

中华人民共和国出入境检验检疫行业标准

SN/T 5440—2022

出口食品中双炔酰菌胺、噻唑菌胺、吡唑磺 菌胺等多种酰胺类杀菌剂残留量的测定 液相色谱-质谱/质谱法

Determination of multiple amide fungicides including
mandipropamid, ethaboxam, amisulbrom etc in food for
export—LC-MS/MS method

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前 言

本文件按照 GB/T 1.1—2020《标准化工作导则 第 1 部分：标准化文件的结构和起草规则》要求进行编制。

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。

本文件由中华人民共和国海关总署提出并归口。

本文件起草单位：中华人民共和国杭州海关技术中心。

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以正式出版文本为准

出口食品中双炔酰菌胺、噻唑菌胺、吡唑磺菌胺、硅噻菌胺、吡噻菌胺等多种酰胺类杀菌剂残留量的测定

液相色谱-质谱/质谱法

1 范围

本文件规定了出口食品中双炔酰菌胺、噻唑菌胺、吡唑磺菌胺、硅噻菌胺、吡噻菌胺残留量的液相色谱-质谱/质谱测定方法。

本文件适用于葡萄、白菜、芹菜、黄瓜、番茄、草莓、花生、猪肉中双炔酰菌胺、噻唑菌胺、吡唑磺菌胺、硅噻菌胺、吡噻菌胺残留量的定量测定和确证。

2 规范性引用文件

下列文件中的内容通过文中的规范性引用而构成本文件必不可少的条款。其中，注日期的引用文件，仅该日期对应的版本适用于本文件；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 术语和定义

本文件没有需要界定的术语和定义。

4 方法提要

试样中残留的双炔酰菌胺、噻唑菌胺、吡唑磺菌胺、硅噻菌胺、吡噻菌胺经1%乙酸乙腈溶液均质提取后，采用基质分散固相萃取净化，液相色谱-质谱/质谱测定，外标法定量。

5 试剂和材料

除特殊说明外，所有试剂均为分析纯，水为符合GB/T 6682规定的一级水。

5.1 乙腈：色谱纯。

5.2 甲醇：色谱纯。

5.3 甲酸：色谱纯。

5.4 1%乙酸乙腈溶液：取1.0 mL乙酸，用乙腈稀释并定容至100 mL。

5.5 0.15%甲酸溶液：取1.5 mL甲酸，用水稀释并定容至1 000 mL。

5.6 标准物质：双炔酰菌胺、噻唑菌胺、吡唑磺菌胺、硅噻菌胺、吡噻菌胺，纯度均大于98.0%，5种酰胺类化合物的中文名称、英文名称、CAS号、分子式和分子量参见附录A。

5.7 标准储备溶液：分别准确称取适量的标准物质(5.6)，用乙腈分别配制成浓度为1 000 $\mu\text{g}/\text{mL}$ 标

准储备液,于一18℃避光保存。

5.8 混合标准中间液:分别准确移取适量的标准储备溶液(5.7),用乙腈稀释配制成浓度为10 μg/mL的混合标准中间液,于一18℃避光保存。

5.9 基质标准工作液:准确移取混合标准中间液适量(5.8),用空白样品基质溶液配制成浓度为1.0 ng/mL、2.0 ng/mL、5.0 ng/mL、10.0 ng/mL、20.0 ng/mL、40.0 ng/mL、60.0 ng/mL的基质标准工作液,现用现配。

5.10 基质分散固相萃取剂:4 g 无水硫酸镁,1 g 氯化钠,1 g 柠檬酸钠和0.5 g 柠檬酸氢二钠。

5.11 基质分散固相净化剂:150 mg 无水硫酸镁,25 mg N-丙基乙二胺,75 mg 石墨化碳黑。

5.12 微孔滤膜:0.22 μm,有机滤膜。

6 仪器和设备

6.1 液相色谱-质谱/质谱仪:带电喷雾离子源(ESI)。

6.2 分析天平:感量为0.0001 g和0.01 g。

6.3 离心机:转速大于等于8 000 r/min。

6.4 均质机。

6.5 涡旋混合器。

6.6 离心管:50 mL 聚四氟乙烯离心管。

7 样品制备与保存

取代表性样品部分约500 g,将其可食部分先切碎,经多功能食品搅拌机充分捣碎均匀,试样均分为两份,装入洁净容器,密封,并标明标记,于一18℃以下冷冻存放。

在制样过程中,应防止样品污染或发生残留物含量的变化。

8 分析步骤

8.1 提取

8.1.1 蔬菜、水果

称取10 g 试样(精确到0.01 g)于50 mL 离心管中,加入20 mL 1%乙酸乙腈溶液,用均质机以9 000 r/min 均质1 min,加入基质分散固相萃取剂(5.10),涡旋1 min,以8 000 r/min 离心5 min,上清液待净化。

8.1.2 肉制品、坚果类

称取5 g 试样(精确到0.01 g)于50 mL 离心管中,加入15 mL 1%乙酸乙腈溶液,用均质机以9 000 r/min 均质1 min,以8 000 r/min 离心5 min,上清液转移至25 mL 比色管中,再加入10 mL 1%乙酸乙腈溶液至残渣中,涡旋1 min,以8 000 r/min 离心5 min,上清液转移至25 mL 比色管中,用1%乙酸乙腈定容至25 mL,待净化。

8.2 净化

取2 mL 待净化液至含有基质分散固相净化剂(5.11)的离心管中,涡旋1 min,以8 000 r/min 离心5 min。取0.5 mL 上清液,加入0.5 mL 水,涡旋1 min,过0.22 μm 滤膜,供液相色谱-质谱/质谱仪

测定。

8.3 测定

8.3.1 液相色谱条件如下：

- a) 色谱柱:Accucore XL C₁₈ 柱, 4.6 mm×150 mm (内径), 粒度 4.0 μm, 或相当者;
- b) 流动相:A, 甲醇;B, 0.15%甲酸水溶液, 梯度洗脱程序见表 1;

表 1 梯度洗脱条件

时间/min	0.15%甲酸水溶液/%	甲醇/%	流速/(mL/min)
0	55.0	45.0	0.4
1.0	55.0	45.0	0.4
3.0	20.0	80.0	0.4
8.0	5.0	95.0	0.4
12.0	5.0	95.0	0.4
13.0	55.0	45.0	0.4
17.0	55.0	45.0	0.4

- c) 流速:0.4 mL/min;
- d) 柱温:40 ℃;
- e) 进样量:10 μL。

8.3.2 质谱条件如下：

- a) 离子源:电喷雾离子源;
- b) 扫描方式:正离子扫描;
- c) 检测方式:多反应监测模式;
- d) 其他参考质谱条件参见附录 B。

8.3.3 定性测定

按照上述测定样品和基质标准工作液, 样品中待测物质的保留时间与基质标准工作液的保留时间偏差在±2.5%之内; 定性离子对的相对丰度与浓度相当的基质标准工作液的相对丰度一致, 相对丰度偏差不超过表 2 规定的范围, 则可判定为样品中存在相应的待测物。

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
允许的相对偏差/%	±20	±25	±30	±50

8.3.4 定量测定

根据样液中被测物的含量情况, 选取响应值相近的基质标准工作液进行色谱分析。基质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。

在上述仪器条件下,5种酰胺类杀菌剂的参考保留时间参见表B.1,5种酰胺类标准溶液多反应监测(MRM)色谱图参见附录C。

8.3.5 空白试验

除不加试样外,均按上述操作步骤进行。

9 结果计算和表述

用色谱数据处理软件或按式(1)计算试样中药物的残留含量。计算结果需扣除空白值:

$$X_i = \frac{c_i \times V}{m} \dots\dots\dots(1)$$

式中:

X_i —— 试样中药物残留量,单位为微克每千克($\mu\text{g}/\text{kg}$);

c_i —— 从标准曲线中测得的药物浓度,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 —— 最终样液代表的试样质量,单位为克(g)。

10 方法的定量限和回收率

10.1 定量限

本方法中双炔酰菌胺、噻唑菌胺、吡唑磺菌胺、硅噻菌胺、吡噻菌胺定量限均为 $10.0 \mu\text{g}/\text{kg}$ 。

10.2 回收率

5种酰胺类杀菌剂在葡萄、白菜、芹菜、黄瓜、番茄、草莓、花生、猪肉中的回收率的实验数据参见附录D。

附 录 A
(资料性)
标准物质的基本信息

表 A.1 酰胺类标准物质的基本信息

序号	中文名称	英文名称	CAS号	分子式	分子量
1	噻唑菌胺	ethaboxam	162650-77-3	$C_{14}H_{16}N_4OS_2$	320.43
2	双炔酰菌胺	mandipropamid	374726-62-2	$C_{23}H_{22}ClNO_4$	411.88
3	吡噻菌胺	penthiopyrad	183675-82-3	$C_{16}H_{20}F_3N_3OS$	359.41
4	硅噻菌胺	silthiopham	175217-20-6	$C_{13}H_{21}NOSSi$	267.46
5	吡唑磺菌胺	amisulbrom	348635-87-0	$C_{13}H_{13}BrFN_5O_4S_2$	466.31

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附 录 B
(资料性)
质谱参考条件¹⁾

质谱参考条件如下：

- a) 电喷雾电压:3 000 V；
- b) 鞘气压力:3 500 kpa (20 arb)；
- c) 毛细管温度:275 ℃；
- d) 干燥气压力:1 400 kpa (8 arb)；
- e) 干燥气温度:20 ℃；
- f) 其他质谱参数参见表 B.1。

表 B.1 5 种酰胺类杀菌剂保留时间、定性离子对、定量离子对、去簇电压和碰撞气能量

化合物	保留时间/min	离子对/(m/z)	去簇电压/V	碰撞能量/V
噻唑菌胺	6.61	321.0/183.0 *	96	22
		321.0/200.0		26
双炔酰菌胺	7.38	412.0/328.0 *	88	14
		412.0/124.9		35
吡噻菌胺	8.38	360.0/176.9 *	82	35
		360.0/275.9		14
硅噻菌胺	8.49	268.0/138.9 *	82	19
		268.0/73.0		32
吡唑磺菌胺	9.51	466.0/226.7 *	81	22
		466.0/148.1		46
* 为定量离子对。				

1) 非商业性声明:附录 B 所列质谱条件是在 TSQ Vantage 质谱仪上完成的,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采用不同厂家或型号的仪器。

附录 C
(资料性)
标准物质的多反应监测色谱图

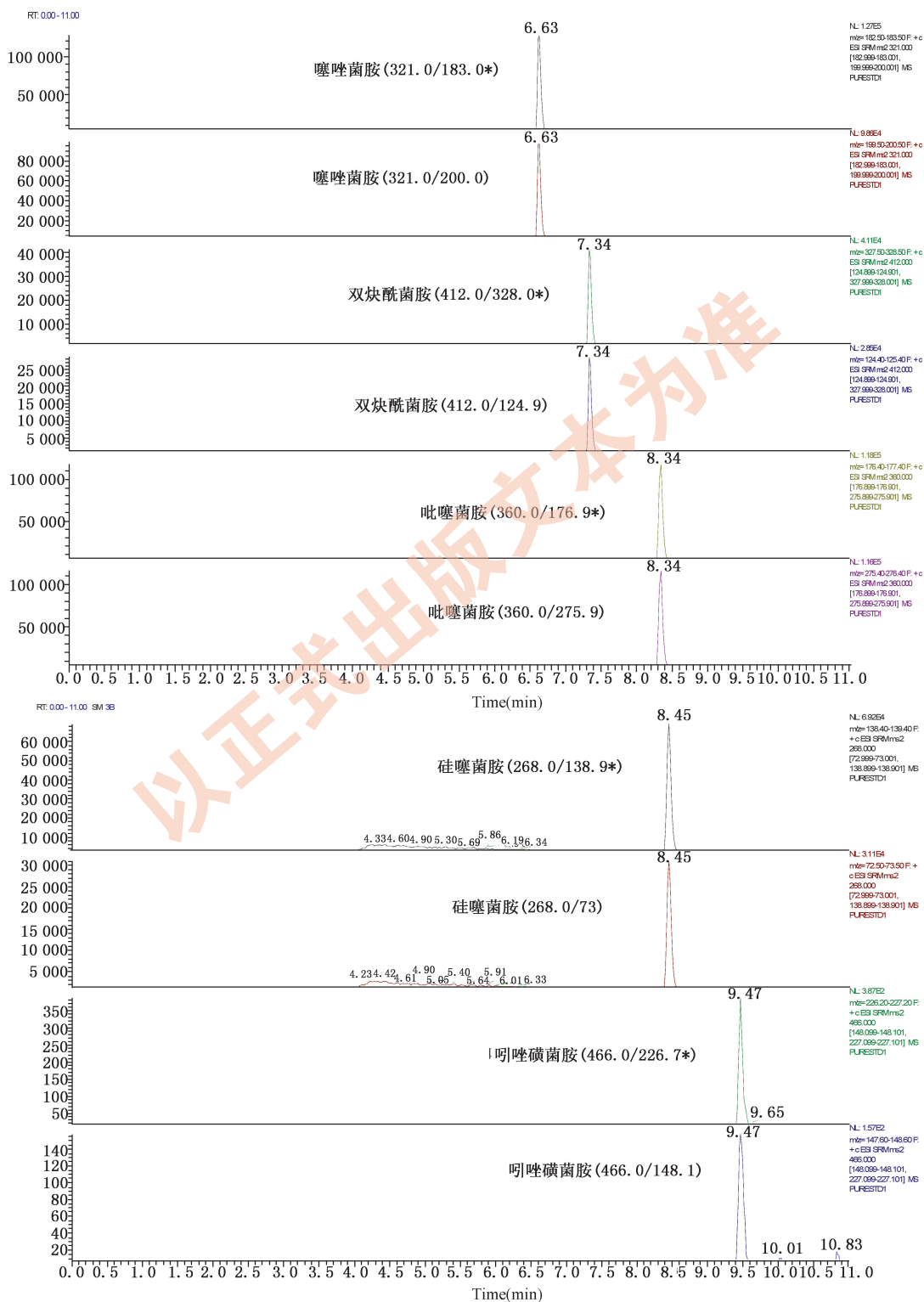


图 C.1 5 种酰胺类杀菌剂标准溶液的多反应监测色谱图(1.0 ng/mL)

附 录 D
(资料性)
添加回收试验数据

表 D.1 葡萄中 5 种酰胺类杀菌剂回收率范围

化合物	添加水平/($\mu\text{g}/\text{kg}$)	回收率范围/%	相对标准偏差/%
噻唑菌胺	10	98.0~102.0	1.7
	20	86.0~97.0	4.6
	100	102.7~107.0	1.6
	5 000	80.0~89.6	4.5
双炔酰菌胺	10	94.0~105.0	4.9
	20	104.0~115.5	3.7
	100	114.4~121.8	2.6
	5 000	76.8~90.6	6.9
吡噻菌胺	10	96.0~110.0	5.2
	20	102.0~107.5	1.8
	100	105.2~112.2	2.2
	5 000	80.1~92.0	5.3
硅噻菌胺	10	92.0~105.0	4.4
	20	98.0~108.5	3.6
	100	105.6~112.2	2.8
	5 000	80.5~97.6	7.7
吡唑磺菌胺	10	72.0~100.0	14.3
	20	86.0~94.5	3.7
	100	105.9~108.9	1.0
	5 000	91.7~111.0	6.4

表 D.2 白菜中 5 种酰胺类杀菌剂回收率范围

化合物	添加水平/($\mu\text{g}/\text{kg}$)	回收率范围/%	相对标准偏差/%
噻唑菌胺	10	84.0~95.0	4.6
	20	88.5~92.5	1.9
	100	87.0~94.4	3.1
	5 000	83.5~94.5	4.8

表 D.2 (续)

化合物	添加水平/($\mu\text{g}/\text{kg}$)	回收率范围/%	相对标准偏差/%
双炔酰菌胺	10	78.0~83.0	2.4
	20	76.5~79.5	1.3
	100	77.3~85.1	4.3
	5 000	85.6~95.3	4.1
	25 000	80.8~91.2	5.0
吡噻菌胺	10	87.0~91.0	1.6
	20	84.5~88.5	2.1
	100	83.9~94.5	4.9
	5 000	83.8~92.7	4.8
硅噻菌胺	10	90.0~98.0	2.8
	20	87.5~94.5	2.9
	100	86.3~93.0	2.6
	5 000	81.2~88.3	3.4
吡唑啉菌胺	10	84.0~95.0	4.4
	20	71.0~82.5	6.6
	100	72.8~86.9	7.1
	5 000	92.1~99.3	2.9

表 D.3 芹菜中 5 种酰胺类杀菌剂回收率范围

化合物	添加水平/($\mu\text{g}/\text{kg}$)	回收率范围/%	相对标准偏差/%
噻唑菌胺	10	92.0~102.0	4.2
	20	90.5~95.5	2.0
	100	87.3~94.6	3.1
	5 000	108.3~111.1	1.0
双炔酰菌胺	10	84.0~101.0	6.3
	20	80.0~98.0	6.8
	100	92.1~96.2	1.7
	5 000	85.6~92.1	3.0
	10 000	93.1~97.4	2.2
吡噻菌胺	10	92.0~102.0	3.7
	20	90.5~99.0	3.0
	100	91.6~96.1	2.2
	5 000	84.7~92.3	3.2
	20 000	93.3~99.5	2.3

表 D.3 (续)

化合物	添加水平/($\mu\text{g}/\text{kg}$)	回收率范围/%	相对标准偏差/%
硅噻菌胺	10	92.0~102.0	3.9
	20	91.5~100.0	3.3
	100	92.8~96.4	1.7
	5 000	93.9~97.3	1.2
吡唑磺菌胺	10	75.0~97.0	10.9
	20	81.0~99.0	7.2
	100	87.6~98.8	4.9
	5 000	95.3~102.0	2.7

表 D.4 黄瓜中 5 种酰胺类杀菌剂回收率范围

化合物	添加水平/($\mu\text{g}/\text{kg}$)	回收率范围/%	相对标准偏差/%
噻唑菌胺	10	92.0~102.0	3.5
	20	93.0~97.5	1.9
	100	100.2~104.6	1.4
	5 000	83.8~92.1	3.5
双炔酰菌胺	10	105.0~113.0	2.7
	20	99.0~102.5	1.2
	100	99.4~125.3	8.1
	5 000	72.5~89.8	8.1
吡噻菌胺	10	102.0~112.0	3.3
	20	100.5~104.5	1.4
	100	97.4~101.9	1.6
	5 000	100.2~112.6	4.1
硅噻菌胺	10	103.0~115.0	4.3
	20	100.5~107.0	2.4
	100	103.4~106.7	1.5
	5 000	89.3~97.0	3.8
吡唑磺菌胺	10	96.0~122.0	8.7
	20	98.0~110.0	4.6
	100	102.7~108.0	1.7
	5 000	99.5~117.6	5.9

表 D.5 番茄中 5 种酰胺类杀菌剂回收率范围

化合物	添加水平/($\mu\text{g}/\text{kg}$)	回收率范围/%	相对标准偏差/%
噻唑菌胺	10	98.0~106.0	3.0
	20	97.0~99.0	0.9
	100	100.4~103.4	1.1
	5 000	88.9~90.8	0.8
双炔酰菌胺	10	100.0~114.0	5.5
	20	101.5~106.0	1.8
	100	97.6~99.7	0.8
	5 000	86.1~97.8	4.3
吡噻菌胺	10	99.0~113.0	5.7
	20	97.0~102.0	2.0
	100	95.6~99.4	1.5
	5 000	86.0~90.9	2.2
硅噻菌胺	10	98.0~107.0	3.2
	20	95.0~103.0	3.0
	100	96.7~99.5	1.2
	5 000	89.7~91.4	0.9
吡唑磺菌胺	10	86.0~98.0	6.4
	20	80.0~98.0	8.0
	100	91.2~100.0	3.2
	5 000	95.9~101.2	2.1

表 D.6 草莓中 5 种酰胺类杀菌剂回收率范围

化合物	添加水平/($\mu\text{g}/\text{kg}$)	回收率范围/%	相对标准偏差/%
噻唑菌胺	10	108.0~115.0	2.3
	20	101.0~110.0	3.5
	100	97.8~105.8	2.8
	5 000	80.3~88.1	3.5
双炔酰菌胺	10	103.0~126.0	6.9
	20	99.0~108.5	3.3
	100	100.9~105.6	1.9
	5 000	85.3~100.0	5.8

表 D.6 (续)

化合物	添加水平/($\mu\text{g}/\text{kg}$)	回收率范围/%	相对标准偏差/%
吡嗪菌胺	10	103.0~119.0	5.5
	20	99.5~104.0	1.9
	100	73.4~105.2	13.0
	5 000	76.0~86.6	5.6
硅噻菌胺	10	103.0~116.0	4.1
	20	97.5~104.0	2.3
	100	89.6~101.0	4.1
	5 000	86.3~89.7	1.8
吡啶磺菌胺	10	105.0~125.0	6.7
	20	99.0~108.5	3.5
	100	77.3~102.0	9.5
	5 000	95.1~100.6	2.7

表 D.7 猪肉中 5 种酰胺类杀菌剂回收率范围

化合物	添加水平/($\mu\text{g}/\text{kg}$)	回收率范围/%	相对标准偏差/%
噻唑菌胺	10	73.0~98.0	10.1
	20	87.0~99.5	5.4
	100	87.8~94.5	2.5
双炔酰菌胺	10	65.0~82.0	8.4
	20	80.0~98.4	9.3
	100	95.1~111.2	6.2
吡嗪菌胺	10	90.0~107.0	7.8
	20	90.5~111.5	8.4
	100	103.8~117.4	5.0
硅噻菌胺	10	100.0~115.0	5.0
	20	98.5~110.0	4.6
	100	96.4~105.0	3.2
吡啶磺菌胺	10	59.0~83.0	14.2
	20	64.5~82.1	9.3
	100	72.0~78.4	3.3

表 D.8 花生中 5 种酰胺类杀菌剂回收率范围

化合物	添加水平/($\mu\text{g}/\text{kg}$)	回收率范围/%	相对标准偏差/%
噻唑菌胺	10	71.0~77.0	5.8
	20	77.5~83.5	3.0
	100	80.7~84.3	2.3
双炔酰菌胺	10	72.0~86.0	5.9
	20	82.5~93.5	4.3
	100	82.5~93.1	4.4
吡噻菌胺	10	79.0~88.0	5.7
	20	90.5~94.0	2.0
	100	90.2~94.7	1.7
硅噻菌胺	10	75.0~80.0	9.3
	20	82.5~87.5	5.6
	100	89.2~91.8	2.8
吡唑磺菌胺	10	66.0~82.0	9.1
	20	70.0~76.0	3.2
	100	72.2~76.2	3.5

Foreword

This document is accordance with GB/T 1.1—2020 《Directives for standardization~part 1: rules for the structure and drafting of standardizing documents》 and SN/T 0001—2016 《Rules for standards of physical and chemical analysis method for the determination in food and cosmetics for export》.

Attention is required to the certain contents of this text which might be related to some patents. This file is not responsible to identify these.

This document was proposed by and is under the charge of General Administration of Customs P. R. China.

This document was drafted by Hangzhou Customs District P. R. China.

This document was mainly drafted by Zhu Zitong, Xie Wen, Lei Meikang, Jiang Zhiying, Hou Jianbo, Ye Youbiao, Zhang Yingjun.

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Determination of multiple amide fungicides including mandipropamid, ethaboxam, amisulbrom etc in food for export—LC-MS/MS method

1 Scope

This standard specifies the method of sample determination of mandipropamid, ethaboxam, amisulbrom, silthiopham, penthiopyrad in food for export by LC-MS/MS.

This standard is applicable to the determination and confirmation of mandipropamid, ethaboxam, amisulbrom, silthiopham, penthiopyrad residues in grape, cabbage, celery, cucumber, tomato, strawberry, peanut and pork for export by LC-MS/MS.

2 Normative references

The contents of the following documents constitute essential provisions of this document through normative references in the text. Among them, the date of the reference document, only the date of the corresponding version is applicable to this document. No date of the reference document, its latest version (including all amendments) is applicable to this document.

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

3 Term and definition

There is no need of term and definition in this document.

4 Principle

The residues of mandipropamid, ethaboxam, amisulbrom, silthiopham, penthiopyrad in sample are extracted with 1% acetic acid acetonitrile solution by homogenization, cleaned up with dispersive solid-phase extraction (QuEChERS) method and determined by LC-MS/MS, quantified by external standard method.

5 Reagents and materials

Unless otherwise specified, all reagents used should be of analytically pure, “Water” is the first grade water complied with GB/T 6682.

5.1 Acetonitrile: chromatography pure.

5.2 Methanol: chromatography pure.

5.3 Formic acid: chromatography pure.

5.4 1% acetic acid acetonitrile solution: pipette 1.0 mL acetic acid dilute to 100 mL with acetonitrile.

5.5 0.15% formic acid solution: pipette 1.5 mL formic acid dilute to 1 000 mL with water.

5.6 Standard substances: mandipropamid, ethaboxam, amisulbrom, silthiopham, penthiopyrad, purity $\geq 98.0\%$, The name, CAS number, molecular formula and molecular weight of 5 kind of amide fungicides are listed in appendix A.

5.7 Standard stock solution: accurately weigh appropriate standard(5.6), dissolve with acetonitrile. The concentration of the standard stock solution is 1 000 $\mu\text{g/mL}$. The solution should be stored at $-18\text{ }^{\circ}\text{C}$ in dark.

5.8 Mixed medium standard solution: respectively pipette appropriate standard stock solution (5.7), dilute with acetonitrile. The concentration of this mixed medium standard solution is 10 $\mu\text{g/mL}$. The solution should be stored at $-18\text{ }^{\circ}\text{C}$ in dark.

5.9 Matrix matched mixed standard working solution: accurately pipette mixed medium standard solution, dilute with blank sample extract to obtain 1.0 ng/mL , 2.0 ng/mL , 5.0 ng/mL , 10.0 ng/mL , 20.0 ng/mL , 40.0 ng/mL , 60.0 ng/mL of matrix matched mixed standard working solution. The matrix matched mixed standard working solution should be prepared before using.

5.10 Extract agent: 4 g of magnesium sulfate, 1 g sodium chloride, 1 g sodium citrate (TSCD), 0.5 g sodium hydrogen citrate (DHS).

5.11 Cleaning-up agent: 150 mg of magnesium sulfate, 25 mg of primary secondary amine (PSA), 75 mg of graphitized carbon black (GCB).

5.12 Membrane filter: 0.22 μm , organic type.

6 Apparatus and equipment

6.1 LC-MS/MS: equipped with electrospray ion source.

6.2 Analytical balance: accurate to 0.000 1 g, 0.01 g.

6.3 Centrifuge: speed of no less than 8 000 r/min.

6.4 Homogenizer.

6.5 Vortex mixer.

6.6 Centrifuge tube: polypropylene, 50 mL.

7 Preparation and storage of test sample

Take representative samples of 500 g, triturate the sample with a comminuter after chopping the edible part. And then place in clean containers, seal and indicate the tag, storage below $-18\text{ }^{\circ}\text{C}$.

In the course of sample preparation, precaution must be taken avoid the contamination or any factors which may cause the change of residue content.

8 Procedure

8.1 Extraction

8.1.1 Vegetable and fruit

Weigh 10 g (accurate to 0.01 g) of the test sample into a 50 mL centrifuge tube, add 20 mL 1% acetic acid acetonitrile solution and homogenize at 9 000 r/min for 1 min, add extractant (5.10), vortex blending for 1 min, and then centrifuge the sample at 8 000 r/min for 5 min. The supernatant is ready for cleanup.

8.1.2 Meat product and nuts

Weigh 5 g (accurate to 0.01 g) of the test sample into a 50 mL centrifuge tube, add 15 mL 1% acetic acid acetonitrile solution and homogenize at 9 000 r/min for 1 min, and then centrifuge the sample at 8 000 r/min for 5 min. The supernatant is taken into a 25 mL colorimetric tube. Another 10 mL 1% acetic acid acetonitrile solution is added, vortex blending for 1 min, and then centrifuge the sample

at 8 000 r/min for 5 min. Combine the supernatant into the same colorimetric tube. The supernatant is ready for cleanup.

8.2 Cleaning-up

Transfer 2 mL of the supernatant into a 10 mL centrifuge tube, add 150 mg of MgSO₄, 25 mg of PSA, 75 mg of GCB into the centrifuge tube. Vibrate vigorously for 1 min and centrifuge at 8 000 r/min for 5 min. Transfer 0.5 mL of the supernatant and make up to the volume of 1 mL with water, vortex blending for 1 min. Filter the analysis solution through 0.22 μm organic membrane, the final solution is ready for analysis by LC-MS/MS.

8.3 Determination

8.3.1 Liquid chromatography condition

- a) Column: C₁₈ 4.6 mm × 150 mm (i. d.), 4.0 μm particle size or other equivalent;
- b) Mobile phase: A: methanol, B: 0.15% formic acid-water, the elution gradient is listed in Table 1;
- c) Flow rate: 0.4 mL/min;
- d) Column temperature: 40 °C;
- e) Injection volume: 10 μL;

Table 1—Mobile phase and elution gradient

Time/min	Mobile phase A /%	Mobile phase B/%
0	45.0	55.0
1.0	45.0	55.0
3.0	80.0	20.0
8.0	95.0	5.0
12.0	95.0	5.0
13.0	45.0	55.0
17.0	45.0	55.0

8.3.2 Mass spectrometry condition

- a) Ion source: electrospray ionization source (ESI);
- b) Scanning mode: positive ion mode;

- c) Monitoring model; multiple reaction monitoring (MRM);
- d) Other reference mass operating conditions are listed in appendix B.

8.3.3 Qualitative analysis

Under the same LC-MS/MS operating conditions, the matrix matched mixed standard working solution and sample solution is injected. If the retention time of sample chromatogram peaks are consistent with that of standard solution with the difference less than $\pm 2.5\%$. The relative intensity of sample transitions shall correspond to those of standard solution transitions for confirmation. The concentration of standard solution should be the 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

8.3.4 Quantitative analysis

According to the amount of analytes in the sample liquid, selected response value of similar matrix matched mixed standard working solution. The responses of the 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, retention time of 5 kind of amide fungicides is listed in Table B. 1. The multiple reaction monitoring (MRM) chromatograms of standard working solution are listed in Annex C.

8.3.5 Blank test

The operation of the blank test is the same as that described in the method of determination, but with the omission of sample addition.

9 Calculation and expression of the result

Calculate the content of amide fungicide residues in the test sample by the chromatogram data processing software or by the followed formula (1). The bank values should be deducted from the calculation result.

$$X_i = \frac{C_i \times V}{m} \dots\dots\dots (1)$$

Where:

X_i —the content of analyte in the test sample, $\mu\text{g}/\text{kg}$;

c_i —the concentration of analyte obtained from the matrix standard working solution, ng/mL ;

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

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

10 Limit of quantification and recovery

10.1 Limit of quantification

The limit of quantification is $10.0 \mu\text{g}/\text{kg}$ in the method for mandipropamid, ethaboxam, amisulbrom, silthiopham, penthiopyrad.

10.2 Recovery

The ranges of recovery in grape, cabbage, celery, cucumber, tomato, strawberry, peanut and pork are showed in appendix D.

Annex A
(informative)

The information of 5 kinds of amide fungicide

Table A. 1—The information of 5 kinds of amide fungicide

Name	CAS Number	Molecular formula	Molecular weight
ethaboxam	162650-77-3	$C_{14}H_{16}N_4OS_2$	320.43
mandipropamid	374726-62-2	$C_{23}H_{22}ClNO_4$	411.88
penthiopyrad	183675-82-3	$C_{16}H_{20}F_3N_3OS$	359.41
silthiopham	175217-20-6	$C_{13}H_{21}NOSSi$	267.46
amisulbrom	348635-87-0	$C_{13}H_{13}BrFN_5O_4S_2$	466.31

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Annex B
(Informative)
Reference mass condition¹⁾

Reference conditions are as follows:

- a) Ion spray voltage: 3 000 V;
- b) Sheath gas pressure: 3 500 kPa (20 arb);
- c) Capillary temperatures: 275 °C;
- d) Drying gas pressure: 1 400 kPa (8 arb);
- e) Drying gas temperatures: 20 °C;
- f) The other mass parameter see the Table B. 1.

Table B. 1—Quality ions, quantity ions, declustering potential voltage and collision energy of analyte

Compound	Retention time (min)	Ion pairs (m/z)	Declustering potential (v)	Collision energy(v)
ethaboxam	6.61	321.0/183.0 *	96	22
		321.0/200.0		26
mandipropamid	7.38	412.0/328.0 *	88	14
		412.0/124.9		35
penthioopyrad	8.38	360.0/176.9 *	82	35
		360.0/275.9		14
silthiopham	8.49	268.0/138.9 *	82	19
		268.0/73.0		32
amisulbrom	9.51	466.0/226.7 *	81	22
		466.0/148.1		46

* mark is the quantification ion pair.

1) Non-commercial statement: Parameters listed in Appendix B are accomplished by ThermoFisher TSQ Vantage 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 equipment of different corporation or different type.

Annex C
(Informative)
MRM chromatogram of standard

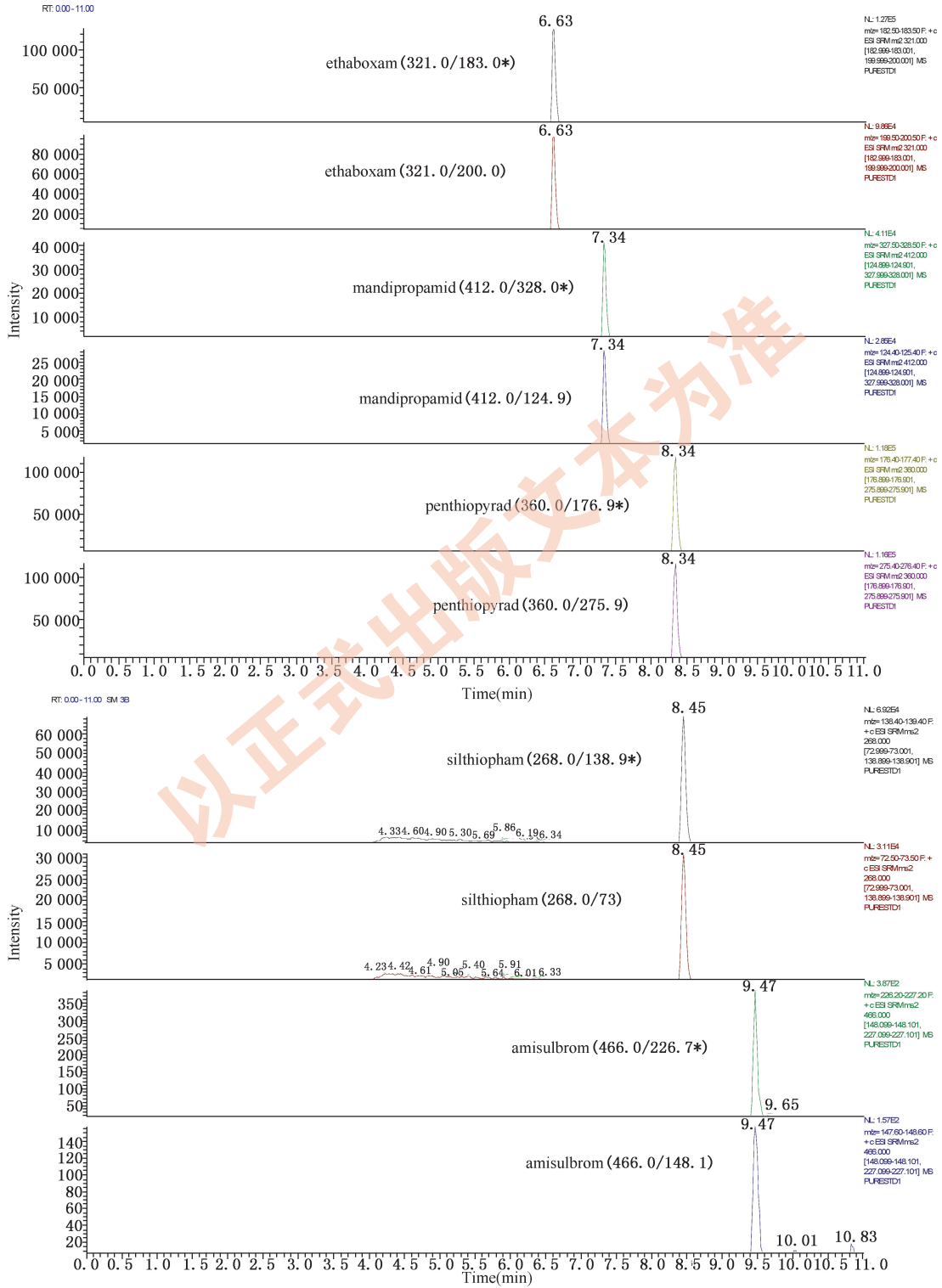


Figure C.1 MRM Chromatography of 5 kinds of amide fungicide (1.0 ng/mL)

Annex D
(Informative)

The fortifying concentration and the range of recovery of analyte

Table D. 1—Recovery range of analyte in grape

Compound	Spike level($\mu\text{g}/\text{kg}$)	Range of recovery/%	RSD/%
ethaboxam	10.0	98.0~102.0	1.7
	20.0	86.0~97.0	4.6
	100.0	102.7~107.0	1.6
	5 000.0	80.0~89.6	4.5
mandipropamid	10.0	94.0~105.0	4.9
	20.0	104.0~115.5	3.7
	100.0	114.4~121.8	2.6
	5 000.0	76.8~90.6	6.9
penthioapyrad	10.0	96.0~110.0	5.2
	20.0	102.0~107.5	1.8
	100.0	105.2~112.2	2.2
	5 000.0	80.1~92.0	5.3
silthiopham	10.0	92.0~105.0	4.4
	20.0	98.0~108.5	3.6
	100.0	105.6~112.2	2.8
	5 000.0	80.5~97.6	7.7
amisulbrom	10.0	72.0~100.0	14.3
	20.0	86.0~94.5	3.7
	100.0	105.9~108.9	1.0
	5 000.0	91.7~111.0	6.4

Table D. 2—Recovery range of analyte in cabbage

Compound	Spike level($\mu\text{g}/\text{kg}$)	Range of recovery/%	RSD/%
ethaboxam	10.0	84.0~95.0	4.6
	20.0	88.5~92.5	1.9
	100.0	87.0~94.4	3.1
	5 000.0	83.5~94.5	4.8

Table D.2 (continued)

Compound	Spike level($\mu\text{g}/\text{kg}$)	Range of recovery/%	RSD/%
mandipropamid	10.0	78.0~83.0	2.4
	20.0	76.5~79.5	1.3
	100.0	77.3~85.1	4.3
	5 000.0	85.6~95.3	4.1
	25 000	80.8~91.2	5.0
penthioopyrad	10.0	87.0~91.0	1.6
	20.0	84.5~88.5	2.1
	100.0	83.9~94.5	4.9
	5 000.0	83.8~92.7	4.8
silthiopham	10.0	90.0~98.0	2.8
	20.0	87.5~94.5	2.9
	100.0	86.3~93.0	2.6
	5 000.0	81.2~88.3	3.4
amisulbrom	10.0	84.0~95.0	4.4
	20.0	71.0~82.5	6.6
	100.0	72.8~86.9	7.1
	5 000.0	92.1~99.3	2.9

Table D.3—Recovery range of analyte in celery

Compound	Spike level($\mu\text{g}/\text{kg}$)	Range of recovery/%	RSD/%
ethaboxam	10.0	92.0~102.0	4.2
	20.0	90.5~95.5	2.0
	100.0	87.3~94.6	3.1
	5 000.0	108.3~111.1	1.0
mandipropamid	10.0	84.0~101.0	6.3
	20.0	80.0~98.0	6.8
	100.0	92.1~96.2	1.7
	5 000.0	85.6~92.1	3.0
	10 000.0	93.1~97.4	2.2
penthioopyrad	10.0	92.0~102.0	3.7
	20.0	90.5~99.0	3.0
	100.0	91.6~96.1	2.2
	5 000.0	84.7~92.3	3.2
	20 000.0	93.3~99.5	2.3

Table D.3 (continued)

Compound	Spike level($\mu\text{g}/\text{kg}$)	Range of recovery/%	RSD/%
silthiopham	10.0	92.0~102.0	3.9
	20.0	91.5~100.0	3.3
	100.0	92.8~96.4	1.7
	5 000.0	93.9~97.3	1.2
amisulbrom	10.0	75.0~97.0	10.9
	20.0	81.0~99.0	7.2
	100.0	87.6~98.8	4.9
	5 000.0	95.3~102.0	2.7

Table D.4—Recovery range of analyte in cucumber

Compound	Spike level($\mu\text{g}/\text{kg}$)	Range of recovery/%	RSD/%
ethaboxam	10.0	92.0~102.0	3.5
	20.0	93.0~97.5	1.9
	100.0	100.2~104.6	1.4
	5 000.0	83.8~92.1	3.5
mandipropamid	10.0	105.0~113.0	2.7
	20.0	99.0~102.5	1.2
	100.0	99.4~125.3	8.1
	5 000.0	72.5~89.8	8.1
penthioopyrad	10.0	102.0~112.0	3.3
	20.0	100.5~104.5	1.4
	100.0	97.4~101.9	1.6
	5 000.0	100.2~112.6	4.1
silthiopham	10.0	103.0~115.0	4.3
	20.0	100.5~107.0	2.4
	100.0	103.4~106.7	1.5
	5 000.0	89.3~97.0	3.8
amisulbrom	10.0	96.0~122.0	8.7
	20.0	98.0~110.0	4.6
	100.0	102.7~108.0	1.7
	5 000.0	99.5~117.6	5.9

Table D.5—Recovery range of analyte in tomato

Compound	Spike level($\mu\text{g}/\text{kg}$)	Range of recovery/%	RSD/%
ethaboxam	10.0	98.0~106.0	3.0
	20.0	97.0~99.0	0.9
	100.0	100.4~103.4	1.1
	5 000.0	88.9~90.8	0.8
mandipropamid	10.0	100.0~114.0	5.5
	20.0	101.5~106.0	1.8
	100.0	97.6~99.7	0.8
	5 000.0	86.1~97.8	4.3
penthiopyrad	10.0	99.0~113.0	5.7
	20.0	97.0~102.0	2.0
	100.0	95.6~99.4	1.5
	5 000.0	86.0~90.9	2.2
silthiopham	10.0	98.0~107.0	3.2
	20.0	95.0~103.0	3.0
	100.0	96.7~99.5	1.2
	5 000.0	89.7~91.4	0.9
amisulbrom	10.0	86.0~98.0	6.4
	20.0	80.0~98.0	8.0
	100.0	91.2~100.0	3.2
	5 000.0	95.9~101.2	2.1

Table D.6—Recovery range of analyte in strawberry

Compound	Spike level($\mu\text{g}/\text{kg}$)	Range of recovery/%	RSD/%
ethaboxam	10.0	108.0~115.0	2.3
	20.0	101.0~110.0	3.5
	100.0	97.8~105.8	2.8
	5 000.0	80.3~88.1	3.5
mandipropamid	10.0	103.0~126.0	6.9
	20.0	99.0~108.5	3.3
	100.0	100.9~105.6	1.9
	5 000.0	85.3~100.0	5.8

Table D.6 (continued)

Compound	Spike level($\mu\text{g}/\text{kg}$)	Range of recovery/%	RSD/%
penthioopyrad	10.0	103.0~119.0	5.5
	20.0	99.5~104.0	1.9
	100.0	73.4~105.2	13.0
	5 000.0	76.0~86.6	5.6
silthiopham	10.0	103.0~116.0	4.1
	20.0	97.5~104.0	2.3
	100.0	89.6~101.0	4.1
	5 000.0	86.3~89.7	1.8
amisulbrom	10.0	105.0~125.0	6.7
	20.0	99.0~108.5	3.5
	100.0	77.3~102.0	9.5
	5 000.0	95.1~100.6	2.7

Table D.7—Recovery range of analyte in pork

Compound	Spike level($\mu\text{g}/\text{kg}$)	Range of recovery/%	RSD/%
ethaboxam	10.0	73.0~98.0	10.1
	20.0	87.0~99.5	5.4
	100.0	87.8~94.5	2.5
mandipropamid	10.0	65.0~82.0	8.4
	20.0	80.0~98.4	9.3
	100.0	95.1~111.2	6.2
penthioopyrad	10.0	90.0~107.0	7.8
	20.0	90.5~111.5	8.4
	100.0	103.8~117.4	5.0
silthiopham	10.0	100.0~115.0	5.0
	20.0	98.5~110.0	4.6
	100.0	96.4~105.0	3.2
amisulbrom	10.0	59.0~83.0	14.2
	20.0	64.5~82.1	9.3
	100.0	72.0~78.4	3.3

Table D. 8—Recovery range of analyte in peanut

Compound	Spike level($\mu\text{g}/\text{kg}$)	Range of recovery/%	RSD/%
ethaboxam	10.0	71.0~77.0	5.8
	20.0	77.5~83.5	3.0
	100.0	80.7~84.3	2.3
mandipropamid	10.0	72.0~86.0	5.9
	20.0	82.5~93.5	4.3
	100.0	82.5~93.1	4.4
pentiopyrad	10.0	79.0~88.0	5.7
	20.0	90.5~94.0	2.0
	100.0	90.2~94.7	1.7
silthiopham	10.0	75.0~80.0	9.3
	20.0	82.5~87.5	5.6
	100.0	89.2~91.8	2.8
amisulbrom	10.0	66.0~82.0	9.1
	20.0	70.0~76.0	3.2
	100.0	72.2~76.2	3.5

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