SN

中华人民共和国出入境检验检疫行业标准

SN/T 0683—2014
代替 SN 0683—1997

出口粮谷中三环唑残留量的测定 液相色谱-质谱/质谱法

Determination of tricyclazole 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 0683—1997《出口粮谷中三环唑残留量检验方法》。

与 SN 0683—1997 相比，本标准技术变化如下：

——前处理方法由原先的人工填料层析柱净化改为固相萃取柱净化法；

——仪器方法由原先的气相色谱-氮磷检测器法和气相色谱-质谱方法确证，改为高效液相色谱-质谱/质谱法；

——检测基质范围由原来的稻谷扩充为玉米、大麦、糙米、大米、小麦。

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、中华人民共和国西藏出入境检验检疫局。

本标准主要起草人：周瑶、王君、次仁拉珍、曹晨、曹晓钢、曲栗、郭德华、朱坚。

本标准所代替标准的历次版本发布情况为：

——SN 0683—1997。

出口粮谷中三环唑残留量的测定 液相色谱-质谱/质谱法

1 范围

本标准规定了出口粮谷中三环唑残留量的测定方法。

本标准适用于玉米、大麦、糙米、大米、小麦中三环唑残留量的测定和确证。

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

SN/T 0800.1 进出口粮油、饲料检验抽样和制样方法

3 原理

试样中残留的三环唑用丙酮振荡提取，过中性氧化铝固相萃取柱净化后，供液相色谱-质谱/质谱仪测定，外标法定量。

4 试剂和材料

除另有规定外，试剂均为分析纯，水为 GB/T 6682 规定的一级水。

4.1 丙酮：色谱纯。

4.2 二氯甲烷：色谱纯。

4.3 甲醇：色谱纯。

4.4 乙腈：色谱纯。

4.5 甲酸：色谱纯。

4.6 醋酸铵：色谱纯。

4.7 5 mmol/L 醋酸铵水溶液(含有 0.1%甲酸)：称取 0.385 g 醋酸铵，加入 1 L 水溶解后，加入 1 mL 甲酸混匀。

4.8 二氯甲烷-甲醇溶液(99+1，体积比)。

4.9 乙腈-水溶液(1+1，体积比)。

4.10 乙腈-5 mmol/L 醋酸铵水溶液(含有 0.1%甲酸)(1+1，体积比)。

4.11 标准品：三环唑(Tricyclazole，CAS No. 41814-78-2)，纯度≥99%。

4.12 标准储备液：称取适量三环唑标准品，用乙腈配制成为 10 μg/mL 的标准储备液，0 ℃～4 ℃保存，有效期 12 个月。用乙腈-5 mmol/L 醋酸铵水溶液(含有 0.1%甲酸)(1+1，体积比)配置所需标准中间液和标准工作液。

4.13 中性氧化铝固相萃取柱：1 g/3 mL。

4.14 注射器和 0.22 μm 有机相针式过滤器。

5 仪器和设备

5.1 粉碎机或相当的设备。
5.2 液相色谱-串联质谱仪，配有电喷雾离子源。
5.3 均质器。
5.4 涡旋振荡器。
5.5 离心机：5 000 r/min。
5.6 分析天平：感量 0.1 mg，0.001 g。
5.7 固相萃取装置。
5.8 50 mL 离心管。
5.9 10 mL 容量瓶。

6 试样制备与保存

制样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。将样品按 SN/T 0800.1 的规定缩分至 1 kg，全部磨碎并通过 ϕ 1 mm 圆孔筛，均分成两份试样，装入洁净容器中，密封，并标明标记，于 −5 ℃以下避光存放。

7 分析步骤

7.1 提取

称取 1 g 样品(准确至 0.001 g)于 50 mL 离心管中，加入 5 mL 丙酮，用涡旋振荡器充分振荡提取 5 min，在 4 400 r/min 转速下离心 4 min 后，将上清液倒入小试管中。重复上述步骤残渣再用 5 mL 丙酮提取一次。合并两次提取的上清液于 10 mL 容量瓶中，用丙酮定容。视样品中三环唑含量高低，取 1 mL～10 mL 提取液于 35 ℃水浴中氮吹至干，再准确加入 4 mL 二氯甲烷溶液充分振荡溶解、混匀。

7.2 净化

在重力流速下，用 6 mL 二氯甲烷活化中性氧化铝固相萃取柱，然后将 2.0 mL 上述提取液过柱，再用 6 mL 二氯甲烷溶液淋洗，最后用 8 mL 二氯甲烷-甲醇溶液(99+1，体积比)洗脱，将所收集的洗脱液于 35 ℃水浴中氮吹至干，残余物用 2.0 mL 乙腈-5 mmol/L 醋酸铵水溶液(含有 0.1%甲酸)(1+1，体积比)定容，混匀后过 0.22 μm 有机相针式滤膜供液相色谱-串联质谱仪检测。

7.3 测定

7.3.1 高效液相质谱条件

高效液相质谱条件如下：

a) 色谱柱：C_{18}柱，长 50 mm，内径 2.1 mm，粒径 3.0 μm 或相当者；
b) 流动相：A：5 mmol/L 醋酸铵水溶液(含有 0.1%甲酸)，B：乙腈溶液；流速：0.3 mL/min，梯度洗脱参数见附录 A 表 A.1；
c) 柱温：30 ℃；
d) 进样量：10 μL；
e) 质谱参数条件参见附录 A。

7.3.2 定量测定

根据试样中被测物的药物含量，选取响应值相近的标准工作液进行分析。标准工作液和待测液中药物的响应值均应在仪器线性响应范围内。如果含量超过标准曲线范围，应用 2.0 mL 乙腈-5 mmol/L 醋酸铵水溶液（含有 0.1%甲酸）（1+1，体积比）稀释到合适浓度后分析。在上述色谱条件下的三环唑的参考保留时间为 7.3 min，标准溶液的多反应监测色谱图参见附录 B 中图 B.1。

7.3.3 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，如果检测的质量色谱峰保留时间与标准品一致，定性离子对的相对丰度，是用相对于最强离子丰度的强度百分比表示，应当与浓度相当标准工作溶液的相对丰度一致，相对丰度允许偏差不超过表 1 规定的范围，则可判断样品中存在对应的被测物。

表 1 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

8 空白试验

除不称取试样外，均按上述操作步骤进行。

9 结果计算和表述

用色谱数据处理机或按式（1）计算样品中待测药物残留量，报告结果时以样品中可提取的三环唑残留量报告结果，计算结果需扣除空白值。

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

式中：

X ——样品中待测组分残留量，单位为微克每千克（μg/kg）；

c ——标准工作溶液中药物的浓度，单位为纳克每毫升（ng/mL）；

A_s ——标准工作溶液中药物的峰面积；

V ——样品溶液最终定容体积，单位为毫升（mL）；

A ——样液中药物的峰面积；

m ——最终样液代表的试样质量，单位为克（g）。

10 测定低限、回收率

10.1 测定低限

本方法测定低限为 20.0 μg/kg。

10.2 回收率

添加回收率实验数据参见附录 C 中表 C.1。

附 录 A
（资料性附录）
Agilent 6410B 液相色谱-四级杆质谱/质谱仪参数[1)]

A.1 梯度洗脱程序表

见表 A.1。

表 A.1 梯度洗脱程序表

梯度时间/min	流动相 A/%	流动相 B/%
1	98	2
4	1	99
13	1	99
13.1	98	2
15	98	2

A.2 质谱/质谱仪参数

质谱/质谱仪参数如下：

a) 离子源：ESI，正离子；
b) 雾化气：氮气；
c) 气流速(Gas Flow)：10 L/min；
d) 干燥气温度：350 ℃；
e) 雾化气(Nebulizer)：275.8 kPa(40 psi)；
f) 电子倍增器电压(Delta EMV)：400 V；
g) 扫描方式：正离子扫描；
h) 毛细管电压：4 000 V；
i) 检测方式：多反应监测(MRM)参数见表 A.2。

表 A.2 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)	去簇电压/V	碰撞能量/V	色谱保留时间 t_r/min
三环唑	190→163	190→163	80	25	7.3
	190→136			30	

1) 非商业性声明：附录 A 所列参数是在 Agilent 6410B 质谱仪完成的，此处列出试验用仪器型号仅是为了提供参考，并不涉及商业目的，鼓励标准使用者尝试不同厂家和型号的仪器。

附 录 B
（资料性附录）
三环唑标准品多反应监测（MRM）色谱图

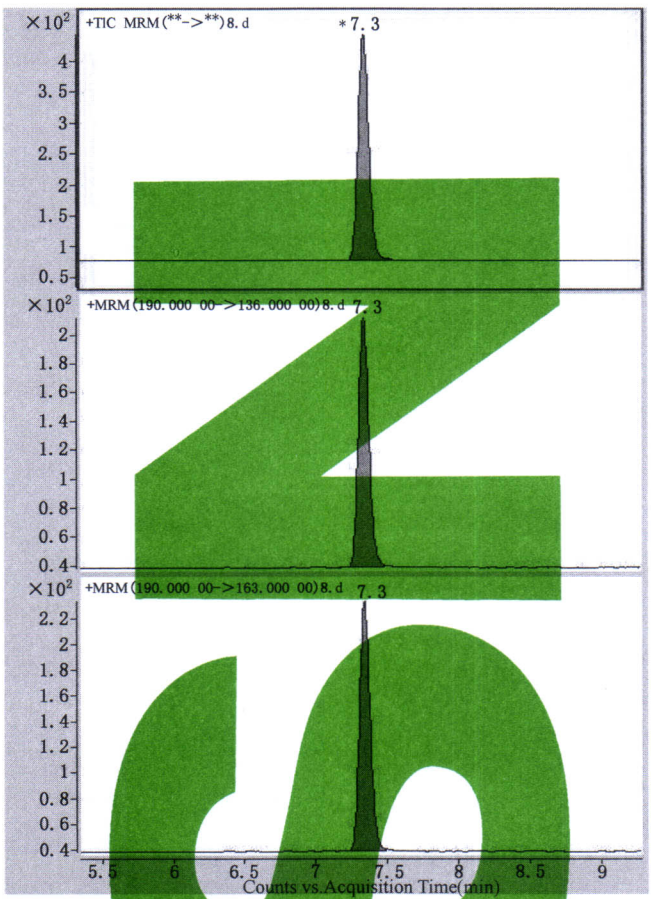


图 B.1 三环唑标准品多反应监测（MRM）色谱图

附 录 C
（资料性附录）
液相色谱-串联质谱法添加回收率实验数据

表 C.1 液相色谱-串联质谱法添加回收率实验数据

基质	添加水平/（mg/kg）	回收率范围/%
玉米	0.02	78.1～93.7
	1.00	80.3～91.2
	3.00	79.6～90.0
大麦	0.02	80.8～95.6
	1.00	85.4～95.4
	3.00	85.7～94.1
糙米	0.02	80.6～94.1
	1.00	82.1～97.3
	3.00	84.3～96.6
大米	0.02	73.0～86.1
	1.00	80.2～91.3
	3.00	81.5～90.2
小麦	0.02	73.1～87.6
	1.00	80.5～90.4
	3.00	83.8～88.7

Foreword

The standard was drafted according to GB/T 1.1—2009.

The standard is replace of SN 0683—1997"Method for the determination of tricyclazole residue in cereals for export".

The main improvement from SN 0683—1997:

—The sample pretreatment is changed to solid phase extraction (SPE), compared to the former edition, which adopted manually loaded chromatographic column.

—The instrument method is changed to HPLC-MS/MS compared to the former edition, which adopted GC-NPD and GC-MS.

—The matrix scope was enlarged to corn, barley, brown rice, rice and wheat.

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 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, Xizang Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Zhou Yao, Wang Jun, Ciren Lazhen, Cao Chen, Cao Xiaogang, Qu Li, Guo Dehua, Zhu Jian.

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

—SN 0683—1997.

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

Determination of tricyclazole in cereals for export—HPLC-MS/MS method

1 Scope

The standard specifies the method of determination of tricyclazole in cereals for export.

This standard is applicable to determination of tricyclazole in corn, barley, brown rice, rice and wheat.

2 Normative references

The following documents is necessary for this standard. For dated references , 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

SN/T 0800.1 Inspection of cereals, oils and feedstuffs for import and export—Methods of sampling and preparation of samples

3 Principles

The tricyclazole residue in samples is extracted by acetone, cleaned on N-Alumina SPE column, and eluted by dichloromethane-methanol mixture. The extract is determined by HPLC-MS/MS after condensation and reconstitution, and quantified by external standard method.

4 Reagents and materials

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

4.1 Acetone: HPLC grade.

4.2 Dichloromethane: HPLC grade.

4.3 Methanol: HPLC grade.

4.4 Acetonitrile:HPLC grade.

4.5 Formic acid:HPLC grade.

4.6 Ammonium acetate:HPLC grade.

4.7 5mmol/L ammonium acetate (containing 0.1% formic acid): Dissolve 0.385 g ammonium acetate in 1 L water,add 1 mL formic acid,mix thoroughly.

4.8 Dichloromethane-methanol (99+1, V/V).

4.9 Acetonitrile-water (1+1, V/V).

4.10 Acetonitrile-5 mmol/L ammonium acetate (containing 0.1% formic acid) (1+1, V/V).

4.11 Tricyclazole (CAS No.41814-78-2),purity ≥99%.

4.12 Stock standard solution:Accurately weigh appropriate standard 4.11,dissolve it in acetonitrile to 10 μg/mL.Store it refrigerated at 0 ℃~4 ℃,valid for 12 months.According to the requirement, prepare a standard working solution of appropriate concentration.

4.13 N-Alumina SPE column:1 g/3 mL.

4.14 Syringe and 0.22 μm membrane filter.

5 Apparatus and equipment

5.1 Grinder or the equivalent instrument.

5.2 Liquid chromatograhy combined with electrospray ionization mass spectrometry.

5.3 Homogenizer.

5.4 Vortex mixer.

5.5 Centrifuger:5 000 r/min.

5.6 Balances:0.1 mg,0.001 g.

5.7 SPE device.

5.8 50 mL centrifuge tube.

5.9 10 mL volumetric flask.

6 Preparation and storage of test sample

In the course of sample preparation, precautions shall be taken to avoid contamination or any factors that may cause the change of residue content. According to SN/T 0800.1, take 1 kg representative portion from the whole primary sample, then grind thoroughly and let it pass through a ϕ1 mm sieve, and divide into 2 equal portions. Keep the sample into a clean vessel, seal and label. The test sample is stored below −5 ℃ and kept away from light.

7 Test procedures

7.1 Extraction

Weigh 1 g (0.001 g) sample in a 50 mL centrifuge tube, add 5 mL acetone (4.1), homogenize them by vortex mixer for 5 min. The mixture was centrifuged at 4 400 r/min for 4 min. Pour the supernatant into a tube. Repeat the procedures before. Combine the supernatant in the volumetric flask and dilute to the mark with acetone. According to the concentration level of tricyclazole in sample, take 1 mL～10 mL extract to be dried by N_2 in 35 ℃ water bath. Dilute the residue with 4 mL dichloromethane and mix them thoroughly.

7.2 Clean up

Activate the N-Alumina SPE column with 6 mL dichloromethane, and then load 2.0 mL extract on the column. Wash the column with 6 mL dichloromethane, and then use 8 mL dichloromethane- methanol (99 + 1, *V*/*V*) to elute the analyte. Dry the eluate by N_2 in 35 ℃ water bath and reconstitute the analyte in 2.0 mL acetonitrile-5 mmol/L ammonium acetate (1 + 1, *V*/*V*). Mix them thoroughly for further HPLC-MS/MS analysis.

7.3 Determination

7.3.1 HPLC operating conditions

HPLC operating conditions are as following:

a) Column: C_{18}, 50 mm × 2.1 mm(i.d.), 3 μm, or the equivalent.

b) Mobile phase: A: 5 mmol/L Ammonium Acetate-0.1% formic acid, B: Acetonitrile. Flow rate: 0.3 mL/min. The gradient program is listed in the Table A.1 of Annex A.

c) Column temperature: 30 ℃.

d) Injection volume: 10 μL.

e) Mass parameters are listed in the Annex A.

7.3.2 Quantification of HPLC-MS/MS

According to the above HPLC-MS/MS operating condition, determine the sample solution and the standard working solution simultaneously. The responses of the analyte in the standard working solution and the sample solution shall be within the linear range of the instrument detection. Quantified by external standard. Under the above HPLC-MS/MS operating condition, the retention time of tricyclazole is 7.3 min. The MRM chromatograms of the standards are presented in the Annex B.

7.3.3 Confirmation of HPLC-MS/MS

Determine the sample under the established LC/MS-MS conditions, and calculate the intensity ratio of two selected ion pairs of the sample solution and the standard working solutions. If the retention time of sample chromatogram peak are consistent with that of working solution, and the relative abundance ratio tolerance meets the requirements listed in the Table 1, it can be concluded that this compound does exist in the sample.

Table 1 Maximum permitted tolerances for relative ion intensities while conformation

Relative ion intensities/%	>50	>20~50	>10~20	≤10
Permitted relative tolerances/%	±20	±25	±30	±50

8 Blank test

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

9 Calculation and expression of result

Calculate the content of benzodiazepine residues in the test sample by HPLC-MS/MS data processor or according to the formula (1). The blank value should be subtracted from the above result of calculation.

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

Where:

X —the residue content in the test sample, μg/kg;
c —the concentration in standard working solution, ng/mL;
A_s — the peak area of residue in standard working solution;
V —the final volume of the sample solution, mL;
A —the peak area of residue in sample solution;
m —mass of test sample of final sample solution, g.

10 Limit of quantitation and recovery

10.1 Limit of quantification

The limit of quantification for tricyclazole is 20.0 μg/kg.

10.2 Recovery

According to the experimental data, the corresponding recovery of each fortifying level is listed in the Table C.1 of Annex C.

Annex A
(Informative annex)
Main Mass Parameters of Agilent 6410B[1)]

A.1 Gradient program of mobile phase

See table A.1.

Table A.1 Gradient program of mobile phase

Time/min	Mobile phase A/%	Mobile phase B/%
1	98	2
4	1	99
13	1	99
13.1	98	2
15	98	2

A.2 MS/MS parameters

MS/MS parameters see the followings as reference:

a) Ion Source:ESI,Positive;

b) Nebulizer gas:N_2;

c) Gas Flow:10 L/min;

d) Ion source temperature:350 ℃;

e) Nebulizer:275.8 kPa(40 psi);

f) Delta EMV:400 V;

g) Scan mode:Positive;

h) Electro spray capillary voltage:4 000 V;

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1) Non-commercial statement: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.

i) Monitor mode:Multiple reaction monitoring(MRM)(seeing table A.2).

Table A.2 Related parameters and qualifier and quantifier in MRM

Compound name	Transitions for qualification (*m/z*)	Transition for quantification (*m/z*)	Cone Voltage/ V	Collision energy/ V	Retention time/ min
Tricyclazole	190→163	190→163	80	25	7.3
	190→136			30	

Annex B
(Informative annex)
Selected ion chromatograms of tricyclazole (MRM)

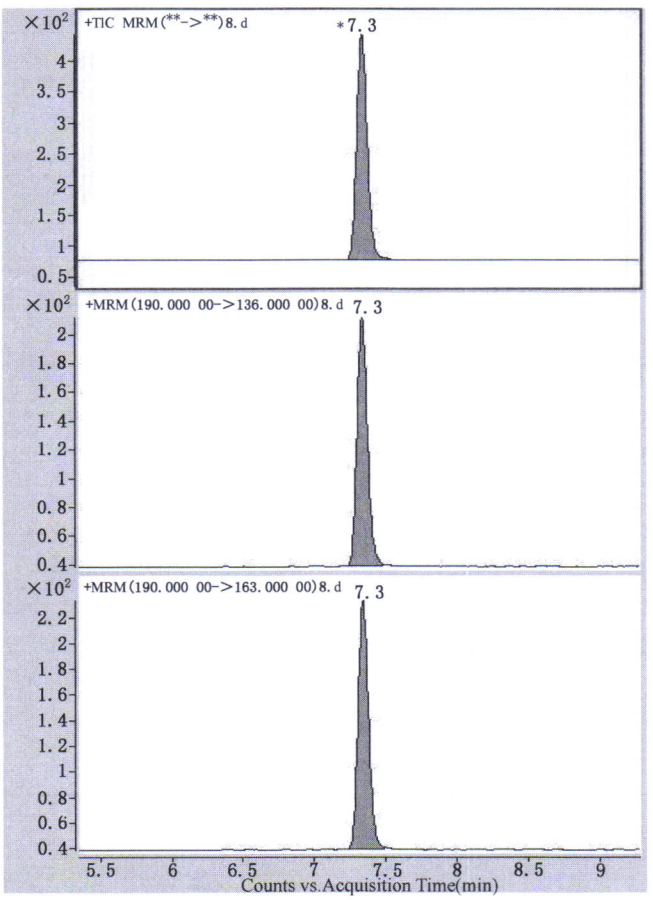


Figure B.1 Selected ion chromatograms of tricyclazole (MRM)

Annex C
(Informative annex)

Table C. 1 The recovery ranges of tricyclazole residue

Matrix	Fortified level/(mg/kg)	Recovery range/%
Corn	0.02	78.1～93.7
	1.00	80.3～91.2
	3.00	79.6～90.0
Barley	0.02	80.8～95.6
	1.00	85.4～95.4
	3.00	85.7～94.1
Brown Rice	0.02	80.6～94.1
	1.00	82.1～97.3
	3.00	84.3～96.6
Wheat	0.02	73.0～86.1
	1.00	80.2～91.3
	3.00	81.5～90.2
Rice	0.02	73.1～87.6
	1.00	80.5～90.4
	3.00	83.8～88.7