SN

中华人民共和国出入境检验检疫行业标准

SN/T 0527—2012
代替 SN 0527—1996

出口粮谷中甲硫威(灭虫威)及代谢物残留量的检测方法　液相色谱-质谱/质谱法

Determination of methiocarb and its metabolite residues in cereals for export—LC-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 0527—1996《出口粮谷中灭虫威残留量检验方法》。

本标准与 SN 0527—1996 相比，主要技术变化如下：

——标准适用范围由糙米扩展到大米、玉米、糙米、大麦和小麦；

——将甲硫威代谢物：甲硫威亚砜、甲硫威砜分别进行测定；

——提取液改为乙腈；

——略去了抽样步骤。

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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、上海质量监督检验技术研究院。

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本标准所代替标准的历次版本发布情况为：

——SN 0527—1996。

出口粮谷中甲硫威(灭虫威)及代谢物残留量的检测方法　液相色谱-质谱/质谱法

1　范围

本标准规定了出口粮谷中甲硫威及代谢物(甲硫威亚砜和甲硫威砜)残留量的液相色谱-质谱/质谱测定方法。

本标准适用于出口大米、玉米、糙米、大麦和小麦中甲硫威、甲硫威亚砜和甲硫威砜残留量的定性定量测定。

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　方法提要

采用乙腈提取试样中残留的甲硫威及代谢物,提取液经无水硫酸镁脱水和石墨化碳净化后,浓缩,采用液相色谱-质谱/质谱检测,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　乙腈:色谱纯。

4.2　甲酸:色谱纯。

4.3　甲醇:色谱纯。

4.4　甲酸胺:色谱纯。

4.5　无水硫酸镁:优级纯。

4.6　乙腈溶液(1+1,体积比):量取 50 mL 乙腈和 50 mL 水,混合均匀。

4.7　20 mmol/L 甲酸铵水溶液(含 0.1%甲酸):准确称取 1.261 2 g 甲酸铵,加入 1 mL 甲酸,用水溶解并转移至 1 000 mL 容量瓶中,定容至刻度。

4.8　0.1%甲酸溶液:1 mL 甲酸溶解于水中,并定容至 1 L。

4.9　农药标准物质:甲硫威(Methiocarb,CAS 号:2032-65-1),甲硫威亚砜(Methiocarb sulfoxid,CAS 号:2635-10-1),甲硫威砜(Methiocarb sulfone,CAS 号:2179-25-1)纯度均大于 98%。

4.10　标准储备液的配制:分别准确称取适量的标准物质,用甲醇配制成浓度为 100 mg/L 的标准储备溶液。该溶液在-18 ℃冰箱中保存。有效期为 1 个月。

4.11　混合标准中间溶液的配制:分别准确移取一定体积的标准储备液,用甲醇稀释成 10.0 mg/L 度的混合标准中间工作液。该溶液应配制于棕色容量瓶中,-18 ℃以下避光可保存 5 d。

4.12　混合标准工作溶液的配制:分别移取适量混合标准中间工作溶液,用空白样品基质溶液稀释,使

所得该溶液为混合标准使用曲线工作溶液，现用现配。

4.13 空白样品基质溶液：称取均质空白样品，按 7.1 和 7.2 步骤进行操作。

4.14 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(ESI)。

5.2 点子天平：感量分别为 0.01 g，0.001 g。

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：25 mL。

5.7 玻璃试管：10 mL，具刻度。

5.8 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。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

称取均质样品 1 g(精确至 0.01 g)，置于 25 mL 聚丙烯离心管中，加入 10 mL 乙腈，涡旋混合 2 min。超声振荡 20 min，在 3 000 r/min 下离心 5 min，取出上清液于另一 50 mL 聚丙烯离心管中。残渣再用 10 mL 乙腈同上操作提取一次。合并上清液于 50 mL 聚丙烯离心管中，混匀，待净化。

7.2 净化

在 7.1 中所得溶液中加入 0.5 g 无水硫酸镁和 0.5 g 石墨化碳。经涡旋混合 2 min 后，在 3 000 r/min 下离心 5 min，将上清液转移到 100 mL 鸡心瓶中。于 40 ℃水浴上减压旋转蒸发至近干。加入 1 mL 乙腈溶液(1+1，体积比)，经涡旋混合 2 min 后，通过 0.22 μm 有机滤膜过滤。及时供液相色谱-质谱/质谱仪测定。

7.3 测定

7.3.1 液相色谱条件

液相色谱条件如下：

a) 色谱柱：C_{18}色谱柱，长 50 mm，内径 2.0 mm，粒径 3.0 μm，或相当者；

b） 柱温：30 ℃；

c） 进样量：20 μL；

d） 流动相、流速及梯度洗脱条件见表 1。

表 1 流动相、流速及梯度洗脱条件

时间/ min	流速/ (mL/min)	20 mmol/L 甲酸铵水溶液(含 0.1%甲酸)/ %	乙腈/ %
0	0.40	50	50
3	0.40	5	95
5.5	0.40	5	95
10.0	0.40	50	50

7.3.2 质谱条件

质谱条件如下：

a） 离子化模式：电喷雾离子源(ESI)，正离子模式；

b） 质谱扫描方式：多反应监测(MRM)；

c） 其他参考质谱条件参见附录 A。

7.3.3 液相色谱-串联质谱测定

7.3.3.1 定性测定

在相同实验条件下，样品中待测物质的保留时间，与基质标准溶液的保留时间偏差在±2.5%之内；每种化合物的质谱定性离子至少应包括一个母离子和两个子离子，且样品中各组分定性离子的相对丰度与浓度接近的基质混合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 2 规定的范围，则可判定为样品中存在对应的待测物。

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
允许的最大偏差/%	±20	±25	±30	±50

7.3.3.2 定量测定

在仪器最佳工作条件下，对混合基质标准工作溶液进样，以峰面积为纵坐标，混合基质标准工作溶液浓度为横坐标绘制标准工作曲线，用标准工作曲线对样品进行定量，样品溶液中待测物的响应值均应在仪器测定的线性范围内。甲硫威及代谢物标准溶液的多反应监测(MRM)色谱图参见图 B.1。

8 结果计算和表述

按式(1)计算试样中检测目标物残留量(mg/kg)：

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

式中：

X_i ——试样中甲硫威及代谢物残留含量，单位为毫克每千克(mg/kg)；

A_i ——样液中甲硫威及代谢物的峰面积；

c_i ——标准工作溶液中甲硫威及代谢物的浓度，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)；

A_{si}——标准工作溶液中甲硫威及代谢物的峰面积；

m ——最终样液代表的试样量，单位为克(g)。

9 测定低限和回收率

9.1 测定低限

本方法的测定低限：甲硫威 10.0 μg/kg，甲硫威亚砜 10.0 μg/kg，甲硫威砜 10.0 μg/kg。

9.2 回收率

在三个添加浓度范围内，大米、玉米、糙米、大麦和小麦中甲硫威及代谢物的回收率数据参见附录 C。

附 录 A
（资料性附录）
LC-MS/MS 参考质谱条件[1)]

质谱条件：

a） 毛细管电压：4 000 V；

b） 雾化气流速：9 L/min；

c） 碰撞气：氮气；

d） 离子源温度：350 ℃；

e） 雾化气压力：310.28 kPa(45 psi)；

f） ΔEMV：300 V；

g） 定性离子对、定量离子对，采集时间、去簇电压及碰撞电压见表 A.1。

h） 质谱扫描方式：多反应监测(MRM)。

表 A.1 甲硫威及代谢物测定的质谱参数

电离模式	化合物名称	参考保留时间/min	监测离子 m/z	采集时间/ms	去簇电压/V	碰撞电压/V
ESI+	甲硫威亚砜	1.29	242.1>185.1[a]	200	106	8
			242.1 >122.1	200	106	28
	甲硫威砜	1.76	258.1 >122.1[a]	200	70	16
			258.1> 107.0	200	70	40
	甲硫威	4.44	226.1 >169.1[a]	200	70	4
			226.1> 121.1	200	70	16

[a] 为定量离子。

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。

附 录 B
（资料性附录）
标准的多反应监测（MRM）色谱图

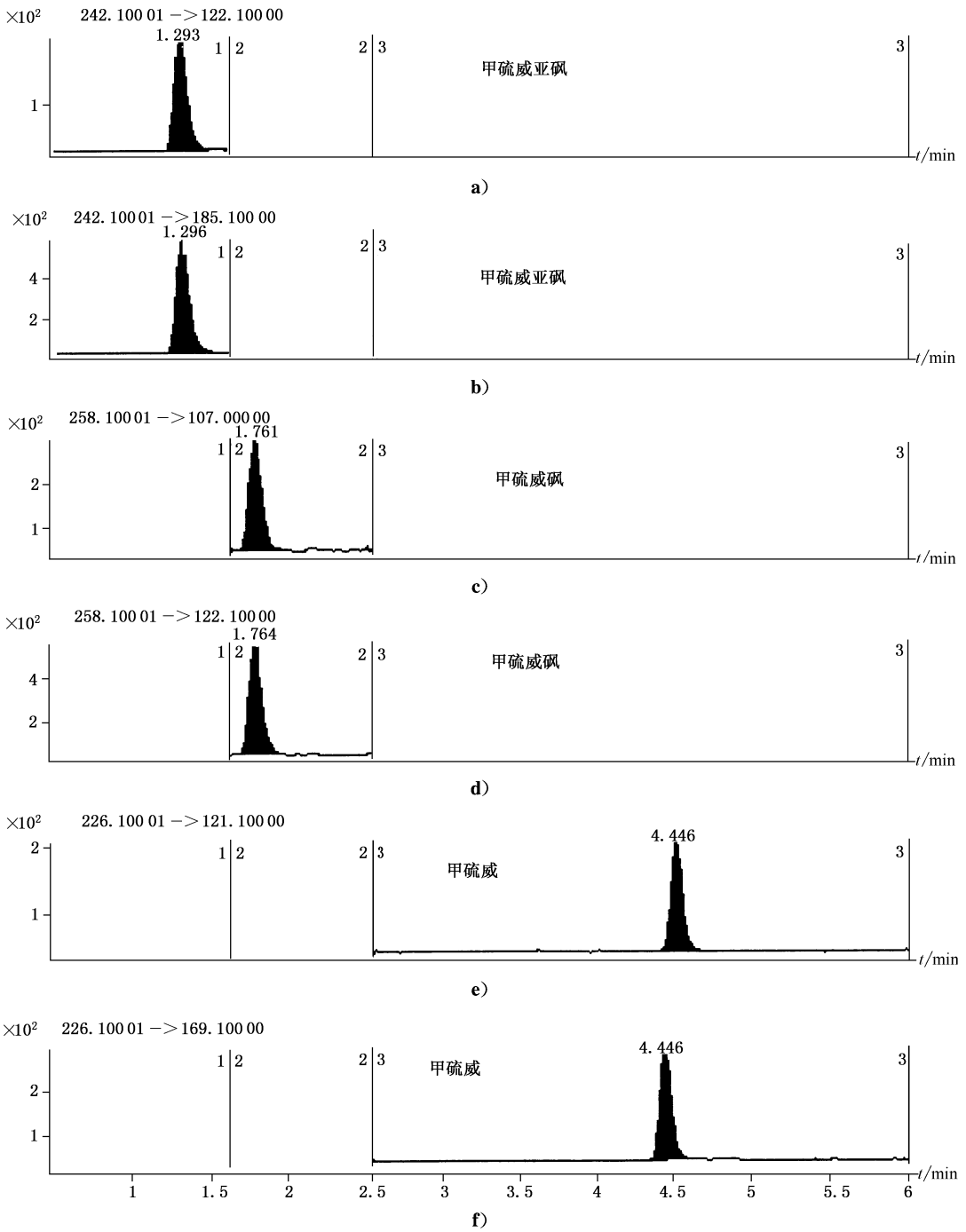

图 B.1 甲硫威及代谢物（10.0 μg /mL）标准溶液的多反应监测（MRM）图

附 录 C
（资料性附录）
回 收 率

表 C.1 大米、玉米、糙米、小麦和大麦中甲硫威及代谢物不同添加水平回收率数据

化合物名称	甲硫威亚砜		甲硫威砜		甲硫威	
	添加水平/（μg/kg）	回收率范围/%	添加水平/（μg/kg）	回收率范围/%	添加水平/（μg/kg）	回收率范围/%
大米	10.0	86.6～119	10.0	76.3～96.7	10.0	75.6～99.6
	50.0	77.0～99.5	50.0	80.3 ～100	50.0	76.6～99.1
	100.0	79.9 ～112	100.0	77.9～99.1	100.0	75.6 ～92.3
玉米	10.0	76.7～102	10.0	74.6～113	10.0	78.9 ～111
	50.0	77.9～102	50.0	74.9 ～101	50.0	77.6 ～102
	100.0	77.8～102	100.0	79.0 ～105	100.0	76.4～89.6
大麦	10.0	79.4～100	10.0	75.1 ～99.9	10.0	78.3 ～86.5
	50.0	77.6～100	50.0	78.7～101	50.0	82.5～98.6
	100.0	76.4～94.3	100.0	77.5～100	100.0	76.6 ～92.1
小麦	10.0	76.7～99.0	10.0	75.2～103	10.0	76.2 ～93.4
	50.0	76.9～101	50.0	77.9 ～102	50.0	80.0 ～101
	100.0	75.2～96.6	100.0	86.4 ～119	100.0	75.6 ～90.4
糙米	10.0	78.6～95.6	10.0	77.8～95.7	10.0	75.4 ～97.8
	50.0	79.7～107	50.0	82.4～113	50.0	80.0～104
	100.0	76.2～104	100.0	75.9～98.3	100.0	76.1 ～85.6

Foreword

The standard is drafted according to GB/T 1. 1—2009 principle.

The standard is replace of SN 0527—1996 Method for the determination of methiocarb residues in cereals for export.

The main improvement from SN 0527—1996:

—This standard is applicable to the determination of residue content of methiocarb and methiocarb-sulfone and methiocarb-sulfoxide in rice,corn,brown rice,barley and wheat l for export.

—The metabolite residue is also determined.

—The test sample is extracted with acetonitrile.

—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 Inspection and Technical Research.

The main drafters of this standard are Yang huiqin,Lin yikan,Yi xionghai,Zhu jian,Guo dehua,Den gxiaojun,Chen di.

The standard replaces the standard previous versions published as:

—SN 0527—1996.

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

Determination of methiocarb and its metabolism residues in cereals for export—LC-MS/MS method

1 Scope

This standard specifies the method of testing methiocarb and metabolism (methiocarb-sulfone and methiocarb-sulfoxide) residue in cereals for export by LC-MS/MS method.

This standard is applicable to the determination of residue content of methiocarb and methiocarb-sulfone and methiocarb-sulfoxide in rice, corn, brown rice, barley and wheat I for export.

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 Principle

The test sample is extracted with acetonitrile, cleaned up by anhydrous magnesium sulfate and graphite powder. The residues are 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 double distilled water.

4.1 Acetonitrile: HPLC grade.

4.2 Methanol: HPLC grade.

4.3 Formic acid: HPLC grade.

4.4 Ammonium formate .

4.5 Anhydrous magnesium sulfate.

4.6 Acetonitrile:water(1+1, *V*/*V*):50 mL acetonitrile and 50 mL distilled water, mixed well.

4.7 20 mmol/L ammonium formate(contain 0.1% formic acid):Weigh ammonium acetate 1.261 2 g, add 1 mL formic acid. Dissolve with water and dilute to 1 000 mL.

4.8 0.1% formic acid:1 mL formic acid dissolve water and dilute to 1 L.

4.9 Methiocarb(CAS No.:2032-65-1)and methiocarb-sulfone (CAS No.:2635-10-1)and methiocarb-sulfoxide(CAS No.:2179-25-1):Purity≥98%.

4.10 Standard stock solution:Accurately weigh certain amount of every standards and dissolve it with methanol to make the standard stock solution of 100 mg/L. The solution is stored in a refrigerator at −18 ℃. This standard can be used within one month.

4.11 Standard medium stock solution:accurately weigh certain amount of every stock solution and dissolve it with methanol to make the standard stock solution to certain mount. The solution is stored in a refrigerator at −18 ℃. This standard can be used within 5 d.

4.12 Standard working solution: dilute the standard medium stock solution with blank sample extracts to the required concentration to make the standard working solution.

4.13 Blank sample extracts:Weigh of the test sample, for 7.1 and 7.2 procedure.

4.14 Membrane filter:0.22 μm.

5 Apparatus and equipment

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, 0.001 g.

5.3 Vortex mixer.

5.4 Solid phase extraction set.

5.5 Nitrogen evaporator.

5.6 Centrifuge tube:25 mL Electronic balanc.

5.7 Glass tube:10 mL with graduation.

5.8 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 shall be stored at room temperature. While sampling and preparing sample, please avoid contamination or any factors that may change residue content.

7 Procedure

7.1 Extraction

Weigh 1 g(accurate to 0.01 g) of the test sample into a 25 mL centrifuge tube. Add 10 mL acetonitrile. Homogenize for 2 min. Ultrsonic extract for 20 min and centrifuge for 5 min at 3 000 r/min. Take the uplayer of acetonitrile to another tube, the residue was reextracted with 10 mL acetonitrile. Combine all the extracts into 50 mL concentrate bottle.

7.2 Cleaning-up

Add 0.5 g anhydrous magnesium sulfate and 0.5 g graphite powder. Vortex mix for 2 min and centrifuge for 5 min at 3 000 r/min. Transfer the uplayer into 100 mL concentrate bottle, Evaporate the extract to dryness under 40 ℃. Add 1 mL sample dilute solution to redissolve the extracts. Vortex mix for 2 min. Filter the solution with 0.22 μm membrane The solution is ready for LC-MS/MS determination.

7.3 Determination

7.3.1 LC operating conditions

LC 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) Column temperature:30 ℃;

c) Injector volume:20 μL;

d) Mobile phase:acetonitrile;the elution gradient is listed in table 1.

Table 1—Elution gradient

Time/ min	Flow rate/ (mL/min)	20 mMammonium formate(contain 0.1% formic acid)/ %	Acetonitrile/ %
0	0.40	50	50
3.0	0.40	5	95
5.5	0.40	5	95
10.0	0.40	50	50

7.3.2 MS/MS operating conditions

MS/MS operating conditions are as follows:

a) Scanning model:positive ion(ESI);

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

c) Referenced conditions seen annex A.

7.3.3 Confirmation of LC-MS/MS

7.3.3.1 LC-MS/MS Determination

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 transiyions 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, then 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

7.3.3.2 LC-MS/MS Determination

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. Reconstituted(MRM)chromatogram of standard working solution is listed in Figure B.1.

8 Calculation and expression of the result

Calculate the content of methiocarb residue in the test sample by LC-MS/MS data processor or according to the followed formula.

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

where:

X_i —the residue content of methiocarb and its metabolism in the test sample, mg/kg;

A_i —the peak area of methiocarb and its metabolism in the sample solution;

c_i —the total concentration of methiocarb and its metabolism in the standard working solution, μg/mL;

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

A_{si} —the total peak area of methiocarb and its metabolism in the standard working solution;

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

9 Detection limit and recovery

9.1 Limit of determination

The method detection limit for methiocarb, methiocarb-sulfone and methiocarb-sulfoxide is 10.0 μg/kg, 10.0 μg/kg and 10.0 μg/kg respectively.

9.2 Range of fortification and recovery

The range of fortification and recovery of this method is shown in annex C.

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

Referenced conditions are as follows:

a) Capillary voltage:4 000 V;

b) Drying gas flow:9 L/min;

c) Nebulizer gas:N_2;

d) Drying gas temperature:350 ℃;

e) Nebulizer pressure:310.28 kPa(45 psi);

f) ΔEMV:300 V;

g) Precursor ions, fragementor(V), Collision energy(V), Transitons for confirmation(*m/z*) and Transitons for quantititation (*m/z*), see table A.1;

h) Monitoring model:multiple reaction monitoring(MRM).

Table A.1—Transition for confirmation and quantitiation

Scanning model	Compound	RT/min	MRM IONS (*m/z*)	Dwell time/ ms	Fragementor/ V	CE/ V
ESI+	methiocarb-sulfoxide	1.29	242.1>185.1[a]	200	106	8
			242.1>122.1	200	106	28
	methiocarb-sulfone	1.76	258.1>122.1[a]	200	70	16
			258.1>107.0	200	70	40
	methiocarb	4.44	226.1>169.1[a]	200	70	4
			226.1>121.1	200	70	16
[a] represents the quantitative transition.						

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.

Annex B
(informative)
MRM Chromatogram of the standards

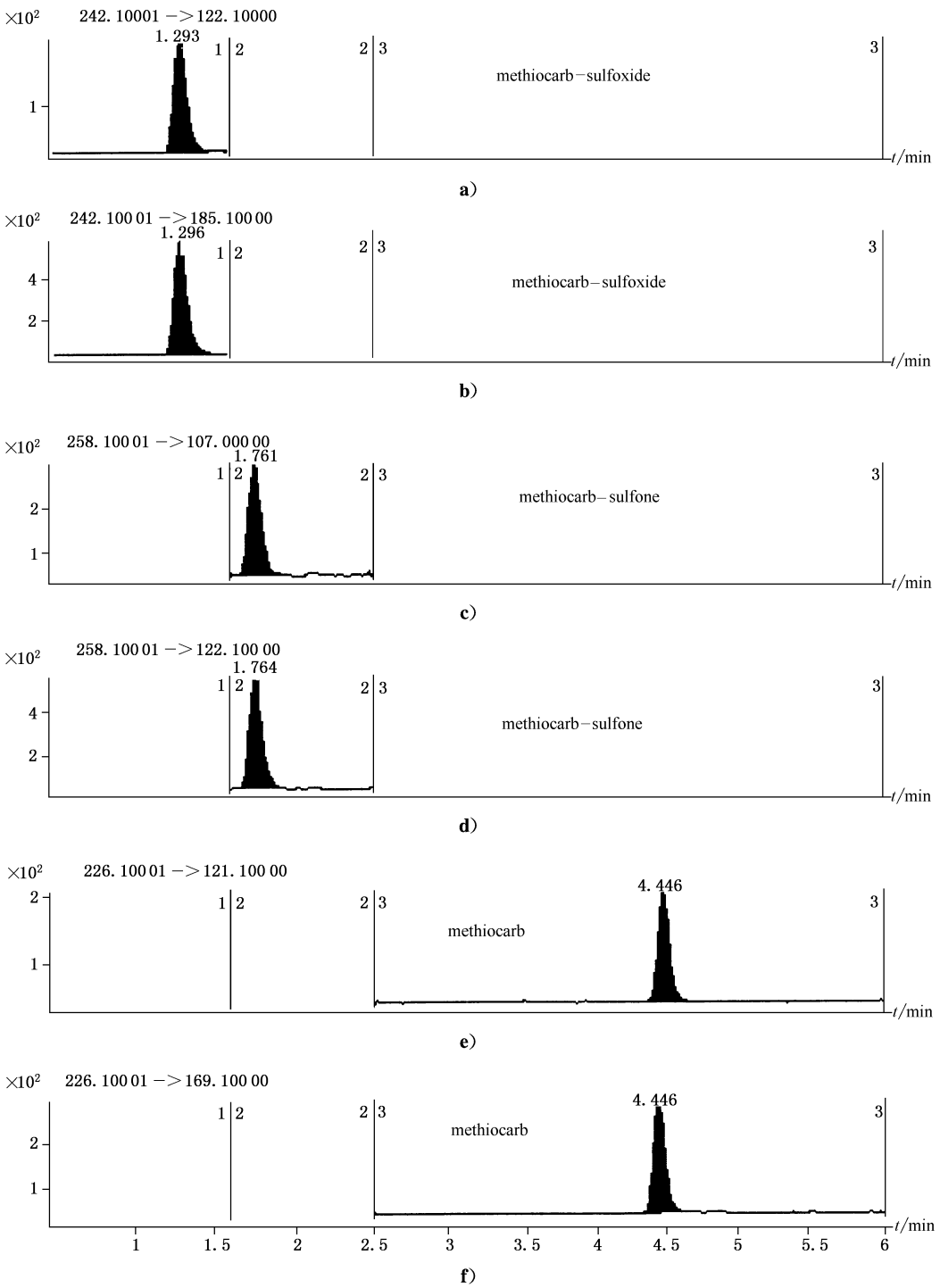

Figure B. 1—MRM chromatogram of methiocarb and metabolism(methiocarb-sulfone and methiocarb-sulfoxide)standards(10. 0 μg /mL)

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Annex C
(informative)
Recouery

Table C. 1—Range of fortification and recovery of this method

Compound	Methiocarb-sulfoxide		Methiocarb-sulfone		Methiocarb	
	Spike level/ (μg/kg)	Recovery/ %	Spike leve/ (μg/kg)	Recovery/ %	Spike level/ (μg/kg)	Recovery/ %
rice	10.0	86.6～119	10.0	76.3～96.7	10.0	75.6～99.6
	50.0	77.0～99.5	50.0	80.3 ～100	50.0	76.6～99.1
	100.0	79.9 ～112	100.0	77.9～99.1	100.0	75.6 ～92.3
corn	10.0	76.7～102	10.0	74.6～113	10.0	78.9 ～111
	50.0	77.9～102	50.0	74.9 ～101	50.0	77.6 ～102
	100.0	77.8～102	100.0	79.0 ～105	100.0	76.4～89.6
brown rice	10.0	79.4～100	10.0	75.1 ～99.9	10.0	78.3 ～86.5
	50.0	77.6～100	50.0	78.7～101	50.0	82.5～98.6
	100.0	76.4～94.3	100.0	77.5～100	100.0	76.6 ～92.1
barley	10.0	76.7～99.0	10.0	75.2～103	10.0	76.2 ～93.4
	50.0	76.9～101	50.0	77.9 ～102	50.0	80.0 ～101
	100.0	75.2～96.6	100.0	86.4 ～119	100.0	75.6 ～90.4
wheat	10.0	78.6～95.6	10.0	77.8～95.7	10.0	75.4 ～97.8
	50.0	79.7～107	50.0	82.4～113	50.0	80.0～104
	100.0	76.2～104	100.0	75.9～98.3	100.0	76.1 ～85.6