

SN

中华人民共和国进出口商品检验行业标准

SN 0595—1996

出口粮谷中赤霉烯酮检验方法

Method for the determination of zearalenone in cereals for export

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中华人民共和国国家进出口商品检验局 发布

前 言

本标准是根据 GB/T 1.1—1993《标准化工作导则 第1单元：标准的起草与表述规则 第1部分：标准编写的基本规定》及 SN/T 0001—1995《出口商品中农药、兽药残留量及生物毒素检验方法标准编写的基本规定》的要求进行编写的。其中测定方法是参考国内外有关文献，经研究、改进和验证后而制定的。本标准同时制定了抽样和制样方法。

测定低限是根据国际上对粮谷中赤霉烯酮残留量的最高限量和测定方法的灵敏度而制定的。

本标准的附录 A 是提示的附录。

本标准由中华人民共和国国家进出口商品检验局提出并归口。

本标准起草单位：中华人民共和国辽宁进出口商品检验局和中国科学院大连化学物理研究所。

本标准主要起草人：随凯、张军、田苗、林乐明、宋文斌。

本标准系首次发布的行业标准。

1 范围

本标准规定了出口粮谷中赤霉烯酮检验的抽样、制样和薄层色谱测定方法。

本标准适用于出口玉米中赤霉烯酮的检验。

2 抽样和制样

2.1 检验批

散积玉米以不超过 200 t 为一检验批；袋装玉米（每袋约 90 kg）以约 2 200 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、规格和等级等。

2.2 抽样数量

2.2.1 袋装货品

按式(1)计算抽样袋数：

$$a = \sqrt{N} \dots\dots\dots (1)$$

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。

2.2.2 散积货品

货堆高度不超过 2 m。按货堆面积划区设点。以 50 m² 为一个取样区，每区设中心及四角（距边线 1 m 处）5 个点。每增加一个取样区，增设 3 个点。

2.3 抽样工具

2.3.1 金属双套管取样器：全长分 1 m、2 m（均包括手柄）两种。内、外管同部位分段开几个槽口，每个槽口长 15~20 cm，口宽 2.0~2.5 cm。内管的内径为 2.5~3.0 cm；取样器的探头长约 7 cm。

2.3.2 取样铲。

2.3.3 分样板。

2.3.4 盛样器：筒或袋，可密封。

2.3.5 分样布或适用铺垫物。

2.4 抽样方法

2.4.1 袋装抽样

2.4.1.1 倒包抽样：从堆垛的各部位随机抽取 2.2.1 规定的应抽样袋数的 10%（每批一般不少于 3 袋），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° 倾角，倒拖约 1 m，使袋内货物全部倒出。查看袋内和袋间品质是否均匀，确认情况正常后，用取样铲随机在各部位抽取样品，并立即将样品倒入盛样器内。每袋抽取样品的量应基本一致。

2.4.1.2 袋内抽样：按 2.2.1 规定的应抽样袋数（扣除倒包抽样袋数），在堆垛四周上、中、下各层以曲

线形走向随机抽取。然后用下述方法进行取样：用 1 m 长的金属双套管取样器(2.3.1)，关闭槽口，从每袋一角依斜对角方向插入袋内，然后旋转内管以开启槽口，待样品流满内管后，再旋转内管以关闭槽口。抽出取样器，立即将样品倒入盛样器内。每袋所取样品的量应与 2.4.1.1 基本一致。

每批所抽取的样品总量应不少于 4 kg。

2.4.2 散积抽样：按 2.2.2 规定的取样点，逐点抽取样品。将取样器(2.3.1)槽口关闭，以倾斜 45°角度插入货堆至相应深度，旋转取样器内管以开启槽口，待样品流满内管后，再旋转内管以关闭槽口。抽出取样器，立即将样品倒入盛样器内。从各点所抽取的样品量应基本一致。

每批所抽取的样品总量应不少于 4 kg。

2.4.3 大样缩分

2.4.3.1 袋装样品：合并从袋内和倒包抽样所取全部样品，倒于分样布上，用分样板按四分法缩分样品至不少于 2 kg，盛于盛样器内，加封后标明标记，并及时送交实验室。

2.4.3.2 散积样品：将抽取的全部样品倒于分样布上，以下按上述袋装样品方法进行。

2.5 试样制备

将样品按四分法缩分至 1 kg，全部磨碎并通过 20 目筛。混匀，均分成两份作为试样，分装入洁净的盛样器内，密封，标明标记。

2.6 试样保存

试样于 -5℃ 以下避光保存。

注：在抽样和制样的操作过程中，必须防止样品受到污染或发生残留物含量的变化。

3 测定方法

3.1 方法提要

试样中赤霉烯酮用乙腈和氯化钾溶液提取，提取液经用异辛烷脱脂、无水硫酸钠柱脱水、浓缩近干。残渣用三氯甲烷溶解，溶液经硅胶柱净化，然后进行薄层板分离，用薄层扫描仪测定，外标法定量。

3.2 试剂和材料

所用试剂除注明外均为分析纯，水为蒸馏水。

3.2.1 三氯甲烷。

3.2.2 氯化钾。

3.2.3 盐酸溶液：0.1 mol/L。

3.2.4 乙腈。

3.2.5 异辛烷。

3.2.6 丙酮。

3.2.7 苯。

3.2.8 乙酸。

3.2.9 展开剂：三氯甲烷-丙酮-苯-乙酸(18+2+8+1)。

3.2.10 无水硫酸钠：650℃ 灼烧 4 h，冷却后贮于干燥器中备用。

3.2.11 硅胶：100~200 目，青岛海洋化工厂产或等效产品。用前在 110℃ 烘 1 h，按 2%(m/m)比例加入蒸馏水，密封，振荡 5 h，平衡过夜备用。

3.2.12 薄层板：薄层层析用硅胶 G 板，100 mm×200 mm，层厚 0.20~0.25 mm，上海信谊仪器厂产或等效产品，用甲醇展洗薄层板后，吹干，在 120℃ 活化 1 h 后，置于干燥器内备用。

3.2.13 氯化钾溶液：取 4 g 氯化钾加 96 mL 0.1 mol/L 盐酸溶液，混匀。

3.2.14 赤霉烯酮标准品：纯度 ≥ 99%。

3.2.15 赤霉烯酮标准溶液：准确称取适量的赤霉烯酮标准品，用三氯甲烷配制成 0.13 mg/mL 浓度的标准储备液。根据需要再用三氯甲烷配成适当浓度的标准工作液。

注：避光于-5℃以下储存。

3.3 仪器与设备

3.3.1 粉碎机。

3.3.2 抽滤漏斗：90 mm(内径)。

3.3.3 振荡器。

3.3.4 旋转蒸发器：配有 200 mL 心形瓶。

3.3.5 恒温水浴锅。

3.3.6 无水硫酸钠柱：筒形漏斗，22 mm(内径)，内装 5 cm 高的无水硫酸钠。

3.3.7 分液漏斗：250 mL。

3.3.8 锥形瓶：具塞，500 mL。

3.3.9 抽滤瓶：250 mL。

3.3.10 样品管：具磨口塞，棕色，5 mL。

3.3.11 硅胶净化柱：250 mm×10 mm(内径)玻璃柱，装入 6 g 硅胶。

3.3.12 刻度移液管：50 mL、1 mL(精确至 0.01 mL)。

3.3.13 层析槽：250 mm×150 mm×50 mm(立式，具磨口)。

3.3.14 自动点样仪：1~99 μ L。

3.3.15 薄层色谱扫描仪：配有 Hg 灯光源。

3.4 测定步骤

3.4.1 提取

称取约 50 g 试样(精确至 0.1 g)，置于锥形瓶中，加 180 mL 乙腈和 20 mL 氯化钾溶液(3.2.13)。振荡 30 min 后，用抽滤漏斗过滤，经 3×20 mL 乙腈洗涤残渣，合并滤液于 250 mL 容量瓶，定容至 250 mL，取滤液 50 mL，置 250 mL 分液漏斗中，加 70 mL 异辛烷。振摇 2 min 后，静置 15 min 使分层。弃去异辛烷层。用 2×60 mL 异辛烷重复两次。收集乙腈层，过无水硫酸钠柱(3.2.6)到心形瓶中，于 50℃水浴中浓缩近干。用 1 mL 三氯甲烷溶解残留物。

3.4.2 净化

将 3.4.1 中提取所得溶液置于硅胶净化柱(3.3.11)中，用 2×0.5 mL 三氯甲烷洗涤心形瓶并倒入上述柱中。注入 50~60 mL 三氯甲烷-丙酮(8+2)混合液进行洗脱(流速为每秒 1~2 滴)，收集前 30 mL 洗脱液于心形瓶中。于 50℃水浴中浓缩至约 2 mL，定量转移至样品管中，继续浓缩至干。准确加入 0.50 mL 三氯甲烷使溶液供测定。

3.4.3 测定

3.4.3.1 薄层扫描条件

- a) 光源：高压汞灯；
- b) 激发波长：313 nm；发射波长：400 nm；
- c) 激发狭缝与发射狭缝：可根据斑点大小进行调节；
- d) 检测方式：反射荧光；
- e) 扫描速度：20 mm/min；
- f) 扫描方式：直线式。

3.4.3.2 薄层色谱测定

根据样液中赤霉烯酮含量情况，选定峰面积相近的标准工作溶液。标准工作溶液和样液中赤霉烯酮响应值均应在仪器检测线性范围内。

取等体积的标准工作溶液和样液在薄层板距下端 1.5~2 cm 基线上，用薄层色谱点样仪参插点样，每点间隔可根据点样范围来确定。

将薄层板放入用三氯甲烷-丙酮-苯-乙酸(18+2+8+1)预饱和的层析槽中展开，至前沿达约

180 mm时,取出吹干。按薄层扫描条件进行测定。标准品薄层色谱扫描图,见附录 A 中图 A1。

3.4.4 空白试验

除不加试样外,按上述测定步骤进行。

3.4.5 结果计算和表述

用色谱数据处理器或按式(2)计算。

$$X = \frac{A \cdot c \cdot V}{A_s \cdot m} \dots\dots\dots (2)$$

式中: X ——试样中赤霉烯酮的含量,mg/kg;

A ——样液斑点峰面积的积分单位;

A_s ——标准工作液斑点峰面积的积分单位;

c ——标准工作溶液中赤霉烯酮的浓度, $\mu\text{g/mL}$;

V ——最终样液的体积,mL;

m ——最终样液所相当的试样量,g。

注:计算结果需扣除空白值。

4 方法的测定低限、回收率

4.1 测定低限

本方法的测定低限为 0.1 mg/kg。

4.2 回收率

回收率的实验数据:

赤霉烯酮添加浓度在 0.1 mg/kg 时,回收率为 93.9%;

赤霉烯酮添加浓度在 1.0 mg/kg 时,回收率为 93.8%;

赤霉烯酮添加浓度在 2.0 mg/kg 时,回收率为 93.9%。

附 录 A
(提示的附录)
标准品薄层色谱扫描图

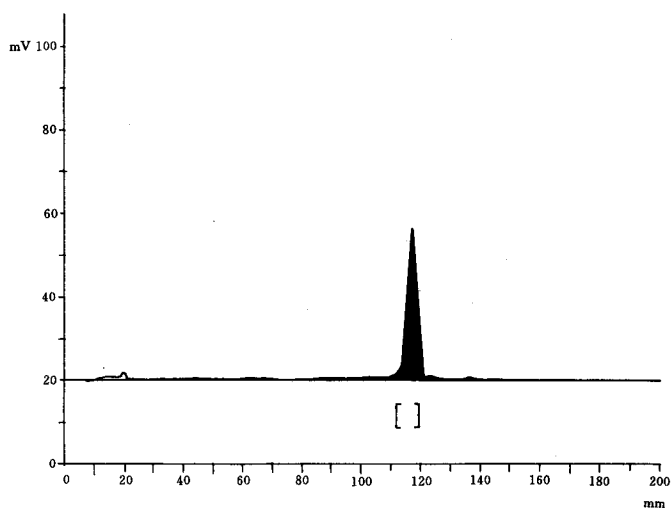


图 A1 赤霉烯酮标准品薄层色谱扫描图

Foreword

This standard was drafted in accordance with the requirements of GB/T 1.1—1993“Directives for the work of standardization—Unit 1;Drafting and presentation of standards—Part 1;General rules for drafting standards”and SN/T 0001—1995“General rules for drafting the standard methods for the determination of pesticide, veterinary drug residues and biotoxins in commodities for export”. The method of determination of this standard was drafted by referring to the relevant domestic and foreign literatures through research,modification and verification. In addition,the methods of sampling and sample preparation are also specified in this standard.

The limit of determination in this standard is defined on the bases of the current international maximum limit for zearalenone residues in cereals and the sensitivity of the method.

Annex A of this standard is an informative annex.

This standard was proposed by and is under the charge of the State Administration of Import and Export commodity Inspection of the People's Republic of China.

This standard was drafted by Liaoning Import and Export Commodity Inspection Bureau of the People's Republic of China,Dalian Institute of Chemical Physics of Chinese Academy of Science.

The main drafters of this standard are Sui Kai,Zhang Jun,Tian Miao,Lin Leming,Song Wenbin.

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

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

**Method for the determination of zearalenone SN 0595—1996
residues in cereals for export**

1 Scope

This standard specifies the methods of sampling, sample preparation and determination of zearalenone residues by thin-layer chromatography in cereals for export.

This standard is applicable to the determination of zearalenone residue content in maize for export.

2 Sampling and sample preparation

2.1 Inspection lot

For the cargo in bulk, each inspection lot should not exceed 200 t; for the cargo in bags, each inspection lot is about 2200 bags.

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

2.2 Quantity of sample taken

2.2.1 Cargo in bags

Calculate the number of bags to be taken by formula(1);

$$a = \sqrt{N} \dots\dots\dots (1)$$

where

a —number of bags to be taken;

N —total number of bags in a lot.

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

2.2.2 Cargo in bulk

The height of the cargo pile should not exceed 2 m. Set up areas and spots for sampling on the pile surface, 50 m² is considered as an area, in which 5 spots shall be fixed, one in the centre and four at the corners (1 m from the margins) of the area. For an additional area, 3 more sampling spots should be fixed.

2.3 Sampling tools

2.3.1 Metallic double casing sampler; Length 1 m or 2 m (both including handle) with some slots on different sections and respectively at the same heights for both inner and outer casings, length of slots: 15—20 cm; width: 2.0—2.5 cm; inside diameter of the inner casing: 2.5—3.0 cm; probe length of the sampler: ca 7 cm.

2.3.2 Sampling shovel.

Approved by the State Administration of
Import and Export Commodity Inspection of
the People's Republic of China on Nov. 15, 1996

Implemented from May 1, 1997

2.3.3 Plate for quartering.

2.3.4 Sample container; Can or bag, which can be sealed.

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

2.4 Sampling procedure

2.4.1 For cargo in bags

2.4.1.1 Sampling by emptying out; Draw 10 percent of the number of bags specified in 2.2.1 (not less than 3 bags) at random and proceed as follows: Unseam and open the bag, and lay it on a clean cloth sheet (or other clean sheet). Crasp 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. Check whether the quality of the goods is uniform within and between the bags. After confirming the goods are in normal condition, scoop up the sample from different parts of the out-poured content with a shovel at random, and place in a sample container promptly. The quantity of the sample drawn from each bag should be basically the same.

2.4.1.2 Sampling from inside the bags; Draw the samples from the number of the bags specified in 2.2.1 (by deducting the number of the bags drawn in 2.4.1.1) as follows: Along the sine wave of the pile, draw samples from the bags of the upper, middle and lower parts around the pile at random. Insert the sampler (2.3.1), with its slots closed, diagonally into each bag. Turn the inner casing to open the slots so that the sample may fill up the inner tube. Again turn the inner casing to close the slots and draw out the sampler, promptly pour the sample into a sample container. The quantity of the sample drawn from each bag shall be basically the same as in 2.4.1.1.

The total weight of the sample of each lot should be not less than 4 kg.

2.4.2 Sampling from the cargo in bulk; Insert the sampler (2.3.1) successively into the pile at the spots specified in 2.2.2 to an appropriate depth at 45° (the slots shall be closed while inserting in). Turn the inner casing to open the slots so that the sample may fill up the inner tube. Again turn the inner casing to close the slots and draw out the sampler. Promptly pour the sample into a sample container. The quantity of the sample drawn from all the spots shall be basically the same.

The total weight of the sample of each lot should be not less than 4 kg.

2.4.3 Reduction of gross sample

For cargo in bags; Pour all the samples (from both 2.4.1.1 and 2.4.1.2) onto a clean sheet, reduce to not less than 2 kg with a plate by quartering. Place in a sample container, seal, label and send to the laboratory in time.

For cargo in bulk; Pour all the samples onto a clean sheet and proceed as for cargo in bags described above.

2.5 Preparation of test sample

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

2.6 Storage of test sample

The test samples should be stored below -5°C and kept away from light.

Note: In the course of sampling and sample preparation, precaution must be taken to avoid contamination or any factors that may cause the change of residue content.

3 Method of determination

3.1 Principle

The zearalenone is extracted from test sample with acetonitrile and solution of potassium chloride. Remove the fat by iso-octane. After removal of water by sodium sulfate, concentrate to near dryness, and dissolve the residue and dilute to definite volume with chloroform. Clean up through the silica gel chromatographic column and separated by thin layer chromatography (TLC). The thin layer plate is determined by thin layer scanning meter, using external standard calibration method.

3.2 Reagents and materials

All reagents are of analytical grade unless other wise specified. "Water" is distilled water.

3.2.1 Chloroform

3.2.2 Potassium chloride.

3.2.3 Hydrochloric acid solution; 0.1 mol/mL.

3.2.4 Acetonitrile.

3.2.5 Iso-octane.

3.2.6 Acetone.

3.2.7 Benzene.

3.2.8 Acetic acid.

3.2.9 Developing reagent:

Chloroform-acetone-benzene-acetic acid (18+2+8+1).

3.2.10 Anhydrous sodium sulfate;

Ignite at 650°C for 4 h. Store in a desiccator.

3.2.11 Silica gel; For chromatographic use, 100—200 mesh, Heat at 110°C for 1 h, add distilled water (2% *m/m*). Seal, vibrate for 5 h, and let stand overnight before use.

3.2.12 Thin layer plate;

Silica gel G plates, 0.20—0.25 mm thickness, 100 mm×200 mm. Develop the thin layer plate with methanol, take out and blow to dryness, activate at 120°C for 1 h, store in a desiccator, ready for use.

3.2.13 Solution of potassium chloride;

Add 4 g of potassium chloride to 96 mL of 0.1 mol/L hydrochloric acid solution, mix thoroughly.

3.2.14 Zearalenone standard; purity ≥ 99%.

3.2.15 Zearalenone standard solution;

Accurately weigh a suitable amount of standard zearalenone and dissolve in chloroform (3.2.1) and prepare a solution of 0.13 mg/mL in concentration as the standard stock solution. According to the requirement, prepare a standard working solution of appropriate concentration.

Note: the zearalenone standard solution shall be stored below -5°C and kept away from light.

3.3 Apparatus and equipment

3.3.1 Grinder

3.3.2 Suction filter funnel; 90 mm (id).

3.3.3 Reciprocating shaker.

3.3.4 Rotatory evaporator; With 200 mL heart-shaped bottle.

3.3.5 Constant temperature water-bath.

3.3.6 Column of anhydrous sodium sulfate;

A cylinder shape funnel, 22 mm (id), filled with 5 cm height of anhydrous sodium sulfate.

3.3.7 Separatory funnel; 250 mL.

3.3.8 Conical flask; 500 mL, with glass stopper.

3.3.9 Suction bottle; 250 mL.

- 3.3.10 Sample vial; 5 mL, brown, with ground glass stopper.
 3.3.11 Silica gel clean-up column; 250 mm×10 mm(id) glass column, containing 6 g of silica gel.
 3.3.12 Graduated pipet; 50 mL, 1 mL (accurate to 0.01 mL).
 3.3.13 Chloromatographic jar; 250 mm×150 mm×50 mm (upright, with ground mouth).
 3.3.14 Auto-spotting meter; 1—99 μ L.
 3.3.15 Thin layer chromatography scanner; with Hg light source.

3.4 Procedure

3.4.1 Extraction

Weight ca 50 g of the test sample (accurate to 0.1 g) into a conical flask. Add 180 mL of acetonitrile and 20 mL of solution of potassium chloride (3.2.13), shake for 30 min. Filter the extract with suction, rinse the residues with 3×20 mL of acetonitrile and filter, combine the filtrates and exactly diluted to 250 mL. Pipet accurately 50 mL of the filtrates, and transfer into a 250 mL separatory funnel, add 70 mL of iso-octane and shake vigorously for 2 min. Let stand for 15 min to separate. Discard the iso-octane layer, and add 60 mL of iso-octane again, repeat the above step twice. Collect the acetonitrile layer. The acetonitrile layer is passed through a column packed with anhydrous sodium sulfate (3.3.6) into a heart-shaped bottle. Concentrate to near dryness in a water-bath at 50°C. Dissolve the residue with 1 mL of chloroform.

3.4.2 Cleanup

Transfer the solution from 3.4.1 onto a silica gel column (3.3.11), rinse the heart-shaped bottle with 2×0.5 mL of chloroform, pour the rinsings onto the same column. Then pass 50—60 mL of chloroform-acetone (8+2) through the column, draining speed; 1—2 drop/s. Collect the first 30 mL of eluate into a heart-shaped bottle. Concentrate to ca 2 mL in a 50°C water-bath and transfer quantitatively into a sample vial. Continue to concentrate to dryness. Dissolve in exactly 0.50 mL of chloroform, the solution is used for determination.

3.4.3 Determination

3.4.3.1 TLC scanning conditions

- Light source; mercury lamp;
- Wave length of excitation; 313 nm; Wave length of emission; 440 nm;
- Slit for excitation and slit for emission; Adjustment in accordance with spot;
- Photo mode; Fluorescence reflection;
- Rate of scanning; 20 mm/min;
- Scanning mode; Straight line.

3.4.3.2 TLC Determination

According to the approximate concentration of zearalenone in the sample solution, select the standard working solution, with similar peak area to that of sample solution. The responses of zearalenone in the standard solution and sample solution should be in the linear range of the instrumental detection. The standard working solution is spotted on the TLC plate, 1.5—2 cm from the baseline and in-between the test sample solution of equal volume is spotted by autospotting meter. According to the range of spot, select the interval between the spots. Place the plate in a chromatographic jar which is presaturated with chloroform-acetone-benzene-acetic acid (18+2+8+1) for developing. When the front reaches 180 mm, take out and blow to dryness for determination according to the scanning condition.

For TLC scanning diagram of zearalenone standard, see fig. A1 of annex A.

3.4.4 Blank test

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

3.4.5 Calculation and expression of result

The calculation of result is carried out by data processor or according to the following formula (2):

$$X = \frac{A \cdot c \cdot V}{A_s \cdot m} \dots\dots\dots (2)$$

where

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

A —the peak area of sample solution spotted on the plate, [i. u.];

A_s —the peak area of standard working solution spotted on the plate, [i. u.];

c —the concentration of zearalenone in the standard working solution, $\mu\text{g/mL}$;

V —the final volume of sample solution, mL;

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

Note: The blank value should be subtracted from the above result of calculation.

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 0.1 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentrations of zearalenone and its corresponding recoveries are:

0.1 mg/kg, the recovery 93.9%;

1.0 mg/kg, the recovery 93.8%;

2.0 mg/kg, the recovery 93.9%.

Annex A
(Informative Annex)
TLC scanning diagram of the standard

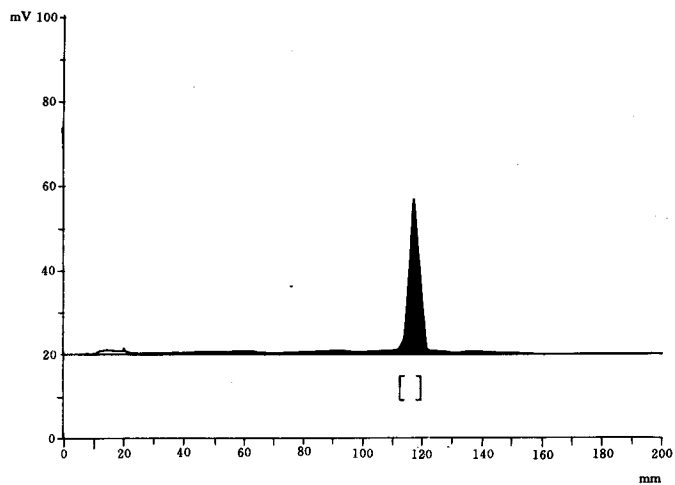


Fig. A1 The TLC scanning diagram of zearalenone standard