SN

中华人民共和国出入境检验检疫行业标准

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进出口中药材及其制品中五氯硝基苯残留量检测方法　气相色谱-质谱法

Determination of quintozene residues in medicinal plant and their products for import and export—GC-MS method

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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

本标准的附录A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：中华人民共和国天津出入境检验检疫局。

本标准主要起草人：林安清、许泓、张曼、古珑、何佳、张骏、吴延晖、肖亚兵。

本标准系首次发布的检验检疫行业标准。

进出口中药材及其制品中五氯硝基苯残留量检测方法　气相色谱-质谱法

1　范围

本标准规定了中药材及其制品中五氯硝基苯残留量气相色谱-质谱检测方法。

本标准适用于人参、人参口服液中五氯硝基苯残留量的测定。

2　方法提要

试样用正己烷-丙酮混合溶液提取，经浓硫酸磺化，气相色谱-质谱法测定，外标法定量。

3　试剂和材料

除另有规定外，试剂均为分析纯，水为一级水。

3.1　正己烷：色谱纯。

3.2　丙酮：色谱纯。

3.3　浓硫酸：优级纯。

3.4　无水硫酸钠：于650℃灼烧4 h，置于干燥器中备用。

3.5　硫酸钠溶液：40 g/L。

3.6　五氯硝基苯(Quintozene，$C_6Cl_5NO_2$，CAS：82-68-8)标准品：纯度大于等于99%。

3.7　五氯硝基苯标准溶液：准确称取适量的五氯硝基苯标准品，用正己烷配制成浓度为100 μg/mL标准储备液。根据需要用正己烷稀释成适当浓度的标准工作溶液。于0℃～4℃冰箱中保存。

3.8　无水硫酸钠柱：80 mm×40 mm(内径)筒形漏斗，底部垫约5 mm高脱脂棉，再装10 g无水硫酸钠(3.4)。

4　仪器和设备

4.1　气相色谱-质谱仪，配电子轰击源(EI)。

4.2　涡旋混合器。

4.3　高速均质器：24 000 r/min。

4.4　离心机：3 000 r/min。

4.5　旋转蒸发器。

4.6　药材粉碎机。

5　样品制备与保存

取有代表性人参样品约500 g，充分粉碎后过2.0 mm筛。取有代表性样品人参口服液约500 g，混匀，装入洁净容器内，密封并标明标记，于4℃冷藏保存。

在抽样和制样的操作过程中，应防止样品受到污染或发生五氯硝基苯残留物含量的变化。

6　测定步骤

6.1　提取

6.1.1　人参样品

称取5 g(精确至0.01 g)试样于100 mL离心管中，加入50 mL丙酮＋正己烷(2＋8，体积比)溶液，

于高速均质器 14 000 r/min 均质 5 min,3 000 r/min 离心 3 min,过滤至 150 mL 浓缩瓶中,重复上述操作一次。合并提取液,于 50℃水浴旋转蒸发至约 50 mL,转移至 150 mL 分液漏斗中。

6.1.2 人参口服液样品

称取 5 g(精确至 0.01 g)试样于 100 mL 离心管中,加入 50 mL 丙酮+正己烷(2+8,体积比)提取液,涡旋混合 5 min,转移至 500 mL 分液漏斗中。加入 300 mL 水,振摇,静置分层,弃去水相。再加入 300 mL 水,重复上述操作一次。

6.2 净化

在上述分液漏斗中加入 10 mL 浓硫酸(3.3),轻轻振摇 0.5 min 后,静置分层,弃去下层酸液。再重复净化 3 次～4 次(净化至下层酸液呈无色)。再用 2×100 mL 硫酸钠溶液(3.5)洗涤两次,静置分层后,弃去水相。将净化液通过无水硫酸钠柱(3.8),用 10 mL 正己烷洗涤无水硫酸钠柱,收集正己烷至浓缩瓶中,于 50℃水浴旋转蒸发至干,用 1.0 mL 正己烷溶解残渣,供气相色谱-质谱仪测定。

6.3 测定

6.3.1 气相色谱-质谱条件

a) 色谱柱:DB-35MS 石英毛细管柱 25 m×0.25 mm(内径),膜厚 0.25 μm,或相当者;

b) 柱温程序:100℃ $\xrightarrow{25℃/min}$ 175℃(3 min) $\xrightarrow{10℃/min}$ 210℃(3 min);

c) 进样口温度:280℃;

d) 接口温度:250℃;

e) 离子源温度:200℃;

f) 载气:氦气,纯度大于等于 99.999%,流速 1.0 mL/min;

g) 进样方式:无分流,1.0 min 后开阀;

h) 溶剂延迟:2.8 min;

i) 进样量:2 μL;

j) 电子轰击源 EI 能量:70 eV;

k) 监测方式:选择离子监测方式(SIM);

l) 选择离子(m/z):定量 295,定性 142、214、237、297。

6.3.2 气相色谱-质谱测定

根据样液中被测物含量情况,选定峰面积相近的标准工作溶液,标准工作溶液和待测样液中五氯硝基苯的响应值均应在仪器检测的线性范围内。对标准工作溶液与样液等体积参插进样测定。

如果样液与标准工作溶液的选择离子色谱图中,在相同保留时间有色谱峰出现,同时所选择离子的丰度比与标准样品相关离子的相对丰度一致(m/z 142、214、237、295、297,其丰度比 100∶73∶85∶51∶36),相似度在允差之内(见表 1),根据选择离子 m/z 295 对其进行外标法定量。在上述气相色谱-质谱条件下,五氯硝基苯的参考保留时间约为 8.0 min。五氯硝基苯标准品总离子流图和质谱图参见附录 A 中图 A.1 和图 A.2。

表 1 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

6.4 空白试验

除不加试样外,均按上述测定步骤进行。

7 结果计算和表述

用色谱数据处理机或按式(1)计算试样中五氯硝基苯残留量,计算结果应将空白值扣除:

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

式中：

X——试样中五氯硝基苯的残留量，单位为毫克每千克(mg/kg)；

A——样液中五氯硝基苯的峰面积；

A_s——标准工作液中五氯硝基苯的峰面积；

c_s——标准工作溶液中五氯硝基苯的浓度，单位为微克每毫升(μg/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量，单位为克(g)。

8 方法的测定低限、回收率

8.1 测定低限

本方法测定低限为：0.004 mg/kg。

8.2 回收率

本方法添加浓度范围及回收率见表2。

表2 样品的添加浓度及回收率的实验数据

样品	添加浓度/(μg/kg)	回收率范围/%	样品	添加浓度/(μg/kg)	回收率范围/%
人参	4	75.0～95.0	人参口服液	4	85.0～97.5
	10	80.0～94.0		10	88.0～97.0
	20	83.0～100.0		20	90.3～97.0

附 录 A
（资料性附录）
五氯硝基苯标准品气相色谱-质谱图

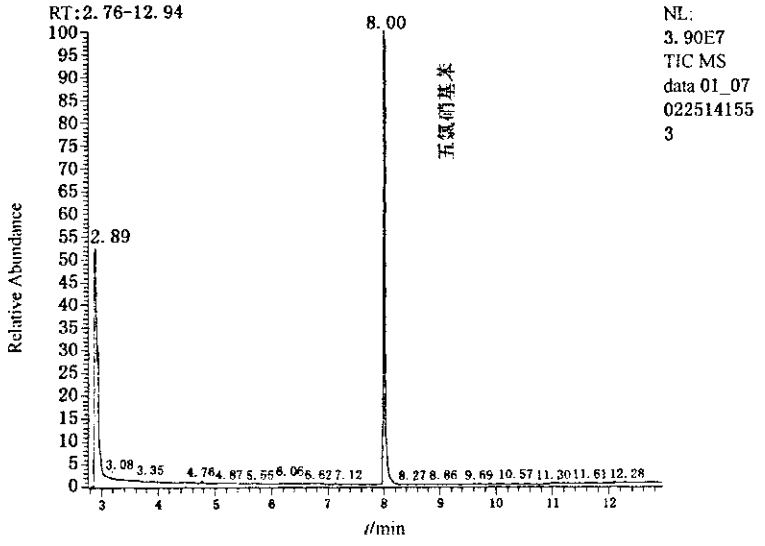

图 A.1 五氯硝基苯标准品总离子流图(TIC)

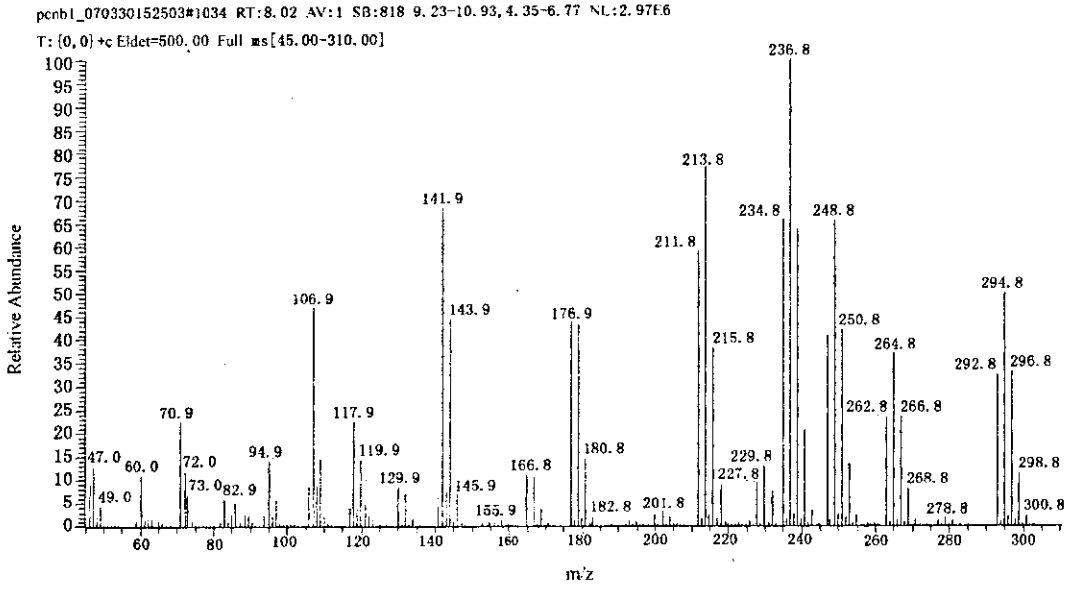

图 A.2 五氯硝基苯标准品质谱图

Foreword

Annex A of this standard is an informative annex.

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

This standard was drafted by tianjin entry-exit inspection and quarantine bureau of the People's republic of China.

The main drafters of this standard are Lin Anqing, Xu Hong, Zhang Man, Gu Long, He Jia, Zhang Jun, Wu Yanhui and Xiao Yabing.

This standard is a professional standard promulgated for the first time.

Determination of quintozene residues in medicinal plant and their products for import and export —GC-MS method

1 Scope

This standard specifies the methods of determination by GC-MS of quintoze residue in medicinal plants and theirs products.

This standard is applicable to the determination of quintoze residue in panax, gen-seng.

2 Principle method of determination

The Quintozene residue were extracted with acetone-*n*-hexane, sulfonaped by Oil of vitriol, determinated by GC-MS. Calculated by comparing peak of the sample with corresponding standard peak area.

3 Reagents and materials

Unless otherwise specified, all reagents used should be analytically pure. "Water" is the first-degree water.

3.1 *n*-Hexane: HPLC grade.

3.2 Acetone: HPLC grade.

3.3 Oil of vitriol: Guaranteed regent.

3.4 Anhydrous sodium sulfate: ignite at 650℃ for 4 h, and store in air-tight container.

3.5 Anhydrous sodium sulfate solution: 40 g/L.

3.6 Quintozene standard ($C_6Cl_5NO_2$, CAS-No: 82-68-8): Purity≥99%.

3.7 Quintozene standard solution: Weigh appropriate quintozene, dissolve in *n*-Hexane and prepare a solution of 100 μg/mL as standard stock solution. Dilute the quintozene standard stock solution to the required concentration as the standard working solution with *n*-Hexane. It should be stored in refrigeratory at 0℃～4℃.

3.8 Anhydrous sodium sulfate column: 80 mm × 40 mm(i. d.)funnel, filled with 10 g anhydrous sodium sulfate(3.4) upon 5 mm.

4 Apparatus and equipment

4.1 GC-MS: Equipped with EI.

4.2 Minishaker

4.3 Homogenizer: 24 000 r/min.

4.4 Vortex mixer: 3 000 r/min.

4.5 Rotation evaporator.

4.6 Pulverizer.

5 Sample preparation and storage

Reduce the sample of panax to ca 500 g, churn up, let pass through a 2.0 mm sieve. Reduce the sample of gen-seng to ca 500 g. Mix thoroughly, place in clean containers, seal and label, stored below 4℃.

In the course of sample preparation, precaution should be taken to aviod contamination or any factors which may cause the change of quintozene residue content.

6 Procedure of determination

6.1 Extraction

6.1.1 Panax sample

Weigh 5 g(accurated to 0.01 g) of the test sample into 100 mL centrifuge tube, adding 50 mL acetone-*n*-hexane (2+8, *V*/*V*), homogenize for 5 min under 14 000 r/min, centrifuge for 3 min under 3 000 r/min. The extraction liquid was filterated to 150 mL erlenmeyer flake, then repeated above operation. Combine the extraction liquid, rotary evaporate the extraction liquid at 50℃ to almost 50 mL. Transfer above solution to 150 mL separator funnel.

6.1.2 Gen-seng sample

Weigh 5 g(accurated to 0.01 g) of the test sample into 100 mL centrifuge tube, adding 50 mL acetone-*n*-hexane(2+8, *V*/*V*),minishaking 5 min, Transfer above solution to 500 mL separator funnel, adding 300 mL water, shaking, let it stand for separately comperately. Abandon the awater phase. Adding 300 mL water ,then repeated above operation.

6.2 Clean up

Add 10 mL oil of vitriol (3.3), shaking lightly for 0.5 min. Let it stand for separately comperately, abandon the acid phase. Repeat above cleaning three or four times(until the acid phase show achromaticity). Add 2×100 mL anhydrous sodium sulfate solution (3.5), let it stand for separately comperately, abandon the water phase. Pass this liquid through the column of anhydrous sodium sulfate (3.8) to another 150 mL erlenmeyer flake. Rinse the cloumn of anhydrous sodium sulfate three times with 10 mL *n*-Hexan. Collect *n*-Hexane phase,rotary evaporate the extraction liquid at 50℃ to dry. Make up to 1.0 mL with *n*-hexane for GC-MS determination.

6.3 Determination

6.3.1 GC-MS operating conditions

a) Column: DB-35MS 25 m×0.25 mm(i.d.), film thickness 0.25 μm or equivalent;

b) Column temperature: 100℃ $\xrightarrow{25℃/min}$ 175℃ (3 min) $\xrightarrow{10℃/min}$ 240℃ (3 min);

c) Injection port temperature:280℃;

d) Interface temperature:250℃;

e) Ion source temperature:200℃;

f) Carrier gas:Helium,purity≥99.999%,flow rate:1.0 mL/min;

g) Injection mode:Splitless, open the valve after 1.0 min;

h) Solvent protection delay:2.8 min;

i) Injection volumn:2 μL;

j) Ionization energy:70 ev;

k) Detection mode:SIM;

l) Sclected ions(m/z):determinded by 295,confirmed by 142,214,237,297.

6.3.2 GC-MS determination

According to the approximate concentration of quintozene residuces in sample solution, select the standard working solution with similar peak area to that of the sample solution. The standard working solution should be randomly injected in between the injection of sample solution of equal volumn.

If there is a peak appeared at the same retention time for both of the sample solution and standard working solution in the mass spectrogram figure. The accordance between the retention time of the measured sample solution and the time of standard, the appearance of all selected ions in the chromatogram of the sample while background is deducted, the consistency between the abundance ratio of the selected ions from the sample and ions from standard(m/z 142,214,237,295 and 297) . The abundance ratio is 100 : 73 : 85 : 51 : 36, and the similarity degree of their relative abundance ratio in permitted tolerance(See table 1). Calculated the ratio of the monitoring ions(m/z 295) with corresponding standard peak area. Under the above GC-MS conditions, the retention time of quintozene is about 8.0 min. See figureA.1 and figureA.2 in annex A.

Table 1—Maximum permitted tolerance for relative ion intensities using a range of mass spectrometric techniques

Relative intensity/%	>50	>20~50	>10~20	≤10
GC-MS(relative)/%	±20	±25	±30	±50

6.4 Blank test

Perfore the blank test with the same procederes as that described in the method of determination but without addition of test sample.

7 Calculation and expression of the results

The calculation of quintozene content in the sample is carried out by GC-MS data processor or according to the following formula(1). The blank value should be subtracted from the above result of calculation:

$$X = \frac{A \cdot c_S \cdot V}{A_S \cdot m} \qquad \cdots\cdots(1)$$

where

X—the residue of quintozene, mg/kg;

A—the peak area of quintozene of the sample solution;

A_S—the peak area of quintozene the Standard working solution;

c_S—the concentration of quintozene in the Standard working solution, μg/mL;

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

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

8 Limit of determination and recovery

8.1 Limit of determination

The limit of determination of this method is 0.004 mg/kg.

8.2 Recovery

The range of fortification and recovery of this method is shown in table 2

Table 2—The fortifying concentration of chlorfenapyr in samples and its corresponding recoveries

Sample	Fortify cncentration/ (μg/kg)	Recovery/%	Sample	Fortify cncentration/ (μg/kg)	Recovery/%
Panax	4	75.0～85.0	gen-seng	4	85.0～97.5
	10	80.0～94.0		10	88.0～97.0
	20	85.0～100.0		20	90.3～97.0

Annex A
(informative annex)
quintozene of standard

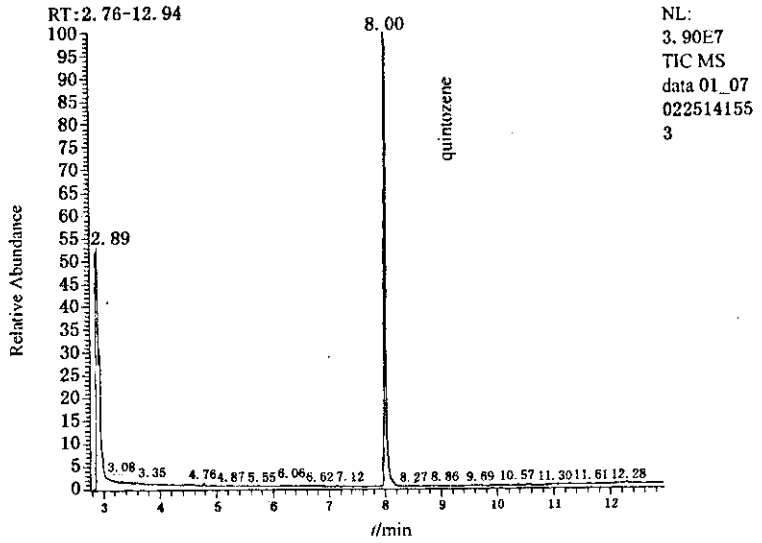

Figure A. 1—GC-MS of TIC quintozene standard

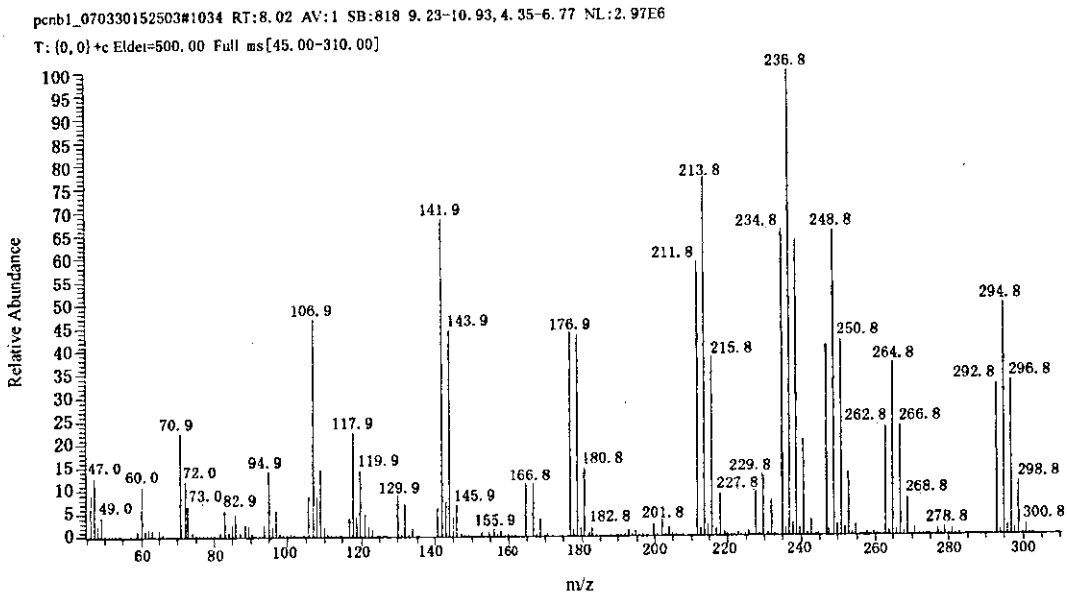

Figure A. 2—MS of quintozene standard

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