

SN

中华人民共和国进出口商品检验行业标准

SN 0652—1997

出口水果中对酞酸铜残留量 检 验 方 法

Method for the determination of copper terephthalate
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水果中对酞酸铜残留量的最高限量和本测定方法的灵敏度而规定的。

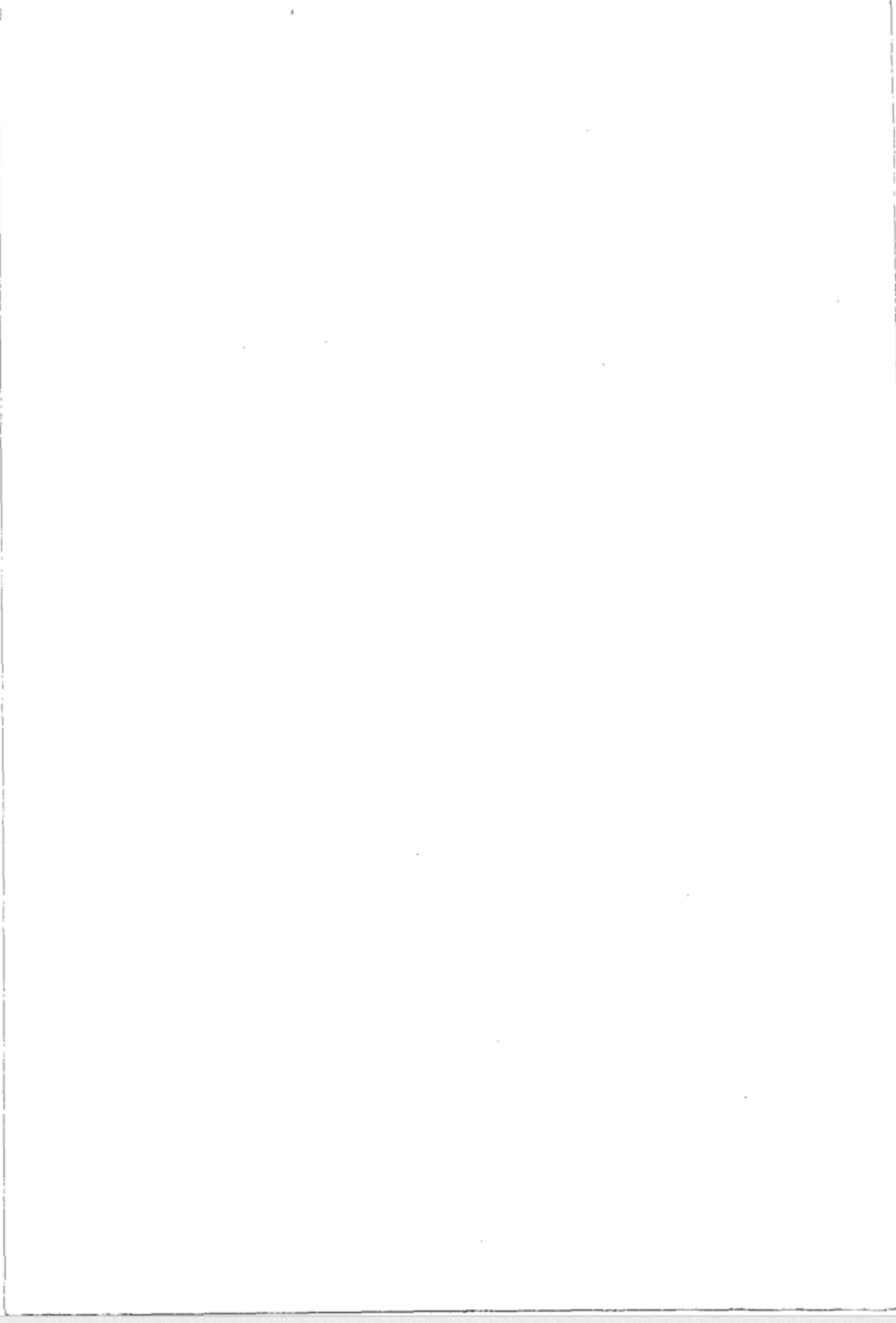
本标准附录 A 为提示的附录。

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天津进出口商品检验局负责起草。

本标准主要起草人：唐翊、佟晖、高晓敏。

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

出口水果中对酞酸铜残留量 检 验 方 法

SN 0652—1997

Method for the determination of copper terephthalate
residues in fruits for export

1 范围

本标准规定了出口水果中对酞酸铜残留量检验的抽样、制样和液相色谱测定方法。
本标准适用于出口柑桔和苹果中对酞酸铜残留量的检验。

2 抽样和制样

2.1 检验批

以不超过1500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
251~1500	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500g作为原始样品,原始样品总量不得少于2kg,放入清洁容器内,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1kg,取可食部分,用组织捣碎机捣碎,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

试样于氢氧化钠溶液中加热回流,溶液经酸化后,用乙酸乙酯提取被分解出的对酞酸,提取物经甲酯化后,再经C₁₈柱净化,乙腈-水混合液洗脱,洗脱液用配有紫外检测器的液相色谱仪检测,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。
- 3.2.2 无水乙醇。
- 3.2.3 甲醇:液相色谱用。
- 3.2.4 乙腈:液相色谱用。
- 3.2.5 硫酸溶液:3 mol/L。
- 3.2.6 盐酸溶液:12 mol/L。
- 3.2.7 硫酸钠溶液:2%。
- 3.2.8 磷酸氢二钠溶液:2%。
- 3.2.9 氢氧化钠溶液:0.03 mol/L。
- 3.2.10 无水硫酸钠:650℃灼烧4 h,贮于密闭容器中备用。
- 3.2.11 氯化钠。
- 3.2.12 洗脱液:
 - a) 乙腈-水(2+8);
 - b) 乙腈-水(2+1)。
- 3.2.13 甲酯化剂:三氟化硼乙醚-甲醇溶液(1+4),0~4℃储存,每月新配。
- 3.2.14 对苯二甲酸(即对酞酸)标准品:纯度≥99%。
- 3.2.15 对苯二甲酸标准溶液:准确称取适量的对苯二甲酸标准品,用热乙醇(约40℃)溶解,冷却后用乙醇定容,配成浓度为1.00 mg/L的标准贮备液。根据需要再用乙醇稀释成适当浓度的标准工作溶液。
- 3.3 仪器和设备
- 3.3.1 高效液相色谱仪:配有紫外检测器。
- 3.3.2 捣碎机。
- 3.3.3 高速离心机。
- 3.3.4 玻璃抽滤瓶。
- 3.3.5 微孔滤膜抽滤器:0.45 μm。
- 3.3.6 离心管:玻璃,具塞,50 mL。
- 3.3.7 净化柱:Sep-Pak C₁₈, No. 51910, Waters,或相当者。使用前顺次用5 mL 乙腈、30 mL 水洗涤。
- 3.3.8 恒温水浴。
- 3.3.9 微量注射器:10 μL。
- 3.3.10 回流设备。

3.4 测定步骤

3.4.1 提取

称取40 g试样(精确到0.1 g)于500 mL蒸馏瓶中,加入150 mL氢氧化钠溶液(0.03 mol/L),于100℃水浴中加热回流1 h。用少量水洗涤冷凝器,洗液合并于蒸馏瓶中,冷却后抽滤。用氢氧化钠溶液(0.03 mol/L)洗涤蒸馏瓶及滤纸上的残渣。合并滤液和洗液,并用氢氧化钠溶液(0.03 mol/L)定容至200 mL。

吸取10 mL上述样液(相当于2 g样品)于100 mL离心管中,加入1 mL硫酸溶液(3 mol/L),轻微旋摇后加入10 mL水。然后加入25 mL乙酸乙酯,盖塞,剧烈振摇约2 min,于2 500 r/min离心约3 min。将上层溶液转移至另一个100 mL离心管中。向第一个管中再加入15 mL乙酸乙酯,重复上述操作。合并乙酸乙酯提取液,再分别用20 mL、10 mL磷酸氢二钠溶液(2%)进行两次液-液提取。弃去乙酸乙酯层,合并水层于100 mL的离心管中。于水层中加入2 mL盐酸溶液(12 mol/L),5 g氯化钠,30 mL乙酸乙酯,激烈振荡约2 min,离心,分取有机层。在水层中再加15 mL乙酸乙酯,同上述操作,分取有机层,合并于同一容器。适量无水硫酸钠脱水后,将该有机层转移至250 mL心形瓶中,于40℃水浴旋转浓

缩至近干。

3.4.2 甲酯化

向上述心形瓶中加入 2 mL 甲酯化剂(3.2.13),充分溶解残留物,将溶液转移至 25 mL 密封试管中,旋紧盖,于 70℃ 水浴酯化 1.5 h。冷却后,加入 10 mL 硫酸钠溶液(2%),分别用 5 mL 乙酸乙酯提取两次。合并乙酸乙酯层,经适量无水硫酸钠脱水后,于 40℃ 水浴旋转浓缩至近干。

3.4.3 净化

加入 1 mL 乙腈于上述残渣中,溶解。再加 4 mL 水,混匀。将该溶液注入净化柱(3.3.7)。依次用 5 mL 水,5 mL 洗脱液(3.2.12a))洗涤,弃去流出液。最后用 2.0 mL 洗脱液(3.2.12b))进行洗脱,收集洗脱液供液相色谱测定。

3.4.4 对苯二甲酸标准工作溶液的处理

准确吸取适当浓度的对苯二甲酸标准工作溶液于一个 25 mL 密封试管中,用氮气流将溶剂吹除。按 3.4.2 甲酯化后,用 2.0 mL 洗脱液(3.2.12b))溶解后供液相色谱测定。

3.4.5 测定

3.4.5.1 色谱条件

- a) 色谱柱: Adsorbosphere XL C₁₈, 250 mm×4.6 mm(内径), 5 μm, 或相当者。
- b) 流动相: 乙腈-水(5+5)。配制后,经 0.45 μm 滤膜抽滤,脱气。
- c) 流速: 0.7 mL/min。
- d) 柱温: 25℃。
- e) 检测波长: 242 nm。
- f) 进样量: 20 μL。

3.4.5.2 色谱测定

根据样液中对苯二甲酸二甲酯含量情况,选定峰高相近的对苯二甲酸二甲酯标准工作溶液。标准工作溶液和样液中对苯二甲酸二甲酯响应值均应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,对苯二甲酸二甲酯的保留时间约为 11 min。标准品的液相色谱图,见附录 A 中图 A1。

3.4.6 空白试验

除不加试样外,均按上述步骤进行。

3.5 结果计算和表述

用色谱数据处理机或按式(1)计算试样中对酞酸铜残留含量:

$$X = \frac{h \cdot c \cdot V}{h_s \cdot m} \times 1.371 \quad \dots\dots\dots (1)$$

式中: X——试样中对酞酸铜残留含量,mg/kg;

h——样液中对苯二甲酸二甲酯的峰高,mm;

h_s——标准工作溶液中对苯二甲酸二甲酯的峰高,mm;

c——标准工作溶液中对苯二甲酸的浓度,μg/mL;

V——最终样液的体积,mL;

m——最终样液所相当的试样量,g;

1.371——对酞酸铜[C₈H₄(COO)₂Cu]与对苯二甲酸[C₈H₄(COOH)₂]分子量之比(即 227.7/166.1=1.371)。

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

对酞酸铜添加浓度及其回收率的实验数据：

在柑桔中，浓度在 0.5 mg/kg 时，回收率为 86.8%；
浓度在 5.0 mg/kg 时，回收率为 91.7%；
浓度在 10.0 mg/kg 时，回收率为 88.8%。
在苹果中，浓度在 0.5 mg/kg 时，回收率为 83.5%；
浓度在 5.0 mg/kg 时，回收率为 86.2%；
浓度在 10.0 mg/kg 时，回收率为 85.1%。

附录 A
(提示的附录)
标准品色谱图

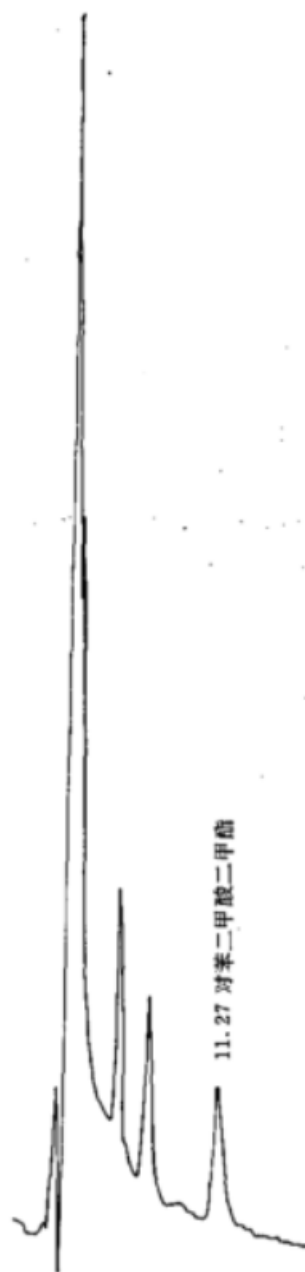


图 A1 对苯二甲酸二甲酯标准品液相色谱图

Foreword

This standard was drafted in accordance with the requirements of GB/T 1.1—1993 "Directives for the work of 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 limit for copper terephthalate residues in the 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 drafted by Tianjin Import and Export Commodity Inspection Bureau of the People's Republic of China.

The main drafters of the standard are Tang Yi, Tong Hui, Gao Xiaomin.

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**

SN 0652—1997

**Method for the determination of copper
terephthalate residues in fruits for export**

1 Scope

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

This standard is applicable to the determination of copper terephthalate residues in oranges and apples 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. From each at least 500 g shall be taken as a primary sample. The total weight of all primary samples 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 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 samples should be stored under -18°C .

Note, In the course of sampling and sample preparation, precautions 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 Aug. 15, 1997

Implemented from Jan. 1, 1998

3 Method of determination

3.1 Principle

The test sample is refluxed with sodium hydroxide solution. After acidifying, the terephthalic acid set free in the solution is extracted with ethyl acetate. The extract is methylated and cleaned up by passing through a C_{18} cartridge. Then elute with acetonitrile-water mixture. The eluate is analyzed by HPLC with UV detector, using external standard method.

3.2 Reagents and materials

Unless otherwise specified, all reagents used should be of analytical grade, "water" is distilled water.

3.2.1 Ethyl acetate.

3.2.2 Ethyl alcohol, absolute.

3.2.3 Methanol, HPLC grade.

3.2.4 Acetonitrile, HPLC grade.

3.2.5 Sulfuric acid solution; 3 mol/L.

3.2.6 Hydrochloric acid solution; 12 mol/L.

3.2.7 Sodium sulfate solution; 2%.

3.2.8 Disodium hydrogen phosphate solution; 2%.

3.2.9 Sodium hydroxide solution; 0.03 mol/L.

3.2.10 Anhydrous sodium sulfate; Ignite at 650°C for 4 h and store in a desiccator.

3.2.11 Sodium chloride.

3.2.12 Eluent;

a) Acetonitrile-water (2+8);

b) Acetonitrile-water (2+1).

3.2.13 Methylating agent; Boron trifluoride etherate-methanol solution (1+4), store at 0—4°C, reprepare monthly.

3.2.14 Terephthalic acid (i.e. *p*-phthalic acid) standard; Purity $\geq 99\%$.

3.2.15 Terephthalic acid standard solution; Accurately weigh a suitable amount of standard and dissolve with hot ethyl alcohol (about 40°C). After cooling, prepare a solution of 1.00 mg/mL in concentration as the standard stock solution with ethyl alcohol. According to the requirement, prepare a standard working solution of appropriate concentration by diluting the stock solution with ethyl alcohol.

3.3 Apparatus and equipment.

3.3.1 Liquid chromatograph; Equipped with UV detector.

3.3.2 Blender.

3.3.3 High speed centrifuge.

3.3.4 Glass suction flask.

3.3.5 Vacuum filtrating device with 0.45 μm filter membrane.

3.3.6 Centrifuge tube; Glass, with stopper, 50 mL.

3.3.7 Cleanup cartridge; Sep-Pak C_{18} , No. 51910, waters or equivalent. Rinse with 5 mL of acetonitrile and 30 mL of water before use.

3.3.8 Water-bath; Thermostatic.

3.3.9 Micro-syringe; 10 μL .

3.3.10 Reflux apparatus.

3.4 Procedure

3.4.1 Extraction

Weigh 40 g (accurate to 0.1 g) of the test sample into a 500 mL distillation flask, add 150 mL of sodium hydroxide solution (0.03 mol/L), and reflux for 1 h in a water-bath of 100°C. Wash the condenser with a small amount of water, collect the washings into the flask. Stand for cooling, then filter by suction, wash the flask and the filter paper with sodium hydroxide solution (0.03 mol/L). Combine the washings, and adjust exactly the volume to 200 mL.

Pipet 10 mL of the above solution (equivalent to 2 g of test sample) into a 100 mL centrifuge tube, add 1 mL of sulphuric acid solution (3 mol/L), shake gently and add 10 mL of water. Add 25 mL of ethyl acetate to the tube, stopper and shake vigorously for about 2 min. Centrifuge for 3 minutes at 2 500 r/min and transfer the supernatant into another 100 mL centrifuge tube. Add 15 mL of ethyl acetate to the former tube, repeat the procedure. Combine the ethyl acetate supernatants, then extract twice with 20 mL and 10 mL of disodium hydrogen phosphate solutions (2%). Discard the ethyl acetate phases. Combine the aqueous phases into a 100 mL centrifuge tube, add 2 mL of hydrochloric acid solution (12 mol/L), 5 g of sodium chloride, and 30 mL of ethyl acetate. Shake vigorously for about 2 min, centrifuge, and collect the organic phase. Add 15 mL of ethyl acetate into aqueous phase, repeat the above process. Combine the organic phase, and dehydrate with a suitable amount of anhydrous sodium sulfate, then transfer into a 250 mL heart-shaped flask. Evaporate the solvent to near dryness with a rotary evaporator in a water-bath of 40°C.

3.4.2 Methylation

Add 2 mL of methylating agent (3.2.13) to the above heart-shaped flask, dissolve the residue thoroughly. Transfer the solution into a 25 mL test-tube, stopper and heat in a water-bath for 1.5 h at 70°C. Cool, add 10 mL of sodium sulfate solution (2%). Extract with 2×5 mL of ethyl acetate. Combine the ethyl acetate extracts, and dehydrate with suitable amount of anhydrous sodium sulfate. Evaporate the solvent to near dryness with a rotary evaporator in a water-bath of 40°C.

3.4.3 Cleanup

Dissolve the residue of 3.4.2 with 1 mL of acetonitrile. Add 4 mL of water, mix well. Let the solution pass through a pre-washed C₁₈ cartridge (3.3.7), discard the effluent. Wash the cartridge with 5 mL of water, 5 mL of eluent (3.2.12a) in sequence. Discard the washings. Finally, elute with 2.0 mL of eluent (3.2.12b), collect the eluate into a bottle. The solution is ready for HPLC analysis.

3.4.4 Treatment of terephthalic acid standard working solution

Accurately pipet 2.0 mL of terephthalic acid standard working solution of appropriate concentration into a 25 mL tube, remove the solvent under a gentle stream of nitrogen, proceed as described in section 3.4.2 for methylation. Dissolve the residue with 2.0 mL of eluent (3.2.12b), and the solution is ready for HPLC analysis.

3.4.5 Determination

3.4.5.1 LC operating condition:

- a) Column: Adsorbosphere XL C₁₈, 250 mm×4.6 mm (id), 5 μm, or equivalent;
- b) Mobile phase: Acetonitrile-water (5+5). Filter through 0.45 μm filter membrane and degas;
- c) Flow rate: 0.7 mL/min;
- d) Column temperature: 25°C;
- e) Detector wavelength: 242 nm;
- f) Injection volume: 20 μL.

3.4.5.2 LC determination

According to the approximate concentration of dimethyl terephthalate (i. e. dimethyl *p*-phthalate) in the sample solution, select the dimethyl terephthalate standard working solution with similar peak height to that of the sample solution. The responses of dimethyl terephthalate 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 chromatographic condition, the retention time of dimethyl terephthalate is about 11 min. For chromatogram of the dimethyl terephthalate standard, see fig A1 in annex A.

3.4.6 Blank test

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

3.5 Calculation and expression of the result

The calculation of copper terephthalate residue content in the test sample is carried out by LC data processor or according to formula (1);

$$X = \frac{h \cdot c \cdot V}{h_s \cdot m} \times 1.371 \quad \dots\dots\dots (1)$$

where

X —the residue content of copper terephthalate in the test samples, mg/kg;

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

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

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

V —the final volume of the sample solution, mL;

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

1.371—the molecular weight ratio (i. e. $227.7/166.1 = 1.371$) of copper terephthalate [$\text{C}_8\text{H}_4(\text{COO})_2\text{Cu}$] to terephthalic acid [$\text{C}_8\text{H}_4(\text{COOH})_2$].

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 copper terephthalate and its corresponding recoveries are:

In orange, 0.5 mg/kg, the recovery 86.8%;

5.0 mg/kg, the recovery 91.7%;

10.0 mg/kg, the recovery 88.8%.

In apple, 0.5 mg/kg, the recovery 83.5%;

5.0 mg/kg, the recovery 86.2%;

10.0 mg/kg, the recovery 85.1%.

Annex A
(informative)
Chromatogram of the standard



Fig. A1 Liquid chromatogram of dimethyl terephthalate standard

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