SN

中华人民共和国出入境检验检疫行业标准

SN/T 1902—2007

水果蔬菜中吡虫啉、吡虫清残留量的测定 高效液相色谱法

Determination of imidacloprid and acetamiprid residues in fruits and vegetables—HPLC

2007-05-23 发布　　2007-12-01 实施

中华人民共和国
国家质量监督检验检疫总局　发布

前　言

本标准的附录A为资料性附录。

本标准由国家认证认可监督管理委员会提出并归口。

本标准由中华人民共和国江苏出入境检验检疫局负责起草。

本标准主要起草人：何松涛、俞晔、乙小娟、张爱东、刘一军、孙建刚。

本标准系首次发布的检验检疫行业标准。

水果蔬菜中吡虫啉、吡虫清残留量的测定 高效液相色谱法

1 范围

本标准规定了水果蔬菜中吡虫啉、吡虫清残留量的高效液相色谱测定方法。

本标准适用于番茄、黄瓜、柑橘中吡虫啉、吡虫清残留量的检验。

2 测定方法

2.1 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，均分成两份，装入洁净容器内，作为试样，密封，并标明标记。

2.2 试样保存

将试样于－18℃以下冷冻保存。

注：在试样制备过程中，应防止样品受到污染或发生残留物含量的变化。

2.3 方法提要

试样中残留的吡虫啉、吡虫清用乙腈提取，提取液经弗罗里矽柱净化，浓缩、定容后，用配有紫外检测器或二极管阵列检测器的高效液相色谱仪测定。

2.4 试剂和材料

除另有规定外，所用试剂均为分析纯，水为蒸馏水或去离子水。

2.4.1 乙腈：HPLC 级。

2.4.2 正己烷。

2.4.3 丙酮。

2.4.4 氯化钠：140℃烘烤 4 h。

2.4.5 吡虫啉标准品：纯度大于等于 96%。

2.4.6 吡虫清标准品：纯度大于等于 96%。

2.4.7 吡虫啉、吡虫清标准溶液：准确称取适量的吡虫啉、吡虫清标准品，用乙腈配制成 100 μg/mL 的单个标准储备液，根据需要再用乙腈＋水(30＋70)稀释成适当浓度的混合标准工作溶液。

2.4.8 固相萃取柱，弗罗里矽柱(Florisil)，体积 3 mL 小柱，填充物 500 mg。

2.5 仪器设备

2.5.1 高效液相色谱仪：配有紫外检测器或二极管阵列检测器。

2.5.2 振荡器。

2.5.3 氮吹仪。

2.5.4 旋转蒸发仪。

2.5.5 漩涡混合器。

2.5.6 微量注射器：100 μL。

2.6 测定步骤

2.6.1 提取

称取 25 g 试样(精确至 0.01 g)，置于 250 mL 具塞锥形瓶中，准确加入 50.0 mL 乙腈，放置 2 h，振荡提取 30 min 后用滤纸过滤。滤液收集到装有 5 g～7 g 氯化钠的 100 mL 具塞量筒中，盖上塞子，剧

烈振荡 1 min，在室温下静止 10 min，使乙腈相和水相分层。

从 100 mL 具塞量筒中吸取 10.0 mL 乙腈溶液，移入 125 mL 梨形瓶中，于 50℃水浴中旋转浓缩至近干。加入 10 mL 丙酮＋正己烷(10＋90)溶解。

2.6.2 净化

将弗罗里矽柱用 2 mL 正己烷预淋洗，当溶剂液面到达柱吸附层表面时，立即倒入样品溶液，弃去流出液。用 5 mL 丙酮＋正己烷(10＋90)涮洗梨形瓶后淋洗弗罗里矽柱，弃去流出液。用 10 mL 丙酮＋正己烷(20＋80)分两次淋洗弗罗里矽柱，用 15 mL 刻度离心管接收洗脱液。将盛有洗脱液的离心管置于氮吹仪上，在 50℃水浴下，氮吹至近干，用乙腈＋水(30＋70)定容至 2.0 mL，在漩涡混合器上混匀，经0.45 μm 滤膜过滤后，待测。

2.6.3 测定

2.6.3.1 液相色谱参考条件

a) 色谱柱：ODS-C_{18}，5 μm，250 mm×4 mm(内径)或相当柱；

b) 色谱柱温度：40℃；

c) 流动相：乙腈＋水，梯度：0 min，(5＋95)；15 min，(25＋75)；20 min，(5＋95)；

d) 流速：1.0 mL/min；

e) 检测波长：258 nm；

f) 进样量：20 μL。

2.6.3.2 色谱测定

根据样液中被测农药残留限量要求，选定浓度相近的混合标准工作溶液。混合标准工作溶液和待测液中农药的含量在仪器检测的线性范围内，按上述色谱条件进行测定，吡虫啉和吡虫清的保留时间约为 13 min 和 15 min。标准品的液相色谱图参见附录 A。

2.6.4 空白试验

除不加试样外，按上述测定步骤进行。

2.7 结果的计算和表达

按式(1)计算试样中吡虫啉、吡虫清的残留量，计算结果需将空白值扣除。

$$X = \frac{A \times c \times V}{A_s \times m} \qquad \cdots\cdots(1)$$

式中：

X——试样中吡虫啉、吡虫清含量，单位为毫克每千克(mg/kg)；

A——试样中吡虫啉、吡虫清的峰面积；

c——标准工作液中吡虫啉、吡虫清的浓度，单位为微克每毫升(μg/mL)；

V——样液最终定容体积，单位为毫升(mL)；

A_s——标准工作液中吡虫啉、吡虫清的峰面积；

m——最终样液所代表的试样量，单位为克(g)。

3 测定低限、回收率

3.1 测定低限

本方法测定低限为 0.02 mg/kg。

3.2 回收率

3.2.1 番茄中吡虫啉、吡虫清的添加浓度及其回收率的实验数据：

——添加浓度在 0.02 mg/kg 时，吡虫啉回收率为 91.4%～109%，吡虫清回收率为95.5%～108%；

——添加浓度在 0.05 mg/kg 时，吡虫啉回收率为 89.2%～101%，吡虫清回收率为90.0%～

102%；

——添加浓度在 0.10 mg/kg 时，吡虫啉回收率为 80.7%～88.5%，吡虫清回收率为85.5%～103%。

3.2.2 黄瓜中吡虫啉、吡虫清的添加浓度及其回收率的实验数据：

——添加浓度在 0.02 mg/kg 时，吡虫啉回收率为 98.5%～106%，吡虫清回收率为94.0%～106%；

——添加浓度在 0.05 mg/kg 时，吡虫啉回收率为 95.0%～106%，吡虫清回收率为90.0%～102%；

——添加浓度在 0.10 mg/kg 时，吡虫啉回收率为 81.4%～97.2%，吡虫清回收率为96.2%～109%。

3.2.3 柑橘中吡虫啉、吡虫清的添加浓度及其回收率的实验数据：

——添加浓度在 0.02 mg/kg 时，吡虫啉回收率为 94.5%～102%，吡虫清回收率为 90.0%～99.0%；

——添加浓度在 0.05 mg/kg 时，吡虫啉回收率为 88.0%～98.4%，吡虫清回收率为 84.4%～93.8%；

——添加浓度在 0.10 mg/kg 时，吡虫清回收率为 84.9%～109%，吡虫清回收率为 87.3%～99.5%。

附 录 A
（资料性附录）
标准品高效液相色谱图

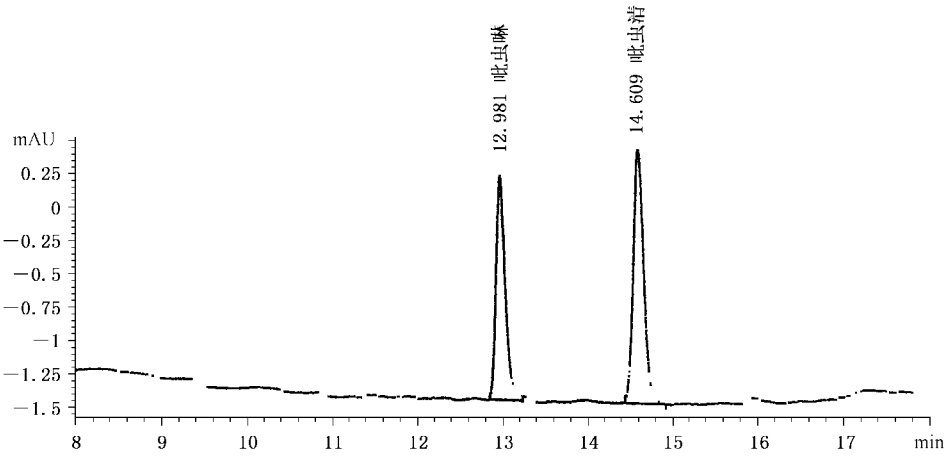

图 A.1 标准品液相色谱图(0.08 μg/mL)

Foreword

Annex A of this standard is an informative one.

This standard was proposed by and is under the charge of National Regulatory Commission for Certification and Accreditation.

This standard was drafted by Jiangsu Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are He songtao, Yu ye, Yi Xiaojuan, Zhang aidong, Liu yijun, Sun jiangang.

This standard is an inspection and quarantine professional standard promulgated for the first time.

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

Determination of imidacloprid and acetamiprid residues in fruits and vegetables —HPLC

1 Scope

This standard specifies the method of determination of imidacloprid and acetamiprid residues in fruits and vegetables by HPLC.

This standard is applicable to the determination of imidacloprid residue in tomatoes, cucumbers and oranges.

2 Method of determination

2.1 Preparation of test sample

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

2.2 Storage of sample

The test sample should be stored below −18℃.

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

2.3 Principle

The imidacloprid and acetamiprid residues in the test sample are extracted with acetonitrile. The extract is cleaned up by Florisil SPE tube and evaporated. The residue is dissolved and made up to a definite volume. Determination is made by means of a HPLC with UV detector or DAD.

2.4 Reagents and materials

Unless otherwise specified, all the reagents used should be analytically pure. "Water" is distilled water or de-ionized water.

2.4.1 Acetonitrile: HPLC grade.

2.4.2 *N*-hexane.

2.4.3 Acetone.

2.4.4 sodium chloride: baked at 140℃ for 4 h.

2.4.5 Imidacloprid standard: Purity no less than 96%.

2.4.6 Acetamiprid standard: Purity no less than 96%.

2.4.7 Imidacloprid and acetamiprid standard solution: Accurately weigh an appropriate amount of imidacloprid or acetamiprid standard, dissolve in acetonitrile to prepare a single standard stock solution of 100 μg/mL. Dilute the standard stock solution with acetonitrile-water (30 + 70) to the required concentration as the mixed standard working solution.

2.4.8 SPE tube, Florisil, 3 mL, 500 mg.

2.5 Apparatus and equipment

2.5.1 High performance liquid chromatograph equipped with UV detector or DAD.

2.5.2 Shaker.

2.5.3 Nitrogen evaporator.

2.5.4 Rotary evaporator.

2.5.5 Vortex mixer.

2.5.6 Micro-syringe: 100 μL.

2.6 Procedure

2.6.1 Extraction

Weigh 25 g (accurate to 0.01 g) of the test sample into a 250 mL conical flask with stopper, accurately add 50.0 mL acetonitrile and set aside for 2 h. Filtrate after shaking for 30 min. Gather filtrate into a 100 mL measuring cylinder in which 5 g～7 g sodium chloride are placed. Stopper the measuring cylinder and shake vigorously for ca 1 min, then let stand for separating completely.

Transfer 10.0 mL acetonitrile layer into a pear-shaped flask, condense it near to dryness with rotary evaporator in a bath of 50℃, dissolve the residue with 10 mL acetone-n-hexane (10 + 90).

2.6.2 Clean up

Condition the SPE tube with 2 mL n-hexane, add the sample solution into the SPE tube when the above solution arrive the sorbent surface, reject the effluents. Rinse the pear-shaped flask with 5 mL acetone + N-hexane (10 + 90), reject the effluents. Then rinse the SPE tube with 5 mL acetone + N-hexane (20 + 80) and once again, collect the elution with 15 mL scale centrifugal tube. Condense near to dryness with nitrogen evaporator in a bath of 50℃. The residue is dissolved with 2.0 mL acetonitrile + water (30 + 70), and mixed with the vortex mixer, filtered through a 0.45 μm membrane and ready for HPLC determination.

2.6.3 Determination

2.6.3.1 HPLC operating condition

a) Column: ODS-C_{18} (5 μm), 250 mm × 4 mm (i.d.) or equivalent;

b) Column temperture: 40℃;

c) Mobile phase: acetonitrile + water, gradient: 0 min, 5%; 15 min, 25%; 20 min, 5%;

d) Flow rate: 1.0 mL/min;

e) Detector wavelength: 258 nm;

f) Injection volume: 20 μL.

2.6.3.2 HPLC determination

According to the approximate concentration of the pesticide in the sample solution, select the mixed standard working solution with similar peak area to that of the sample solution. The responses of pesticide in the mixed standard working solution and sample solution should be within the linear range of the instrumental detection. Under the above chromatographic condition, the retention time of imidacloprid and acetamiprid is ca 13 min and 15 min. For HPLC chromatogram of the standard, see also annex A.

2.6.4 Blank test

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

2.7 Calculation and expression of the result

Calculate the content of imidacloprid or acetamiprid in the test sample according to the formula (1),

the blank value should be substracted from the result of calculation.

$$X = \frac{A \times c \times V}{A_s \times m} \quad \cdots\cdots(1)$$

where

X—the residue content of imidacloprid or acetamiprid in the test sample, mg/kg;

A—the peak area of imidacloprid or acetamiprid in the sample soluion;

c—the concentration of imidacloprid or acetamiprid in the standard working solution, μg/mL;

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

A_s—the peak area of imidacloprid or acetamiprid in the standard working solution;

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

3 Limit of determination and recovery

3.1 Limit of determination

The limit of determination of this method is 0.02 mg/kg.

3.2 Recovery

3.2.1 According to experimental data, the fortifying concentrations of imidacloprid and acetamiprid in tomato and their corresponding recoveries are:

—0.02 mg/kg, the recovery of imidacloprid and acetamiprid is 91.4%～109% and 95.5%～108%;
—0.05 mg/kg, the recovery of imidacloprid and acetamiprid is 89.2%～101% and 90.0%～102%;
—0.10 mg/kg, the recovery of imidacloprid and acetamiprid is 80.7%～88.5% and 85.5%～103%.

3.2.2 According to experimental data, the fortifying concentrations of imidacloprid and acetamiprid in cucumber and their corresponding recoveries are:

—0.02 mg/kg, the recovery of imidacloprid and acetamiprid is 98.5%～106% and 94.0%～106%;
—0.05 mg/kg, the recovery of imidacloprid and acetamiprid is 95.0%～106% and 90.0%～102%;
—0.10 mg/kg, the recovery of imidacloprid and acetamiprid is 81.4%～97.2% and 96.2%～109%.

3.2.3 According to experimental data, the fortifying concentrations of imidacloprid and acetamiprid in orange and their corresponding recoveries are:

—0.02 mg/kg, the recovery of imidacloprid and acetamiprid is 94.5%～102% and 90.0%～99.0%;
—0.05 mg/kg, the recovery of imidacloprid and acetamiprid is 88.0%～98.4% and 84.4%～93.8%;
—0.10 mg/kg, the recovery of imidacloprid and acetamiprid is 84.9%～109% and 87.3%～99.5%.

Annex A
(Informative)
HPLC chromatogram of the standards

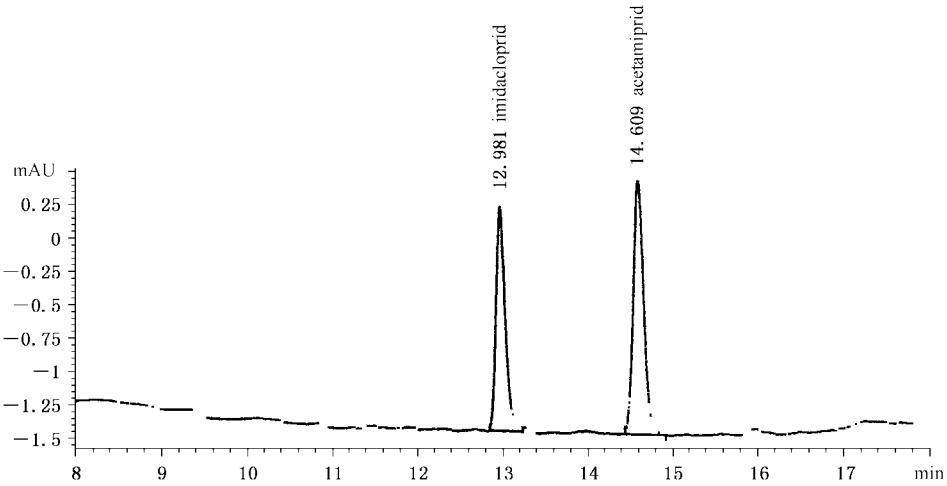

Figure A.1—HPLC chromatogram of the standards (0.08 μg/mL)

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*
中 国 标 准 出 版 社 出 版
北京复兴门外三里河北街 16 号
邮政编码:100045
网址 www.spc.net.cn
电话:68523946 68517548
中国标准出版社秦皇岛印刷厂印刷
*
开本 880×1230 1/16 印张 1 字数 17 千字
2007 年 9 月第一版 2007 年 9 月第一次印刷
印数 1—2 000
*
书号:155066·2-18046 定价 10.00 元

SN/T 1902-2007