SN

中华人民共和国进出口商品检验行业标准

SN 0211—93

出口粮谷中棕曲霉毒素 A 的检验方法

Method for determination of ochratoxin A in grains for export

1993-06-04 发布　　1993-08-01 实施

中华人民共和国国家进出口商品检验局　发布

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1 主题内容与适用范围

本标准规定了出口粮谷中棕曲霉毒素 A 检验的抽样、制样和薄层扫描测定方法。

本标准适用于出口大米、玉米、面粉和大豆中棕曲霉毒素 A 的检验。

2 抽样和制样

2.1 检验批

以不超过 4 000 袋为一检验批。

同一检验批的商品应具有相同的特征，如包装、标记、产地、规格、等级等。

2.2 抽样数量

按一批总袋数的平方根抽取。

$$a = \sqrt{N} \qquad \cdots\cdots(1)$$

式中：N——全批袋数；

a——抽样袋数。

注：a 值取整数，小数部分向前进位为整数。

2.3 抽样工具

2.3.1 金属单管取样器：全长 55 cm(包括手柄)，直径 1.5～2.5 cm，沟槽长度应超过袋对角线长度的二分之一。

2.3.2 取样铲：见图 1。

2.3.3 分样板：见图 2。

2.3.4 样品筒(袋)：可密封。

2.3.5 分样布或适用铺垫物。

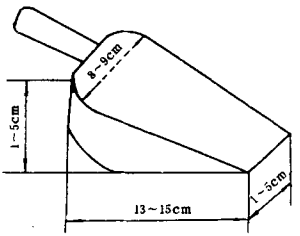


图 1 取样铲

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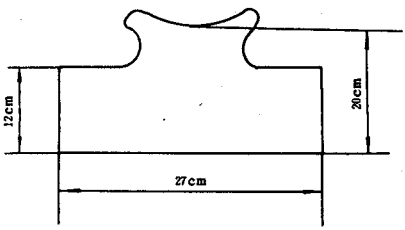


图 2 分样板

2.4 抽样方法

2.4.1 袋内抽样：按 2.2 规定计算抽样袋数，在堆垛四周上、中、下各部位以曲线形走向，随机抽取。将扦槽朝下，从每袋一角依斜对角方向插入袋内，然后将扦槽旋转朝上，抽出扦样器，立即倒入盛样容器内。每袋扦取样品数量应基本一致。

2.4.2 倒包抽样：将袋口缝线全部拆开，平置于分样布或其他洁净的铺垫物上，双手紧握袋底两角提起约成 45°倾斜角，倒拖 1 m 以上，使袋内货物全部倒出后，用取样铲在各部位扦取样品约 100 g，立即倒入盛样容器内。

2.4.3 大样缩分：集中袋内或倒包所取的样品，倒于分样布上，使用分样板按四分法缩分样品不少于 4 kg，加封后，标明标记并及时送实验室。

2.5 试样制备

将样品缩分到 1 kg，全部磨碎，通过 20 目筛，混匀，均分成两份，装入洁净容器内，密封，标明标记。

2.6 试样保存

将试样于 5 ℃下避光保存。

3 测定方法

3.1 方法提要

样品中的棕曲霉毒素 A 经提取、浓缩、薄层展开后，用碳酸氢钠-乙醇溶液喷雾，再对标准点和样液点进行荧光强度的扫描定量测定。

3.2 试剂和材料

除另有规定外，试剂均系分析纯，除乙腈外均需重蒸馏。水指蒸馏水或相适应的去离子水。

3.2.1 三氯甲烷。

3.2.2 正己烷或石油醚(沸程 30～60 ℃)。

3.2.3 无水乙醇。

3.2.4 苯-乙腈(98+2)。

3.2.5 乙酸-苯溶液(1+99)。

3.2.6 甲醇-水(55+45)。

3.2.7 氯化钠溶液：40 g/L。称 40.0 g 氯化钠溶于 1 000 mL 水中。

3.2.8 碳酸氢钠-乙醇溶液：将 6.0 g 碳酸氢钠溶于 100 g 水中，并加入 20 mL 无水乙醇。

3.2.9 棕曲霉毒素 A 标准贮备液：准确称取棕曲霉毒素 A 标准品(准确至 0.000 1 g)，配成浓度为 10 μg/mL的乙酸-苯溶液，作为贮备液。用紫外分光光度计标定浓度(最大吸收峰的波长为 333 nm，分子量 403，摩尔消光系数 5 550)，并作硅胶薄层色谱纯度鉴定，避光，于低温冰箱中保存。

3.2.10 棕曲霉毒素 A 标准工作液：用乙酸-苯溶液将 10 μg/mL 的棕曲霉毒素 A 标准贮备液精确稀释成浓度为 0.5 μg/mL 的标准工作液。避光，于低温冰箱中保存。

3.2.11 展开剂

3.2.11.1 乙醚：经无水硫酸钠脱水。

3.2.11.2 乙酸乙酯-甲苯-甲酸-甲醇溶液(25+60+10+5)。

3.2.11.3 乙酸-甲醇-苯溶液(5+5+90)。

3.2.12 重铬酸钾-硫酸洗液：100 g/L。

3.2.13 无水硫酸钠。

3.3 仪器和设备

3.3.1 薄层扫描仪：备有荧光检测器。

3.3.2 紫外光灯：100～125 W；带有波长 365 nm 滤光片。

3.3.3 硅胶 G 薄层板。

3.3.4 展开槽。

3.3.5 展开缸。

3.3.6 玻璃喷雾器。

3.3.7 微量注射器：10 μL、20 μL、2 μL。

3.3.8 全玻璃浓缩器。

3.3.9 电动振荡器。

3.3.10 小型粉碎机。

3.4 测定步骤

3.4.1 提取

称取 20.0 g 粉碎过筛样品，置于 250 mL 具塞锥形瓶中，加 30 mL 正己烷或石油醚和 100 mL 甲醇水溶液，湿润瓶塞，盖紧，振摇 30 min，静置分层。以快速定性滤纸过滤于 250 mL 分液漏斗中。等下层甲醇-水溶液分清后，放出 20 mL 甲醇-水溶液于一个带刻度的 125 mL 分液漏斗中，加 25 mL 三氯甲烷，轻摇提取 2 min。静置分层后，将三氯甲烷提取液放入另一分液漏斗中，并将甲醇-水层再用 10 mL 三氯甲烷重复提取(提取过程如出现乳化现象可滴加少许甲醇促其分层)。合并三氯甲烷提取液，并在合并液中加入氯化钠水溶液(加入量视样品不同而异，面粉 75 mL，其余 50 mL 左右)。振荡放置分层后，将三氯甲烷层通过装有约 20 g 无水硫酸钠的玻璃漏斗流入全玻璃浓缩瓶中，再用数毫升三氯甲烷洗涤无水硫酸钠层，洗涤液一并收集于浓缩瓶内。在 60～70 ℃水浴中减压浓缩至干后，用苯-乙腈溶液准确定容至 0.4 mL，即为分析样液。

3.4.2 点板

于 20 cm×20 cm 的薄板上，按图点板。将薄板自左向右 12 cm 处划一条垂线即将薄板分为左右两个区域。从薄板的左底两边各以距边缘 3 cm 为基线，在两基线的交点处滴加样液点 A。滴加量依样品中毒素含量高低而定。再于距样液点向右 10 cm 水平基线上以 1 cm 间距滴加 5 个标准点，依次滴加标准工作液 2,4,6,8,10 μL(相当于 1,2,3,4,5 ng)。

3.4.3 双向展开

按图所示方向将薄板先以乙醚为展开剂在展开缸内做第Ⅰ方向展开至 12 cm 垂线处取出，吹干，再以 3.2.11.2 或 3.2.11.3 展开剂作第Ⅱ方向展开至 13 cm 处。取出薄板，吹干。

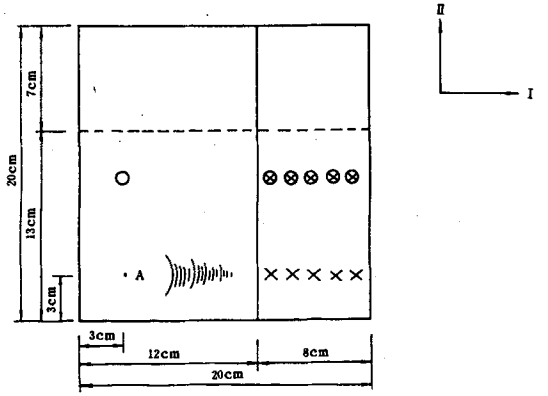


图 3 点板与双向展开示意图

———样品与标准点展开区域分界线；· A—样液点；×—标准点；

⊗—展开后标准点；○—展开后样液中棕曲霉毒素 A 荧光点

3.4.4 观察与确证

将展开薄板置于紫外光灯下(波长 365 nm)，观察与标准点比移值相同的蓝绿色荧光点，用喷雾剂 3.2.8 对准各荧光点进行喷雾后吹干，观察荧光点变为蓝紫色且荧光强度明显加强时可确证为棕曲霉毒素 A。于荧光点下方作针刺标记，即可扫描定量。

3.4.5 测定

3.4.5.1 薄层扫描工作条件：

薄层扫描仪(带荧光检测器)；

光源：汞灯；

激发波长：365 nm；

荧光波长：450 nm；

检测方式：反射；

狭缝：1.4×9 mm；

扫描方式：线性扫描。

3.4.5.2 工作曲线绘制：以标准毒素含量(ng)为横坐标，以峰面积积分值(mm^2)为纵坐标，绘制工作曲线。

3.4.5.3 结果计算和表述

用样液荧光点峰面积扫描积分值从工作曲线上查出毒素含量(ng)，再按式(2)计算：

$$X = \frac{c \cdot V_1}{m \cdot V_2} \qquad \cdots\cdots(2)$$

式中：X——试样中棕曲霉毒素 A 含量，μg/kg；

c——从工作曲线上查得样液点上相应的棕曲霉毒素 A 的含量，ng；

V_1——样液最后定容体积，μL；

V_2——样液点样体积，μL；

m——最后提取液相当样品质量，g。

4 测定低限、回收率

4.1 测定低限

本方法测定低限为 5 μg/kg。

4.2 回收率

回收率的实验数据：棕曲霉毒素 A 浓度在 5～20 μg/kg 范围内，回收率为 78.6%～104.1%。

附加说明：

本标准由中华人民共和国国家进出口商品检验局提出。

本标准由中华人民共和国山东进出口商品检验局负责起草。

本标准主要起草人李寅宾、解志海、张艺兵、林涛。

Professional Standard of the People's Republic of China for Import and Export Commodity Inspection

SN 0211—93

Method for determination of ochratoxin A in grains for export

1 Scope and field of application

This standard specifies the method of sampling, sample preparation and determination by TLC scanner of ochratoxin A in grains for export.

This method is applicable to the determination of ochratoxin A in grains for export.

2 Sampling and sample preparation

2.1 Each inspection lot should not exceed 4 000 bags.

The characteristics of the acrgo within the same inspection lot, such as packing, mark, origin, specification and grade, should be the same.

2.2 Quantity of sample taken

The square root of the total number of bags in a lot shall be taken as the number of bags to be sampled:

$$a = \sqrt{N} \qquad \cdots\cdots(1)$$

where

N—totalnumber of bags in a lot;

a—number of bags to be sampled.

Note: If value a is with decimal, round off the decimal part, which is added as unity to the integral part of a.

2.3 Sampling tools

2.3.1 Metallic sampler: Length (including handle): 55 cm; diameter: 1.5—2.5 cm; groove length ≥ half of the bag's diagonal length.

2.3.2 Sampling shovel: See fig. 1.

2.3.3 Plate for quartering: See fig. 2.

2.3.4 Sample can (bag): Which can be sealed.

2.3.5 Cloth (or other material) sheet: For sample dividing (quartering).

Approved by the State Administration of Import and Export Commodity Inspection of the People's Republic of China on Jun. 4, 1993

Implemented from Aug. 1, 1993

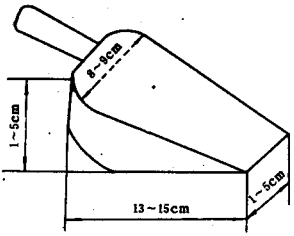


Fig. 1 Sampling shovel

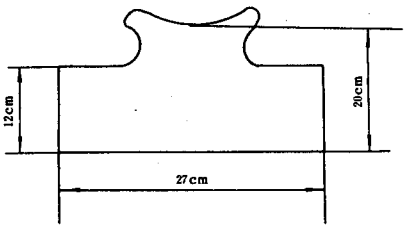


Fig. 2 Plate for quartering

2.4 Sampling procedure

2.4.1 Sampling from the bag

Draw the samples from a number of bags specified in 2.2 as follows: Along the sine wave of the pile, draw samples from the bags of the upper, middle and lower parts of the pile at random. Insert the sampler, with its groove facing downward, diagonally into each bag, then turn the sampler by 180°, draw out the sampler, and promptly the sample into a container. The quantity of the sample drawn from each bag shall be basically the same. Mix all the samples from different bags to form a gross sample.

2.4.2 Sampling by emptying out

Unseam and open the bag, and erect it on a clean cloth sheet (or other clean sheet). Grasp tight two corners of the bag bottom and raise up to an angle of 45°, tug backward for ca 1 m until all content of the bag is emptied out. Scoop up the sample from different parts of the out-poured content with a shovel, totalling ca 100 g, and place in a sample container promptly. Mix all the samples from different bags to form a gross sample.

2.4.3 Reduction of gross sample

Pour the sample (either from 2.4.1 or 2.4.2)on a clean sheet for quartering, reduce to not less than 4 kg with a plate by quartering. Place in a sample container, seal, label and send to the laboratory in time.

2.5 Preparation of test sample

Reduce the sample to ca 1 kg, grind thoroughly and let pass through a 20 mesh sieve, divide into 2 equal portions, place in clean containers, seal and label.

2.6 Storage of test sample

The test sample shall be stored at 5 C and kept away from light.

3 Method of determination

3.1 Principle

The ochratoxin A in the sample is extracted, concentrated and developed by thin layer chromatography. Both the spots of test solution and standard solution are sprayed with bicarbonate-ethanol solution, and the fluorescence intensitis of the spots are measured quantitatively by scanning with fluorescence detector.

3.2 Reagents and materials

Unless otherwise specified, all the reagents shall be analytically pure and must be redistilled (except acetonitrile), "water" is distilled water or the corresponding de-ionized water.

3.2.1 Chloroform.

3.2.2 n-Hexane or petroleum ether(30—60 ℃).

3.2.3 Absolute ethanol.

3.2.4 Benzene-acetonitrile(98+2).

3.2.5 Acetic acid-benzene(1+99).

3.2.6 Methanol-water(55+45).

3.2.7 Sodium chloride solution: 40 g/L. Weigh 40.0 g sodium chloride, dissolve in 1 000 mL water.

3.2.8 Sodium bicarbonate-ethanol solution: Weigh 5.0 g sodium bicarbonate, dissolve in 100 g water, and add 20 mL absolute ethanol in the solution.

3.2.9 Ochratoxin A standard stock solution: Accurately weigh an adequate amount of standard ochratoxin A (accurate to 0.000 1 g), dissolve in acetic acid-benzene and prepare a solution of 10 μg/mL as the standard stock solution. Determine the concentration with ultraviolet spectrophotometer (maximum absorption peak is 333 nm, molecule weight is 403, molal extinction coefficient is 5 550), and determine the purity with TLC, reserve in a freezer and avoid light.

3.2.10 Ochratoxin A standard working solution: Dilute the ochratoxin A standard stock solution of 10 μg/mL to 0.5 μg/mL with acetic acid-benzene, reserve in a freezer and avoid light.

3.2.11 Developing solvent

3.2.11.1 Ethyl ether: Dehydrated with anhydrous sodium sulfate.

3.2.11.2 Ethyl acetate-toluene-formic acid-methanol(25+60+10+5).

3.2.11.3 Acetic acid-methanol-benzene(5+5+90).

3.2.12 Potassium dichromate-sulfuric acid solution: 100 g/L.

3.2.13 Anhydrous sodium sulfate.

3.3 Apparatus and equipment

3.3.1 TLC scanner, equipped with fluorescence detector.

3.3.2 Fluorescent lamp: 100—125 W, with 365 nm wave-length filter.

3.3.3 Thin-layer plate of silica gel G.

3.3.4 Developing vessel.

3.3.5 Developing tank.

3.3.6 Glass sprayer.

3.3.7 Micro-syringe: 10 μL, 20 μL, 2 μL.

3.3.8 All-glass concentrator.

3.3.9 Vibrator, electric.

3.3.10 Mini-grinder.

3.4 Procedure

3.4.1 Extraction

Weigh 20.0 g of ground and screened sample in a 250 mL stoppered Erlenmeyer flask. Add 30 mL of n-hexane or petroleum ether and 100 mL methanol-water solution, moisten the stopper, stopper tightly, shake for 30 min, let stand and separate. Filter the solution through rapid quantitative filter paper into a 250 mL separatory funnel, allow the low methanol-water layer to separate clearly. Pipet 20 mL methanol-water solution to a graduated 125 mL separatory funnel, add 25 mL chloroform, shake for 2 min and allow the phases to separate. If emulsion occurs, a few drops of methanol may be added to promote separation. Transfer the chloroform layer to another separatory funnel. Extract the methanol-water layer again with 10 mL chloroform, collect and combine the chloroform layers. Adding sodium chloride solution (for flour, 75 mL; for others 50 mL) in collections, after shake, stand for separation. Pass the chloroform layer through a glass funnel with about 10 g of anhydrous sodium sulfate into a glass bottle for concentrating. Wash the sodium sulfate layer with a few mL of chloroform. Combine the filtrate with that in the concentrating bottle, concentrate in a water-bath (60—70 °C) under reduced pressure to dryness. Dissolve the residue with benzene-acetonitrile solution (98+2) and dilute exactly to 0.4 mL as the test solution for analysis.

3.4.2 Spotting on the plate

On a 20 cm×20 cm plate, spot the standard working solution and the test solution as shown in graph. Scribe a vertical line 12 cm from the left edge. Then scribe a baseline 3 cm from left and the bottom edge. On the cross of the two lines, spot the test solution spot A. The amount of test solution spotted depends on the ochratoxin A content of the sample. Spot the ochratoxin A standard working solution 2, 4, 6, 8, 10 μL (equivalent to 1, 2, 3, 4, 5 ng) respectively on the horizontal baseline, the first spot is 10 cm apart from the sample spot A, the successive spots are 1 cm apart from each other.

3.4.3 Two-dimensional development

As graphic expression, in the first direction, develop with ethyl ether to the 12 cm vertical line. In the second direction, develop with 3.2.11.2 or 3.2.11.3 developing solvent to 13 cm along the second direction.

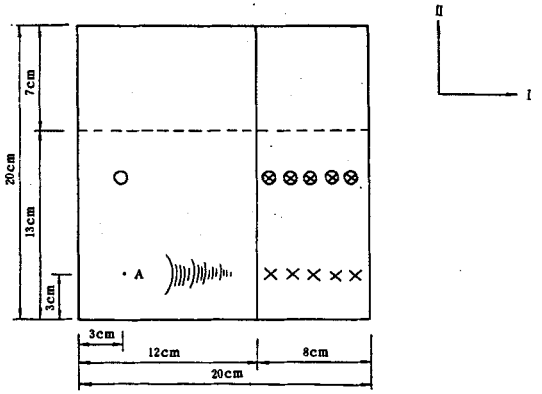


Fig. 3 Spotting and Developing Diagrammatic Sketch

— — — Demarcation line for the developing aera of the sample and standard spots ;

· A — Sample spot ; × — Standard spot ; ⊘ — Developed standard spot ;

◯ —Ochratoxin A fluorescence spot in developed sample spot

3.4.4 Observation and confirmation

Under UV-light(wavelength 365 nm),as the test solution spot is at the same r_f level of the standard spot,it shows blue-green fluorescent spot. Spray 3.2.8 spraying agent on each fluorescent spot and dry by blowing. If the fluorescent spots become blue-purple and the fluorescent intensity stronger obviously,ochratoxin A is confirmed. Gently mark with a needle under the spots for scanning determination.

3.4.5 Determination

3.4.5.1 Conditions of the TLC scanning

TLC scanner(equipped with fluorescence detector)

Light source:mercury lamp;

Wave length of excitation:365 nm;

Wave length of fluorescence:450 nm;

Type of examination:reflection;

Narrow slot:1.4×9 mm;

Type of scanning:linear scan.

3.4.5.2 Plottig of the working curve

Plot the working curve with standard ochratoxin A content(ng) as abscissa against the integral value(mm^2) of the peak area as ordinate.

3.4.5.3 Calculation and expression of the result

Obtain the equivalent ochratoxin A content(ng) from the working curve with respect to the scan integral value of the fluorescence spot's peak area,and calculate with following formula:

$$X = \frac{c \cdot V_1}{m \cdot V_2} \qquad \text{.....(2)}$$

where

X—Content of ochratoxin A in test sample, μg/kg;

c—Mass of ochratoxin A on the sample spot, obtained by referring to the working curve, ng;

V_1—Volume of final test solution, μL;

V_2—Volume of test solution spotted, μL;

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

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 5 μg/kg.

4.2 Recovery

According to the experimental data, when the concentration of ochratoxin A is in the range of 5—20 μg/kg, the recovery is 78.6%—104.1%.

Additional Explanations:

This standard was proposed by the State Administration of Import and Export Commodity Inspection of the People's Republic of China.

This standard was drafted by the Shandong Import and Export Commodity Inspection Bureau of the People's Republic of China.

This standard was mainly drafted by Li Yinbin, Xie Zhihai, Zhang Yibing, Lin Tao.

Note: This English version, a translation from the Chinese text, is solely for guidance.