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中华人民共和国出入境检验检疫行业标准

SN/T 2483—2010

进出口粮谷中柄曲菌素含量检测方法 液相色谱法

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

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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：胡小钟、赵晓亚、林雁飞、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。

进出口粮谷中柄曲菌素含量检测方法 液相色谱法

1 范围

本标准规定了进出口粮谷中柄曲菌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、小麦中柄曲菌素含量的检测。

2 方法提要

样品中的柄曲菌素用乙腈提取，C_{18}固相萃取小柱净化，用液相色谱仪配紫外或二极管阵列检测器测定，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 甲醇：色谱纯。

3.2 乙腈：色谱纯。

3.3 乙酸：优级纯。

3.4 酸化乙腈溶液：色谱纯乙腈，用乙酸调 pH 值至 5.0。

3.5 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。

3.6 柄曲菌素标准物质(sterigmatocystin，$C_{18}H_{12}O_6$，CAS NO：10048-13-2)：纯度大于或等于 97%。

3.7 柄曲菌素标准储备液：精密称取适量标准品(精确至 0.000 1 g)，用乙腈溶解配制浓度为 1.0 mg/mL 的标准储备液，保存于－18 ℃冰箱内。

3.8 柄曲菌素标准工作液：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。保存于 4 ℃冰箱内。

4 仪器和设备

4.1 高效液相色谱仪：配有二极管阵列检测器或紫外检测器。

4.2 振荡器。

4.3 离心机：4 000 r/min。

4.4 旋转蒸发器。

4.5 真空固相萃取装置。

4.6 氮吹仪。

4.7 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 2 份，装入洁净容器内，密封并标识。在制样过程中，应防止样品受到污染或发生残留物含量的变化。

6 测定步骤

6.1 提取

准确称取样品 5.0 g(精确至 0.01 g)于 50 mL 离心管内,向其中加入 15 mL 酸化乙腈溶液,振荡提取 30 min。在 3 500 r/min 下离心 3 min,转移上层有机相至 250 mL 梨形瓶中,用 15 mL 酸化乙腈溶液重复提取两次,合并于 250 mL 梨形瓶中。45 ℃下,减压浓缩至干。加入 5 mL 酸化乙腈溶液溶解,溶解液再加入 20 mL 水。

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 5 mL 甲醇洗脱,接取全部洗脱液。在 40 ℃下,洗脱液用氮气吹干,再以 1.0 mL 流动相溶解残渣,混匀,过 0.45 μm 滤膜,滤液供液相色谱测定。

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-水(55+45,V/V),乙酸调 pH5.5;

c) 流速:1.0 mL/min;

d) 进样量:20 μL;

e) 柱温:28 ℃;

f) 检测波长:325 nm。

6.3.2 色谱测定

根据样液中被测柄曲菌素含量情况,选定峰面积相近的标准工作溶液。标准工作溶液和样液中柄曲菌素响应值均应在仪器检测线性范围内。对标准工作溶液和样液等体积参插进样测定。在上述色谱条件下,柄曲菌素保留时间约为 9.0 min,标准物质色谱图参见图 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)计算试样中柄曲菌素质量分数,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——试样中柄曲菌素质量分数,单位为毫克每千克(mg/kg);

A——样液中柄曲菌素的峰面积;

A_s——标准工作溶液中柄曲菌素的峰面积;

c——标准工作溶液中柄曲菌素的浓度,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

粮谷中柄曲菌素检测的添加回收率数据见表 1。

表 1　回收率数据

基体	添加浓度/(mg/kg)	回收率/%
大米	0.01	88.4～91.7
	0.025	88.0～91.4
	0.05	79.3～94.3
大麦	0.01	78.4～93.7
	0.025	77.1～96.2
	0.05	76.8～92.6
燕麦	0.01	78.6～95.6
	0.025	76.4～93.5
	0.05	78.9～94.2
小麦	0.01	76.9～94.3
	0.025	78.5～91.8
	0.05	81.4～96.5

附　录　A
（资料性附录）
标准物质色谱图

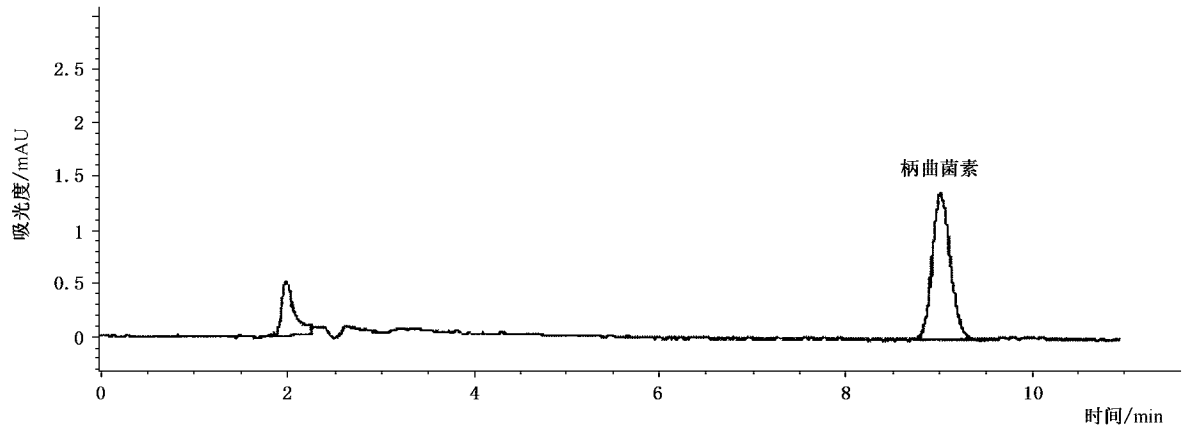

图 A.1　柄曲菌素标准物质(100 ng/mL)的液相色谱图

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.

The main drafters of this standard are Hu Xiaozhong, Zhao Xiaoya, Lin Yanfei, 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.

Note: This English version, a translation from the Chinese text, is only for reference.

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

1 Scope

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

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

2 Principle

The contents of sterigmatocystin in cereal are extracted with acetonitrile. Thereafter being cleaned up by the C_{18} SPE column,the contents are determined by HPLC with UV or DAD 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 Methanol:Chromatographic pure.

3.2 Acetonitrile:Chromatographic pure.

3.3 Acetic acid:Guaranteed reagent.

3.4 Acetonitrile solution:Chromatographic pure Acetonitrile,Adjusted pH value to 5.0 by acetic acid.

3.5 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.

3.6 Sterigmatocystin standard chemicals($C_{18}H_{12}O_6$,CAS NO:10048-13-2),Purity was 97% above.

3.7 Standard stock solution:Accurately weigh appropriate sterigmatocystin(3.6),dissolve and

quantitatively with acetonitrile. The concentration of the solution is 1.0 mg/mL. The stock solution should be stored at −18 ℃.

3.8 Standard working solution: according to the requirement, accurately add 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. The solution should be stored at 4 ℃ refrigeratory.

4 Apparatus and equipment

4.1 High performance liquid chromatography: equipped with diode-array detector detection.

4.2 Oscillator.

4.3 Centrifuge.

4.4 Rotary evaporator.

4.5 Solid phase extraction with vaccum pump.

4.6 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 5.0 g of the sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 15 mL acetonitrile solution(3.4), mix intensely and extract with ultrasonic extractor 30 min. Then centrifuge for 3 min at 3 500 r/min. Transfer the acetonitrile layer into a 250 mL pear-shape bottle. Another 15 mL acetonitrile solutiong(3.4) is added and the mixture is extracted again. Combine the extracts into the same pear-shape bottle and evaporate under N_2 condition to dryness at 45 ℃. The residue is reconstituted with 5 mL acetonitrile solutiong(3.4), dilute with 20 mL water.

6.2 Cleanup procedure

Draw the above solution through a per-contioned C_{18} SPE column. Wash the column with 5 mL water. Then the column is dried by air for at 3 min. The analyst contents are eluted with 5 mL methane. 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 0.45 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-water (55 + 45, *V*/*V*), adjusted pH value to 5.5 by acetic acid.

c) Flow rate: 1.0 mL/min;

d) Injection volume: 20 μL;

e) Column temperature: 28 ℃;

f) Detection wavelength: 325 nm.

6.3.2 HPLC determination

According to the approximate concentration of sterigmatocystin in the sample solution, select the standard working solution with similar peak area to that of the sample solution. The responses of sterigmatocystin 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 sterigmatocystin is about 9.0 min. For chromatogram of the standard, see fig. 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 sterigmatocystin contents in the test sample by LC data processor or according to the formula (1):

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

where

X—the contents of sterigmatocystin in the test sample, mg/kg;

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

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

c—the concentration of sterigmatocystin 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 0.010 mg/kg.

8.2 Recovery

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

Table 1 Recoveries of spiked samples

sample	Levels/(mg/kg)	Recoveries/%
rice	0.01	88.4～91.7
	0.02	88.0～91.4
	0.05	76.3～94.3
barley	0.01	80.6～94.1
	0.02	82.6～93.5
	0.05	85.5～98.2

Table 1（continued）

sample	Levels/（mg/kg）	Recoveries/%
oats	0.01	79.8～95.2
	0.02	81.9～96.7
	0.05	78.5～91.6
wheat	0.01	84.3～95.6
	0.02	89.8～93.5
	0.05	81.4～95.8

Annex A
（informative）
Chromatogram of the standard

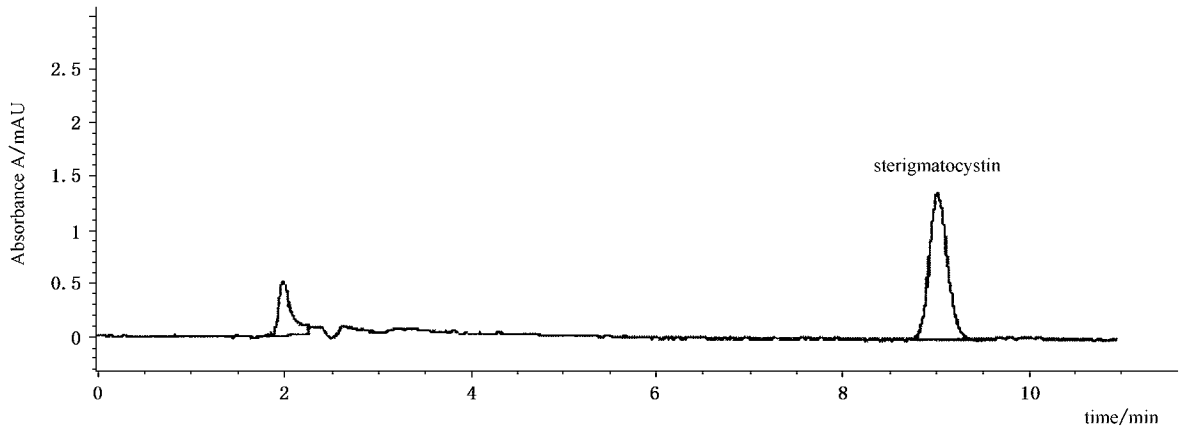

Fig A.1 Liquid chromatogram of sterigmatocystin（100 ng/mL）standard

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