SN

中华人民共和国出入境检验检疫行业标准

SN/T 2426—2010

进出口粮谷中桔霉素含量检测方法 液相色谱法

Determination of citrinin contents in cereals for import and export—HPLC method

2010-01-10 发布　　2010-07-16 实施

中华人民共和国
国家质量监督检验检疫总局　发布

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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：中华人民共和国湖北出入境检验检疫局。

本标准主要起草人：林雁飞、赵晓亚、胡小钟、王鹏、李晶、付晓芳。

本标准系首次发布的出入境检验检疫行业标准。

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进出口粮谷中桔霉素含量检测方法 液相色谱法

1 范围

本标准规定了进出口粮谷中桔霉素含量液相色谱检测方法。

本标准适用于进出口大米、大麦、燕麦、小麦中桔霉素含量的检测。

2 方法提要

用乙腈-异丙醇-水的混合溶液提取试样中桔霉素，C_{18}固相萃取小柱净化，用配荧光检测器的液相色谱仪测定，外标法定量。

3 试剂和材料

除另有规定外，试剂均为分析纯，水为蒸馏水或相当纯度的水。

3.1 异丙醇：色谱纯。

3.2 乙腈：色谱纯。

3.3 磷酸：优级纯。

3.4 提取溶剂：乙腈-异丙醇-水(35＋10＋55，体积比)，用磷酸调 pH 为 1.5。

3.5 磷酸溶液：取 5.6 mL 磷酸，以水定容至 1 000 mL。

3.6 流动相：乙腈-异丙醇-0.08 mol/L 磷酸(35＋10＋55，体积比)。

3.7 C_{18}固相萃取柱：500 mg，3 mL，或相当者。使用前分别用 5 mL 甲醇和 5 mL 水预淋洗并保持柱体湿润。

3.8 桔霉素标准物质(citrinin，$C_{13}H_{14}O_5$，CAS 编号：518-75-2)：纯度大于等于 97%。

3.9 桔霉素标准储备液：称取适量桔霉素标准物质，用乙腈溶解并定容至 1.0 mg/mL，0 ℃～4 ℃保存。

3.10 桔霉素标准工作液：根据需要用流动相将标准储备液稀释成 25 ng/mL、50 ng/mL、100 ng/mL、500 ng/mL、1 000 ng/mL 的标准工作溶液。

3.11 玻璃纤维滤纸：直径 11 cm，孔径 1.5 μm。

4 仪器和设备

4.1 高效液相色谱仪：配有荧光检测器。

4.2 振荡器。

4.3 离心机：4 000 r/min。

4.4 真空固相萃取装置。

4.5 氮吹仪。

4.6 分析天平。

5 试样制备和保存

取有代表性样品 500 g，用粉碎机粉碎并通过 830 μm 圆孔筛，混匀，分成两份，装入洁净容器内，密封并标识。在制样的操作过程中，应防止样品受到污染或发生残留物含量的变化。

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6 测定步骤

6.1 提取

称取试样约 5 g(精确至 0.01 g)于 50 mL 离心管中,加 10 mL 提取溶剂(3.4),在振荡器上振荡提取 30 min。于 3 500 r/min 离心 4 min,上清液转入另一离心管中。在残渣中再加入 5 mL 提取溶剂(3.4),重复上述操作,合并上清液。在提取液中加水至 40 mL,并用磷酸调 pH 为 1.5,过玻璃纤维滤纸(3.11),待净化。

6.2 净化

将上述溶液过预淋洗好的 C_{18} 固相萃取柱,用 5 mL 水淋洗柱子。待淋洗液全部流出柱子后,减压抽干 3 min。用 10 mL 甲醇以 1.0 mL/min 的速度洗脱,收集全部洗脱液,在 40 ℃下,N_2 吹干,再以 1.0 mL 流动相溶解,过 0.2 μm 滤膜,供液相色谱测定。

6.3 测定

6.3.1 液相色谱条件

a) 色谱柱:C_{18} 柱,250 mm×4.6 mm(内径),粒径 5 μm,或相当者;

b) 流动相:乙腈-异丙醇-0.08 mol/L 磷酸(35+10+55,体积比);

c) 流速:1.0 mL/min;

d) 进样量:50 μL;

e) 柱温:28 ℃;

f) 检测波长:Ex=331 nm,Em=500 nm。

6.3.2 色谱测定

根据样液中被测桔霉素含量情况,选定峰面积相近的标准工作溶液。标准工作液和样液中桔霉素响应值均应在仪器检测线性范围内。对标准工作液和样液等体积参插进样测定。在上述色谱条件下,桔霉素保留时间约为 9.1 min,标准物质色谱图参见附录 A 中图 A.1。

6.3.3 空白试验

除不加试样外,均按上述操作步骤进行。

7 结果计算和表述

用色谱数据处理软件或按式(1)计算试样中桔霉素含量,计算结果需将空白值扣除:

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

式中:

X——试样中桔霉素含量,单位为毫克每千克(mg/kg);

A——样液中桔霉素的峰面积;

A_s——标准工作液中桔霉素的峰面积;

c——标准工作液中桔霉素的浓度,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 测定低限、回收率

8.1 测定低限

本方法的测定低限为 0.01 mg/kg。

8.2 回收率

粮谷中桔霉素检测的添加回收率数据见表 1。

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表 1　回收率数据

基　　体	添加浓度/(mg/kg)	回收率/%
大米	0.01	78.9～90.2
	0.05	88.0～91.4
	0.10	74.8～96.6
大麦	0.01	75.2～96.8
	0.05	79.1～94.2
	0.10	75.8～97.6
燕麦	0.01	74.6～90.5
	0.05	76.2～89.7
	0.10	75.9～91.3
小麦	0.01	77.9～96.2
	0.05	79.9～93.1
	0.10	80.4～95.9

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附　录　A
（资料性附录）
标准物质色谱图

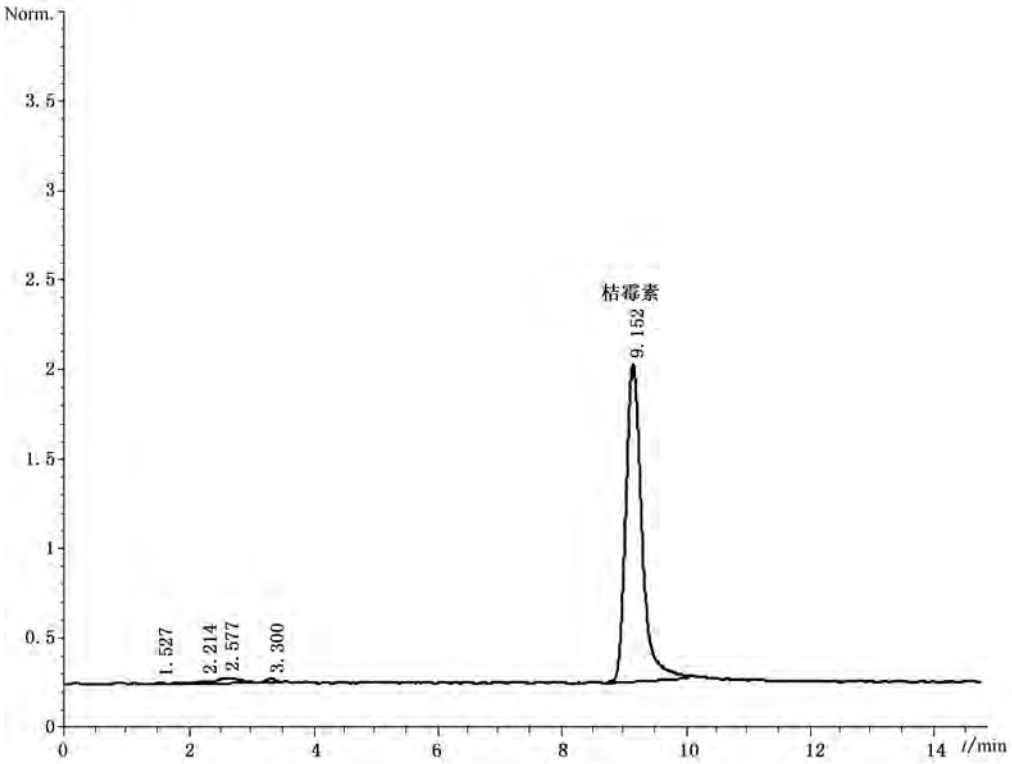


图 A.1　桔霉素标准物质(100 ng/mL)的液相色谱图

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Foreword

Annex A of this standard is an informative annex.

This standard was proposed by and is under the jurisdiction of Certification and Accreditation Administration of the People's Republic of China.

This standard is drafted by Hubei Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

Main drafters of this standard are: Lin Yanfei, Zhao Xiaoya, Hu Xiaozhong, Wang Peng, Li Jing, Fu Xiaofang.

This standard is a professional standard for Entry-Exit inspection and quarantine of the People's Republic of China promulgated for the first time.

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Determination of citrinin contents in cereals for import and export—HPLC method

1 Scope

This standard specifies the method of determination of citrinin in cereals by high performance liquid chromatography (HPLC).

This standard is applicable to the determination of citrinin contents in rice, barley, oats and wheat for import and export.

2 Principle

The contents of citrinin in the test sample is extracted with acetonitrile-isopropanol-water mixed solution. Thereafter being cleaned up by the C_{18} SPE column, the contents are determined by HPLC with FLD detector, quantified by external standard method.

3 Reagents and materials

Unless specified, all reagents used should be of analytical grade; "water" is the double distilled water.

3.1 Isopropanol: Chromatography pure.

3.2 Acetonitrile: Chromatography pure.

3.3 Phosphoric acid: Guaranteed reagent.

3.4 Extraction solution: acetonitrile-isopropanol-water (35 + 10 + 55, *V*/*V*/*V*), pH value is adjusted to 1.5 by phosphoric acid.

3.5 H_3PO_4 solution: pipette 5.6 mL phosphoric acid and dilute to 1 000 mL with water.

3.6 Mobile phase: acetonitrile-isopropanol-0.08 mol/L phosphoric acid (35 + 10 + 55, *V*/*V*/*V*).

3.7 C_{18} SPE Cartridge: 500 mg, 3 mL. or equivalent. Condition C_{18} SPE cartridge with 5 mL methanol and 5 mL water before using.

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3.8 Citrinin standard chemicals ($C_{13}H_{14}O_5$, CAS No:518-75-2), Purity was 97% above.

3.9 Standard stock solution: Accurately weigh appropriate citrinin (3.6), dissolve and quantitatively with acetonitrile. The concentration of the solution is 1.0 mg/mL. The stock solution should be stored at 0 ℃～4 ℃ refrigeratory.

3.10 Standard working solution: according to the requirement, accurately measure different volumes of standard stock solution to a 10 mL volumetric flask, dilute with mobile phase to make different concentration of the standard solution such as 25 ng/mL, 50 ng/mL, 100 ng/mL, 500 ng/mL, 1 000 ng/mL.

3.11 Glass Microfiber: 11 cm, 1.5 μm。

4 Apparatus and equipment

4.1 High performance liquid chromatography: equipped with fluorescence detector detection.

4.2 Oscillator.

4.3 Centrifuge: 4 000 r/min.

4.4 Solid phase extraction with vaccum pump.

4.5 Nitrogen concentrator.

5 Sample preparation and storage

The sample is about 500 g, grind thoroughly and let pass through a 830 μm sieve. Keep the prepared sample into a clean container, seal and label. In the course of sample preparation, precautions should be taken to avoid the contamination or any factors which may cause the change of residue content.

6 Procedure

6.1 Extraction procedure

Accurately weight 5 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 10 mL extracted solution (3.4), mix intensely and extract with ultrasonic extractor 30 min. Then centrifuge for 4 min at 3 500 r/min. The supernatant is taken into other 50 mL centrifuge tube. Another 5 mL extracted solution (3.4) is added and the mixture was extracted again. Combine the supernatant into the same centrifuge tube and add water to 40 mL, adjusted pH value to 1.5 by phosphoric acid.

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6.2 Cleanup procedure

Draw the above solution through a per-conditioned C_{18} SPE column. Wash the column with 5 mL water. Then the column is dried by air for at 3 min, and eluted with 10 mL methanol. The eluted solution is evaporated to near dryness at 40 ℃ and dried under nitrogen flow, then 1.0 mL mobile phase is added to reconstitute the residue. After being filtrated with a 0.2 μm filter, the final solution is ready for analysis by high performance liquid chromatography.

6.3 Determination

6.3.1 LC operating conditions

a) LC column: C_{18}, 5 μm, 4.6 mm (i.d.) × 250 mm, or equivalent;

b) Mobile phase: acetonitrile-isopropanol-0.08 mol/L phosphoric acid (35 + 10 + 55, *V*/*V*/*V*);

c) Flow rate: 1.0 mL/min;

d) Injection volume: 50 μL;

e) Column temperature: 28 ℃;

f) Detection wavelength: Ex = 331 nm, Em = 500 nm.

6.3.2 HPLC determination

According to the approximate concentration of citrinin in the sample solution, select the standard working solution with similar peak area to that of the sample solution. The responses of citrinin in the standard working solution and sample solution should be within the linear range of the instrumental detection. The standard working solution should be randomly injected in-between the injections of the sample solution of equal volume. Under the above operating condition, the retention time of citrinin is about 9.1 min. For chromatogram of the standard, see figure A.1 in annex A.

6.3.3 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.

7 Calculation and expression of the result

Calculate the content of citrinin contents in the test sample by LC data processor or according to the formula (1):

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

Where:

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

A—the peak area of citrinin in the sample solution;

A_s—the peak area of citrinin in the standard working solution;

c—the concentration of citrinin in the standard working solution, μg/mL;

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

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

8 Limit of determination and recovery

8.1 Limit of determination

The limit of determination of this method is 0.01 mg/kg.

8.2 Recovery

According to the experimental date, the fortifying concentration of citrinin in cereals and its corresponding recoveries see table 1.

Table 1—Recoveries of spiked samples

Sample	Levels/(mg/kg)	Recoveries/%
Rice	0.01	78.9～90.2
	0.05	88.0～91.4
	0.10	74.8～96.6
Barley	0.01	75.2～96.8
	0.05	79.1～94.2
	0.10	75.8～97.6
Oats	0.01	74.6～90.5
	0.05	76.2～89.7
	0.10	75.9～91.3
Wheat	0.01	77.9～96.2
	0.05	79.9～93.1
	0.10	80.4～95.9

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Annex A
（informative）
Chromatogram of the standard

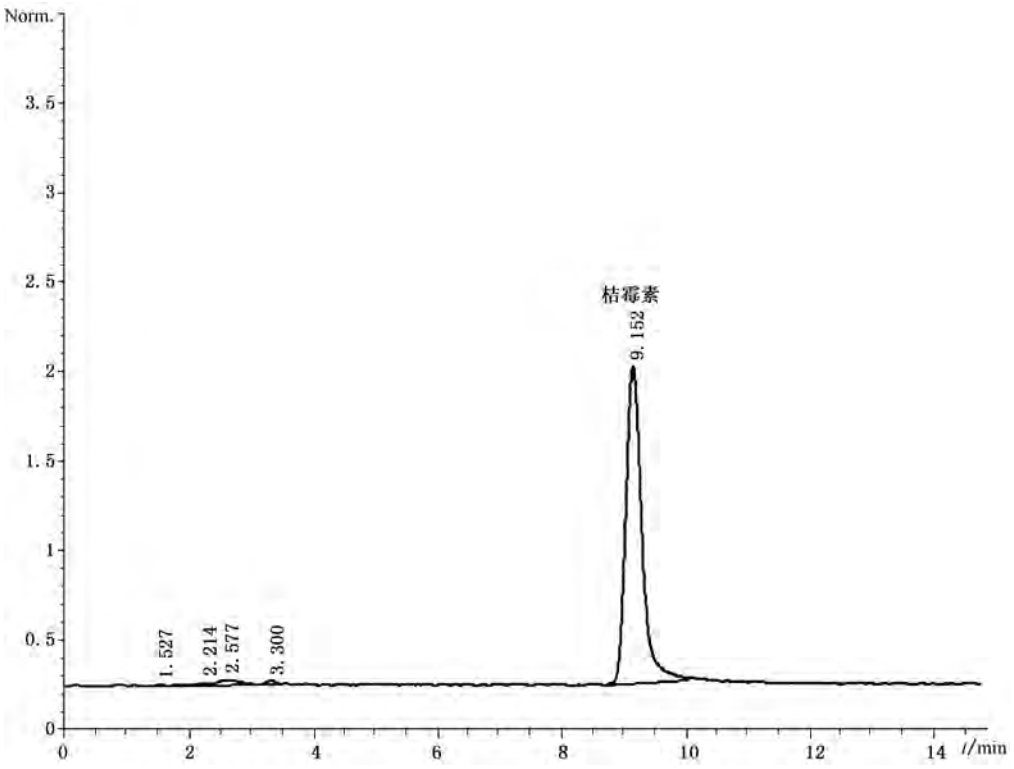


Figure A.1—Liquid chromatogram of citrinin（100 ng/mL）standard

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中 国 标 准 出 版 社 出 版
北京复兴门外三里河北街 16 号
邮政编码:100045
网址 www.spc.net.cn
电话:68523946 68517548
中国标准出版社秦皇岛印刷厂印刷
*
开本 880×1230 1/16 印张 1 字数 19 千字
2010 年 4 月第一版 2010 年 4 月第一次印刷
印数 1—1 600
*
书号:155066·2-20737 定价 18.00 元

SN/T 2426-2010

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