SN

中华人民共和国出入境检验检疫行业标准

SN/T 0220—2016
代替 SN 0220—1993

出口水果中多菌灵残留量的检测方法

Determination of carbendazim residue in fruits for export

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中华人民共和国
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前　言

本标准按照 GB/T 1.1—2009、GB/T 20001.4—2001 和 SN/T 0001—1995《出口商品中农药、兽药残留量及生物毒素检验方法标准编写的基本规定》给出的规则起草。

本标准代替 SN 0220—1993《出口水果中多菌灵残留量检验方法》，本标准与 SN 0220—1993 相比主要修改如下：

——修改了标准名称；

——标准适用范围增加了苹果、猕猴桃、西瓜、葡萄(见第 1 章)；

——将 1993 年版中“2　抽样和制样”修改为“3.4　试样的制备和保存”；

——将 1993 年版“3.4.1　提取、净化”修改为“3.5　样品处理”，对提取溶剂、提取方式、净化用固相萃取条件进行了修改；

——增加“液相色谱-质谱/质谱法”(见第 4 章)。

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：中华人民共和国江西出入境检验检疫局。

本标准主要起草人：朱春燕、吕小丽、郭平、陈林、万建春、祝建新、占春瑞、桂家祥。

本标准所代替标准的历次版本发布情况为：

——SN 0220—1993。

出口水果中多菌灵残留量的检测方法

1 范围

本标准规定了出口水果中多菌灵残留量测定的高效液相色谱法和液相色谱-质谱/质谱法。

本标准适用于苹果、猕猴桃、脐橙、西瓜、葡萄中多菌灵残留量的测定。

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 第一法 高效液相色谱法(HPLC 法)

3.1 方法提要

试样中多菌灵在弱碱性条件下经乙酸乙酯振荡提取，混合型强阳离子交换固相萃取柱净化，高效液相色谱测定，外标法定量。

3.2 试剂和材料

除特殊注明外，所有试剂均为分析纯，水为 GB/T 6682 规定的一级水。

3.2.1 乙腈：色谱纯。

3.2.2 甲醇：色谱纯。

3.2.3 乙酸乙酯：色谱纯。

3.2.4 盐酸：36%～38%。

3.2.5 氨水：25%～28%。

3.2.6 氢氧化钠。

3.2.7 1.0 mol/L 氢氧化钠溶液：称取 4 g 氢氧化钠，加水溶解并定容至 100 mL。

3.2.8 0.1 mol/L 盐酸溶液：取 9 mL 盐酸(3.2.4)，加水定容至 1 000 mL。

3.2.9 氨水-甲醇溶液：取 4 mL 氨水(3.2.5)用甲醇定容至 100 mL。

3.2.10 乙腈-水溶液：乙腈-水(20+80，体积比)。

3.2.11 标准品：多菌灵(Carbendazim)，CAS 登录号：10605-21-7，纯度大于等于 99%。

3.2.12 标准储备液(100 mg/L)：准确称取多菌灵标准品(折合成多菌灵含量为 10.0 mg)，用甲醇溶解并定容至 100 mL，混匀。－18 ℃以下避光保存。

3.2.13 标准中间液(10 mg/L)：取 1.0 mL 标准储备液(3.2.12)用甲醇稀释至 10 mL，4 ℃以下避光保存。

3.2.14 标准工作液：临用前取适量标准中间液(3.2.13)，用流动相稀释至 0.1 μg/mL、0.25 μg/mL、0.5 μg/mL、1.0 μg/mL、2.0 μg/mL、5.0 μg/mL、10.0 μg/mL 标准工作液。

3.2.15 混合型强阳离子交换固相萃取柱(Mixed-mode strong cation ion exchange SPE):Oasis MCX[1] 6 mL,150 mg 或相当者。使用前依次用 6 mL 甲醇(3.2.2)、6 mL 水和 6 mL 0.1 mol/L 盐酸(3.2.8)对柱子进行活化。

3.2.16 0.22 μm 微孔滤膜:有机系。

3.3 仪器和设备

3.3.1 高效液相色谱仪:配有紫外检测器或二极管阵列检测器。

3.3.2 分析天平:感量为 0.01 g 和 0.1 mg。

3.3.3 旋涡混合器。

3.3.4 振荡提取器。

3.3.5 离心机:4 500 r/min。

3.3.6 固相萃取装置。

3.3.7 组织捣碎机。

3.3.8 氮吹浓缩仪。

3.3.9 超声波清洗器。

3.4 试样的制备与保存

3.4.1 试样制备

取有代表性样品约 500 g,用组织捣碎机将样品匀浆,混合均匀,装入洁净容器内密封并做好标识。

3.4.2 试样保存

试样于−18 ℃冰箱内保存。制样和样品保存过程中,应防止样品受到污染和待测物损失。

3.5 样品处理

3.5.1 提取

称取 5 g 试样(精确至 0.01 g)于 50 mL 具塞塑料离心管中,加入 0.5 mL 1.0 mol/L 氢氧化钠溶液(3.2.7)和 20 mL 乙酸乙酯涡旋混匀后,振荡提取 30 min,4 500 r/min 离心 5 min,收集上清液到 150 mL 浓缩瓶中。残渣再加入 20 mL 乙酸乙酯,重复提取一次,合并上清液,于 45 ℃下旋转蒸发浓缩至 5 mL 左右,转移至 25 mL 离心管中,于 45 ℃下氮气吹干,用 10 mL 0.1 mol/L 盐酸溶液(3.2.8)超声 5 min 溶解,待净化。

3.5.2 净化

将 3.5.1 所得待净化溶液全部转移至活化好的混合型强阳离子交换固相萃取柱(3.2.15)中,再依次用 6 mL 0.1 mol/L 盐酸溶液(3.2.8)、6 mL 甲醇淋洗小柱,弃去淋洗液;真空抽干 2 min,用 6 mL 氨水-甲醇溶液(3.2.9)进行洗脱,收集洗脱液。于 45 ℃下氮气吹干,准确加入 1.0 mL 乙腈(3.2.1),超声 2 min 溶解残渣,涡旋 30 s,过 0.22 μm 微孔滤膜,供高效液相色谱仪测定。

1) 非商业性声明:本标准采用的固相萃取柱为 Waters 公司产品,此处列出仅为提供参考,并不涉及商业目的,鼓励标准使用者尝试不同厂家的固相萃取柱。

3.6 高效液相色谱条件

3.6.1 液相色谱参考条件

液相色谱参考条件如下：

a) 色谱柱：C_{18}柱，250 mm(柱长)× 4.6 mm(内径)，粒度 5 μm，或相当者；

b) 流动相：乙腈-水(20+80，体积比)；

c) 流速：1.0 mL/min；

d) 柱温：35 ℃；

e) 进样量：10 μL；

f) 波长：286 nm。

3.6.2 液相色谱测定

按 3.6.1 液相色谱条件测定标准工作溶液和样液，外标法定量。如果样品中多菌灵的含量超出标准曲线范围，应用流动相稀释后再进行分析。在上述色谱条件下，多菌灵的色谱峰保留时间约为 9.8 min。多菌灵标准色谱图参见图 A.1。

3.7 结果计算

用色谱数据处理机或按式(1)计算样品中多菌灵的残留量。

$$X=\frac{(c-c_0)\times V}{m}\times\frac{1\,000}{1\,000} \quad \cdots\cdots(1)$$

式中：

X ——试样中待测物的浓度，单位为毫克每千克(mg/kg)；

c ——试样终溶液中待测物的浓度，单位为微克每毫升(μg/mL)；

c_0 ——空白试验溶液中待测物的浓度，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)；

m ——试样终溶液代表的试样量，单位为克(g)。

3.8 空白试验

除不加试样外，均按上述操作步骤进行。

3.9 测定低限

本方法的测定低限为 0.05 mg/kg。

3.10 回收率

不同样品基质中多菌灵的回收率试验数据见参见表 B.1。

4 第二法 液相色谱-质谱/质谱法(LC-MS/MS 法)

4.1 方法提要

试样中的多菌灵在弱碱性条件下经乙酸乙酯振荡提取，混合型强阳离子交换固相萃取柱净化，液相色谱-质谱/质谱仪(LC-MS/MS)测定，外标法定量。

4.2 试剂和材料

除标准工作液按照4.2.1外，其他试剂和材料的要求与3.2一致。除特殊注明外，所有试剂均为分析纯，水为GB/T 6682规定的一级水。

标准工作液：临用前取适量标准中间液(3.2.13)，用流动相稀释至5.0 ng/mL、10.0 ng/mL、20.0 ng/mL、40.0 ng/mL、100.0 ng/mL、200.0 ng/mL标准工作液。

4.3 仪器和设备

除检测仪器按照4.3.1规定外，其他仪器和设备的要求与3.3一致。

液相色谱-质谱/质谱仪：配电喷雾离子源(ESI)。

4.4 试样的制备与保存

试样的制备与保存同3.4。

4.5 样品处理

4.5.1 提取

按3.5.1规定进行提取。

4.5.2 净化

按3.5.2规定处理至“于45 ℃下氮气吹干”，加入5.0 mL乙腈-水溶液(3.2.10)超声2 min溶解残渣，混匀后，取部分溶解液过0.22 μm微孔滤膜后，供液相色谱-质谱/质谱仪测定。

4.6 测定

4.6.1 液相色谱条件

液相色谱条件如下：

a) 色谱柱：C_{18}柱，150 mm(柱长)× 2.1 mm(内径)，粒度5 μm，或相当者；

b) 流动相：乙腈-水(20+80，体积比)；

c) 流速：300 μL/min；

d) 柱温：35 ℃；

e) 进样量：5 μL。

4.6.2 质谱/质谱条件

质谱/质谱条件如下：

a) 离子化模式：电喷雾电离(ESI)正离子模式；

b) 扫描方式：多反应监测(MRM)；

c) 分辨率：单位质量分辨率(Unit)；

d) 其他质谱条件参见附录C。

4.6.3 定性测定

按照4.6.1和4.6.2规定的仪器条件测定样液和标准工作溶液，在相同实验条件下，样品中待测物质的保留时间与标准溶液的保留时间偏差在±2.5%之内；且样品中各组分定性离子的相对丰度与浓度接近的标准工作溶液中对应的定性离子的相对丰度进行比较，偏差不超过表1规定的范围，则可判定为

样品中存在对应的待测物。

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

4.6.4　定量测定

在仪器最佳工作条件下，对样液及标准工作溶液(线性浓度范围多菌灵为：5.0 ng/mL～200.0 ng/mL)测定，外标法定量。样液中待分析物的响应值均应在仪器测定的线性范围内，若超出线性范围可用流动相适当稀释。标准溶液多反应监测(MRM)色谱图参见图 A.2。

4.7　空白试验

除不加试样外，均按 4.5 和 4.6 规定的操作步骤进行。

4.8　结果计算与表述

用色谱数据处理机或按式(2)计算样品中多菌灵的残留量。

$$X=\frac{(c-c_0)\times V}{m}\times\frac{1\,000}{1\,000} \qquad \cdots\cdots(2)$$

式中：

X ——试样中待测物的浓度，单位为微克每千克(μg/kg)；

c ——试样终溶液中待测物的浓度，单位为纳克每毫升(ng/mL)；

c_0 ——空白试验溶液中待测物的浓度，单位为纳克每毫升(ng/mL)；

V ——样液最终定容体积，单位为毫升(mL)；

m ——试样终溶液代表的试样量，单位为克(g)。

4.9　测定低限(LOQ)

本方法的测定低限为 0.01 mg/kg。

4.10　回收率

不同样品基质中多菌灵的回收率试验数据见参见表 B.2。

附 录 A
（资料性附录）
待测物色谱图

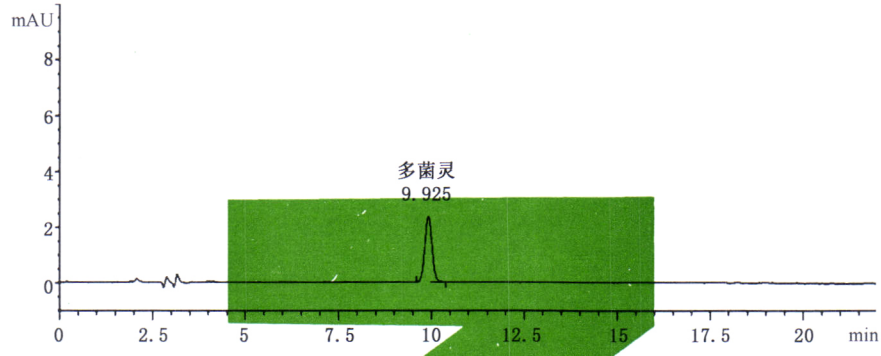
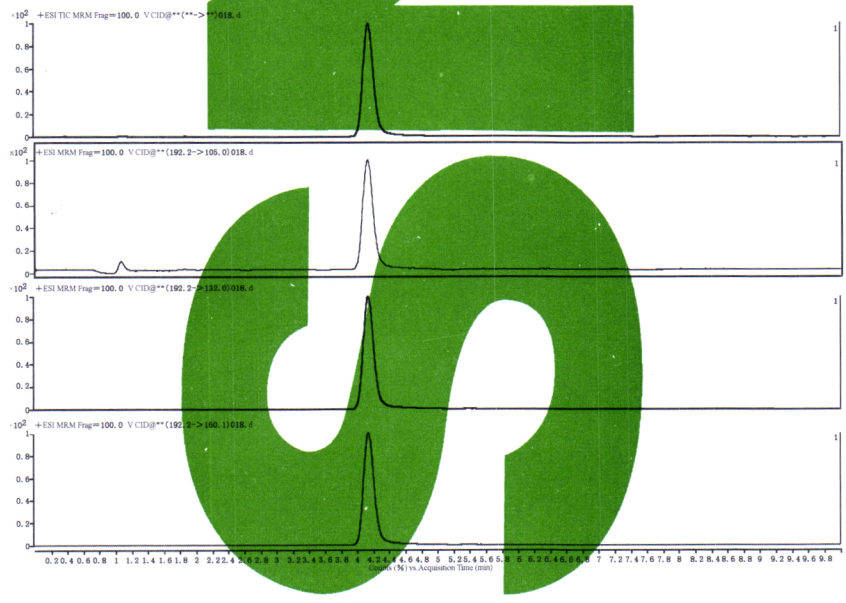


图 A.1 多菌灵标准溶液液相色谱图(0.25 μg/mL)



注：从上至下分别是多菌灵标准溶液总离子流图、三个子离子的色谱图。

图 A.2 多菌灵标准溶液多反应监测(MRM)色谱图(10.0 ng/mL)

附 录 B
（资料性附录）
多菌灵残留的添加回收率

表 B.1 高效液相色谱法多菌灵残留的添加回收率试验数据

添加浓度 mg/kg	回收率范围/%				
	苹果	猕猴桃	脐橙	西瓜	葡萄
0.05	82.8～95.6	84.0～96.0	85.3～92.4	87.2～99.2	86.4～92.4
0.10	93.4～98.2	91.0～95.4	89.8～96.6	80.4～92.8	88.8～95.0
0.20	91.6～99.3	86.4～98.3	82.6～96.5	89.2～99.5	85.3～94.3
7.00	91.1～99.4	84.2～96.7	85.6～91.4	87.9～100.4	80.8～96.7

表 B.2 液相色谱-质谱/质谱法多菌灵残留的添加回收率试验数据

添加浓度 mg/kg	回收率范围/%				
	苹果	猕猴桃	脐橙	西瓜	葡萄
0.01	88.1～97.4	93.1～101.0	87.8～94.2	82.6～102.4	86.3～96.6
0.02	92.1～97.6	85.9～104.0	85.4～95.3	86.0～91.1	87.1～104.6
0.04	93.3～99.1	84.6～99.6	84.0～92.5	82.9～105.1	89.9～109.5

附 录 C
（资料性附录）
参考质谱条件[2)]

C.1 毛细管电压：3 500 V。

C.2 干燥气温度：350 ℃。

C.3 干燥气流量：10 L/min。

C.4 雾化器压力：241 kPa(35 psi)。

C.5 碰撞气：氮气。

C.6 其他质谱参数见表 C.1。

表 C.1 主要参考质谱参数

化合物	母离子(m/z)	子离子 (m/z)	驻留时间/ms	裂解电压/V	碰撞能量/V
多菌灵	192.2	160.1[a]	100	100	17
		132.0	100	100	28
		105.0	100	100	28
注：对于不同质谱仪器，仪器参数可能存在差异，测定前应将质谱参数优化到最佳。					
[a] 定量离子。					

2) 非商业性声明：附录 C 所列参考质谱条件是在 Agilent 6410 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。

Foreword

This standard is drafted according to GB/T 1.1—2009、GB/T 20001.4—2001 and SN/T 0001—1995. This standard is replace of SN 0220—1993 Method for the determinaton of carbendazim residue in fruits for export.compared with SN 0220—1993, the main modifications of this standard are as follows:

—Modified the standard name;

—Enlarged the scope of application: "apple, kiwi, watermellon and grape"(see chapter 1);

—Modified "2 sampling and preparation"in 1993 Edition to "3.4 sample preparation and storage";

—Modified "3.4.1 sampling and preparation" in 1993 Edition to "3.5 sample treatment";Modified the extraction solvent, extraction method and SPE conditiong for purification.

—Added " LC-MS/MS Method"(see chapter 4).

This standard is propased by and is under the charge of the National Regulation Commission for Certification and Accreditation.

This standard is drafted by Jiangxi Entry-Exit and Quarantine Bureau of the People's Repubulie of China.

This standard is mainly drafted by Zhu Chunyan, Lv Xiaoli, Guo Ping, Chen Lin, Wan Jianchun, Zhu Jianxin, Zhan Chunrui, Gui Jiaxiang.

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

—SN 0220—1993.

Determination of Carbendazim Residue in Fruits for Export

1 Scope

This standard specifies determination of carbendazim residue in fruits for export by HPLC and LC-MS/MS.

This standard is applicable to the determinaton of carbendazim residue in apple, kiwi, navel orange, watermellon and grape.

2 Normative Reference

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

3 Method Ⅰ High Performance Liquid Chromatography

3.1 Method Principle

Under the week alkaline conditions, Carbendazim in the test samples is extracted with ethyl acetate, and purified by Mixed-mode strong cation ion exchange SPE, then determinded by HPLC, quantified by external standard method.

3.2 Chemicals Reagents and Materials

All the reagents used should be analytically pure unless otherwise specified. "Water" is first class water, as defined in GB/T 6682.

3.2.1 Acetronitrile, chromatographic grade.

3.2.2 Methanol, chromatographic grade.

3.2.3 Ethyl acetate, chromatographic grade.

3.2.4 Hydrochloric acid: 36%～38%.

3.2.5 Ammonium hydroxide: 25%～28%.

3.2.6 Sodium hydroxide.

3.2.7 1.0 mol/L Sodium hydroxide solution: Weigh 4 g sodium hydroxide in 100 mL volumetric flask, dissolve and make up to the mark with water.

3.2.8 0.1 mol/L hydrochloric acid: slowly add 9 mL of hydrochloric acid (3.2.4) to 1 000 mL of water.

3.2.9 Ammonium methanol solution: Pipette 4 mL ammonium hydroxide (3.2.5) in 100 mL volumetric flask, make up to the mark with methanol.

3.2.10 Acetonitrile solution: acetonitrile/water (20+80, volume ratio).

3.2.11 Standard substance: Carbendazim, CAS 10605-21-7, purity≥99%.

3.2.12 Standard stock solution of 100 mg/L: accurately weigh a small quantity of standard substance (equal to 10.0 mg Carbendazim) in a 100 mL volumetric flask, dissolve and make up the mark with methanol. The solution should be kept away from light and preserved at −18 ℃.

3.2.13 Intermediate standard solution (10 mg/mL): Pipette 1.0 mL of standard stock solution(3.2.12), make up the solution to 10 mL with methanol. The solution is kept away from light and below 4 ℃.

3.2.14 Working standard solution: Dilute 10 mg/L of intermediate solution (3.2.13) to 0.1 μg/mL, 0.25 μg/mL, 0.5 μg/mL, 1.0 μg/mL, 2.0 μg/mL, 5.0 μg/mL, 10.0 μg/mL of working standard solution with mobile phase. Freshly prepared.

3.2.15 Mixed-mode strong cation ion exchange column: Oasis MCX[1)] 6 mL, 150 mg or equivalent. Activate the SPE column with 6 mL methnol (3.2.2), 6 mL water and 6 mL 0.1 mol/L of hydrochloric acid (3.2.8) successively before use.

3.2.16 0.22 μm millipore filter: hydrophobicity

1) Non-commercial statement: The corporation of the SPE used in the standard is produced by Waters company. The SPE involved in the standard method is only for reference and not related to commercial aims, and it is encouraged to use the SPE of different corporation or different type.

3.3 Instruments and Apparatus

3.3.1 HPLC with UV detector or DAD detector.

3.3.2 Analytical balance, capable of accurately weighing to 0.01 g and 0.1 mg.

3.3.3 Vortex mixer.

3.3.4 Water Bath-Heated Shaker.

3.3.5 Centrifuge: 4 500 r/min.

3.3.6 SPE Vacuum Manifolds.

3.3.7 Blender.

3.3.8 Nitrogen evaporator.

3.3.9 Utrasonic cleaner.

3.4 Sample Preparation and Preservation

3.4.1 Sample Preparation

Weigh about 500 g of representive sample, homogenate with a tissue triturator, mix uniformity and put in a clean container which is sealed and labeled.

3.4.2 Sample Preservation

Store the sample at −18 ℃. During this process, it must be protected from contamination and avoided the loss of the analyte.

3.5 Sample Preparation

3.5.1 Extraction

Weigh 5 g sample(accurate to 0.01 g) in 50 mL plastic centrifuge tube, add 0.5 mL of 1.0 mol/L sodium hydroxide solution (3.2.7) and 20 mL of ethyl acetate, cap the tube, vortex swirl and shake for 30 min, centrifuge for 5 min at 4 500 r/min, collect the supernatant into a 150 mL conical tube. Add 20 mL of ethyl acetate into residue for extraction once more. Merge and concentrate the extract to about 5 mL with rotary vacuum evaporator (Keep the temperature at 45 ℃). The residue is transferred to a 25 mL centrifuge tube and dried with nitrogen evaporator at 45 ℃, add 10 mL 0.1 mol/L hydrochloric acid (3.2.8), place in ultrasonic bath for 5 min to disperse the colorant.

3.5.2 Purification

Transfer all the solution get from the section 3.5.1 into a mixed-mode strong cation ion exchange SPE column (3.2.15) which has been activated, flush the SPE column with 6 mL hydrochloric acid, 6 mL methanol (3.2.2) successively, discard those solutions, vacuum for 2 min, place 6 mL ammonium methanol solution into the syringe, collect the elution into a 15 mL conical tube. Dry with nitrogen evaporator at 45 ℃, add 1.0 mL acetonitrile (3.2.1) accurately, put the tube in ultrasonic bath for 2 mins to disperse the residue. After vortex 30 s, filter the solution through 0.22 μm millipore filter prior to HPLC analysis.

3.6 HPLC system

3.6.1 Chromatographic Conditions are as followed (Recommended)

Chromatographic Conditions are as follows:

a) Column: C_{18}, 250 mm × 4.6 mm id, 5 μm particle size or equivalent;

b) Mobile Phase: acetonitrile/water (20 + 80, volume ratio);

c) Flow Rate: 1.0 mL/min;

d) Column Temperature: 35 ℃;

e) Injection Volume: 10 μL;

f) Detected Wavelength: 286 nm.

3.6.2 Analysis by LC

Using conditions established in sec.3.6.1, analyze working standard solution and samples and calculate the content of carbendazim with external standard method. If the concentration exceeds the linear range of the calibration curve, the final solution should be diluted with mobile phase and reanalyzed. Under the above chromatographic conditions, the retention time of carbendazim is about 9.8 mins. For the standard chromatogram of carbendazim, refer to figure.A.1.

3.7 Result Calculation

The levels of Carbendazim residue in samples are calculated by HPLC data processor or according to the following equation.

$$X = \frac{(c - c_0) \times V}{m} \times \frac{1\,000}{1\,000} \quad \cdots\cdots(1)$$

Where:

X—Concentration of the analytes in sample, in mg/kg;

c—Final concentration of the analytes in sample solution, in μg/mL;

c_0—Concentration of the analytes in the solution of blank experiment, in μg/mL;

V—Volume of the final sample solution, in mL;

m—Weigh of the sample in the final solution, in g.

3.8 Blank experiment

Refer to the above procedure, but without adding sample.

3.9 Limit of Quantitation

The limit of quantitation for this method is 0.05 mg/kg.

3.10 Percentage Recovery

The percentage recovery of carbendazim in different sample matrix refer to Table.B.1.

4 Method Ⅱ Liquid Chromatography-Mass/Mass

4.1 Method Principle

Under the weak alkaline conditions, Carbendazim in the test samples is extracted with ethyl acetate, and purified by mixed-mode strong cation ion exchange SPE, then determinded by Liquid Chromatography-Mass/Mass(LC-MS/MS), quantified by external standard method.

4.2 Reagent and materials

Prepare working standard solution from clause 4.2.1. In addition, prepare other reagents and materials from clause 3.2. All the reagents used should be analytically pure unless otherwise specified. "Water" is first class water, as defined in GB/T 6682.

Working standard solution: Dilute intermediate standard solution (3.2.13) to 5.0 ng/mL, 10.0 ng/mL, 20.0 ng/mL, 40.0 ng/mL, 100.0 ng/mL, 200.0 ng/mL of working standard solution with mobile phase.

Freshly prepared.

4.3 Instruments and Apparatus

Analytical apparatus are the same as clause 4.3.1, preparation of the other instruments and apparatus from clause 3.3.

LC-MS/MS: ESI.

4.4 Sample Preparation and preservation

Preparation and preservation of sample from clause 3.4.

4.5 Sample preparation

4.5.1 Extraction

Refer to clause 3.5.1.

4.5.2 Purification

Preparation from clause 3.5.2, after drying with nitrogen evaporator at 45 ℃, add 5.0 mL acetonitrile, disperse the residue in ultrasonic bath for 2 mins to homogenize it, filter it through 0.22 μm millipore filter prior to LC-MS/MS analysis.

4.6 Analysis

4.6.1 HPLC conditions

HPLC conditions are as follows:

a) Column: C_{18}, 150 mm × 2.1 mm ID, 5 μm particle size or equivalent;

b) Mobile Phase: acetonitrile/water (20 + 80, volume ratio);

c) Flow Rate: 300 μL/min;

d) Column Temperature: 35 ℃;

e) Injection Volume: 5 μL.

4.6.2 MS/MS conditions

MS/MS conditions are as follows:

a) ESI mode: ESI^+;

b) Scan mode: MRM;

c) Acquisition Rate: Unit;

d) the other MS conditions refer to Appendix C.

4.6.3 Qualitative analysis

Analyze the samples and working standard solution under the above conditions listed in 4.6.1 and 4.6.2.Under the same experiment condition,the devation between the retention time of the analytes and standard solution is under ±2.5%; Compared the devation of relative abundance of the analytes qualitative ions to that of working standard solution,it is confirmed that the analyte is contained in sample if standard solution is under the range defined as Table.1.

Table.1 The maximum allowable deviation of relative ion abundance

Relative ion abundance/%	>50	>20～50	>10～20	≤10
maximum allowable misalignment/%	±20	±25	±30	±50

4.6.4 Quantitative analysis

Under the optimized condition,determind working standard solution and samples (The linear ranges of Carbendazim is from 5.0 ng/mL～200.0 ng/mL),quantify by external standard method. The response values should be within the linear range. If the response value exceeds the linear range of the calibration curve,the final solution should be diluted appropriately with mobile phase and reanalyzed. For the multiple reaction monitoring (MRM) chromatogram of carbendazim standard,refer to Figure.A.2.

4.7 Blank experiment

Refer to the 4.5 and 4.6 procedure,but without adding sample.

4.8 Result Calculation

The levels of Carbendazim residue in samples are calculated by LC-MS/MS data processor or according to the following equation.

$$X = \frac{(c - c_0) \times V}{m} \times \frac{1\,000}{1\,000} \quad \text{.....(2)}$$

Where:

X—Concentration of the analytes in sample, in μg/kg;

c—Final concentration of the analytes in sample solution, in ng/mL;

c_0—Concentration of the analytes in the solution of blank experiment, in ng/mL;

V—Volume of the final sample solution, in mL;

m—Weigh of the sample in the final solution, in g.

4.9 Limit of Quantitation

The limit of quantitation for this method is 0.01 mg/kg.

4.10 Recovery

The percentage recovery of Carbendazim in different sample matrix refer to Table.B.2.

Annex A
(Informative appendix)
Chromatogram of the analyte

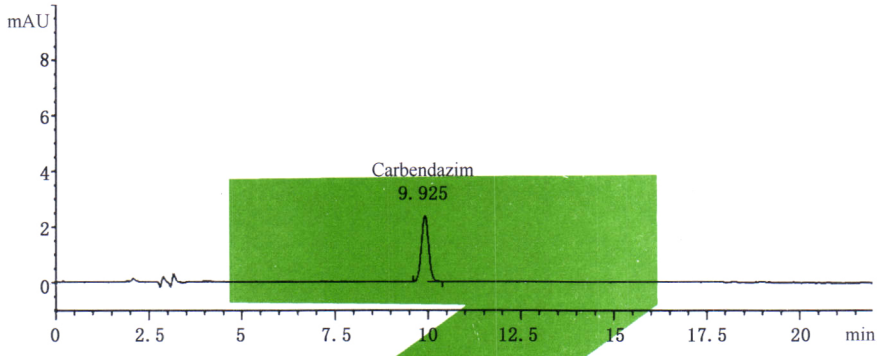
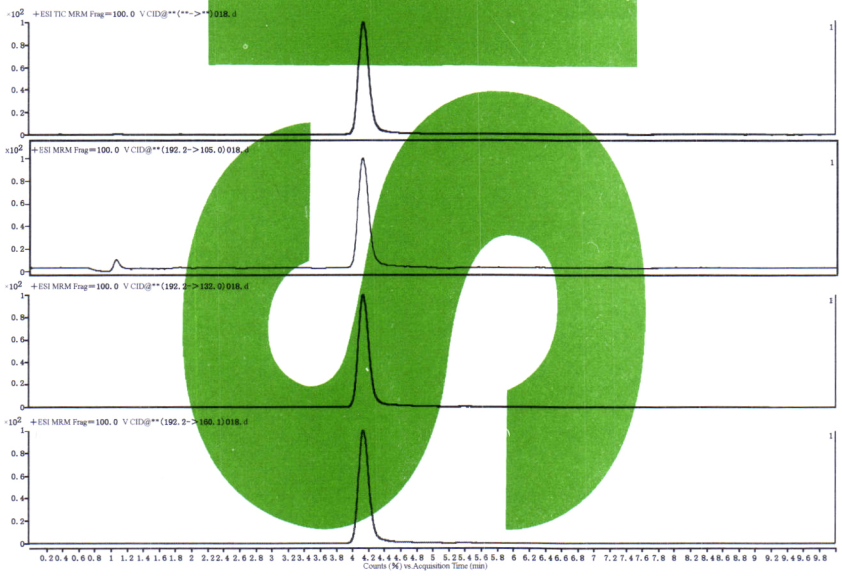


Figure A.1 The chromatogram of carbendazim standard solution(0.25 μg/ mL)



NOTE: The figure from top to bottom respectively refer to the TIC, the chromatograms of three ions.

Figure. A.2 The multiple reaction monitoring (MRM) chromatogram of carbendazim standard(10.0 ng/mL)

Annex B
(Informative appendix)
The recovery test data

Table B.1 The recovery test data of HPLC

fortified concentration/ (mg/kg)	The recovery range/%				
	apple	kiwi fruit	navel orange	watermelon	grape
0.05	82.8～95.6	84.0～96.0	85.3～92.4	87.2～99.2	86.4～92.4
0.10	93.4～98.2	91.0～95.4	89.8～96.6	80.4～92.8	88.8～95.0
0.20	91.6～99.3	86.4～98.3	82.6～96.5	89.2～99.5	85.3～94.3
7.00	91.1～99.4	84.2～96.7	85.6～91.4	87.9～100.4	80.8～96.7

Table B.2 The recovery test data of LC-MS/MS

fortified concentration/ (mg/kg)	The recovery range/%				
	apple	kiwi fruit	navel orange	watermelon	grape
0.01	88.1～97.4	93.1～101.0	87.8～94.2	82.6～102.4	86.3～96.6
0.02	92.1～97.6	85.9～104.0	85.4～95.3	86.0～91.1	87.1～104.6
0.04	93.3～99.1	84.6～99.6	84.0～92.5	82.9～105.1	89.9～109.5

Annex C
(Informative appendix)
MS/MS Conditions(recommended)[2)]

C. 1 capillary voltage: 3 500 V.

C. 2 drying gas temperature: 350 ℃.

C. 3 Gas flow: 10 L/min.

C. 4 Nebulizer: 241 kPa (35 psi).

C. 5 collision gas: nitrogen.

C. 6 other mass spectrum parameters are shown in Table. C. 1.

Table C. 1 Main reference spectrum parameter

compound	parent ion (*m/z*)	daughter ion (*m/z*)	residence time/ms	cracking voltage/V	collision energy/V
carbendazim	192. 2	160. 1[a]	100	100	17
		132. 0	100	100	28
		105. 0	100	100	28
Note: for different mass spectrometer, there may be differences between the instrument parameters, before determination, mass spectrum parameter should be optimizated to the best.					
[a] quantitative ion.					

2) Non-commercial statement: Referenced conditions were obtained from Agilent 6410 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.