SN

中华人民共和国出入境检验检疫行业标准

SN/T 0218—2014
代替 SN 0218—1993

出口粮谷中天然除虫菊素残留总量的检测方法　气相色谱-质谱法

Determination of pyrethrins residues in cereals for export—GC-MS method

2014-01-13 发布　　2014-08-01 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

本标准按照 GB/T 1.1—2009 给出的规则起草。

本标准代替 SN 0218—1993《出口粮谷中天然除虫菊素残留量检验方法》。

本标准与 SN 0218—1993 相比，主要修改如下：

——适用范围由大米扩展到大麦、小麦、玉米、大米、糙米和高粱；

——略去了抽样步骤。

请注意本文件的某些内容可能涉及专利。本文件的发布机构不承担识别这些专利的责任。

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：中华人民共和国上海出入境检验检疫局、上海市标准化研究院、中华人民共和国佛山出入境检验检疫局。

本标准主要起草人：杨惠琴、杨瑞钰、陈丹萍、伊雄海、朱坚、郭德华。

本标准所代替标准的历次版本发布情况为：

——SN 0218—1993。

出口粮谷中天然除虫菊素残留总量的检测方法　气相色谱-质谱法

1　范围

本标准规定了粮谷中天然除虫菊素残留量的气相色谱-质谱检测方法。

本标准适用于大麦、小麦、玉米、大米、糙米和高粱中天然除虫菊素(除虫菊素Ⅰ、除虫菊素Ⅱ、瓜叶菊素Ⅰ、瓜叶菊素Ⅱ、茉莉菊素Ⅰ、茉莉菊素Ⅱ)残留总量的检测和确证。

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,仅注日期的版本适用于本文件。凡是不注日期的引用文件,其最新版本(包括所有的修改单)适用于本文件。

GB/T 6682　分析实验室用水规格和试验方法

3　方法提要

试样中残留的天然除虫菊素用乙醚提取,提取液经弗罗里硅土小柱净化,用气相色谱-质谱仪测定,外标法定性定量。

4　试剂材料

除非另有说明,所用试剂均为分析纯,水为GB/T 6682规定的一级水。

4.1　乙醚:色谱纯。

4.2　正己烷:色谱纯。

4.3　丙酮:色谱纯。

4.4　无水硫酸钠:于650 ℃下灼烧4 h,贮于密闭容器中备用。

4.5　乙醚-丙酮-正己烷(4+4+2,体积比)溶液:量取400 mL乙醚、400 mL丙酮和200 mL正己烷至1 000 mL试剂瓶中。

4.6　农药混合标准物质:除虫菊素Ⅰ(pyrethrin Ⅰ,CAS:121-21-1)、除虫菊素Ⅱ(pyrethrin Ⅱ,CAS:121-29-9)、瓜叶菊素Ⅰ(cinerin Ⅰ,CAS:25402-06-6)、瓜叶菊素Ⅱ(cinerin Ⅱ,CAS:121-20-0)、茉莉菊素Ⅰ(jasmolin Ⅰ,CAS:4466-14-2)、茉莉菊素Ⅱ(jasmolin Ⅱ,CAS:1172-63-0)。

4.7　混合标准储备液的配制:分别称取约0.01 g(精确至0.000 1 g)除虫菊素混合标准品于10 mL的容量瓶中,用丙酮配制成约1 000 μg/mL的标准储备溶液,低于5 ℃避光保存。

4.8　混合标准中间溶液的配制(10.0 μg/mL):各移取上述的标准储备液1.00 mL至100 mL容量瓶中,用丙酮稀释至刻度,低于5 ℃避光保存。

4.9　弗罗里硅土小柱:6 mL(0.5 g),或相当者。

5　仪器与设备

5.1　气相色谱-质谱联用仪:配有(EI)离子源。

5.2 旋转蒸发器。
5.3 涡旋混匀器。
5.4 固相萃取装置。
5.5 氮吹仪。
5.6 离心管:15 mL。
5.7 玻璃试管:10 mL,具刻度。
5.8 均质器。
5.9 恒温水浴锅。

6 试样的制备与保存

6.1 试样制备

将样品用四分法浓缩分至 1 kg,全部磨碎并通过 20 目筛,混匀,均分成两份试样,装入洁净的容器内,密封,标明标记。

6.2 试样保存

试样于常温状态下保存。

7 分析步骤

7.1 提取

称取 10 g 样品(精确至 0.01 g)于 50 mL 离心管中,在离心管中加入 15.0 mL 乙醚,用均质器于 20 000 r/min下均质 2 min。将离心管在 3 000 r/min 下离心 10 min。取出上清液于另一 50 mL 梨型瓶中,残留物再用 15.0 mL 乙醚提取一次。合并提取液 40 ℃下旋转浓缩至近 1.0 mL,待净化。

7.2 净化

弗罗里硅土柱净化。在弗罗里硅土柱子中加入约 0.5 cm 的无水硫酸钠。先用 10 mL 正己烷预淋洗活化,弃去流出液,将上述浓缩液过弗罗里硅土柱子,用 10 mL 乙醚-丙酮-正己烷(4+4+2,体积比)溶液以 2 滴/s 的速度洗脱,洗脱液收集于 10 mL 刻度试管中,于 40 ℃下氮吹干,加入 1 mL 的丙酮定容,供气相色谱-质谱检测。

7.3 测定

7.3.1 色谱测定条件

色谱测定条件如下:

a) 色谱柱:DB-5MS 石英毛细管柱,长 30 m,内径 0.25 mm,膜厚 0.25 μm,或相当者;
b) 柱温:初始温度 70 ℃,保持 1 min,以 20 ℃/min 升温至 270 ℃,保持 1 min,再以 1 ℃/min 升温至 285 ℃,保持 1 min,最后以 20 ℃/min 升温至 300 ℃,保持 15 min;
c) 进样口温度:260 ℃;
d) 载气:氦气,纯度大于等于 99.999%;流量 1.2 mL/min;
e) 进样量:1.0 μL。

7.3.2 质谱条件

质谱条件如下：

a) 离子化模式：EI(260 ℃)；

b) 电离能量：70 eV；

c) 气相色谱/质谱接口温度：280 ℃；

d) 选择离子扫描：监测离子，见表 1。

表 1 除虫菊素的监测离子、其丰度比和保留时间

名 称	监测离子 m/z	监测离子丰度比 %	保留时间 min
瓜叶菊素 I	123*，150，168，316	100∶70∶40∶5	18.797
茉莉菊素 I	123*，69，135，330	100∶75∶50∶30	19.773
除虫菊素 I	123*，81，105，162	100∶40∶30∶6	19.857
瓜叶菊素 II	107*，93，121，167	100∶30∶25∶9	23.370
茉莉菊素 II	107*，93，121，167	100∶90∶60∶30	24.773
除虫菊素 II	123*，91，107，167	100∶70∶50∶10	24.887
注：“*”为定量离子。			

7.3.3 气相色谱-质谱测定

根据样液中被测组分含量，选定浓度相近的标准工作溶液。其响应值均应在仪器检测的线性范围内。对标准工作溶液与样液等体积参插进样测定，以色谱峰面积按外标法定量。在上述色谱条件下，除虫菊素的参考保留时间参见表 1。标准品的选择离子色谱图参见附录 A 中图 A.1。

经确证分析被测物质量色谱峰保留时间与标准品样品相一致，并且在扣除背景的样品图谱中，所选择的离子均出现；同时所选择离子的丰度比于标准品相关离子的相对丰度一致，相似度在允许差之内(参见表 2)，则可判定样品中除虫菊素为阳性。

表 2 定性确证时相对离子丰度的最大允许偏差

相对离子丰度	>50%	>20%～50%	>10%～20%	≤10%
允许的相对偏差	±10%	±15%	±20%	±50%

7.4 空白试验

除不加试样外，均按上述操作步骤进行。

8 结果计算和表述

用色谱数据处理机或按式(1)计算样品中除虫菊素(除虫菊素 I、除虫菊素 II、瓜叶菊素 I、瓜叶菊素 II、茉莉菊素 I、茉莉菊素 II)残留总量。计算结果需扣除空白值。

$$X=\frac{\sum A_i \times c \times V}{\sum A_{si} \times m} \qquad \cdots\cdots(1)$$

式中：

X ——样品中待测组分残留量，单位为毫克每千克(mg/kg)；

$\sum A_i$ ——样液中农药残留的峰面积之和；

c ——标准工作溶液中农药残留的浓度，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)；

$\sum A_{si}$ ——标准工作溶液中农药残留的峰面积之和；

m ——最终样液所代表的试样量，单位为克(g)。

9 测定低限和回收率

9.1 测定低限

本方法测定低限为 0.05mg/kg(除虫菊素残留总量)。

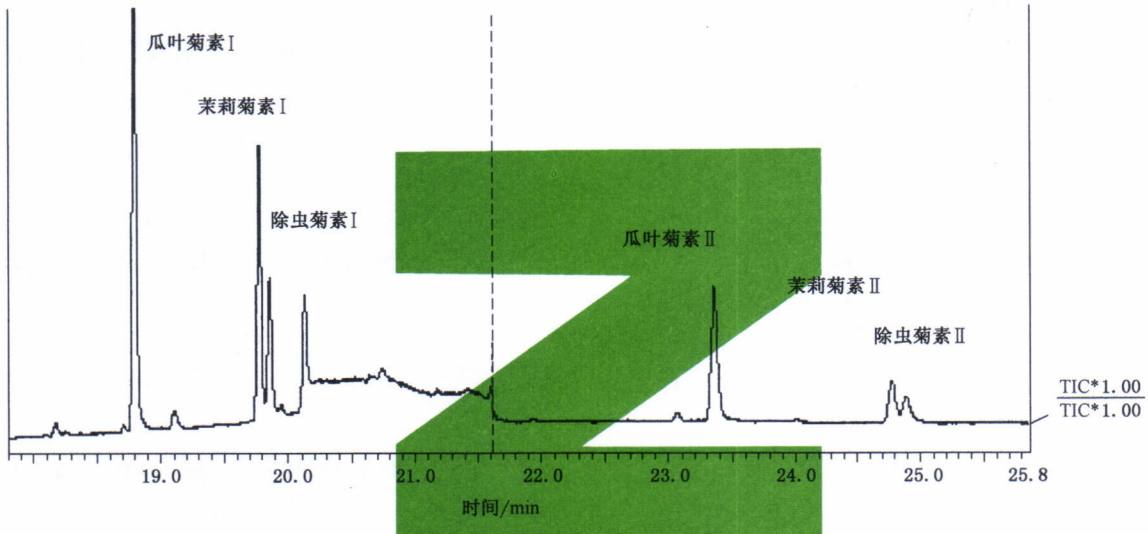
9.2 回收率

回收率详见表 3。

表 3 除虫菊素添加浓度及回收率试验数据

样品名称	添加浓度/(mg/kg)	回收率范围/%
大麦	0.05	78.9～119.0
	0.10	75.5～95.6
	1.0	85.7～104.3
小麦	0.05	84.9～111.2
	0.10	79.3～99.4
	1.0	88.5～115.6
玉米	0.05	83.1～117.9
	0.10	79.2～109.3
	1.0	78.3～118.4
大米	0.05	84.9～111.2
	0.10	81.3～116.4
	1.0	81.5～115.6
糙米	0.05	79.3～119.1
	0.10	76.2～105.5
	1.0	81.3～119.4
高粱	0.05	80.8～115.2
	0.10	75.4～109.7
	1.0	88.6～113.9

附 录 A
（资料性附录）
除虫菊素标准品的气相色谱-质谱选择离子色谱图



注：时间为 18.797 min、19.773min、19.857 min、23.370 min、24.773 min、24.887 min。

图 A.1 除虫菊素标准品的气相色谱-质谱选择离子色谱图

Foreword

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

This standard is replace of SN 0218—1993 Method for the determination of pyrethrins residues in cereals for export.

The main improvements from SN 0218—1993 are as follows:

—This standard is applicable to the determination of residue content of pyrethrins in rice, corn, brown rice, barley, wheat and chinese sorghum for expor;

—The cleanup processure is improved.

Some parts of the standard may have relationship with some patents. The release department have no responsibility to recognize these patents.

This standard was proposed by and is under the charge of the Certification and Accreditation Administration of the People's Republic of China.

This standard was drafted by the Shanghai Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Shanghai Institute of Quality and Standardization, Foshan Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Yang Huiqin, Yang Ruiyu, Chen Danping, Yi Xionghai, Zhu Jian, Guo Dehua.

This standard replaced the following standard:

—SN 0218—1993.

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

Determination of pyrethrins residues in cereals for export—GC-MS method

1 Scope

This standard specifies the method of testing pyrethrins residue in food by GC/MS method.

This standard is applicable to the determination and confirmation of pyrethrins (p yrethrin Ⅰ, pyrethrin Ⅱ, cinerin Ⅰ, cinerin Ⅱ, jasmolin Ⅰ, jasmolin Ⅱ) residues in rice, corn, brown rice, barley, wheat and chinese sorghum.

2 Normative reference

The following documents are necessary for this standard. For dated reference, only dated editions shall apply to this standard. For undated references, the latest edition of the normative document (including subsequent amendments) is referred to applies.

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

3 Principle

The pyrethrins residue from the above food sample of cereals is extracted with aether, then cleaned up with Florisil column. The residual content is determined by GC-MS, and quantified by external standard method.

4 Reagents and materials

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

4.1 Aether: HPLC grade.

4.2 N-hexane: HPLC grade.

4.3 Acetone: HPLC grade.

4.4 Anhydrous sodium sulfate: Ignite at 650 ℃ for 4 h, and keep in a tightly closed container.

4.5 Aether-acetone-n-hexane (4 + 4 + 2, *V/V/V*): combine 400 mL aether, 400 mL acetone and 200 mL n-hexane.

4.6 Standard substance: pyrethrin Ⅰ (CAS: 121-21-1), pyrethrin Ⅱ (CAS: 121-29-9), cinerin Ⅰ (CAS: 25402-06-6), cinerin Ⅱ (CAS: 121-20-0), jasmolin Ⅰ (CAS: 4466-14-2), jasmolin Ⅱ (CAS: 1172-63-0).

4.7 Stock standard solution (1 000.0 μg/mL): accurately weigh 0.01 g ± 0.000 1 g of pyrethrins standard into a 10 mL volumetriv. Mix well. This solution is a stock solution of 1 000 μg/mL pyrethrins in acetone, these solutions stored at <5 ℃, avoid light preservation.

4.8 Intermediate standard solution (10.0 μg/mL): dilute the stock standard solution 1.00 mL into 100 mL volumetric flask separately with acetone to 10.0 μg/mL, these solutions stored at <5 ℃, avoid light preservation.

4.9 Florisil column (6 mL, 0.5 g), equivalent.

5 Apparatus and equipment

5.1 GC/MS with EI.

5.2 Rotray vacuum evaporator.

5.3 Vortex mixer.

5.4 Solid phase extraction set.

5.5 Nitrogen evaporator.

5.6 Plastic centrifuge tube: 15 mL.

5.7 Glass tube: 10 mL with graduation.

5.8 Homogenizer.

5.9 Water bath container.

6 Preparation and storage of test sample

6.1 Preparation of test sample

Take approximately 1 kg of representative sample. Smash thoroughly by a crusher. Mix thoroughly. Put in clean containers. Seal and label them.

6.2 Storage of test sample

The test samples should be stored at room temperature.

7 Procedure

7.1 Extraction

Weigh 10 g±0.01 g of the sample into a 50 mL polypropylene tube, add 15 mL of diethyl ether, blend for 2 min with vortex mixer in 20 000 r/min, then centrifuge for 10 min under 3 000 r/min. Transfer the supernatant to a 50 mL rotary evaporator tube, repeat the extraction procedure again and combine the supernatant. Evaporate the extraction solution to approximately 1 mL with gentle nitrogen evaporator at 40 ℃, and wait for cleanup.

7.2 Florisil column clean up

Add about 0.5 cm anhydrous sodium sulfate into the Florisil column; rinse the column with 10 mL n-hexane before use. Transfer the sample solution to the column, and then elute with 10 mL of diethyl ether-acetone-n-hexane (4+4+2, *V*/*V*/*V*), control the flow rate near 2 drops/s. Collect all elutes in a 10 mL glass tube and evaporate to dryness with gentle nitrogen evaporator at 40 ℃. Dissolve the residue and dilute exactly to 1.0 mL with acetone for GC-MS determination and confirmation.

7.3 Determination

7.3.1 GC condition

GC condition is as following:

a) Column(analysis): DB-5MS 30 m×0.25 mm(i.d)×0.25 μm(film thickness), equivalent;

b) Column temperature: 70 ℃(1 min)—20 ℃/min—270 ℃ (1 min)—1 ℃/min—285 ℃ (1 min)—20 ℃/min—300 ℃ (15 min);

c) Injection port temperature: 260 ℃;

d) Carrier gas: Helium, purity≥99.999%, 1.2 mL/min;

e) Injection volume: 1.0 μL.

7.3.2 MS condition

MS condition is as following:

a) Eledtron ionization mode: EI (260 ℃);

b) Ionization energy: 70 eV;

c) Interface temperature:280 ℃;

d) Selected monitoring ion (m/z): table 1.

Table 1—Monitor ion, relative intensity and retention time for pyrethrins

Name	Monitor ion m/z	Relative intensity %	Retention Time min
cinerin Ⅰ	123* ,150,168,316	100 : 70 : 40 : 5	18.797
jasmolin Ⅰ	123* ,69,135,330	100 : 75 : 50 : 30	19.773
pyrethrin Ⅰ	123* ,81,105,162	100 : 40 : 30 : 6	19.857
cinerin Ⅱ	107* ,93,121,167	100 : 30 : 25 : 9	23.370
jasmolin Ⅱ	107* ,93,121,167	100 : 90 : 60 : 30	24.773
pyrethrin Ⅱ	123* ,91,107,167	100 : 70 : 50 : 10	24.887
Note: "*"stands for quantitation ion.			

7.3.3 Determination and confirmation by GC-MS

According to operating parameters of GC-MS above, sample solution and the standard working solution are determined simultaneously. The responses of the analyte in the standard working solution and the sample solution all should be within the linear range of the instrument detection and quantified by internal standard. The reference retention time of pyrethrins as table 1, GC-MS selected ion chromatogram and mass spectrum of the pyrethrins standards are shown respectively as figure A. 1 in annex A.

If the retention times of sample chromatogram pesks are consistent with the standard, and after subtracted background noise, the relative intensity ratios of each qualitative ions are also consistent with similar concentration standard, and the similarity degree of their relative abundance ratio in permitted tolerance (see table2), we can confirm that are corresponding analyte in the sample.

Table 2—Maximum permitted tolerances for relative ion intensities while confirmation

Relative intensity	>50%	>20%～50%	>10%～20%	≤10%
Permitted tolerances	±10%	±15%	±20%	±50%

7.4 Blank test

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

8 Calculation and expression of result

Calculate the content of pyrethrins (pyrethrin Ⅰ, pyrethrin Ⅱ, cinerin Ⅰ, cinerin Ⅱ, jasmolin Ⅰ, jasmolin Ⅱ) residue in the test sample by GC/MS data processor or according to the formula (1). The blank value should be subtracted from result of calculation above.

$$X = \frac{\sum A_i \times c \times V}{\sum A_{si} \times m} \qquad \cdots\cdots(1)$$

Where:

X —the residue conten of pesticides in the test sample, mg/kg;
$\sum A_i$ —the all peak area of pesticides in the sample solution;
c —the concentration of pesticides in the standard working solution, μg/mL;
V —the final volume of the sample solution, mL;
$\sum A_{si}$ —the all peak area of pesticides in the standard working solution;
m —the corresponding mass of test sample in the final sample solution, g.

9 Limit of quantitation(LOQ) and recovery

9.1 Limit of quantitation

The limit of quantitation (LOQ) of the method is 0.05 mg/kg.

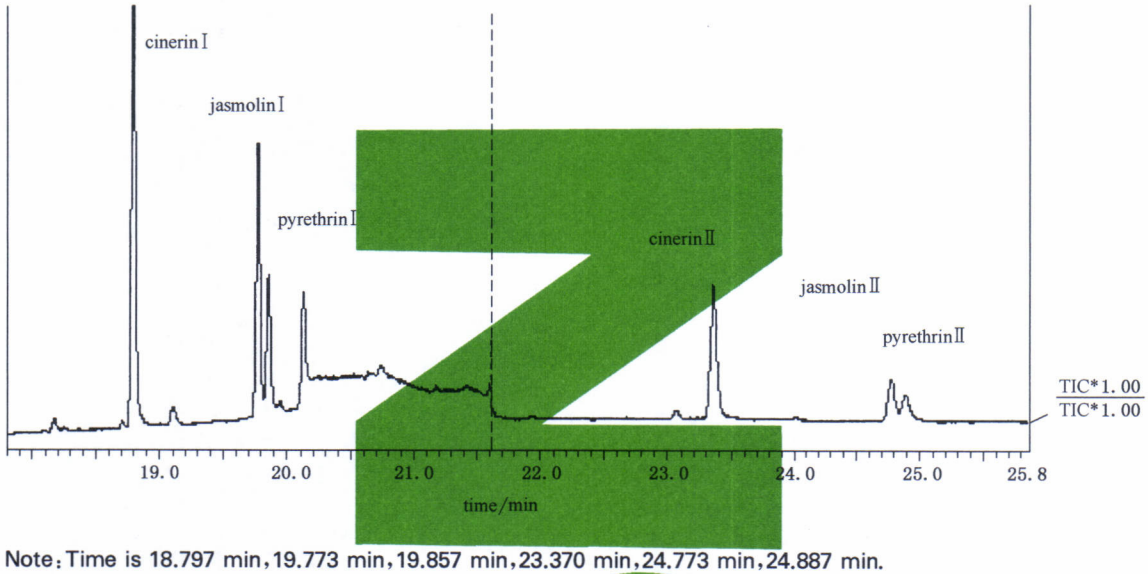
9.2 Recovery

Listed in table 3.

Table 3—Recovery range of pyrethrins

Sample	Spike/(mg/kg)	Recovery/%
barley	0.05	78.9～119.0
	0.10	75.5～95.6
	1.0	85.7～104.3
wheat	0.05	84.9～111.2
	0.10	79.3～99.4
	1.0	88.5～115.6
corn	0.05	83.1～117.9
	0.10	79.2～109.3
	1.0	78.3～118.4
rice	0.05	84.9～111.2
	0.10	81.3～116.4
	1.0	81.5～115.6
brown rice	0.05	79.3～119.1
	0.10	76.2～105.5
	1.0	81.3～119.4
chinese sorghum	0.05	80.8～115.2
	0.10	75.4～109.7
	1.0	88.6～113.9

Annex A
(Informative annex)
GC-MS selected ion chromatogram (TIC) of pyrethrins standards



Note: Time is 18.797 min, 19.773 min, 19.857 min, 23.370 min, 24.773 min, 24.887 min.

Figure A.1—GC/MS chromatogram (TIC) of the pyrethrins pesticides standards