SN

中华人民共和国出入境检验检疫行业标准

SN/T 0647—2013
代替 SN 0647—1997

出口坚果及坚果制品中抑芽丹残留量的测定　高效液相色谱法

Determination of maleic hydrazide residues in nuts and nut products for export—HPLC method

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前　言

本标准按照 GB/T 1.1—2009 给出的规则起草。

本标准代替 SN 0647—1997《出口坚果及坚果制品中抑芽丹残留量检验方法　分光光度法》。本标准与 SN 0647—1997 相比，除编辑性修改外主要技术变化如下：

——标准名称改为《出口坚果及坚果制品中抑芽丹残留量的测定　高效液相色谱法》；

——方法适用范围中增加了板栗及其制品；

——样品前处理方法由蒸馏法改为提取净化法；

——测定方法由分光光度法改为高效液相色谱法。

本标准由国家认证认可监督管理委员会提出并归口。

本标准起草单位：中华人民共和国山东出入境检验检疫局。

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本标准所替代标准的历次版本发布情况为：

——SN 0647—1997。

出口坚果及坚果制品中抑芽丹残留量的测定　高效液相色谱法

1　范围

本标准规定了出口坚果及坚果制品中抑芽丹残留量检测的制样和高效液相色谱测定方法。

本标准适用于核桃及制品、板栗及制品中抑芽丹残留量的测定。

2　规范性引用文件

下列文件对于本文件的应用是必不可少的。凡是注日期的引用文件，仅注日期的文件适用于本文件。凡是不注日期的引用文件，其最新版本(包括所有的修改单)适用于本标准。

GB/T 6682　分析实验室用水规格和试验方法

3　方法提要

试样经正己烷脱脂后，用甲醇提取，再经 C_{18} 柱净化后，高效液相色谱-紫外检测器检测，外标法定量。

4　试剂和材料

除另有规定外，所用试剂均为分析纯，水为 GB/T 6682 中规定的一级水。

4.1　甲醇：色谱纯。

4.2　正己烷：色谱纯。

4.3　乙酸铵。

4.4　氢氧化钠。

4.5　乙酸铵溶液(0.02 mol/L)：称取 1.54 g 乙酸铵，加水定容至 1 000 mL，用前经 0.45 μm 滤膜过滤。

4.6　氢氧化钠溶液(0.01 mol/L)：称取 0.40 g 氢氧化钠，加水定容至 1 000 mL。

4.7　抑芽丹标准物质(Maleic hydrazide，$C_4H_4N_2O_2$，CAS No：123-33-1)：纯度大于 99.9%。

4.8　抑芽丹标准储备溶液：准确称取适量抑芽丹标准品，用 0.01 mol/L 氢氧化钠溶液(4.6)溶解并稀释成浓度为 1 mg/mL 的标准储备液。

4.9　抑芽丹标准工作溶液：吸取 1.0 mL 标准储备溶液(4.8)，用 0.01 mol/L 氢氧化钠溶液(4.6)稀释定容至 100 mL，配制成浓度为 10 mg/L 的标准工作溶液。

4.10　微孔滤膜：0.45 μm，有机相。

4.11　固相萃取柱：C_{18} 小柱，3 mL，500 mg，或相当者。依次用 4 mL 甲醇和 4 mL 0.01 mol/L 氢氧化钠溶液(4.6)活化后备用。

5　仪器和设备

5.1　高效液相色谱仪：配有紫外或二极管阵列检测器。

5.2 电子天平：感量为 0.000 1 g 和 0.01 g。
5.3 旋转蒸发仪。
5.4 均质机：转速不低于 10 000 r/min。
5.5 离心机：转速不低于 6 000 r/min。
5.6 涡旋混合器。
5.7 样品粉碎机。
5.8 筛子：2.0 mm 圆孔筛。
5.9 分样板。

6 试样的制备与保存

6.1 一般要求

在制样和保存过程中，应防止样品受到污染或发生残留物含量的变化。

6.2 试样制备

将原始样品的可食部分用四分法缩分出约 200 g，用样品粉碎机粉碎成可通过 20 mm 圆孔筛的颗粒。充分混匀，均分成两份，装入清洁的容器内，作为试样。密封并标明标记。

6.3 试样保存

将试样于－5 ℃以下避光保存。

7 测定步骤

7.1 去脂肪

称取 2.5 g 试样(精确至 0.01 g)于 50 mL 离心管中，加 5 mL 水，涡旋混匀后浸泡 30 min，加 20 mL 正己烷均质 1 min，以 6 000 r/min 的转速离心 5 min，弃去正己烷层；加 20 mL 正己烷按上述步骤重新脱脂一次，弃去正己烷层。

7.2 提取

向离心管中加 20 mL 甲醇，均质 1 min，以 6 000 r/min 的转速离心 5 min，将甲醇层小心取出过滤到 100 mL 鸡心瓶中；残留物再用 20 mL 甲醇重复提取一次。合并提取液于上述鸡心瓶中，40 ℃旋转蒸发至约 8 mL，用氮气吹至约 2 mL，加 3 mL 0.01 mol/L 氢氧化钠溶液(4.6)混匀待净化。

7.3 净化

将上述混匀的溶液全部过 C_{18} 小柱(4.11)，用 4 mL 0.01 mol/L 氢氧化钠溶液(4.6)洗脱并定容至 10 mL，0.45 μm 滤膜(4.10)过滤，备用。

7.4 高效液相色谱条件

高效液相色谱条件如下：

a) 波长：330 nm；
b) 色谱柱：硅胶柱，3.5 μm，4.6 mm×150 mm，或相当者；
c) 柱温：40 ℃。

d) 流动相:0.02 mol/L 乙酸铵溶液(4.5);

e) 流动相流速:0.60 mL/min;

f) 进样量:20 μL。

7.5 空白试验

除不加样品外,均按上述步骤进行。

7.6 标准曲线绘制

根据样品中抑芽丹含量情况,选定峰面积相近的标准工作溶液。标准工作溶液和试样中抑芽丹响应值均应在仪器线性范围内。将系列标准工作液依次按上述色谱条件上机测定,记录色谱峰面积。以峰面积为纵坐标,浓度为横坐标,绘制标准曲线。

7.7 测定

根据保留时间定性,外标法定量。抑芽丹标准品的液相色谱图参见附录 A 中图 A.1。在上述色谱条件下,抑芽丹的保留时间约为 3.1 min。

8 结果计算与表述

按式(1)计算样品中抑芽丹残留量:

$$X=\frac{c\times V\times 1\,000}{m\times 1\,000} \quad \cdots\cdots(1)$$

式中:

X ——样品中抑芽丹的残留量,单位为毫克每千克(mg/kg);

c ——标准曲线上查出试样溶液中抑芽丹的浓度,单位为毫克每升(mg/L);

V ——最终定容体积,单位为毫升(mL);

m ——所称取试样质量,单位为克(g)。

9 测定低限和回收率

9.1 定量限

本方法抑芽丹的定量限为 0.2 mg/kg。

9.2 回收率

样品的添加浓度及回收率的实验数据见表 1。

表 1 添加浓度及回收率的实验数据

样品名称	添加浓度 mg/kg	回收率 %
核桃	0.2	82.5～95.5
	0.4	81.2～94.8
	2.0	90.2～100.2

表 1（续）

样品名称	添加浓度 mg/kg	回收率 %
蜂蜜核桃	0.2	83.5～96.5
	0.4	80.2～101.2
	2.0	91.0～104.9
板栗	0.2	80.5～94.0
	0.4	83.4～97.8
	2.0	90.2～100.6
糖炒板栗	0.2	81.5～99.5
	0.4	84.2～97.8
	2.0	90.2～102.6

附 录 A
（资料性附录）
抑芽丹标准品谱图

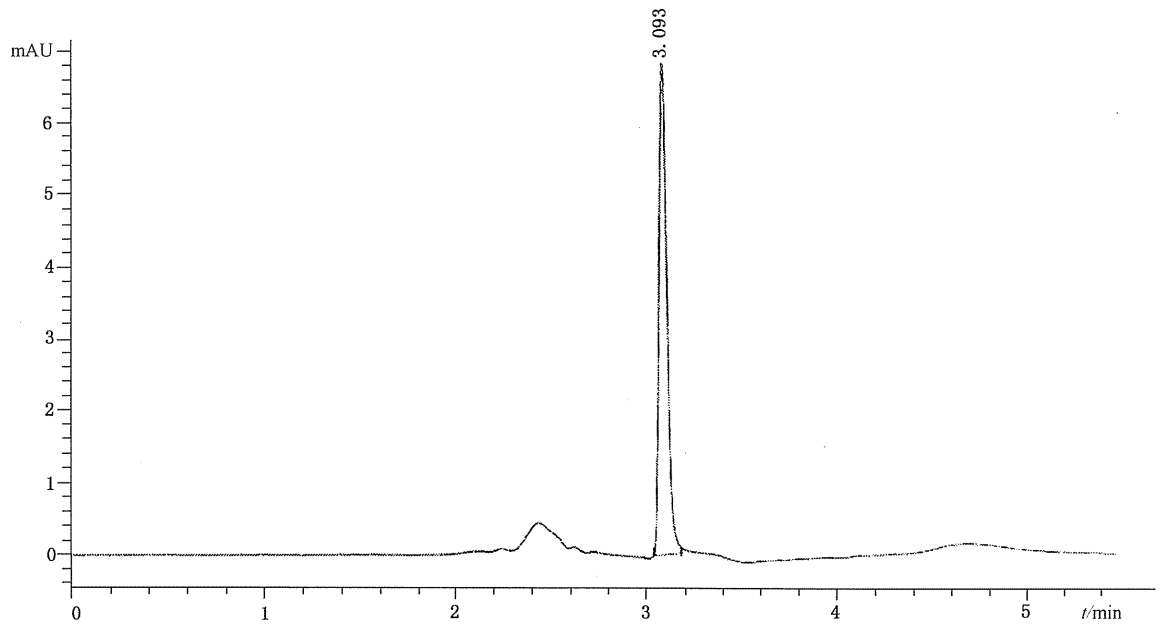

图 A.1 抑芽丹标准品谱图(1.5 mg/L)

Foreword

This standard is drafted according to GB/T 1.1—2009.

This standard substitutes for SN 0647—1997《Method for the Determination of Maleic Hydrazide Residues in Nuts and Nut Procducts for Export—Spectrophotometry》. This standard was modified based on SN 0647—1997:

—The name was in-steaded of"Determination of maleic hydrazide residues in nuts and nut procducts for export—HPLC method".

—Added the determination for chestnut and its products.

—Modified method for the sample pretreatment. The pretreatment method was changed into extraction and purification method.

—The determination method was changed spectrophotometric method into HPLC method.

This standard Was proposed by and is in charge of the National Regulation Commission for Certification and Accreditation.

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

Main drafters of this standard are Cong Weihong, Xu Chenggang, Yang Lijun, Wang Jing, Shi Wenchun, Hu Qiaoru, Liu Yumin, Cui Fengjie, Hou Jianquan, Cong Chao.

This standard replaced the previous version of the release of the standard as follows:

—SN 0647—1997.

Determination of maleic hydrazide residues in nuts and nut procducts for export—HPLC method

1 Scope

This standard specifies the methods of sample preparation and determination by HPLC of maleic hydrazide residues in nuts and nut products for export.

This standard is applicable to the determination of maleic hydrazide residues in walnut, chestnut and their products.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB/T 6682 Water for analytical laboratoy use—Specification and test methods.

3 Principle

The test sample is extracted by methanol after defatted by hexane, and then cleaned up with C_{18} solid phase extraction cartridge, the analyte is determinated and confirmed by High performance liquid chromatography with UV detection, using external standard mehtod.

4 Reagents and materials

Unless otherwise specified, all of the reagents used should be analytical grade, "water" is the first grade water described by GB/T 6682.

4.1 Methanol: HPLC grade.

4.2 Hexane: HPLC grade.

4.3 Ammonium acetate.

4.4 Sodium hydroxide.

4.5 Ammonium acetate solution(0.02 mol/L):1.54 g Ammonium acetate is dissolved in water and diluted to 1 000 mL,and filtered through 0.45 μm filters before using.

4.6 Sodium hydroxide solution(0.01 mol/L):0.4 g sodium hydroxide is dissolved in water and diluted to 1 000 mL.

4.7 Maleic hydrazide standard:CAS No. 123-33-1,$C_4H_4N_2O_2$,Purity>99.9%.

4.8 Maleic hydrazide standard stock solution: Accurarely weigh an acequate amont of maleic hydrazide standard,dilute with 0.01 mol/L sodium hydroxide sulution(4.6)to form a standard stock solution of 1 mg/mL.

4.9 Maleic hydrazide standard working solution:dilute 1.0 mL of the stock solution(4.8) with 0.01 mol/L sodium hydroxide solution(4.6) to 100 mL as the standard working solution,the concentration is 10 mg/L.

4.10 Membrane filter:0.45 μm(organic phase).

4.11 C_{18} columns:Sep-Pak Cartridges,C_{18} columns,3 mL,500 mg,or equivalent. Each column was conditioned with 4 mL methanol followed by 4 mL 0.01 mol/L sodium hydroxide(4.6).

5 Apparatus and equipment

5.1 High-performance liquid chromatography,equipped with UV or DAD.

5.2 Electronic balance:accurate to 0.000 1 g and 0.01 g.

5.3 Rotary vacuum evaporator.

5.4 Homogenizer:≥10 000 r/min.

5.5 Centrifuge:≥6 000 r/min.

5.6 Vortex mixer.

5.7 Sample pulverizer.

5.8 Sieve:2.0 mm round-hole sieve.

5.9 Quartering plate.

6 Preparation and storage of test sample

6.1 Requirement

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

6.2 Reparation

200 g sample is divided from the edible parts of the original sample by the quartering method. The Sample is pulverized into granules, and pass through a 20 mm round-hole sieve with a pulverizer. Mix thoroughly and divide equally into two portions, place in clean sample containers as the test samples, seal and label.

6.3 Storage of test sample

The test samples should be stored below −5 ℃ and away from light.

7 Procedure

7.1 Removal of fat

A sample of 2.5 g(accurate to 0.01 g) was weighed into a plastic centrifuge tube(50 mL), and mixed with 5 mL water. The mixture was swirled and allowed to soak for 30 min. 20 mL hexane was added and the mixture was homogenized for 1 min. Centrifuge for 5 min at 6 000 r/min, and the hexane layer was discarded. Then repeating the operation of homogenate, and the hexane layer was discarded.

7.2 Extraction

20 mL Methanol was added to the Plastic centrifuge tube, and the mixture was homogenised for 1 min, and centrifuged at 6 000 r/min for 5 min. The solvent was decanted, and filtered into a concentrate bottle(100 mL). The sample was re-homogenised with methanol(20 mL). The contents were filtered and collected into the concentrate bottle. Evaporate the mixture to about 8 mL below 40 ℃, and then to 2 mL on N-Evap. The extract was added 3 mL 0.01 mol/L sodium hydroxide sulution(4.6) and mixed.

7.3 Purification

All the extracts was purified using C_{18} columns and colleced(4.11), eluted with 4 mL 0.01 mol/L sodium hydroxide solution(4.6). The extract was quantitatively transferred to 10 mL, and filtered through 0.45 μm membrane(4.10) for HPLC analysis.

7.4 HPLC operating conditions:

HPLC operating conditions in as following:

a) Detector wavelenth:330 nm;

b) HPLC column:Silica gel column,3.5 μm,4.6 mm × 150 mm,or equivalent;

c) Column temperature:40 ℃;

d) Mobile phase:0.02 mol/L Ammonium acetate solution(4.5);

e) Flow rate:0.60 mL/min;

f) Injection volume:20 μL.

7.5 Blank test

Undergo according to the above procedures excluding the sample.

7.6 Stardard curve plotting

According to the Maleic Hydrazide concentration of the sample solution, determine the standard working solution. The responses of the analyte in the standard working solution and the sample solution should both be within the linear range of the instrument detection. The series stardard work solutions are detected as the above HPLC operating conditions, recording the peak area. The curve is drawed by the peak area as the longitudinal axis and the concentration as the horizontal axis.

7.7 Detection

The qualitative method is based on retention time and the quantifiation based on external standard method. The Maleic hydrazide chromatogram of the standard is as Figure A.1 of annex A. As the above HPLC operating conditions, the Maleic hydrazide retention time is about 3.1 minutes.

8 Calculation and expression of the result

Calculate the content of Maleic hydrazide residues in the test sample according to the followed formula(1).

$$X = \frac{c \times V \times 1\,000}{m \times 1\,000} \qquad \cdots\cdots(1)$$

Where

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

c —the concentration of Maleic hydrazide according to the standard curve, mg/L;

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

m —the mass of the test sample, g.

9 Detection limit and recovery

9.1 Limit of quantification

The determination Limit of this method is 0.2 mg/kg.

9.2 Recovery

The fortified content and recovery of this method is shown in table 1.

Table 1—The range of fortification and recovery of this method

Sample	Fortified content mg/kg	Recovery range %
walnut	0.2	82.5～95.5
	0.4	81.2～94.8
	2	90.2～100.2
honey walnut	0.2	83.5～96.5
	0.4	80.2～101.2
	2	91.0～104.9
chestnut	0.2	80.5～94.0
	0.4	83.4～97.8
	2	90.2～100.6
suger fry chestnut	0.2	81.5～99.5
	0.4	84.2～97.8
	2	90.2～102.6

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Annex A
(informative annex)
Maleic hydrazide chromatogram of the standard

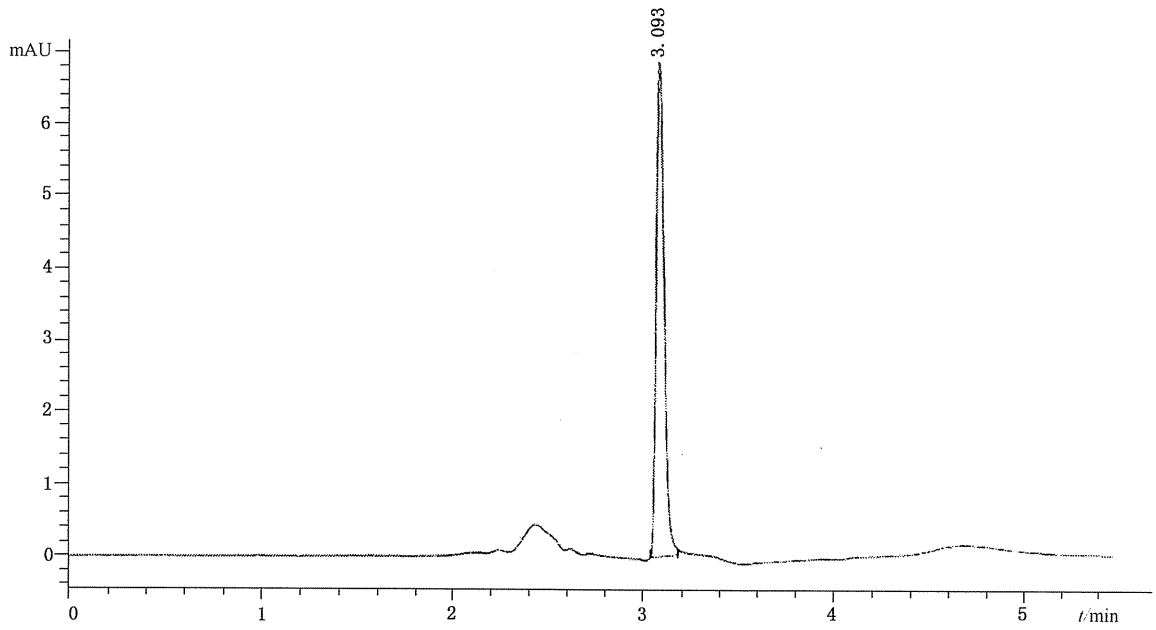

Figure A. 1—Maleic hydrazide chromatogram of the standard(1. 5 mg/L)