SN

中华人民共和国进出口商品检验行业标准

SN 0654—1997

出口水果中克菌丹残留量检验方法

Method for the determination of captan residues in fruits for export

1997-08-15发布　　1998-01-01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、改进和验证后制定的。在标准中同时制定了抽样和制样方法。

测定低限是根据国际上对水果中克菌丹的最高限量和测定方法的灵敏度而制定的。

本标准附录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。

本标准由中华人民共和国河南进出口商品检验局负责起草。

本标准主要起草人：李选臣、李国村、王志明、郑汝喜、高志伟。

本标准系首次发布的行业标准。

中华人民共和国进出口商品检验行业标准

出口水果中克菌丹残留量检验方法

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Method for the determination of captan residues in fruits for export

1 范围

本标准规定了出口水果中克菌丹残留量检验的抽样、制样和高效液相色谱测定方法。

本标准适用于出口苹果中克菌丹残留量的检验。

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

将所取样品缩分出1 kg，取可食部分，经组织捣碎机捣碎，均分为两份，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

试样中残留的克菌丹用丙酮提取，抽滤，提取液经浓缩后，用Sep-Pak C_{18}柱净化，并用甲醇洗脱，洗脱液经浓缩、定容、过滤后，用配有紫外检测器的高效液相色谱仪测定，外标法定量。

3.2 试剂和材料

除另有规定外，所用试剂均为分析纯，水为蒸馏水。

中华人民共和国国家进出口商品检验局1997-08-15批准　　1998-01-01实施

3.2.1 丙酮。

3.2.2 甲醇：紫外光谱纯。

3.2.3 乙腈：紫外光谱纯。

3.2.4 克菌丹标准品：纯度≥99.0%。

3.2.5 克菌丹标准溶液：

3.2.5.1 克菌丹标准储备液：称取 0.1 g(准确至 0.000 2 g)的克菌丹标准品于 100 mL 容量瓶中，用适量的甲醇溶解，再用甲醇稀释至刻度，混匀。配成浓度为 1 mg/mL 的标准储备液。

3.2.5.2 克菌丹标准工作液：根据需要准确移取适量的克菌丹标准储备液，用甲醇稀释成适当浓度的标准工作液。标准工作溶液需每周配制一次。

3.3 仪器和设备

3.3.1 高效液相色谱仪并配有紫外检测器。

3.3.2 组织捣碎机。

3.3.3 振荡器。

3.3.4 微量进样器：25 μL，100 μL。

3.3.5 容量瓶：2 mL。

3.3.6 离心管：10 mL。

3.3.7 净化柱：Sep-Pak C_{18}，用 2 mL 甲醇活化，并用 2 mL 水洗过。

3.3.8 滤膜：孔径 0.5 μm(有机)。

3.4 测定步骤

3.4.1 提取

称取试样约 30 g(精确到 0.1 g)于 100 mL 具塞锥形烧瓶中，加入 50 mL 丙酮，于振荡器上振荡 1 h，提取液用布氏漏斗抽滤，用丙酮约 40 mL 分三次洗涤残渣及烧瓶。合并滤液和洗液，用氮气吹至 20 mL左右。

3.4.2 净化

将浓缩的提取液倒入 Sep-Pak C_{18}净化柱中，用 10 mL 水洗去水溶性杂质。用 10 mL 甲醇洗脱，收集洗脱液于 10 mL 的离心管中，用氮气吹至近干，然后用甲醇转移至 2 mL 容量瓶中，定容。用滤膜过滤，滤液供高效液相色谱测定。

3.4.3 测定

3.4.3.1 高效液相色谱条件

a) 色谱柱：Nova-Pak C_{18}柱，5 μm，25 cm×4 mm(内径)；

b) 流动相：甲醇-乙腈-水(35+35+30)；

c) 流速：0.8 mL/min；

d) 测定波长：254 nm；

e) 柱温：30℃；

f) 进样量：25 μL。

3.4.3.2 高效液相色谱测定

根据样液中克菌丹的含量情况，选定峰高与样液相近的标准工作溶液。标准工作溶液和待测样液中克菌丹的响应值，均应在仪器检测的线性范围内。对标准溶液和样液应等体积参插进样测定。在上述色谱条件下，克菌丹保留时间约为 2.8 min。标准品的色谱图见附录 A 中图 A1。

3.4.4 空白试验

除不称取试样外，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(1)计算试样中克菌丹的残留含量：

$$X = \frac{h \cdot c \cdot V}{h_s \cdot m} \quad \cdots\cdots(1)$$

式中：X——试样中克菌丹残留量，mg/kg；

h——样液中克菌丹的峰高，mm；

h_s——标准工作液中克菌丹的峰高，mm；

c——标准工作液中克菌丹的浓度，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 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 0.3 mg/kg。

4.2 回收率

苹果中克菌丹的添加浓度及其回收率的实验数据：

在 0.3 mg/kg 时，回收率为 81.3%；

在 1.0 mg/kg 时，回收率为 84.6%；

在 3.0 mg/kg 时，回收率为 96.1%。

附 录 A
（提示的附录）
标准品色谱图

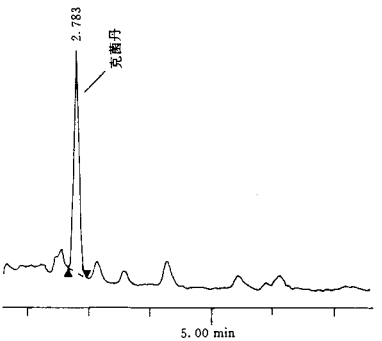

图 A1 克菌丹标准品的液相色谱图

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 detemination in this standard is defined on the basis of the current international maximum limits for captan residues in fruits 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 mainly drafted by Henan Import and Export Commodity Inspection Bureau of the People's Republic of China.

The main drafters of this standard are Li Xuanchen, Li Guocun, Wang Zhiming, Zheng Ruxi, Gao Zhiwei.

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

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 0654—1997

Method for the determination of captan residues in fruits for export

1 Scope

This standard specifies the methods of sampling, sample preparation and determination of captan residues by high performance liquid chromatography in fruits for export.

This standard is applicable to the determination of captan residues in apple 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, 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 the primary sample should not be less than 2 kg, which should be sealed, labeled and sent to laboratory in time.

2.4 Preparation of test sample

The combined primary sample is reduced to 1 kg, the edible portions are blended in a blender, 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 test sample

The test sample should be stored below −18℃.

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

Approved by the State Administration of Import and Export Commodity Imspection of the People's Republic of China on Aug. 15, 1997

Implemented from Jan. 1, 1998

3 Method of determination

3.1 Principle

The captan residues in the test sample are extracted by acetone. The extract is filtered by suction. and then concentrated and cleaned up by Sep-Pak C_{18} cartridge. Elute with methanol, and the eluate is concentrated, made up to volume, and filtered. Determination is made by high performance liquid chromatography equipped with UV 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 Acetone.

3.2.2 Methanol: UV grade.

3.2.3 Acetonitrile: UV grade.

3.2.4 Captan standard: Purity ≥99.0%.

3.2.5 Captan standard solution:

3.2.5.1 Captan standard stock solution: Weigh 0.1 g (accurate to 0.000 2 g) of captan standard into a 100 mL volumetric flask, dissolve with suitable volume of methanol and make up to the mark with methanol, mix thoroughly. The concentration of the standard stock solution is 1.0 mg/mL.

3.2.5.2 Captan standard working solution: According to the requirement prepare standard working solution of appropriate concentration by diluting the standard stock solution with methanol. Prepare the standard working solution freshly every week.

3.3 Apparatus and equipment

3.3.1 High performance liquid chromatograph, equipped with UV detector.

3.3.2 High-speed blender.

3.3.3 Shaker.

3.3.4 Micro-syringe: 25 μL, 100 μL.

3.3.5 Volumetric flask: 2.0 mL.

3.3.6 Centrifuge tube: 10 mL.

3.3.7 Cleanup column: Sep-Pak C_{18} cartridge, activated with 2 mL of methanol and rinsed with 2 mL of water.

3.3.8 Filter membrane: Aperture 0.5 μm (organic).

3.4 Procedure

3.4.1 Extraction

Weigh ca 30 g of the test sample (accurate to 0.1 g) into a 100 mL conical flask with stopper, add 50 mL of acetone, shake for 1 h in a shaker, the extract is filtered by suction through a Buchner funnel, wash the residue and the flask three times with ca 40 mL in total of acetone, combine the filtrate and the washings. The solution is evaporated to ca 20 mL under a stream of nitrogen.

3.4.2 Cleanup

Transfer the concentrated extract into a Sep-Pak C_{18} cartridge, remove the water soluble impurities by rinsing with 10 mL of water. Then elute with 10 mL of methanol through the cartridge, collect the eluate into a centrifuge tube (10 mL) and evaporate nearly to dryness with a stream of nitrogen. Dissolve and transfer the extract to a volumetric flask (2 mL) with methanol and make up to the mark with methanol, filter the solution with the filter membrane (0.5 μm). The filtrate is used for HPLC determination.

3.4.3 Determination

3.4.3.1 HPLC operating condition

a) HPLC column: Nova-Pak C_{18} column, 5 μm, 25 cm×4 mm(id);

b) Mobile phase: Methanol-acetonitrile-water (35+35+30);

c) Flow rate: 0.8 mL/min;

d) Wavelength: 254 nm;

e) Column temperature: 30℃;

f) Injection volume: 25 μL.

3.4.3.2 HPLC determination

According to the approximate concentration of captan in the sample solution select the standard working solution with similar peak height to that of the sample solution. The responses of captan in the standard working solution and sample solution should be in the linear range of the instrumental detection. The standard working solution should be injected in-between the injections of the sample solution of equal volume. Under the above operating condition the retention time of captan is ca 2.8 min. For chromatogram of the standard, see fig. A1 in annex A.

3.4.4 Blank test

The operation of 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

The calculation of captan residues in the test sample is carried out by a data processor or according to formula (1):

$$X = \frac{h \cdot c \cdot V}{h_s \cdot m} \qquad \cdots\cdots(1)$$

where

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

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

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

c—the concentration of captan 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 0.3 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentrations of captan in apple and its corresponding recoveries are:

0.3 mg/kg, the recovery 81.3%;

1.0 mg/kg, the recovery 84.6%;

3.0 mg/kg, the recovery 96.1%.

Annex A

(informative)

Chromatogram of the captan standard

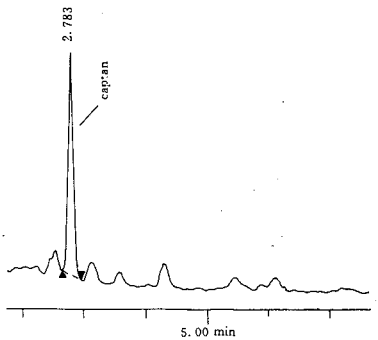

Fig. A1 Liquid chromatogram of the captan standard