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中华人民共和国进出口商品检验行业标准

SN 0220—93

出口水果中多菌灵残留量检验方法

Method for the determination of carbendazim residues in fruits for export

1993-12-28发布　　1994-05-01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

本标准规定了出口水果中多菌灵残留量检验的抽样、制样和液相色谱测定方法。

本标准适用于出口柑桔中多菌灵残留量的检验。

2 抽样和制样

2.1 检验批

以不超过1 500件为一检验批。

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

批量，件	最低抽样数，件
1～25	1
26～100	5
101～250	10
251～1 500	15

2.3 抽样方法

按2.2规定的抽样件数随机抽取，逐件开启。每件至少取500 g作为原始样品，原始样品总量不得少于2 kg。加封后，标明标记，及时送实验室。

2.4 试样制备

将所取原始样品缩分出1 kg，取可食部分，经组织捣碎机捣碎，均分成两份，装入洁净容器内，作为试样。密封，并标明标记。

2.5 试样保存

将试样于－18℃以下冷冻保存。

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

试样用盐酸溶液加热回流，进行提取。提取液经过滤后调至碱性，再用二氯甲烷提取。提取液经浓缩，C_{18}预处理小柱净化，甲醇洗脱。洗脱液浓缩后用液相色谱仪-紫外检测器测定，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 正己烷：优级纯。

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3.2.2 二氯甲烷。

3.2.3 甲醇：色谱纯。

3.2.4 甲醇溶液：20%(*V*/*V*)水溶液。

3.2.5 盐酸溶液：2 mol/L。

3.2.6 氢氧化钠溶液：10 mol/L，2 mol/L。

3.2.7 碳酸氢钠溶液：用水溶解 5 g 碳酸氢钠并稀释至 100 mL。

3.2.8 多菌灵标准品：纯度≥96%。

3.2.9 多菌灵标准溶液：准确称取适量的多菌灵标准品，先用少量的盐酸溶液(3.2.5)微热溶解，再用该盐酸溶液定容，配成浓度为 1.00 mg/mL 的标准储备溶液。根据需要再配成适当浓度的标准工作溶液。

3.3 仪器和设备

3.3.1 液相色谱仪并配有紫外检测器。

3.3.2 离心管：具塞，5 mL，15 mL，50 mL。

3.3.3 离心机：转速 6 000 r/min。

3.3.4 快速混匀器。

3.3.5 高速组织捣碎机。

3.3.6 多功能微量化学样品处理仪(或相当的装置)。

3.3.7 微量注射器：25 μL、100 μL。

3.3.8 玻璃抽滤器。

3.3.9 砂芯漏斗：2 号或 3 号。

3.3.10 C_{18}预处理小柱：先用 3 mL 甲醇，再用同体积水分别淋洗。

3.3.11 注射器：5 mL。

3.4 测定步骤

3.4.1 提取、净化

称取试样约 5 g(精确到 0.1 g)于 50 mL 离心管(3.3.2)内，加 10 mL 盐酸溶液(3.2.5)，装上回流冷凝管后，加热至沸腾，回流 30 min。移出，冷却至室温。将离心管内的样液用砂芯漏斗抽滤，再用 10 mL 盐酸溶液(3.2.5)分数次洗涤残渣。合并滤液，在容量瓶中用盐酸溶液(3.2.5)定容至 25 mL。

准确吸取 2.5 mL 上述滤液，同时吸取与样液中多菌灵浓度相近的标准工作溶液 2.5 mL，分别置于 15 mL 离心管中，加 4 mL 二氯甲烷，在快速混匀器上混匀 2 min，离心(4 000 r/min)3 min。用尖嘴吸管吸出下层二氯甲烷，弃去。在水相中加入适量氢氧化钠溶液(10 mol/L)，使试液接近碱性，然后小心滴加氢氧化钠溶液(2 mol/L)，使试液的 pH 为 10～11。再滴加碳酸氢钠溶液，使试液的 pH 调至 9.5～10。用 3×4 mL 二氯甲烷在快速混匀器中提取 2 min，离心(4 000 r/min)3 min，用尖嘴吸管将二氯甲烷转入另一离心管中。合并三次二氯甲烷提取液，浓缩至 1 mL。

将上述浓缩的提取液通过注射器转入 C_{18}预处理小柱(3.3.10)。用 3 mL 甲醇溶液(3.2.4)洗涤离心管并过 C_{18}预处理小柱，弃去流出液。继用 3 mL 正己烷洗涤离心管并过 C_{18}预处理小柱，弃去流出液。用 2×2.5 mL 甲醇(3.2.3)洗涤离心管并过 C_{18}预处理小柱，收集二次洗脱液并浓缩至 1 mL 以下。用甲醇(3.2.3)定容至 1 mL，供液相色谱测定。

3.4.2 测定

3.4.2.1 色谱条件

a. 色谱柱：μBondapak C_{18}，300 mm×3.9 mm(id)；

b. 流动相：甲醇-水(4+6)；

c. 流速：0.8 mL/min；

d. 检测器：紫外检测器，测定波长 286 nm；

e. 色谱柱温度:室温。

3.4.2.2 色谱测定

根据样液中多菌灵含量情况,选定与样液峰高相近的标准工作溶液。标准工作溶液和样液中多菌灵响应值均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,多菌灵保留时间约为 7 min。

3.4.3 空白试验

除不加试样外,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下式计算试样中多菌灵残留含量:

$$X = \frac{h \cdot c_s}{h_s \cdot c}$$

式中:X——试样中多菌灵残留量,mg/kg;

h——样液中多菌灵的峰高,mm;

h_s——标准工作溶液中多菌灵的峰高,mm;

c_s——标准工作溶液中多菌灵的浓度,μg/mL;

c——最终样液所代表的试样浓度,g/mL。

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 0.7 mg/kg。

4.2 回收率

回收率的实验数据:多菌灵添加浓度在 0.7～12.0 mg/kg 范围内,回收率为 90.9%～102.2%。

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江西进出口商品检验局负责起草。

本标准主要起草人徐文彦、施小珊、黎双珍。

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

Method for the determination of carbendazim residues in fruits for export

SN 0220—93

1 Scope and field of application

This standard specifies the methods for sampling, sample preparation and determination by high performance liquid chromatograph (HPLC) of carbendazim residues in fruits for export.

This standard is applicable to the determination of carbendazim residues in orange for export.

2 Sampling and sample preparation

2.1 Inspection lot

The quantity of an inspection lot should not be more than 1 500 packages.

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

2.2 Quantity of sample taken

Number of packages in each inspection lot	Minimum number of packages to be taken
1—25	1
26—100	5
101—250	10
251—1 500	15

2.3 Sampling procedure

A number of packages specified in 2.2 are taken at random and opened one by one. The sample weight taken as the primary sample from each package should be at least 500 g. The total weight of all primary samples should not be less than 2 kg, which shall be sealed, labeled and sent to laboratory in time.

2.4 Preparation of test sample

The combined primary sample is reduced to ca 1 kg. The edible portions are blended and grided by tissue grider, and then divided into two equal portions. Each portion is placed in a clean container as the test sample, which is then sealed and labeled.

2.5 Storage of the sample

The test samples should be stored below −18℃.

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

Approved by the State Administration of Import and Export Commodity Inspection of the People's Republic of China on Dec. 28, 1993

Implemented from May. 1, 1994

3 Method of determination

3.1 Principle

The sample is refluxed with HCl solution for extraction. The extract is made alkaline and reextracted with dichloromethane. The dichloromethane extract is concentrated, cleaned up by C_{18} chromatographic micro-column and then eluted with methanol. The eluate is concentrated and determined with high performance liquid chromatography, equipped with UV detector, using extrenal standard method.

3.2 Reagents and materials

Unless otherwise specified, all reagents are analytical grade, and "water" is distilled water.

3.2.1 n-Hexane: GR.

3.2.2 Dichloromethane.

3.2.3 Methanol: chromatographically pure.

3.2.4 Methanol solution: 20% aqueous solution (V/V).

3.2.5 Hydrochloric acid solution: 2 mol/L.

3.2.6 Sodium hydroxide solutions: 2 mol/L, 10 mol/L.

3.2.7 Sodium hydrogen carbonate solution: Dissolve 5 g of sodium hydrogen carbonate in water and dilute to 100 mL.

3.2.8 Carbendazim standard: Purity≥96%.

3.2.9 Carbendazim standard solution: Accurately weigh an adequate amount of carbendazim standard, dissolve in few mL of 2 mol/L hydrochloric acid by warming, and dilute to a concentration of 1.00 mg/mL with hydrochloric acid solution as standard stock solution. According to the requirement, prepare the standard working solution of appropriate concentration.

3.3 Apparatus and equipment

3.3.1 High performance liquid chromatograph, equipped with UV detector.

3.3.2 Centrifuge tube: with ground stopper, 5 mL, 15 mL, 50 mL.

3.3.3 Centrifuge: 6 000 r/min.

3.3.4 Homogenizer.

3.3.5 High-speed tissue grinder.

3.3.6 Multifuction sample treatment unit for microchemical method (or equivalent).

3.3.7 Micro-syringe: 25 μL, 100 μL.

3.3.8 Suction filter.

3.3.9 Sintered glass filter: No. 2 or No. 3.

3.3.10 C_{18} chromatographic mico-column: Prewash with 3 mL of methanol and successively with the same volume of water.

3.3.11 Injector: 5 mL.

3.4 Procedure

3.4.1 Extraction, clean up

Weigh ca 5 g of the test sample (accurate to 0.1 g) into a 50 mL centrifuge tube (3.3.2), add 10 mL of hydrochloric acid solution (3.2.5). Connect the tube to a condenser and heated to boiling. The solution is refluxed for 30 min. Take out and let the tube cool to room temperature and then remove the condenser. Filter the suspension through a sintered glass filter by suction. Then rinse the tube and the residue several times with about 10 mL of hydrochloric acid solution (3.2.5). Transfer

the filterate and washings to a 25 mL volumetric flask and dilute to 25 mL with hydrochloric acid solution(3.2.5).

Accurately pipet 2.5 mL of the above test sample solution and 2.5 mL of the standard working solution with similar carbendazim concentration of the sample solution into two 15 mL centrifuge tubes respectively. Add 4 mL of dichlomethane to each centrifuge tube and homogenize on a homogenizer for 2 min, centrifuge for 3 min(under 4 000 r/min). Pipet and discard the lower dichlorimethane layer. Make the water phase to nearly alkaline with several drops of NaOH solution(10 mol/L), then carefully add NaOH solutoin(2 mol/L) dropwise until pH of the solution is 10—11. Finally adjust the pH of the solution to 9.5—10 with $NaHCO_3$ solution. Extract with 4 mL dichloromethane on a homogenizer for 2 min and centrifuge for 3 min (under 4 000 r/min). Pipet the dichloromethane layer into another centrifuge tube. Repeat this extraction as above twice more. Collect the extracts and concentrate to 1 mL.

Pass the above concentrated extract through the C_{18} Chromatographic micro-column(3.3.10) with injector, then wash the centrifuge tube and the C_{18} micro-column with 3 mL of methanol solution (3.2.4). Discard the effluent. Rinse the centrifuge tube and the C_{18} chromatographic micro-column with 3 mL of n-hexane, discard the effluent too. Then, wash the centrifuge tube and let through the C_{18} chromatographic micro-column with 2.5 mL of methanol(3.2.3) twice. Collect two of the eluates, concentrate and dilute to 1.0 mL with methand(3.2.3) for HPLC determination.

3.4.2 Determination

3.4.2.1 HPLC operating condition

a. Chromatographic column: μBondapak C_{18} 300 mm×3.9 mm(id);

b. Mobile phase: Methanol-water(4+6);

c. Flow rate: 0.8 mL/min;

d. Detector: UV-detetor, wave length 286 nm for determination;

e. Column temperature: Room temp.

3.4.2.2 Chromatographic determination

According to the approximate concentration of carbendazim in the sample solution, select the standard working solution with similar peak height to that of sample solution. The responses of carbendazim in the standard solution and sample solution should be in the linear range of the instrumental detection. The standard working solution should be in-jected in-between occasionally with the sample solution of equal volume. Under above chromatographic condition, the retention time of carbendazim is about 7.5 min.

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 omisson of sample addition.

3.5 Calculation and expression of the result

Calculate the content of carbendazim in the sample is carried out by HPLC data processor or according to the following formula:

$$X = \frac{h \cdot c_s}{h_s \cdot c}$$

where

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

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

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

c_s—the concentration of carbendazim in the standard working solution, μg/mL;

c—concentration of sample mass in final test solution, g/mL.

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

4 Limit of determination and recovery

4.1 Limit of defermination

The limit of determination of this method is 0.7 mg/kg.

4.2 Recovery

According to the experimental data, when the fortified concentration of carbendazim is in the range of 0.7—12.0 mg/kg, the recovery is 90.9%—102.2%.

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 Jiangxi Import and Export Commodity Inspection Bureau of the People's Republic of China.

This standard was mainly drafted by Xu Wenyan, Shi Xiaoshan, Li Shuangzheng.

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