SN

中华人民共和国进出口商品检验行业标准

SN 0659—1997

出口蔬菜中邻苯基苯酚残留量检验方法　液相色谱法

Method for the determination of *o*-phenylphenol residues in vegetables for export —Liquid chromatography

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蔬菜中邻苯基苯酚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：中国进出口商品检验技术研究所。

本标准主要起草人：王超、刘瑜。

本标准系首次发布的行业标准。

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1　范围

本标准规定了出口蔬菜中邻苯基苯酚残留量检验的抽样、制样和液相色谱测定方法。

本标准适用于出口番茄及辣椒中邻苯基苯酚残留量的检验。

2　抽样和制样

2.1　检验批

以不超过1 500件为一个检验批。

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约1 kg，取可食部分，经组织捣碎机均浆后分成两份，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

用乙酸乙酯提取试样中残留邻苯基苯酚。提取液经过滤、浓缩后，用流动相定容。用配有荧光检测器的液相色谱仪测定，外标法定量。

3.2　试剂和材料

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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。

3.2.2 甲醇：色谱纯。

3.2.3 乙腈：色谱纯。

3.2.4 磷酸盐缓冲溶液(pH8.0)：称取 1.722 g 磷酸氢二钾与 0.120 g 磷酸二氢钾，溶于水中，然后用水定容至 1 000 mL。

3.2.5 邻苯基苯酚标准品：纯度≥99.5%。

3.2.6 邻苯基苯酚标准溶液：准确称取适量的邻苯基苯酚标准品，用甲醇配成浓度为 500 μg/mL 的标准储备液，根据需要再用流动相稀释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：配有荧光检测器。

3.3.2 注射器：50 μL。

3.3.3 旋转蒸发器。

3.3.4 组织捣碎机。

3.3.5 振荡器。

3.4 测定步骤

3.4.1 提取

称取试样约 10.0 g(精确到 0.1 g)，放入 250 mL 锥形瓶中，加入 50 mL 乙酸乙酯，于振荡器上振荡 30 min。混合物经漏斗过滤，滤液收集在 200 mL 圆底烧瓶中。将残渣再移入原锥形瓶中。加入 30 mL 乙酸乙酯，继续振荡 5 min，过滤。用 10 mL 乙酸乙酯洗涤锥形瓶及漏斗。合并滤液及洗液，于 40℃减压浓缩至约 0.5 mL。用流动相定容至 100 mL。经 0.45 μm 滤膜过滤后，进行液相色谱测定。

3.4.2 测定

3.4.2.1 色谱条件

a) 色谱柱：CLC C_8(M)柱，150 mm×4.6 mm(内径)，或相当者；

b) 保护柱：CLC G-C_8 保护柱，或相当者；

c) 色谱柱温度：35℃；

d) 检测波长：激发波长 285 nm，发射波长 350 nm；

e) 流动相：甲醇-乙腈-磷酸盐缓冲溶液(3+3+4)；

f) 流速：1.0 mL/min。

3.4.2.2 色谱测定

根据样液中邻苯基苯酚含量情况，选定峰高相近的邻苯基苯酚标准工作溶液。标准工作溶液和样液中的邻苯基苯酚的响应值均应在仪器的检测线性范围内。对标准工作液和样液等体积参插进样测定。在上述色谱条件下，邻苯基苯酚的保留时间约为 12.7 min。标准品的色谱图如附录 A 中图 A1。

3.4.3 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)计算试样中邻苯基苯酚的残留含量：

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

式中：X——试样中邻苯基苯酚残留含量，mg/kg；

h——样液中邻苯基苯酚的峰高，mm；

h_s——标准工作液中邻苯基苯酚的峰高，mm；

c——标准工作液中邻苯基苯酚的浓度，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 测定低限、回收率

4.1 测定低限

本方法的测定低限为 0.5 mg/kg。

4.2 回收率

邻苯基苯酚添加浓度及其回收率的实验数据：

在番茄中，在 0.1 mg/kg 时，回收率为 98.9%；

在 0.5 mg/kg 时，回收率为 98.2%；

在 10.0 mg/kg 时，回收率为 99.6%。

在辣椒中，在 0.1 mg/kg 时，回收率为 100.5%；

在 0.5 mg/kg 时，回收率为 91.4%；

在 10.0 mg/kg 时，回收率为 90.2%。

附 录 A
（提示的附录）
标准品色谱图

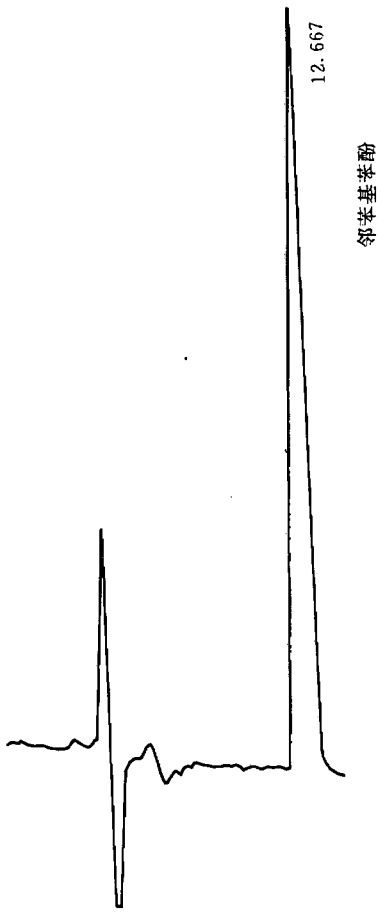

图 A1 邻苯基苯酚标准品液相色谱图

Foreword

This standard was drafted in accordance with the requirements of GB/T 1.1—1993 "Directives for the work of the 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 in this standard is defined on the basis of the current international maximum limits for *o*-phenylphenol residues in vegetables 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 China Import and Export Commodity Inspection Technology Institute.

The main drafters of this standard are Wang Chao, Liu Yu.

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

Method for the determination of *o*-phenylphenol residues in vegetables for export —Liquid chromatography

SN 0659—1997

1 Scope

This standard specifies the methods of sampling, sample preparation and determination of *o*-phenylphenol residues by high performance liquid chromatography (HPLC) in vegetables for export.

This standard is applicable to the determination of *o*-phenylphenol residues in tomato and chilli 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, grade and specification, 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 taken from each package should be at least 500 g as a primary sample. The total weight of all the primary samples should not be less than 2 kg, which shall be placed in a clean container, sealed, labeled, and sent to laboratory in time.

2.4 Preparation of test sample

The combined primary sample is mixed and reduced to ca 1 kg, the edible portions are blended, 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 sample

The test samples should be stored below −18℃.

Note: In the course of sampling and sample preparation, precautions must be taken to avoid contamination or any

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factors which may cause the change of residue content.

3 Method of determination

3.1 Principle

o-Phenylphenol residues in the test sample are extracted with ethyl acetate, and the extract is filtered and concentrated. The residue is leached and diluted to a definite volume by mobile phase, which is then analyzed by HPLC with fluorescence detector, using external standard method.

3.2 Reagents and material

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

3.2.1 Ethyl acutate.

3.2.2 Methanol: LC grade.

3.2.3 Acetonitrile: LC grade.

3.2.4 Phosphate buffer solution (pH8.0): Weigh 1.722 g of K_2HPO_4 and 0.120 g of KH_2PO_4, dissolve in water, then diluted to 1 000 mL.

3.2.5 *o*-Phenylphenol standard: Purity≥99.5%.

3.2.6 *o*-Phenylphenol standard solution: Accurately weigh an adequate amount of *o*-phenylphenol standard, dissolve in methanol to prepare a solution of 500 μg/mL in concentration as the standard stock solution, from which standard working solution of appropriate concentration is prepared by diluting with mobile phase according to the requirement.

3.3 Apparatus and equipment

3.3.1 High performance liquid chromatograph, equipped with fluorescence detector.

3.3.2 Injector: 50 μL.

3.3.3 Rotary evaporator.

3.3.4 Blender.

3.3.5 Shaker

3.4 Procedure

3.4.1 Extraction

Weigh ca 10.0 g (accurate to 0.1 g) of the test sample into a 250-mL conical flask, add 50 mL of ethyl acetate, and shake for 30 min with a shaker. Filter the extract into a 200-mL round bottom flask. Transfer the residue back to the original flask, and add anather 30 mL of ethyl acetate to the residue, shake for 5 min and filter as above. Wash the conical flask and the filter paper with 10 mL of ethyl acetate. Combine the filtrates and washings and evaporate under reduced pressure to ca. 0.5 mL with 40℃ bath temperature. Dissolve the residue and make up to exactly 100 mL with mobile phase, after passing through a 0.45 μm filter, the solution is used for high performance liquid chromatographic determination.

3.4.2 Determination

3.4.2.1 HPLC operating condition

a) LC column: CLC C_8(M), 150 mm×4.6 mm(id), or equivalent;

b) Guard column: CLC G-C_8, or equivalent;

c) Column temperature: 35℃;

d) Wavelength: Ex 285 nm, Em 350 nm;

e) Mobile phase: methanol-acetonitrile-phosphate buffer solution (3+3+4);

f) Flow rate: 1.0 mL/min.

3.4.2.2 HPLC determination

According to the approximate concentration of *o*-phenylphenol in the sample solution, select the standard working solution with similar peak height to that of the sample solution. The responses of *o*-phenylphenol 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 *o*-phenylphenol is about 12.7 min. For the chromatogram of the standard, see fig. A1 of 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 without addition of sample.

3.5 Calculation and expression of the result

Calculate the content of *o*-phenylphenol residues in the test sample by data processor or according to the formula (1):

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

where

X—the residue content of *o*-phenylphenol in test sample, mg/kg;

h—the peak height of *o*-phenylphenol in sample solution, mm;

h_s—the peak height of *o*-phenylphenol in the standard working solution, mm;

c—the concentration of *o*-phenylphenol in standard working solution, μg/mL;

V—the final volume of 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 0.5 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentrations of *o*-phenylphenol and their corresponding recoveries are:

in tomato, 0.1 mg/kg, the recovery 98.9%;
0.5 mg/kg, the recovery 98.2%;
10.0 mg/kg, the recovery 99.6%.

in chilli, 0.1 mg/kg, the recovery 100.5%;
0.5 mg/kg, the recovery 91.4%;
10.0 mg/kg, the recovery 90.2%.

Annex A

(Informative annex)

Chromatogram of the standard

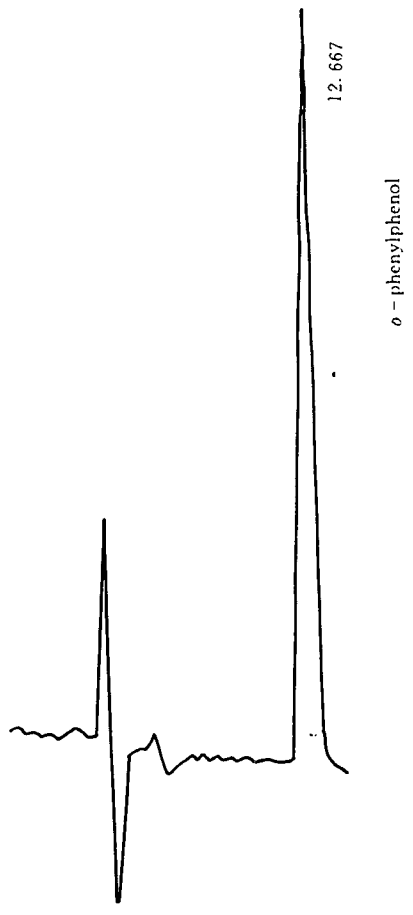

Fig. A1 Liquid chromatogram of *o*-phenylphenol standard