SN

中华人民共和国出入境检验检疫行业标准

SN/T 0525—2012
代替 SN 0525—1996

出口水果、蔬菜中福美双残留量检测方法

Determination of thiram residue in fruit and vegetable for export

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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 0525—1996《出口水果、蔬菜中福美双残留量检验方法》。

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：中华人民共和国深圳出入境检验检疫局、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：林黎、陈波、侯乐锡、谢丽琪、周昱、岳振峰、靳保辉。

本标准所代替标准的历次版本发布情况为：

——SN 0525—1996。

出口水果、蔬菜中福美双残留量检测方法

1 范围

本标准规定了出口水果、蔬菜中福美双残留量的液相色谱和液相色谱-质谱/质谱检测方法。

本标准适用于苹果、梨、香蕉、西瓜、芹菜、茄子和白菜中福美双残留量的测定。

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 原理

样品经乙腈提取，浓缩后用液相色谱仪和液质联用仪进行测定，外标法定量。

4 试剂和材料

除另有说明外，所有试剂均为分析纯，水为 GB/T 6682 规定的一级水。

4.1 乙腈：HPLC 级。

4.2 甲醇：HPLC 级。

4.3 甲醇-水(30＋70，体积比)：取 30 mL 甲醇(4.2)和 70 mL 水混合均匀。

4.4 甲醇-水(50＋50，体积比)：取 50 mL 甲醇(4.2)和 50 mL 水混合均匀。

4.5 甲酸：HPLC 级。

4.6 醋酸铵：HPLC 级。

4.7 0.05% 甲酸的甲醇溶液：在 200 mL 甲醇中加入 0.1 mL 甲酸。

4.8 0.05% 甲酸的甲醇溶液-水(50＋50，体积比)：取 50 mL 0.05% 甲酸的甲醇溶液(4.7)和 50 mL 水混合均匀。

4.9 醋酸铵溶液(0.05% 甲酸)：5 mmol/L。准确称取 0.385 4 g 醋酸铵(4.6)，用水溶解，加 0.5 mL 甲酸(4.5)，用水定容到 1 000 mL。

4.10 无水硫酸钠：分析纯。500 ℃ 灼烧 4 h，置于干燥器中备用。

4.11 福美双标准物质：thiram，纯度大于 99%，CAS 号：137-26-8。

4.12 福美双标准储备溶液：准确称取 12.5 mg 标准品(4.11)，用 0.05% 甲酸的甲醇溶液(4.7)溶解并定容至 25 mL，得到浓度为 500 mg/L 标准储备液，此溶液保存于棕色容量瓶中，可在－18 ℃条件下保存 3 个月。

4.13 标准工作溶液：液相色谱法：根据需要取适量标准储备液，以 0.05%甲酸的甲醇溶液-水(50＋50，体积比)(4.8)稀释成适当的标准工作液；液相色谱-质谱/质谱法：根据需要取适量标准储备液，以空白基质提取液，稀释成适当的标准工作液。

4.14 滤膜：0.45 μm，有机系。

5 仪器和设备

5.1 液相色谱仪，配紫外检测器。
5.2 液相色谱-质谱/质谱联用仪，配电喷雾(ESI)源。
5.3 涡旋振荡器。
5.4 往复式振荡器。
5.5 旋转蒸发仪。
5.6 分析天平：感量 0.1 mg 和 0.001 g。
5.7 低温离心机：可制冷至 4 ℃，5 000 r/min。
5.8 塑料离心管：15 mL 和 50 mL。

6 样品制备与保存

样品至少 500 g，取可食部分后将其切成小块，用组织捣碎机将样品匀浆，混合均匀后分成两份，装入洁净的样品袋内，密闭并标识；在－18 ℃保存避光保存。在制样的操作过程中，应防止样品受到污染或发生残留物含量的变化。

7 测定步骤

7.1 提取

7.1.1 称取 4 g(精确到 0.01 g)均匀试样于 50 mL 离心管中，加入 30 mL 乙腈(4.1)，涡旋振荡 30 s，往复振荡提取 10 min，在 4 ℃下 5 000 r/min 冷冻离心 5 min。上清液过无水硫酸钠(4.10)至一干净鸡心瓶，再用 30 mL 乙腈重复以上步骤，合并提取液于同一鸡心瓶。
7.1.2 提取液于 30 ℃旋转蒸发至 2 mL 左右，将剩余溶液转移至一干净的 15 mL 离心管中，用1.5 mL 甲醇-水(30＋70，体积比)(4.3)洗涤鸡心瓶，洗涤液合并至上述离心管中，用甲醇-水(30＋70，体积比)(4.3)定容到 4 mL，过滤膜，所得样液直接供 HPLC 测定。
7.1.3 将 7.1.2 所得溶液用 0.05％ 甲酸的甲醇溶液-水(50＋50，体积比)(4.8)稀释 20 倍，供 HPLC-MS/MS 测定。

7.2 测定

7.2.1 液相色谱法测定方法

7.2.1.1 液相色谱条件

液相色谱条件如下：
a) 色谱柱：Eclipse XDB-C_{18}，250 mm × 4.6 mm(内径)，5 μm，或相当者；
b) 柱温：25 ℃；
c) 流动相：甲醇-水(50＋50，体积比)；
d) 分析时间：15 min；
e) 流速：1.0 mL/min；
f) 检测波长：270 nm；
g) 进样量：50 μL。

7.2.1.2 液相色谱测定

根据试样中福美双的含量情况，选定响应值适宜的标准工作溶液进行色谱分析。标准工作溶液和待测样液中福美双的响应值均应在仪器线性响应范围内。对标准工作液和样液等体积参插进样测定。在本方法条件下，福美双的参考保留时间为 9.0 min。福美双的液相色谱图参见附录 B 中图 B.1。

7.2.2 液相色谱-质谱/质谱法测定方法

7.2.2.1 色谱条件

色谱条件如下；

a) 色谱柱：Eclipse XDB-C_{18}，150 mm×2.1 mm(内径)，3.5 μm，或相当者；

b) 柱温：25 ℃；

c) 流动相：5 mmol/L 醋酸铵溶液(0.05% 甲酸)(4.9)-甲醇(50+50，体积比)；

d) 分析时间：8 min；

e) 流速 0.3 mL/min；

f) 进样量：10 μL。

7.2.2.2 质谱条件

质谱条件如下：

a) 离子化模式：电喷雾电离正离子模式(ESI^+)；

b) 质谱监测方式：多反应监测(MRM)。

c) 其他参考质谱条件参见表 A.1。

7.2.2.3 液相色谱-质谱/质谱测定

本方法采用外标法定量，定量离子为 $m/z=119.9$。为减少基质对定量的影响，需用空白样液来配制所使用的基质标准工作溶液。根据试样中福美双的含量情况，选定响应值适宜的标准工作溶液进行色谱分析。标准工作溶液和待测样液中福美双的响应值均应在仪器线性响应范围内。对标准工作液和样液等体积参插进样测定。在上述色谱条件下，福美双的保留时间约为 5.1 min 。福美双的多反应监测(MRM)离子色谱图参见图 C.1。

7.3 定性确证

按照上述条件测定样品和标准品，样品中待测物色谱峰保留时间与标准品对应的保留时间偏差应一致，允许偏差小于±2.5%；并且在扣除背景后的样品谱图中，各定性离子的相对丰度与浓度接近的同样条件下得到的标准溶液谱图相比，最大允许相对偏差不超过表 1 中规定的范围，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

7.4 空白实验

除不加待测组分外，按上述测定步骤进行。液相色谱-质谱/质谱测定所用空白基质溶液，是上述空

白溶液用0.05% 甲酸的甲醇溶液-水(50+50,体积比)(4.8)稀释所得。

7.5 回收率试验

阴性样品中添加标准溶液,按步骤7.1～7.2操作,测定后计算样品添加的回收率。

8 结果计算

按式(1)计算试样中福美双的含量,计算结果需扣除空白值。

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

式中:

X ——试样中福美双含量,单位为微克每千克(μg/kg);

A ——样液中福美双的峰面积;

c ——标准工作溶液中福美双的浓度,单位为纳克每毫升(ng/mL);

V ——样品溶液最终定容体积,单位为毫升(mL);

A_s——标准工作溶液中福美双的峰面积;

m ——样品溶液所代表最终试样的质量,单位为克(g)。

结果应保留三位有效数字。

9 测定低限与回收率

9.1 测定低限

液相色谱和液相色谱-质谱/质谱的测定低限:梨、芹菜、香蕉为0.5 mg/kg,苹果、白菜、西瓜、茄子为1.0 mg/kg。

9.2 回收率

9.2.1 福美双液相色谱回收率数据见表2。

表2 福美双的添加回收率数据

样品名称	添加水平/(mg/kg)	回收率范围/%
梨	0.5	80.2～96.8
	5	80.0～93.8
	10	79.0～89.2
芹菜	0.5	78.0～100
	5	87.0～101
	10	83.0～96.0
香蕉	0.5	58.0～75.5
	5.0	70.0～86.0
	10	82.0～100

表 2（续）

样品名称	添加水平/（mg/kg）	回收率范围/%
白菜	1.0	61.2～69.0
	5.0	71.1～76.7
	10	85.2～110
茄子	1.0	85.0～106
	5.0	75.8～88.0
	10	86.3～93.9
西瓜	1.0	53.0～74.1
	5.0	68.7～78.4
	10	78.2～91.0
苹果	1.0	63.2～78.6
	5.0	75.5～90.0
	10	78.3～92.1

9.2.2 福美双液相色谱-质谱/质谱回收率数据见表 3。

表 3 福美双的添加回收率数据

样品名称	添加水平/（mg/kg）	回收率范围/%
梨	0.5	72.0～86.0
	5	76.0～88.0
	10	82.2～92.2
芹菜	0.5	72.0～94.4
	5	78.0～99.2
	10	73.0～87.6
香蕉	0.5	66.1～91.5
	5.0	70.0～89.2
	10	72.3～92.5
白菜	1.0	63.0～94.1
	5.0	76.7～90.9
	10	97.0～118
茄子	1.0	80.5～92.1
	5.0	83.0～89.5
	10	73.0～92.6

表 3（续）

样品名称	添加水平/ （mg/kg）	回收率范围/ %
西瓜	1.0	52.2～76.8
	5.0	74.7～89.7
	10	67.3～103
苹果	1.0	57.3～73.4
	5.0	76.6～83.4
	10	80.3～90.2

附 录 A
（资料性附录）
参考质谱条件[1)]

参考质谱条件：

a） 扫描方式：正离子扫描；

b） 毛细管电压：4.5 kV；

c） 脱溶剂气温度 TEM：450 ℃；

d） 气帘气：68.95 kPa(10 psi)；

e） GS1：206.85 kPa(30 psi)；

f） GS2：482.65 kPa(70 psi)；

g） 其他质谱参数见表 A.1。

表 A.1 福美双的主要质谱参数

母离子 (m/z)	子离子 (m/z)	驻留时间/ ms	碰撞能量/ eV	出口电压/ V	去簇电压/ V	入口电压/ V
241.2	88.0[a]	100	18	4	33	4
	119.9	100	24	4	33	4
[a] 定量检测离子。						

1） 非商业性声明：附录 A 所列参考质谱条件是在 API 4000 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。

附　录　B
（资料性附录）
福美双的液相色谱图

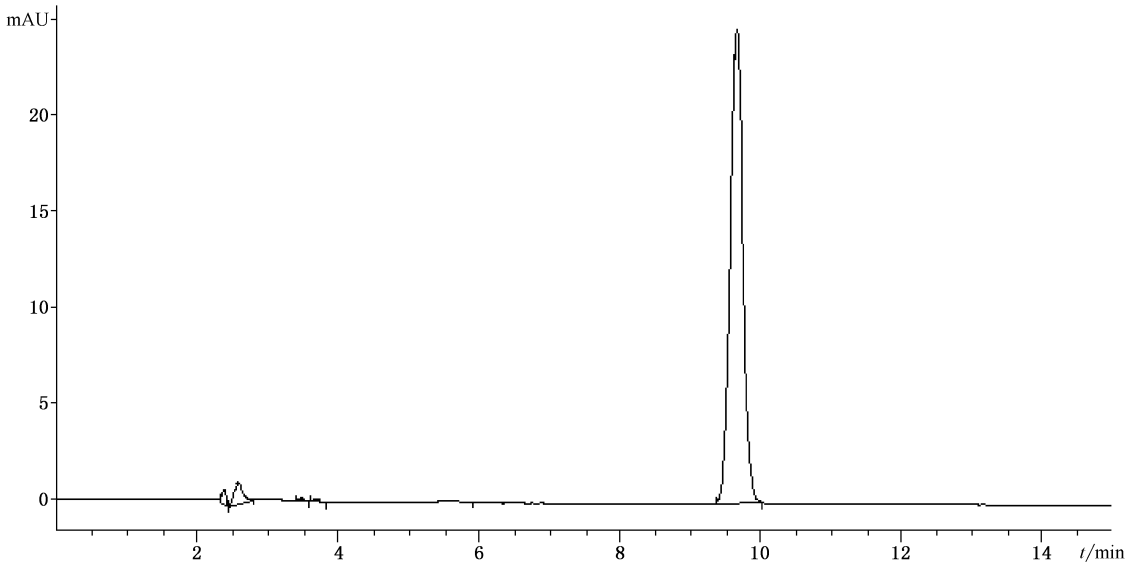
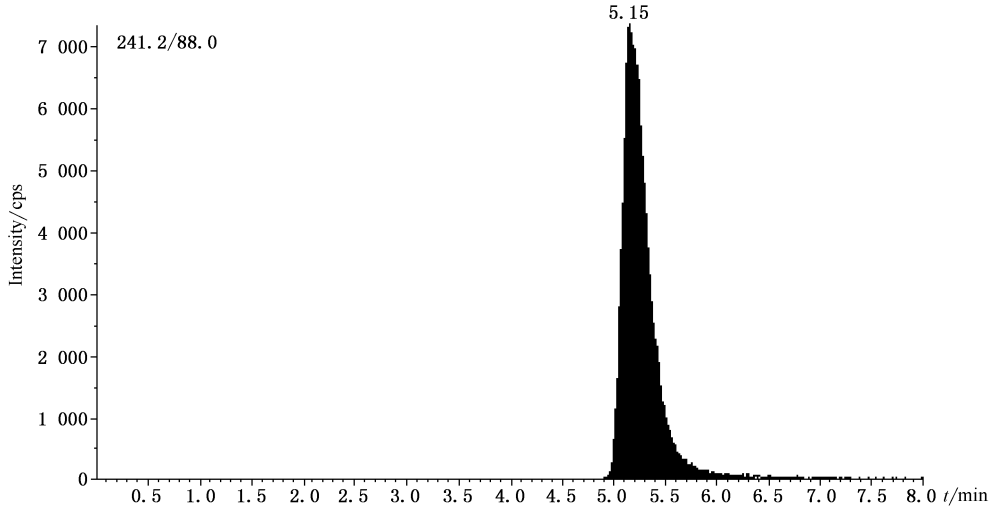
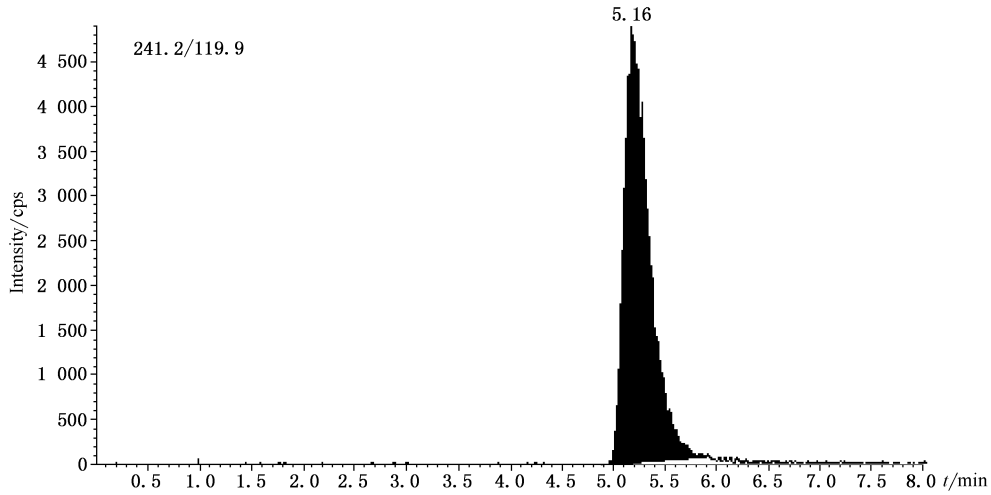


图 B.1　福美双的液相色谱图(浓度为 1 mg/kg)

附 录 C
（资料性附录）
福美双多反应监测（MRM）色谱图



a）



b）

图 C.1 福美双多反应监测（MRM）色谱图（浓度为 1 mg/kg）

Foreword

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

The standard is replale of SN 0525—1996 Method for the determination of thiram residues in fruits and vegetables for export.

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

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

The main drafters of this standard are Lin Li, Chen Bo, Hou Lexi, Xie Liqi, Zhou Yu, Yue Zhenfeng, Jin Baohui.

The standard replaces the standard previous versions published as:

—SN 0525—1996.

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

Determination of thiram residue in fruit and vegetable for export

1 Scope

This standard specifies the methods of determination by HPLC and HPLC-MS/MS of thiram residue.

This standard is applicable to the qualitative and quantitative determination of thiram in apple, pear, banana, watermelon, celery, eggplant and cabbage.

2 Normative references

The following documents is necessary for this standard. For dated refereces, 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 residue is extracted with acetonitrile, then concentrated and determined by HPLC and HPLC-MS/MS, quantified by external standard method.

4 Reagents and materials

Unless otherwise specified, all reagents should be of analytical grade; "Water" is deioned water according to GB/T 6682

4.1 Acetonitrile: HPLC grade.

4.2 Methanol: HPLC grade.

4.3 Methanol-water(30+70, *V*/*V*): Lntensive mixing 30 mL methanol(4.2) with 70 mL water.

4.4 Methanol-water(50+50, *V*/*V*): Intensive mixing 50 mL methanol(4.2) with 50 mL water.

4.5 Formic acid: HPLC grade.

4.6 Ammonium acetate: HPLC grade.

4.7 0.05% formic acid in methanol: Add 0.1 mL formic acid to 200 mL methanol and mix thoroughly.

4.8 0.05% formic acid in methanol-water (50 + 50, *V*/*V*): Intensive mixing 50 mL 0.05% formic acid in methanol (4.7) with 50 mL water.

4.9 5 mmol/L ammonium acetate solution (0.05% formic acid): Weigh 0.385 4 g ammonium acetate (4.6), dissolved with water, transferred into a 1 000 mL volumetric flask, add 0.5 mL formic acid and dilute with water to 1 000 mL.

4.10 Anhydrous sodium sulfate: Drying 4 h at 500 ℃, coolled to room temperature in the desiccator, stored in sealed container before use.

4.11 Thiram standard: Thiram, purity>99%, CAS No.: 137-26-8.

4.12 Standard stock solution: Accurately weigh 12.5 mg standard (4.11), dissolved in 0.05% formic acid in methanol (4.7) and diluted to 25 mL to make a standard stock solution of 500 mg/L in concentration. The solution can be preserved at −18 ℃ for 3 months.

4.13 Standard working solution: HPLC method: according to the requirement, accurately measure an adequate volume of standard stock solution, and diluted with 0.05% formic acid in methanol-water (50 + 50, *V*/*V*) (4.8) before use; HPLC-MS/MS: according to the requirement, accurately measure an adequate volume of standard stock solution, diluted with blank matrix extract before use.

4.14 Membrane: 0.45 μm.

5 Apparatus and equipment

5.1 HPLC, equipped with DAD.

5.2 HPLC-MS/MS, equipped with ESI.

5.3 Vortex mixer.

5.4 Oscillator.

5.5 Evaporator.

5.6 Balances: 0.1 mg and 0.001 g.

5.7 Centrifuge, refrigerated to 4 ℃, 5 000 r/min.

5.8 Polypropylene centrifuge tube: 15 mL and 50 mL, with stopper.

6 Sample preparation and storage

Take approximately 500 g of representative sample. Collect the edible parts(do not wash with water) and cut into minces, crush with a crusher into pulp, mix thoroughly and divided into two parts. Put into clean containers. Seal and label, stored at −18 ℃.

7 Procedure

7.1 Extraction

7.1.1 Accurately weigh 4 g of the test sample(accurate to 0.01 g) in 50 mL centrifuge tube, add 30 mL acetonitril(4.1), blend for 30 s in vortex mixer, oscillating extract for 10 min, refrigerated centrifuge for 5 min(5 000 r/min). Pass the upper extract through anhydrous sodium sulfate(4.10) to a clean heart-shape bottle. Re-extract the test sample with 30 mL acetronitrile and combine the supernatant.

7.1.2 Rotary Evaporate the extract to 2 mL or so, transfer the remain solution to a clean 15 mL centrifuge tube, wash the heart-shape bottle with 1.5 mL methanol-water(30+70, *V*/*V*)(4.3), combine the extract, dilute with Methanol-water(30+70, *V*/*V*)(4.3) to 4 mL. Filter sample through a 0.45 μm membrane for HPLC analysis.

7.1.3 Dilute the extract from 7.1.2 to 20 times with 0.05% formic acid in methanol-water(50+50, *V*/*V*)(4.8), then analysed by HPLC-MS/MS.

7.2 Determination

7.2.1 HPLC operating conditions

7.2.1.1 HPLC parameters

HPLC parameters as follows:

a) Column: Eclipse XDB-C_{18}, 250 mm × 4.6 mm(i.d.), 5 μm, or equivalent.

b) Column temperature: 25 ℃.

c) Mobile phase: methanol-water(50+50, *V*/*V*).

d) Analyse time:15 min.

e) Flow rate:1.0 mL/min.

f) Detecting wavelenth:270 nm.

g) Injection volume:50 μL.

7.2.1.2 HPLC detection

Prepare standard solution at appropriate concentrations according to the analyte in sample extracts. The response of thiram residue in standard work solution and extract should not exceed the linear response range. Standard work solutions and sample extract should be injected with same volume in turn. The referenced retention times for thiram is 9.0 min. Annex B is the HPLC chromatogram of thiram standard solution.

7.2.2 HPLC-MS/MS detection method

7.2.2.1 HPLC parametres

HPLC parametres as follows:

a) Column:Eclipse XDB-C_{18},150 mm×4.6 mm(i.d.),3.5 μm,or equivalent.

b) Column temperature:25 ℃.

c) Mobile phase:5 mmol/L Ammonium acetate solution(0.05% formic acid)(4.9)-water(50+50, *V*/*V*).

d) Analyse time:8 min.

e) Flow rate:0.3 mL/min.

f) Injection volume:10 μL.

7.2.2.2 MS parametres

MS parametres as follows:

a) Ion source:ESI^+;

b) Detection mode:MRM;

c) Other operating conditions are listed in annex A.

7.2.2.3 HPLC-MS/MS determination

Thiram residue is quantified by external standard with quantification ion of m/z = 119.9. Use blank matrix extract to prepare standard solution, in order to minimize the influence of matrix to quantification. Prepare standard solution at appropriate concentrations according to the analyte in sample extracts. The response of thiram residue in standard work solution and extract should not exceed the linear response range. Standard work solutions and sample extract should be injected with same volume in turn. The referenced retention times for thiram is 5.1 min. MRM Chromatogram of thiram is shown as Figure. C.1.

7.3 Qualitative determination

If the deviation of retention time of analyte between test sample and standard solution is within ±2.5 % under the same experiment conditions, and the difference of relative ion ratio of analyte between test sample and standard solution is also within the error allowed(the max deviation allowed for relative ion ratio are listed in table 1), corresponding analyte would be considered to be in the sample.

Table 1—Max deviation allowed for relative ion ratio in qualitative determination

Relative ion ratio/%	>50	>20～50	>10～20	≤10
Max deviation allowed/%	±20	±25	±30	±50

7.4 Blank test

The operation of the blank test is the same as that described in the method of determination, but with omission of sample addition. Blank matrix solution used in HPLC-MS/MS detection is the blank solution metioned above diluted by 0.05% formic acid in methanol-water(50+50, V/V)(4.8).

7.5 Recovery test

Standard spiked blank sample is operated as 7.1～7.2, and then calculate the recovery.

8 Calculation and expression of result

The calculation of thiram residue content in the sample is according to the following formula, the blank value should be subtracted from the result of calculation:

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

Where:

X —the residue content of thiram in the test sample, μg/kg;

A —the peak area of thiram residue in sample extract;

c —the concentration of thiram in standard solution, ng/mL;

V —the final volume of sample solution, mL;

A_s—the peak area of thiram residue in standard solution;

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

Result should keep in three-digit significant figure.

9 Limit of quantification and recovery

9.1 Limit of quantification

The LOQ of HPLC and HPLC-MS/MS: Pear, celery, banana: 0.5 mg/kg;

Apple, cabbage, watermelon and eggplant: 1.0 mg/kg.

9.2 Recovy

9.2.1 The recovery of thiram HPLC method is shown in table 2.

Table 2—Range of recovery of thiram using HPLC method

Sample type	Spike level/ mg/kg	Recovery/ %
pear	0.5	80.2～96.8
	5	80.0～93.8
	10	79.0～89.2
celery	0.5	78.0～100
	5	87.0～101
	10	83.0～96.0

Table 2（continued）

Sample type	Spike level/ mg/kg	Recovery/ %
banana	0.5	58.0～75.5
	5.0	70.0～86.0
	10	82.0～100
cabbage	1.0	61.2～69.0
	5.0	71.1～76.7
	10	85.2～110
eggplant	1.0	85.0～106
	5.0	75.8～88.0
	10	86.3～93.9
watermelon	1.0	53.0～74.1
	5.0	68.7～78.4
	10	78.2～91.0
apple	1.0	63.2～78.6
	5.0	75.5～90.0
	10	78.3～92.1

9.2.2 The recovery of thiram HPLC-MS/MS method is shown in table 3.

Table 3—Range of recovery of thiram using HPLC-MS/MS method

Sample type	Spike level/ （mg/kg）	Recovery/%
pear	0.5	72.0～86.0
	5	76.0～88.0
	10	82.2～92.2
celery	0.5	72.0～94.4
	5	78.0～99.2
	10	73.0～87.6
banana	0.5	66.1～91.5
	5.0	70.0～89.2
	10	72.3～92.5
cabbage	1.0	63.0～94.1
	5.0	76.7～90.9
	10	97.0～118

Table 3（continued）

Sample type	Spike level/ mg/kg	Recovery/ %
eggplant	1.0	80.5～92.1
	5.0	83.0～89.5
	10	73.0～92.6
watermelon	1.0	52.2～76.8
	5.0	74.7～89.7
	10	67.3～103
apple	1.0	57.3～73.4
	5.0	76.6～83.4
	10	80.3～90.2

Annex A
(Informative)
Operating conditions of mass spectrometer[1)]

Operating conditions of mass spectrometer are as follows:

a) Ionization mode:ESI^+.

b) Ionspray voltage:4.5 kV.

c) Desolvation gas temperature:450 ℃.

d) Desolvation gas rate:68.95 kPa(10 psi).

e) GS1:206.85 kPa(30 psi).

f) GS2:482.65 kPa(70 psi).

g) Monitor mode:Multiple reaction monitor,condition setup can be found in table A.1.

Table A.1—Main MS parameters of thiram

Parent (m/z)	Daughter (m/z)	Dwell time/ ms	CE/ eV	CXP/V	DP/V	EP/V
241.2	88.0[a]	100	18	4	33	4
	119.9	100	24	4	33	4
[a] ions for quantitative determination.						

1) Non-commercial statement:the reference mass parameters in Annex A are accomplished by API 4000 LC-MS/MS, the equipment and its type involved in the standard method is only for reference and not related to any commercial aim,and the analysts are encouraged to use equipments of different corporation or different type.

Annex B
(Informative)
HPLC chromatogram of thiram

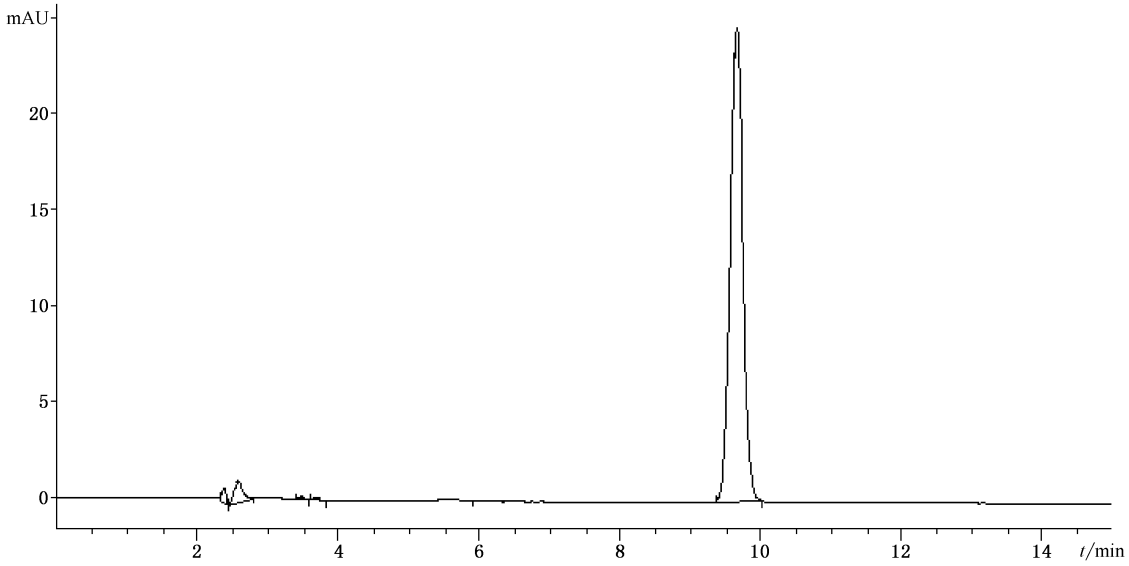
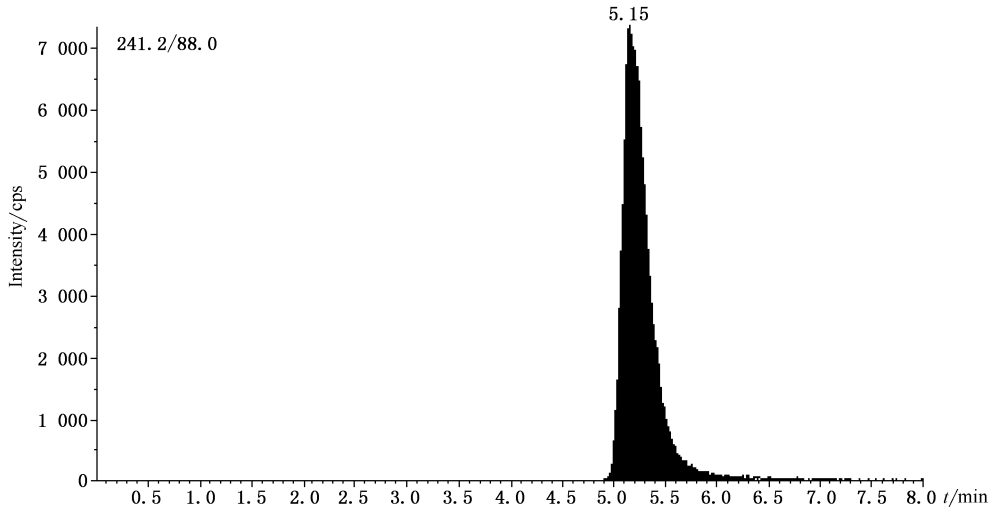
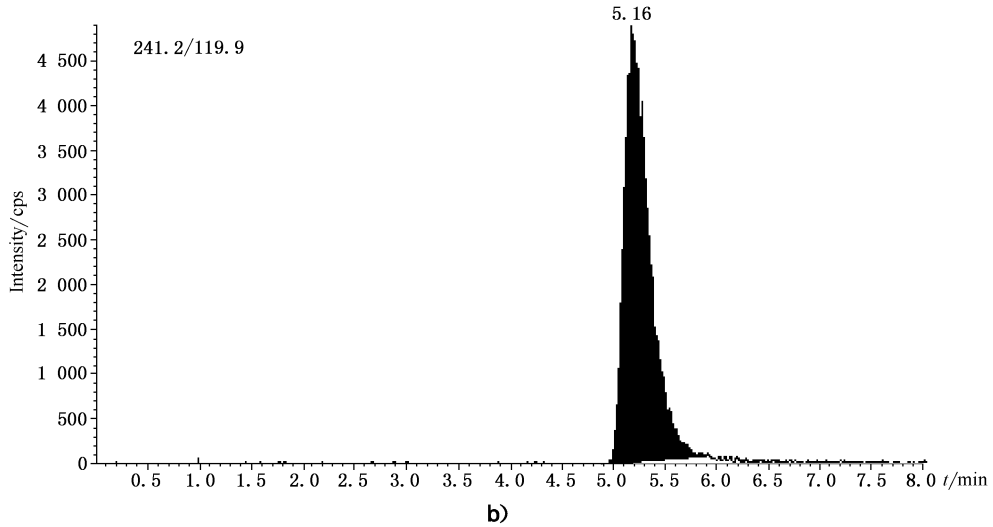


Figure B. 1—HPLC chromatogram of thiram(1 mg/kg)

Annex C
(Informative)
MRM Chromatogram of thiram



a)



b)

Figure C.1—MRM Chromatogram of thiram(1 mg/kg)