SN

中华人民共和国出入境检验检疫行业标准

SN/T 0520—2012
代替 SN 0520—1996

出口粮谷中烯菌灵残留量测定方法 液相色谱-质谱/质谱法

Determination of imazalil in cereals for export—HPLC-MS/MS 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 0520—1996《出口粮谷中烯菌灵残留量检验方法》。

与 SN 0520—1996 相比，除编辑性修改外主要技术变化如下：

——更改了标准名称；

——液相色谱-紫外检测法更改为液相色谱-质谱/质谱法，降低了方法的检出限；

——修改了前处理方法。

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，中华人民共和国厦门出入境检验检疫局。

本标准主要起草人：周瑶、伊雄海、叶鹏、樊祥、盛永刚、唐毅锋、朱坚。

本标准所代替标准的历次版本发布情况为：

——SN 0520—1996。

出口粮谷中烯菌灵残留量测定方法 液相色谱-质谱/质谱法

1 范围

本标准规定了出口粮谷中烯菌灵残留量的测定方法。

本标准适用于小麦、大麦、大米、高粱、玉米、糙米中烯菌灵残留量的液相色谱-质谱/质谱仪检测。

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

试样在无水硫酸钠和氯化钠盐析作用下，经乙腈提取，再经水稀释后用液相色谱-质谱/质谱仪测定，外标法定量。

4 试剂和材料

除非另有说明，所用试剂均为分析纯，水为符合 GB/T 6682 规定的一级水。

4.1 乙腈：高效液相色谱纯。

4.2 无水硫酸钠：分析纯，650 ℃灼烧 4 h，冷却后贮于密闭容器中备用。

4.3 氯化钠。

4.4 甲酸：纯度≥99%。

4.5 乙腈水溶液：水：乙腈(7+3，体积比)。

4.6 0.1%甲酸溶液：1 mL 甲酸溶解于水中，并定容至 1 L。

4.7 农药标准物质：烯菌灵(Imazalil，CAS 号：35554-44-0)：纯度≥98.0%。

4.8 标准储备液的配制：准确称取适量的烯菌灵标准品，用乙腈配制成质量浓度为 1.0 mg/mL 的标准储备溶液。该溶液在－18 ℃冰箱中保存有效期为 12 个月。

4.9 标准中间溶液的配制：用乙腈将稀释标准储备液至终质量浓度约为 1.0 μg/mL，低于 4 ℃避光冷藏保存，有效期为 6 个月。

4.10 标准工作溶液的配制：根据需要，临用时吸取一定量的标准中间溶液，用乙腈水溶液(4.5)配制成适当浓度的混合标准工作溶液。低于 4 ℃避光冷藏保存，现用现配。

4.11 微孔滤膜：0.22 μm，有机系。

5 仪器与设备

5.1 液相色谱-质谱/质谱仪：配有电喷雾离子源(ESI)。

5.2 电子天平：感量分别为 0.01 g，0.000 1 g。
5.3 均质器。
5.4 离心机：400 r/min 以上。
5.5 离心管：50 mL。
5.6 涡旋混匀器。
5.7 容量瓶：20 mL。

6 试样制备与保存

6.1 试样制备

称取小麦、大麦、大米、高粱、玉米、糙米等代表性样品约 500 g，用粉碎机粉碎，过 20 目筛，混匀，装入洁净容器，密封，标明标记。

6.2 试样保存

将试样于－5 ℃以下避光保存。在抽样及制样的操作过程中，应防止样品受到污染或发生残留物含量的变化。

7 分析步骤

7.1 提取

称取试样 2.5 g(精确到 0.01 g)于 50 mL 离心管中，加入 1 g 氯化钠，4 g 无水硫酸钠混匀。加入 10 mL 乙腈，于涡旋混匀器上混合 2 min，在 4 000 r/min 下离心 5 min。将上清液转移至 20 mL 容量瓶。再用 5 mL 乙腈重复提取一次。合并提取液于同一容量瓶中。用水准确定容至 20 mL，混合均匀，过 0.22 μm 滤膜，滤液供液相色谱-质谱/质谱仪测定。

7.2 测定

7.2.1 液相色谱-质谱/质谱条件

液相色谱-质谱/质谱条件如下：

a) 色谱柱：C_{18}柱，长 50 mm，内径 2.0 mm，粒径 3 μm 或相当者；
b) 流动相：A：0.1%甲酸溶液(%)，B：乙腈溶液：流速：0.5 mL/min，梯度洗脱程序见表 1；
c) 柱温：35 ℃；
d) 进样量：10 μL；
e) 离子源：电喷雾(ESI)；
f) 扫描方式：正离子；
g) 监测方式：多反应监测(MRM)；
h) 质谱条件参见附录 A。

表 1 流动相梯度洗脱程序

时间/min	流动相 A/%	流动相 B/%
0	70	30
1.0	5	95
7.0	5	95
7.1	70	30
11	70	30

7.2.2 色谱测定

7.2.2.1 定性测定

按照液相色谱-质谱/质谱条件测定样品和标准工作溶液，样品的质量色谱峰保留时间与标准品中对应的保留时间一致；且样品中各组分定性离子的相对丰度与接近浓度的标准工作溶液中相应的定性离子的相对丰度进行比较，偏差不超过表2规定的范围，则可判定样品中存在对应的被测物。

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	±20%	±25%	±30%	±50%

7.2.2.2 定量测定

在仪器最佳工作条件下，对标准工作溶液进样。用标准工作曲线按外标法定量，样品溶液中被测物的响应值均应在仪器测定的线性范围内。根据试样中被测样液的含量情况，选取响应值相近的标准工作液进行色谱分析。标准工作液和样液中待测物的响应值均应在仪器线性响应范围内。在上述色谱条件下烯菌灵的参考保留时间约为3.2 min，烯菌灵标准品多反应监测(MRM)色谱图参见附录B中图B.1。

7.2.3 空白试验

除不加试样外，均按上述操作步骤进行。

8 结果计算和表达

结果用色谱数据处理机或按式(1)计算试样中烯菌灵的残留量，计算结果应扣除空白值：

$$X_i = \frac{A_i \times c_i \times V}{A_{si} \times m} \quad \cdots\cdots(1)$$

式中：

X_i ——试样中烯菌灵残留含量，单位为毫克每千克(mg/kg)；

A_i ——样液中烯菌灵的峰面积；

c_i ——标准工作溶液中烯菌灵的质量浓度，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_{si}——标准工作溶液中烯菌灵的峰面积；

m ——最终样液代表的试样质量，单位为克(g)。

9 测定低限和回收率

9.1 测定低限

本方法对出口小麦、大麦、大米、高粱、玉米、糙米中烯菌灵残留量的测定低限均为5.00 μg/kg。

9.2 回收率

不同添加浓度范围内回收率的实验数据，见表3。

表 3　本方法添加浓度及回收率范围

基质	添加浓度 mg/kg	回收率范围 %
小麦	0.005	80.1～98.6
	0.010	84.8～107
	0.100	80.6～90.2
大麦	0.005	74.2～83.2
	0.010	72.7～78.9
	0.100	79.4～73.2
大米	0.005	71.2～77.3
	0.010	78.3～83.2
	0.100	78.8～83.5
高粱	0.005	74.9～82.4
	0.010	73.6～84.9
	0.100	78.3～89.2
玉米	0.005	75.0～81.9
	0.010	72.1～79.1
	0.100	74.9～79.5
糙米	0.005	79.9～89.4
	0.010	79.5～84.2
	0.100	74.7～89.2

附 录 A
（资料性附录）
参考质谱条件[1)]

参考质谱条件如下：

a） 电离源方式：电喷雾电离；

b） 扫描方式：正模式；

c） 监测方式：多反应监测；

d） 雾化气：氮气；

e） 气流速：10 L/min；

f） 干燥气温度：350 ℃；

g） 雾化气（Nebulizer）压力：45 psi[2)]；

h） 电子倍增器电压（ΔEMV）：400 V；

i） 分辨率：单位分辨率；

j） 毛细管电压：4 000 V；

k） 定性离子对（m/z）、定量离子对（m/z）、碎裂电压、碰撞能量和保留时间见表 A.1。

表 A.1 多反应监测参数表

组分名称	定性离子对 m/z	定量离子对 m/z	碎裂电压 V	碰撞能量 eV	保留时间 t_r min
烯菌灵	297.1→158.9	297.1→158.9	80	22	3.2
	297.1→200.9			15	

1） 非商业性声明：附录 A 所列参考质谱条件是在 Agilent 6410B LC/MS/MS 型液质联用仪上完成的，此处列出试验用仪器型号仅为提供参考，并不涉及商业目的，鼓励标准使用者尝试不同厂家或型号的仪器。

2） 非法定计量单位，1 psi≈6.895 kPa。

附 录 B
（资料性附录）
烯菌灵标准品多反应监测（MRM）色谱图

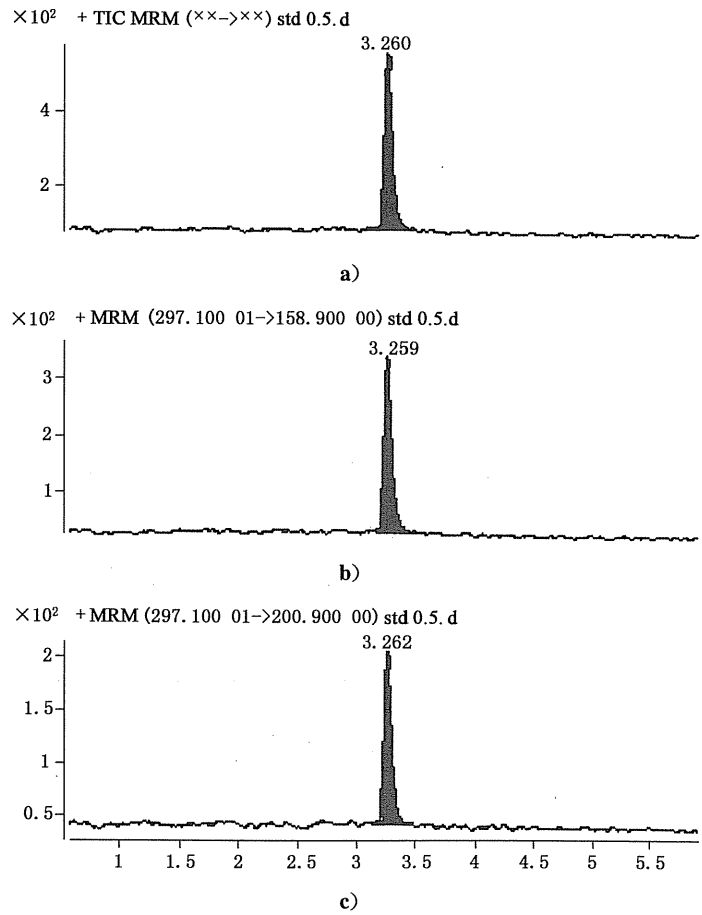

图 B.1 烯菌灵标准品多反应检测（MRM）色谱图（0.5 μg/L）

Foreword

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

This standard is replace of SN 0520—1996 Method for determination of imazalil residues in cereals for export.

The main improvements from SN 0520—1996 are as follows:

—The name of the standard is changed;

—The method is changed to HPLC-MS/MS from HPLC-UV;

—The sample pretreatment 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 the Certification and Accreditation Administration of the People's Republic of China.

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

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

The main drafters of this standard are Zhou Yao, Yi Xionghai, Ye Peng, Fan Xiang, Shen Yonggang, Tang Yifeng and Zhu Jian.

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

—SN 0520—1996.

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

Determination of imazalil residues in cereals for export—LC-MS/MS method

1 Scope

This standard specifies the method for determination of imazalil residue in cereals for export.

This standard is applicable to the determination and confirmation of residue content of imazalil in wheat, barley, rice, broomcorn, corn and brown rice for export.

2 Normative reference

The following documents is 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 referred to applies.

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

3 Principle

The imazalil residue in sample is extracted with acetonitrile in the presence of anhydrous sodium sulfate and sodium cloride, and determined by liquid chromatography-mass/mass spectrometry, quantified by external standard method.

4 Reagents and materials

All the reagents used should be analytically pure unless otherwise specified. "Water" is first class water.

4.1 Acetonitrile: HPLC grade.

4.2 Anhydrous sodium sulfate: dehydrated at 650 ℃ for 4 h, and stored in a tightly closed container.

4.3 Sodium chloride

4.4 Formic acid: Purity≥99%.

4.5 Water:acetonitrile (3+7, *V*/*V*)

4.6 0.1% formic acid solution

4.7 Standards:Imazalil (CAS No:35554-44-0):Purity≥98.0%.

4.8 Standard stock solution:accurately weigh certain amount of Imazalil standard and dissolve with acetonitrile to make the standard stock solution of 1.0 mg/mL. The solution is stored in a refrigerator at −18 ℃.

4.9 Stock standard solution of intermediate standards:dilute stock standard solution to final concentration of 1.0 μg/mL in acetonitrile,store refrigerated at <4 ℃,assign a shelf life of 6 months.

4.10 Working standard solutions:transfer adeuqate intermediate solution of standards to sample blank matrix blank for preparation of calibaration curve,store refrigerated at <4 ℃.

4.11 Membrane filter:0.22 μm,organic phase.

5 Apparatus and equipments

5.1 Liquid chromatography-mass/mass spectrometry, equipped with electrospray ion source and triquadruple mass spectrometer.

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

5.3 Homogenizer.

5.4 Centrifuge:4 000 r/min.

5.5 Plastic centrifuge tube:50 mL.

5.6 Vortex mixer.

5.7 Volumetric flask:20 mL.

6 Preparation and storage of test sample

6.1 Preparation of test sample

Wheat,barley,rice,broomcorn,corn and brown rice and etc.:Take approximately 500 g of representative sample. Smash thoroughly by a crusher,though 20 mesh sieve. Mix thoroughly. Put in clean containers. Seal

and label them.

6.2 Storage of test sample

The test samples should be stored under −5 ℃ and kept away from light While sampling and preparing sample, please avoid contamination or any factors that may change residue content.

7 Procedure

7.1 Extraction

Weigh 2.5 g(accurate to 0.01 g) sample in a 50 mL centrifuge tube, add 1 g sodium chloride, 4 g anhydrous sodium sulfate and mix them well. Add 10 mL acetonitrile, homogenize them by vortex mixer for 2 min. The mixture was centrifuged at 4 000 r/min for 5 min. Transfer the supernatant to a 20 mL volumetric flask. Repeat the extraction step by 5 mL acetonitrile. Combine the extract to the same volumetric flask. Dilute the extract to 20 mL with water, and filter the mixture by 0.22 μm film for HPLC-MS/MS analysis.

7.2 Determination

7.2.1 LC-MS/MS operating conditions

LC-MS/MS operating conditions are as follows:

a) LC column: C_{18} column, 50 mm × 2.0 mm(i.d), 3.0 μm(or other equivalent ones);

b) Mobile phase: A: 0.1% formic acid, B: acetonitrile; the elution gradient at the flow rate of 0.5 mL/min is listed inTable 1;

c) Column temperature: 35 ℃;

d) Injection volume: 10 μL;

e) Ion source: ESI;

f) Scanning model: positive ion;

g) Monitoring model: multiple reaction monitoring(MRM);

h) Referenced conditions seen Annex A.

Table 1—The elution gradient

Time/min	A/%	B/%
0	70	30
1.0	5	95
7.0	5	95
7.1	70	30
11	70	30

7.2.2 LC-MS/MS Determination

7.2.2.1 Confirmation of the method

Under the LC-MS/MS operating conditions, the standard working solution and sample solution is injected. If the retention times of sample chromatogram pecks are consistent with that of standard solution, calibration curve method is used for quantitative measurement. The relative intensities of sample transitions shall correspond to those of standard solution transitions for confirmation. The concentration of standard solution should be same with those of sample solution. The permitted tolerances listed in table 2, the corresponding analyte must be present in sample.

Table 2—Maximum permitted tolerances relative ion intensities while confirmation

relative intensity	>50%	>20%～50%	>10%～20%	≤10%
permitted tolerances	±20%	±25%	±30%	±50%

7.2.2.2 Quantitatin of the method

According to the approximate concentration of analyte in sample solution, select the standard working solution with similar responses to that of sample solution. The responses of analyte in the standard working solution and the sample solution should be within the linear range of the instrument detection. Under the above LC-MS/MS operating conditions, the retention time of imazalil is about 3.2 min. Reconstituted ion chromatogram of standard working solution is listed in Annex B FigureB.1.

7.2.3 Blank test

Undergo according to the above procedures excluding the sample.

8 Calculation and expression of the result

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

The result of calculation should be deducted with blank value:

$$X_i = \frac{A_i \times c_i \times V}{A_{si} \times m} \quad \text{.....(1)}$$

Where:

X_i —the residue content of imazalil in the test sample, mg/kg;

A_i —the peak area of imazalil in the sample solution;

c_i —the total concentration of imazalil in the standard working solution, μg/mL;

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

A_{si}—the total peak area of imazalil in the standard working solution;

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

9 Detection limit and recoveries

9.1 Determination limits

The limits of quantitation for brown rice, corn, barley, wheat, buckwheat, rice, broomcorn and oat, are 5.00 μg/kg.

9.2 Range of fortification and recoveries

The range of fortification and recovery of this method are shown in table 3.

Table 3—The range of fortification and recovery of this method

Sample	Fortified content/(mg/kg)	Recovery range/%
Wheat	0.005	80.1～98.6
	0.010	84.8～107
	0.100	80.6～90.2
Barley	0.005	74.2～83.2
	0.010	72.7～78.9
	0.100	79.4～73.2

Table 3 (continued)

Sample	Fortified content/(mg/kg)	Recovery range/%
Rice	0.005	71.2～77.3
	0.010	78.3～83.2
	0.100	78.8～83.5
Broomcorn	0.005	74.9～82.4
	0.010	73.6～84.9
	0.100	78.3～89.2
Corn	0.005	75.0～81.9
	0.010	72.1～79.1
	0.100	74.9～79.5
Brown rice	0.005	79.9～89.4
	0.010	79.5～84.2
	0.100	74.7～89.2

Annex A
(informative)
Referenced conditions by LC-MS/MS[1)]

LC-MS/MS conditions are as follows:

a) Ion source:ESI;

b) Scanning model:positive ion;

c) Monitoring model:multiple reaction monitoring (MRM);

d) Nebulizer gas:N_2;

e) Drying gas flow:10 L/min;

f) Drying gas temperature:350 ℃;

g) Nebulizer pressure:45 psi[2)];

h) ΔEMV:400 V;

i) Resolving power:Q1(unit)Q3(unit);

j) Ion spray voltage:4 000 V;

k) Transiton for confirmation (m/z)and transiton for quantititation (m/z),fragmentor,collision energy,retention time,see Table A. 1.

Table A. 1—Transition for confirmation and quantitiation

Compound name	Transition for qualification m/z	Transition for quantification m/z	Fragmentor V	Collision energy eV	Retention time min
Imazalil	297. 1→158. 9	297. 1→158. 9	80	22	3. 2
	297. 1→200. 9			15	

1) **Non-commercial statement**:Referenced conditions were obtained from Agilent 6410B LC/MS/MS. The equipments and their types involved in the standard method are not related to commercial aims,and it is encouraged to use equipments of different corporation or different type.

2) 1 psi≈6. 895 kPa

Annex B
(informative)
MRM Chromatogram of the standards

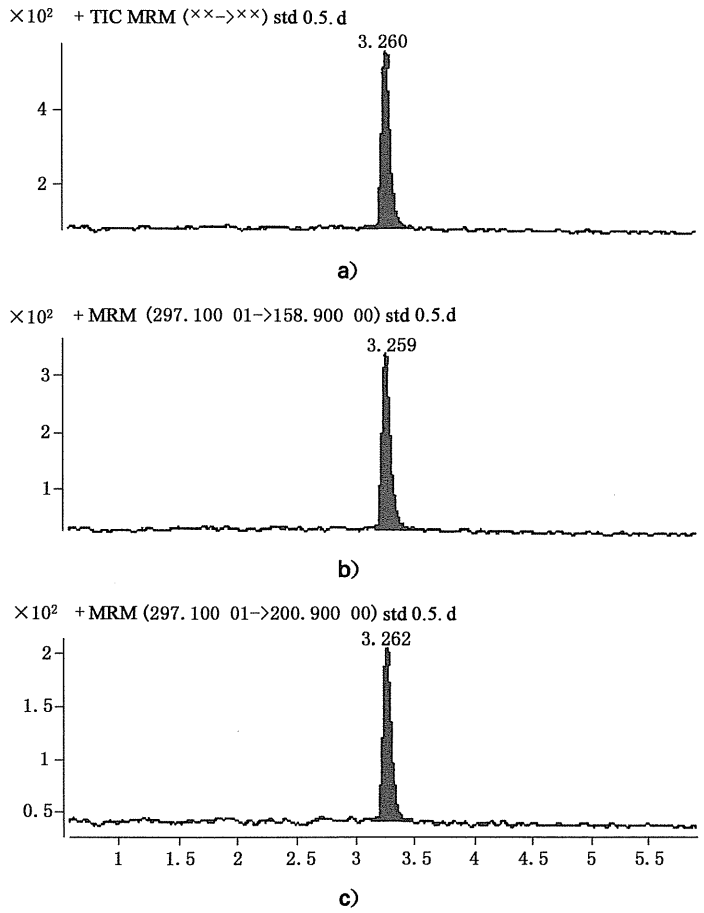

Figure B. 1—Selected ion chromatograms of imazalil(MRM)