SN

中华人民共和国进出口商品检验行业标准

SN 0520—1996

出口粮谷中烯菌灵残留量检验方法

Method for the determination of imazalil residues in cereals for export

1996-04-29发布　　1996-10-01实施

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》的要求而进行编写的。其中测定方法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烯菌灵残留量的最高限量和测定方法的灵敏度而制定的。

附录A为提示的附录。

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：中华人民共和国上海进出口商品检验局。

本标准主要起草人：陈家华。

中华人民共和国进出口商品检验行业标准

出口粮谷中烯菌灵残留量检验方法

SN 0520—1996

Method for the determination of imazalil residues in cereals for export

1 范围

本标准规定了出口粮谷中烯菌灵残留量检验的抽样、制样和液相色谱测定方法。

本标准适用于出口大米中烯菌灵残留量的检验。

2 抽样和制样

2.1 检验批

以不超过 4 000 袋(200 t)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 抽样数量按一批总袋数的平方根〔式(1)〕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 cm,沟槽长度应超过袋对角线长度的一半。

2.3.2 取样铲。

2.3.3 分样板。

2.3.4 样品筒(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 倒包抽样

从堆垛的各部位随机抽取 2.2 规定的应抽样件数的 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 1 m 以上,使袋内货物全部倒出。检查货物的外观、气味、有无发霉、变质等,并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,立即将样品倒入盛样器内。每袋抽取样品数量应基本一致。

2.4.2 袋内抽样

按 2.2 规定的应抽样袋数的 90%,在堆垛四周上、中、下各层以曲线形走向随机抽取。将取样器(2.3.1)管槽朝下,从每袋一角依斜对角方向插入袋内,然后将管槽旋转朝上,抽出取样器,立即将样品倒入盛样容器内。每袋抽取样品数量应与 2.4.1 基本一致。

每批样品总量应不少于 4 kg。

中华人民共和国国家进出口商品检验局 1996-04-29 批准　　1996-10-01 实施

2.4.3 大样缩分

集中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出样品不少于 2 kg，盛于样品筒内，加封后标明标记并及时送交实验室。

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，均分成两份试样，装入洁净的容器内，密封，标明标记。

2.6 试样保存

将试样于−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

以乙酸乙酯提取样品中的烯菌灵，稀硫酸反提取，酸相碱化后用乙酸乙酯提取，蒸去乙酸乙酯，残渣溶解于流动相中。用配有紫外检测器的液相色谱仪测定，外标法定量。

3.2 试剂和材料

除另有规定外，试剂均为分析纯，水为蒸馏水。

3.2.1 乙酸乙酯：重蒸馏。

3.2.2 乙腈：紫外光谱级。

3.2.3 硫酸：优级纯，ρ 约 1.84 g/mL。

3.2.4 硫酸水溶液：0.1 mol/L。

3.2.5 氢氧化钠溶液：1 mol/L。

3.2.6 碳酸钠溶液：5%(m/V)。

3.2.7 磷酸盐缓冲液：分别称取 0.2 mol/L 磷酸二氢钾 68 mL、0.2 mol/L 磷酸氢二钠 32 mL，混匀，pH 调至 7.5。移取 10 mL 置于 1 L 容量瓶中，加入 1 mol/L 氯化钠溶液 2 mL，混匀，用水定容至刻度。

3.2.8 无水硫酸钠：650℃灼烧 4 h，冷却后贮于密闭容器中备用。

3.2.9 烯菌灵标准品：纯度≥99%。

3.2.10 烯菌灵标准溶液：准确称取适量的烯菌灵标准品，用甲醇配制成浓度为 100 μg/mL 的标准储备液。根据需要移取适量储备液，用氮气将甲醇吹去后用流动相配制成适当浓度的标准工作溶液。

3.3 仪器和设备

3.3.1 液相色谱仪：配有紫外检测器。

3.3.2 匀质机：3 000 r/min。

3.3.3 离心机：5 000 r/min。

3.3.4 旋转蒸发器。

3.3.5 微量注射器：25 μL、100 μL。

3.4 测定步骤

3.4.1 提取和净化

称取试样约 5 g(精确至 0.1 g)，置于 50 mL 离心管中。加入 2 g 无水硫酸钠、25 mL 乙酸乙酯，均质 2 min，离心 1 min(5 000 r/min)，将上清液移入 125 mL 分液漏斗中。残渣用 25 mL 乙酸乙酯按上述操作重复处理一次，合并上清液于分液漏斗中。用 20 mL 碳酸钠溶液洗涤乙酸乙酯相，弃去碳酸钠洗液。用 20 mL 蒸馏水洗乙酸乙酯相，弃去水相。乙酸乙酯相分别用 20、10 mL 硫酸溶液(0.1 mol/L)提取，合并硫酸相，弃去乙酸乙酯相。用 10 mL 乙酸乙酯洗涤硫酸相，弃去乙酸乙酯相。用 1 mol/L 氢氧化钠溶液碱化酸溶液(约 10 mL)后，用 2×5 mL 乙酸乙酯提取，合并乙酸乙酯相，并经无水硫酸钠脱水，收集于梨形瓶中，在水浴 50℃的旋转蒸发器上蒸发至近干。准确加入 1 mL 流动相(3.4.2.1c)以溶解残渣，

经 0.45 μm 滤膜过滤后供液相色谱测定。

3.4.2 测定

3.4.2.1 液相色谱条件

a) 色谱柱：径向加压柱 Z-modul Bondapak C_{18}，10 cm×8 mm(id)粒度 10 μm；

b) 检测器：UV，204 nm；

c) 流动相：乙腈-磷酸盐缓冲液(1+1)；

d) 流速：2 mL/min；

e) 色谱柱温度：室温。

3.4.2.2 液相色谱测定

根据样液中烯菌灵含量情况，选定峰高相近的标准工作溶液。标准工作溶液和样液中烯菌灵的响应值均应在仪器的检测线性范围内。对标准工作溶液和样液等体积参插进样测定。在上述色谱条件下烯菌灵的保留时间约为 9 min。标准品色谱图见附录 A 中图 A1。

3.4.3 空白试验

除不加试样外，均按上述测定步骤进行。

3.5 结果计算和表述

用色谱数据处理机或按式(2)计算试样中烯菌灵残留量：

$$X = \frac{h \cdot c \cdot V}{h_s \cdot m} \quad \cdots\cdots(2)$$

式中：X——试样中烯菌灵残留量，mg/kg；

h——样液中烯菌灵的色谱峰高，mm；

h_s——标准工作溶液中烯菌灵的色谱峰高，mm；

c——标准工作溶液中烯菌灵浓度，μg/mL；

V——样液最终定容体积，mL；

m——最终样液所代表的试样量，g。

注：计算结果需扣除空白值。

4 测定低限、回收率

4.1 测定低限

本方法的测定低限为 0.025 mg/kg。

4.2 回收率

回收率的实验数据：

烯菌灵浓度在 0.025 mg/kg 时，回收率为 94.0%；

烯菌灵浓度在 0.050 mg/kg 时，回收率为 101.0%；

烯菌灵浓度在 1.00 mg/kg 时，回收率为 91.0%。

附 录 A
（提示的附录）
标准品色谱图



图 A1 烯菌灵色谱图

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 relevant domestic and foreign literatures through research, modification and verification. In addition, methods of sampling and sample preparation are also specified in this standard.

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

Annex A is the 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 Shanghai Import and Export Commodity Inspection Bureau of the People's Republic of China.

The main drafter of this standard is Chen Jiahua.

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

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

SN 0520—1996

Method for the determination of imazalil residues in cereals for export

1 Scope

This standard specifies the methods of sampling, sample preparation and determination of imazalil residues by liquid chromatography in cereals for export.

This standard is applicable to the determination of imazalil residue content in rice for export.

2 Sampling and sample preparation

2.1 Inspection lot

Each inspection lot should not exceed 4000 bags (200 t).

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

The number of bags to be sampled shall be calculated according to the formula (1):

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

where

N——total number 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 cm; groove length: longer than half the diagonal length of the bag.

2.3.2 Sampling shovel.

2.3.3 Plate for quartering.

2.3.4 Sample can (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 Sampling by emptying out

Draw 10 percent of the number of bags specified in 2.2 (not less than 3 bags) at any part of the pile at random. Unseam and open the bag, and lay 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

Approved by the State Administration of Import and Export Commodity Inspection of the People's Republic of China on Apr. 29, 1996

Implemented from Oct. 1, 1996

the bag is emptied out. Check the appearance, odour of the goods and whether the goods are moldy, rotten etc. Also 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, and place in a sample container promptly. The quantity of the sample drawn from each bag should be basically the same.

2.4.2 Sampling for the cereals in the bag

Draw the samples from 90 percent of the 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(2.3.1), with its groove facing downward, diagonally into each bag, then turn the sampler by 180°, draw out the sampler, promptly pour the sample into a container. The quantity of the sample drawn from each bag shall be basically the same as in 2.4.1.

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

2.4.3 Reduction of gross sample

Pour all the samples (from both 2.4.1 and 2.4.2)on 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.

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 2 equal portions, place in clean containers, seal and label.

2.6 Storage of test sample

The test sample shall be stored below −5℃ 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 imazalil residues in rice are extracted with ethyl acetate, the extract is again extracted with acid solution by liquid partitioning. The acid phase is alkalified and extracted with ethyl acetate. Expel the ethyl acetate, dissolve the residue in the mobile phase, the solution is analyzed by HPLC with ultraviolet detector, using external standard method.

3.2 Reagents and materials

Unless otherwise specified, the reagents should be analytically pure, "water"is distilled water.

3.2.1 Ethyl Acetate: Redistilled.

3.2.2 Acetonitrile: UV grade.

3.2.3 Sulfuric acid, ρ ca 1.84 g/mL.

3.2.4 Sulfuric acid solution: Aqueous solution, 0.1 mol/L.

3.2.5 Sodium hydroxide solution: Aqueous solution, 1 mol/L.

3.2.6 Sodium carbonate solution: Aqueous solution, 5%(m/V).

3.2.7 Phosphate buffer solution: Mix 68 mL of 0.2 mol/L KH_2PO_4 and 32 mL of 0.2 mol/L Na_2HPO_4, adjust to pH7.5 and pipet 10 mL into a 1 L volumetric flask, add 2.0 mL of 1 mol/L sodium chloride solution, mix and dilute to the volume with water.

3.2.8 Anhydrous sodium sulfate: Ignite for 4 h at 650℃, and keep in a tightly closed container.

3.2.9 Imazalil standard: Purity≥99%.

3.2.10 Imazalil standard solution: Accurately weigh an adequate amount of imazalil standard, dissolve in

methyl alcohol and prepare a solution of 100 μg/mL in concentration as the standard stock solution. According to the requirement pipet certain amount of standard stock solution, and prepare a standard working solution of appropriate concentration with the mobile phase after removal of methyl alcohol by a nitrogen current.

3.3 Apparatus and equipment

3.3.1 High performance liquid chromatograph, equipped with UV detector.

3.3.2 Homogenizer: 3 000 r/min.

3.3.3 Centrifuge: 5 000 r/min.

3.3.4 Rotary evaporator.

3.3.5 Micro-syringe: 25 μL; 100 μL.

3.4 Procedure

3.4.1 Extraction and cleanup

Weigh ca 5 g of the test sample (accurate to 0.1 g) into a 50 mL centrifuge tube, add 2 g of andydrous sodium sulfate and 25 mL of ethyl acetate. After homogenizing for 2 min and centrifuging for 1 min (5 000 r/min), transfer the supernatant into a 125 mL separating funnel. Repeat the operation on the residue by adding another 25 mL of ethyl acetate. Combine the supernatant layers in the same separating funnel. Wash the ethyl acetate phase with 20 mL of sodium carbonate solution, and discard the sodium carbonate washings. Wash the ethyl acetate phase again with 20 mL of water, discard the aqueous washings. Extract the ethyl acetate phase with successive 20 mL、10 mL of sulfuric acid solution (0.1 mol/L). Combine the sulfuric acid extracts, and discard the ethyl acetate phase. Wash the sulfuric acid extract with 10 mL of ethyl acetate and discard the ethyl acetate washings. Alkalify the aqueous solution with 1 mol/L sodium hydroxide solution (ca 10 mL). Extract the aqueous solution two times with 5 mL of ethyl acetate each. Combine the ethyl acetate extract and let pass through anhydrous sodium sulfate into a pear-shaped flask. Evaporate the extract nearly to dryness on rotary evaporator at 50℃. Dissolve the residue in 1 mL mobile phase (3.4.2.1c) solution. Filter with 0.45 μm film, the solution is ready for liquid chromatographic determination.

3.4.2 Determination

3.4.2.1 LC operating condition

a) LC column: Z-modul Bondapak C_{18}, 10 cm×8 mm (id), 10 μm (particle size);

b) Detector: UV, 204 nm;

c) Mobile phase: Acetonitrile-phosphate buffer solution (1+1);

d) Flow rate: 2 mL/min;

e) Column temperature: Room temperature.

3.4.2.2 LC determination

According to the approximate concentration of imazalil in the sample solution, select the standard working solution with similar peak height to that of the sample solution. The responses of imazalil 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 imazalil is ca 9 min. For the chromatogram of the standard, see fig. A1 in annex A.

3.4.3 Blank test

The operation of the blank test is the same as that described in the method of determination but with the omission of sample addition.

3.5 Calculation and expression of the result

Calculate the content of imazalil residues in the test sample by LC data processor or according to the formula (2):

$$X = \frac{h \cdot c \cdot V}{h_s \cdot m} \quad \cdots\cdots (2)$$

where

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

h——the peak height of imazalil in the sample solution, mm;

h_s——the peak height of imazalil in the standard working solution, mm;

c——the concentration of imazalil 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.

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 0.025 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentrations of imazalil and their corresponding recoveries are:

0.025 mg/kg, the recovery 94.0%;

0.050 mg/kg, the recovery 101.0%;

1.00 mg/kg, the recovery 91.0%.

Annex A

(informative)

Chromatogram of the standard



Fig. A1 Chromatogram of the imazalil standard