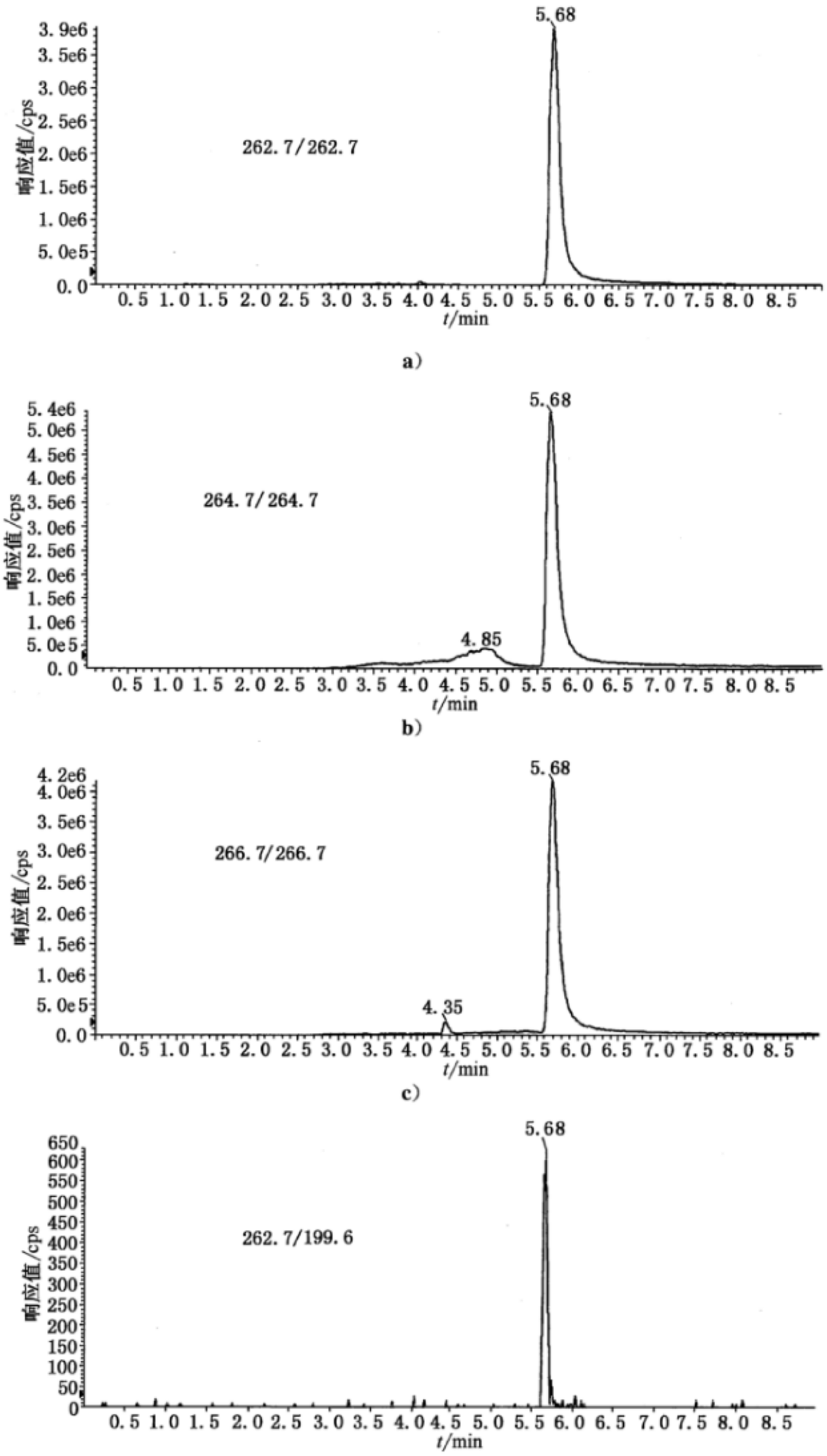

图 B.1 50 ng/mL 五氯酚标准的多反应监测（MRM）色谱图

附 录 C
（资料性附录）
皮革及皮革制品中五氯酚的添加回收率范围

表 C.1 皮革及皮革制品中五氯酚的添加回收率范围

基质	添加浓度/(μg/kg)	回收率/%	标准偏差/%
猪皮	50	81.2～93.4	5.6
	500	88.6～110.2	10.5
	1 000	93.4～100.5	2.7
牛皮	50	81.6～102.0	7.9
	500	91.4～102.0	4.3
	1 000	88.6～99.3	5.0
羊皮	50	82.0～102.6	8.4
	500	80.0～100.6	9.1
	1 000	81.3～99.5	3.9

Foreword

SN/T 0193《Method for Determination of Pentachlorophenol in Leather and Leather Product for Export》 is divided into 2 parts:

——First part: LC-MS/MS method

——Second part: GC method

This part is the first part of SN/T 0193.

The standard was drafted in accordance with the GB/T 1.1—2009.

This standard is replace of SN/T 0193.1—1993《Method for Determination of Pentachlorophenol in Leather and Leather Product for Export-Acetylation-gas chromatography》.

The main improvement from SN/T 0193.1—1993:

——The preparation and equipment is improved, and limit of determination is reduced.

——The sampling processor is omitted.

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

The standard was drafted by Shanghai Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Xinjiang Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China. Jiaxin Quality Supervision Bureau.

The standard was mainly drafted by Zhao Shanzhen, Han li, Quli, Yi Xionghai, Zhou yao, Cao chen, Zhang xin, Zhang yi, Jiang sujie.

The standard replaced the previous version of the release of the standard as follows:

——SN/T 0193.1—1993.

Determination of pentachlorophenol residue in leather and leather products for export —Part 1:HPLC-MS/MS method

1 Scope

The standard specifies the method of sample preparation and determination of pentachlorophenol residue in tannin leathers by HPLC-MS/MS.

The standard is applicable to the determination of pentachlorophenol in tannin pigskin sheepskin cow skin by HPLC-MS/MS.

2 Principle

The pentachlorophenol residue is extracted by 1% NaOH, cleaned up by HLB solid phase column; the pentachlorophenol residue is determined by HPLC-MS/MS and quantified by external standard method.

3 Reagents and materials

Unless otherwise specified, the entire reagent used should be analytical grade, water is deionizer water.

3.1 Acetonitrile: HPLC grade.

3.2 Methanol: HPLC grade.

3.3 Formic acid: HPLC grade.

3.4 Ammonium acate: HPLC grade.

3.5 Sodium hydrate.

3.6 SPE columns: HLB (60 mg, 3 mL), or equalvent.

3.7 1%NaOH: Weight 1.0 g sodium hydrate (précised to 0.01 g)(3.5) in 100 mL water.

3.8 5 mmol/L ammonium acate: Weight 0.385 g ammonium acate (précised to 0.01 g) (3.4) add 1 mL formic acid, dissolved in 1 L water.

3.9 Acetonitrile, contain 0.1% formic acid: measure l mL formic acid (3.3) to 1 L acetonitrile (3.1).

3.10 Water-methanol (95 + 5, V_1 + V_2): Measure 95 mL water, add 5 mL methanol (3.2), mixed well.

3.11 Water-acetonitrile (90 + 10, V_1 + V_2): Measure 90 mL water, add 10 mL acetonitrile (3.1), mixed well.

3.12 Standards: Pentachlorophenol CAS number: 87-86-5, purity ≥95%.

3.13 Stock standard solution: Weight 0.01 g standards (précised to 0.0001 g) (3.12), dissolve in methanol to final concentration of 1.0 mg/mL. The stock standard solution store in refrigerated at −18 ℃ in brown bottle for 12 months.

3.14 Intermediate standard solution: Remove 100 μL of stock standard solution (3.13); dissolve in methanol to final concentration of 1.0 μg/mL. The stock standards store refrigerated at −4 ℃ in brown bottle for six months.

3.15 Working standard solutions: To require as useful, dilute the intermediate standard solution (3.14) to proper concentration as 1.0 ng/mL to 100.0 ng/mL in matrix solution, diluted directly before using.

3.16 Microspore film: 0.22 μm.

4 Apparatus and equipment

4.1 Liquid chromatography combined with electrospray ionization mass spectrometry.

4.2 Balances (0.000 1 g and 0.01 g).

4.3 Vortex mixer.

4.4 Centrifuge: 10 000 r/min.

4.5 Apparatus of SPE.

4.6 Evaporator with nitrogen flow.

5 Preparation and storage of test sample

About 500 g reprehensive sample which is totally minced to about 1 cm^2 and placed into a clean vessel as a test sample, which is sealed and labeled. The test sample should be stored at temperature of below −18 ℃. Casing sample was desalted before using.

Note: in the course of sampling and sample preparation, precaution should be taken to avoid contamination or any factor that may causes the change of residue content.

6 Procedure

6.1 Extraction

Weight 0.5 g (précised to 0.01 g) sample in 50 mL plastic centrifuge tubes, add 25 mL 0.1% NaOH (3.7), mix for 5 min, and then centrifuge at 8 000 r/min for 5 min utes. Collect the supernatants for SPE cleaning up.

6.2 Cleaning up

The HLB SPE cartridge is washed with 3 mL methanol and 3 mL water, before using and keeps the column wetness. Load all the sample solution to a conditioned, concentrated the outflow at 0.5 mL/min, rinse the column with 3 mL water-methanol (3.10), and elute the analysts with 6 mL methanol. Evaporate the elute solution at 40 ℃ ±5 ℃. And dilute to 1.0 mL by water-acetonitrile (3.11). After being filter by 0.22 μm film (3.16), the final solution is ready for HPLC−MS/MS analysis.

7 Determination

7.1 HPLC operating conditions

HPLC operating conditions is as following:

a) Column: C_{18} (50 mm × 2.1 mm, 1.8 μm), or the equvalent.

b) Mobile phase: A: 5 mmol/L ammonium acrate, contain 0.1% formic acid; B: acetonitrile, contain 0.1% formic acid.

c) Rate: 0.4 mL/min, seeing Table 1.

d) Column temperature: room temperature.

e) Injection volume:10 μL.

Table 1 Gradient program of mobile phase

Time/min	Phase A/%	Phase B/%
0	95	5
3	80	20
4	80	20
4.5	95	5
9	95	5

7.2 Mass spectral acquisition

Mass spectral aequisition is as following:

a) Source:ESI,negative mode.

b) Monitor mode:multiple reaction monitoring,MRM.

c) Related parameters and quantifiter MRM:listed as Annex A.

7.3 Quantitation of HPLC-MS/MS

According to the above HPLC-MS/MS operating condition,determined the sample solution and the standard working curve simultaneously. The standard working curve should contained 5 level of concentration. If the determined sample is over the scope of standard working curve,the concentration of determined sample should dilute to a proper concentration by matrix solution. Under the above HPLC-MS/MS operating condition,the retention time is 5.68 min,the MRM chromatograms of the standard are listed as Figure B Figure B.1.

7.4 Confirmation of HPLC-MS/MS

Determinate under the established HPLC-MS/MS conditions,and calculated the intensity ration of two selected ion pairs of the sample solution and the standard working solution. The relative abundance ratio tolerance is the same as listed (Table 2); it is safe to conclude that this compound do exit in the sample.

Table 2 Maximum permitted tolerances for relative ion intensities while conformation

Relative ion intensities %	>50%	>20%~50%	>10%~20%	≤10%
Permitted relative tolerances %	±20%	±25%	±30%	±50%

7.5 Blank test

The operation of the blank test is the same as describe in the method of determination, but without addition the sample.

7.6 Calculation and expression of result

Calculation the content of pentachlorophenol residue in the test sample by HPLC-MS/MS data processor or according to the formula (1). The blank value should be subtracted from the above result of calculation.

$$X_i = \frac{\rho_i \times V}{m} \times \frac{1\,000}{1\,000} \qquad \cdots\cdots(1)$$

Where:

X_i ——the residue content of pentachlorophenol, μg/kg;

ρ_i ——the concentration of pentachlorophenol residue from standard working curve, ng/mL;

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

m ——mass of test sample of final sample solution, g.

8 Limit of quantification (LOQ) and recovery

8.1 Limit of quantification(LOQ)

The limit of quantification of pentachlorophenol is 50 μg/kg.

Because the test results depend on the instrument used, general parameters of LC-MS/MS cannot be given, setting parameters to ensure the effective separation of the components and other components in the chromatographic determination, the following parameters are shown to be feasible.

8.2 Recovery

According to the experimental data, the fortified concentration and recovery ranges of pentachlorophenol are listed in Annex C Table C.1.

Annex A
(Informative)
Main mass parameters of API 4 000[1)]

A.1 Electros pray capillary voltage:positive mode 5 500 V.

A.2 GS1:380 kPa (50 psi).

A.3 CUR:172 kPa (25 psi).

A.4 GS2:310 kPa (45 psi)

A.5 TEM:500 ℃.

A.6 Qualifier and quantifier MRM,Collision Energy (CE),Declustering Potential (DP).

Table A.1 Transitions for confirmation and quantification,CE、DP

Compound	Precursor ion (Q_1)	Product ion (Q_3)	CE/V	DP/V
pentachlorophenol	262.7*	262.7*	−5	−75
	264.7	264.7	−5	−72
	266.7	266.7	−5	−68
	262.7	199.6	−44	−34
Annotation:the symbol"*"represents the quantitative transition.				

1) Non-commercial statement:tile equipment and their types involved in the standard method are Agilent 1200 liquid chromatography and AB API4000 mass spectrum,the test apparatus is provided for reference only not related to commercial aims,and it is encouraged to use equipment of diferent corporation or different type.

Annex B
(Informative)
MRM chromatogram of pentachlorophenol standard

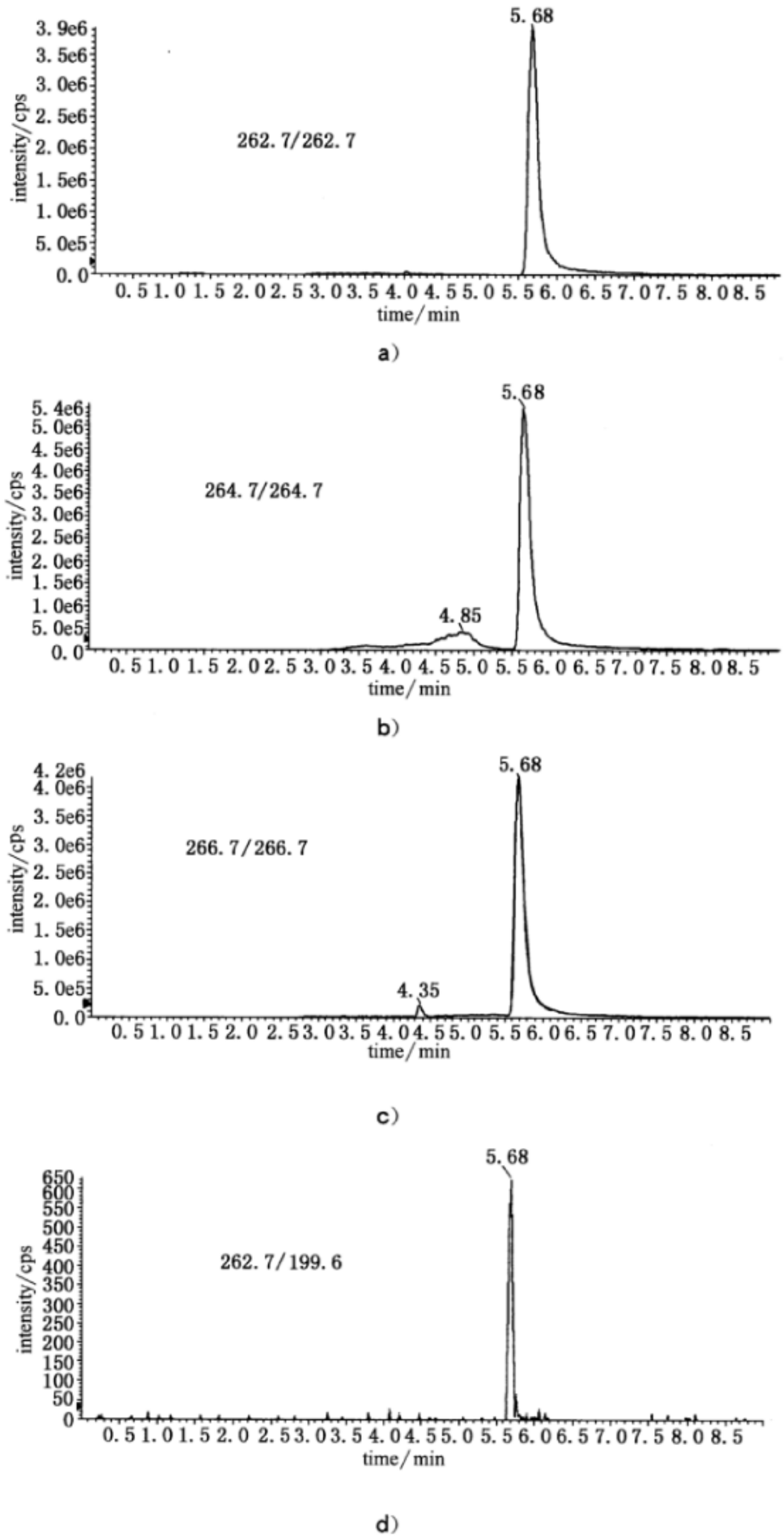

Figure B.1 50 ng/mL MRM chromatogram of pentachlorophenol standard

Annex C
(Informative)
Table C. 1 Recoveries of pentachlorophenol residue

Table C. 1 Recoveries of pentachlorophenol residue

Matrix	Spiked level/(μg/kg)	Recovery/%	Standard deviation/%
pigskin	50	81. 2～93. 4	5. 6
	500	88. 6～110. 2	10. 5
	1 000	93. 4～100. 5	2. 7
cowskin	50	81. 6～102. 0	7. 9
	500	91. 4～102. 0	4. 3
	1 000	88. 6～99. 3	5. 0
sheepskin	50	82. 0～102. 6	8. 4
	500	80. 0～100. 6	9. 1
	1 000	81. 3～99. 5	3. 9