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中华人民共和国出入境检验检疫行业标准

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进出口食品中异稻瘟净残留量的检测方法

Determination of iprobenfos residues in food for import and export

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前　言

本标准的附录A、附录B均为资料性附录。

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：中华人民共和国湖南出入境检验检疫局、中华人民共和国内蒙古出入境检验检疫局、中华人民共和国黑龙江出入境检验检疫局、中华人民共和国福建出入境检验检疫局。

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本标准系首次发布的出入境检验检疫行业标准。

进出口食品中异稻瘟净残留量的检测方法

第一法　气相色谱-质谱法

1　范围

本标准规定了食品中异稻瘟净的气相色谱-质谱检测方法。

本标准适用于茶叶、菠菜、荞头、苹果、板栗、蜂蜜、食醋、大米、鸡肉、牛肉、鱼肉中异稻瘟净残留量的测定和确证。

2　方法提要

试样中残留的异稻瘟净采用丙酮和正己烷(1+2)振荡提取，石墨化碳黑固相萃取柱或中性氧化铝固相萃取柱净化，洗脱液浓缩并定容后，供气相色谱-质谱仪测定和确证，外标法定量。

3　试剂和材料

除另有规定外，所有试剂均为分析纯，水为蒸馏水。

3.1　正己烷：重蒸馏。

3.2　丙酮：重蒸馏。

3.3　氯化钠。

3.4　无水硫酸钠：经650℃灼烧4 h，置干燥器中备用。

3.5　丙酮+正己烷(1+2)溶液。

3.6　丙酮+正己烷(1+1)溶液。

3.7　异稻瘟净标准物质(Iprobenfos，$C_{13}H_{21}O_3PS$，CAS：26087-47-8)：纯度大于99%。

3.8　标准储备溶液：准确称取适量的异稻瘟净标准物质，用丙酮将配制成1 000 μg/mL标准储备液，再根据检测要求用正己烷稀释成相应的标准工作溶液。标准溶液避光于4℃保存。

3.9　石墨化碳黑固相萃取柱：3 mL，125 mg，或相当者。

3.10　中性氧化铝固相萃取柱：3 mL，125 mg，或相当者。

4　仪器和设备

4.1　气相色谱-质谱联用仪，配电子轰击离子源(EI源)。

4.2　组织捣碎机。

4.3　粉碎机。

4.4　涡旋混匀器。

4.5　固相萃取装置，带真空泵。

4.6　多功能微量化样品处理仪，或相当者。

4.7　低速离心机：3 000 r/min。

4.8　离心管：15 mL。

4.9　刻度试管：15 mL。

4.10　微量注射器：10 μL。

5 试样制备与保存

5.1 试样制备

5.1.1 水果或蔬菜

取有代表性样品500 g，将其可食用部分切碎后不可用水洗涤，用捣碎机将样品加工成浆状。混匀，装入洁净的盛样容器内，密封并标明标记。

5.1.2 茶叶及粮谷

取有代表性样品500 g，用粉碎机粉碎并通过2.0 mm圆孔筛。混匀，装入洁净的盛样容器内，密封并标明标记。

5.1.3 肉及肉制品

取有代表性样品500 g，将其切碎后，用捣碎机将样品加工成浆状，混匀，装入洁净的盛样容器内，密封并标明标记。

5.2 试样保存

茶叶、蜂产品、调味品及粮谷类等试样于0℃～4℃保存；水果蔬菜类和肉及肉制品类等试样于－18℃以下冷冻保存。

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。

6 测定步骤

6.1 提取

对于茶叶、板栗、蜂蜜、大米样品，称取1 g均匀试样(精确至0.001 g)，对于菠菜、荠头、苹果、食醋、鸡肉、牛肉、鱼肉样品，称取2 g均匀试样(精确至0.001 g)，将称取的试样置于15 mL离心管中，加入1 g氯化钠，加入2 mL蒸馏水，于混匀器上混匀30 s，放置15 min。加入3 mL丙酮＋正己烷混合液(3.5)，在混匀器上混匀2 min。2 500 r/min离心1 min，吸取上层正己烷萃取液于另一试管中。再分别加入3 mL丙酮＋正己烷混合液重复提取两次，合并提取液。

6.2 净化

6.2.1 茶叶、菠菜、荠头、苹果、板栗、蜂蜜样品：将石墨化碳黑固相萃取柱(柱内填约1 cm高的无水硫酸钠层)安装在固相萃取的真空抽滤装置上，先用1 mL×3丙酮预淋洗萃取柱，再用1 mL×3正己烷预淋洗萃取柱，弃去全部预淋洗液。将正己烷提取液加入到石墨化碳黑固相萃取柱中，待提取液全部流出后，再用3 mL丙酮＋正己烷混合液(3.6)洗脱萃取柱，保持流速1.5 mL/min，收集全部流出液，于45℃下，氮气流吹至近干。最后用正己烷定容至0.5 mL，供GC-MS分析。

6.2.2 食醋、大米、鸡肉、牛肉、鱼肉样品：将中性氧化铝固相萃取柱(柱内填约1 cm高的无水硫酸钠层)安装在固相萃取的真空抽滤装置上，先用3 mL丙酮预淋洗萃取柱，再用3 mL正己烷预淋洗萃取柱，弃去全部预淋洗液。将正己烷提取液加入到中性氧化铝固相萃取柱中，待提取液全部流出后，再用3 mL丙酮＋正己烷混合液(3.6)洗脱萃取柱，保持流速1.5 mL/min，收集全部流出液，于45℃下，氮气流吹至近干。最后用正己烷定容至0.5 mL，供GC-MS分析。

6.3 测定

6.3.1 气相色谱-质谱条件

a) 色谱柱：HP-5MS石英毛细管柱，30 m×0.25 mm(内径)，膜厚0.25 μm，或相当者；

b) 色谱柱温度：初始温度80℃，以7℃/min升至205℃，再以25℃/min升至280℃保持5 min；

c) 进样口温度：280℃；

d) 色谱-质谱接口温度：270℃；

e) 载气：氦气，纯度大于等于99.995%，0.8 mL/min；

f) 进样量：1 μL；

g) 进样方式：无分流进样，1 min 后开阀；

h) 电离方式：EI；

i) 电离能量：70 eV；

j) 检测方式：选择离子监测方式(SIM)；

k) 监测离子(m/z)：203，204，246，288；定量离子：204；

l) 溶剂延迟：10 min。

6.3.2 色谱测定与确证

根据样液中异稻瘟净的含量情况，选定峰面积相近的标准工作溶液，对标准工作液和样液等体积参插进样，测定标准工作溶液和样液中异稻瘟净的响应值均应在仪器检测的线性范围内。

在相同实验条件下，样品中待测物质的质量色谱保留时间与标准工作液相同，并且在扣除背景后的样品质量色谱中，所选离子均出现，经过对比所选择离子的丰度比与标准品对应离子的丰度比，其值在允许范围内(允许范围见表 1)则可判定样品中存在对应的待测物。在第 6.3.1 条规定的色谱条件下，异稻瘟净的参考保留时间是 17.70 min，其监测离子(m/z)丰度比是 203∶204∶246∶288＝17∶100∶15∶19。色谱图和质谱图参见附录 A。

表 1 使用定性气相色谱-质谱时相对离子丰度最大容许误差

相对丰度(基峰)/%	>50	>20～50	>10～20	≤10
GC/MS 时相对离子丰度最大允许误差/%	±10	±15	±20	±50

6.4 空白实验

除不加试样外，均按上述测定步骤进行。

6.5 结果计算和表述

用色谱数据处理机或按式(1)计算试样中异稻瘟净的含量，计算结果须扣除空白值。

$$X=\frac{A\cdot c_S\cdot V}{A_S\cdot m} \qquad \cdots\cdots(1)$$

式中：

X——试样中异稻瘟净的含量，毫克每千克(mg/kg)；

A——样液中异稻瘟净的峰面积；

c_S——标准工作液中异稻瘟净的浓度，微克每毫升(μg/mL)；

A_S——标准工作液中异稻瘟净的峰面积；

V——样液最终定容体积，毫升(ml)；

m——最终样液所代表的试样量，克(g)。

7 测定低限、回收率

7.1 测定低限

本方法的测定低限为 0.005 mg/kg。

7.2 回收率

添加浓度和回收率见表 2。

表 2 回收率数据

样品名称	添加浓度/(mg/kg)	回收率范围/%
大米	0.005	91.9～113.1
	0.01	91.6～108.1
	0.20	97.5～119.0

表 2(续)

样品名称	添加浓度/(mg/kg)	回收率范围/%
茶叶	0.005	74.9～93.3
	0.01	90.8～107.9
	0.02	83.5～108.1
菠菜	0.005	86.8～110.1
	0.01	91.9～110.5
	0.02	107.1～118.9
苹果	0.005	75.3～86.5
	0.01	92.8～119.9
	0.02	95.6～113.1
蜂蜜	0.005	96.8～114.4
	0.01	97.7～115.8
	0.02	91.2～112.8
板栗	0.005	109.9～120.9
	0.01	90.2～108.8
	0.02	88.8～119.5
食醋	0.005	93.4～104.2
	0.01	89.9～100.6
	0.02	95.2～107.2
荞头	0.005	87.6～98.5
	0.01	93.7～100.6
	0.02	82.1～94.3
鱼肉	0.005	81.8～104.9
	0.01	80.9～94.0
	0.02	90.0～106.8
牛肉	0.005	86.4～99.4
	0.01	92.4～111.0
	0.02	94.2～111.9
鸡肉	0.005	70.9～103.0
	0.01	70.2～93.1
	0.02	73.3～92.7

第二法　气相色谱法

8　范围

本标准规定了食品中异稻瘟净残留量的气相色谱测定方法。

本标准适用于大米、菠菜、苹果、牛肉、鸡肉、鱼肉、蜂蜜、板栗、茶叶、食醋等食品中异稻瘟净残留量

的测定。

9 原理

试样中残留的异稻瘟净农药经丙酮＋正己烷(1＋2)和正己烷提取，过固相萃取柱净化，用配备火焰光度检测器的气相色谱仪进行测定，外标法定量。

10 试剂与材料

除另有规定外，所有试剂均为分析纯，水为蒸馏水。

10.1 正己烷：重蒸馏。

10.2 丙酮：重蒸馏。

10.3 无水硫酸钠：经650℃灼烧4 h，置干燥器中备用。

10.4 丙酮＋正己烷(1＋2)溶液。

10.5 异稻瘟净标准物质(Iprobenfos，$C_{13}H_{21}O_3PS$，CAS：26087-47-8)：纯度大于99%。

10.6 标准储备溶液：准确称取适量的异稻瘟净标准物质，用丙酮将配制成1 000 μg/mL标准储备液，再根据检测要求用正己烷稀释成相应的标准工作溶液。标准溶液避光于4℃保存。

10.7 石墨化碳黑固相萃取柱：3 mL，125 mg，或相当者。

10.8 中性氧化铝固相萃取柱：3 mL，125 mg，或相当者。

11 仪器和设备

11.1 气相色谱仪：配火焰光度检测器(FPD)。

11.2 快速混匀器。

11.3 离心机：3 000 r/min。

11.4 多功能微量化样品处理仪，或相当者。

11.5 具塞刻度离心管：5 mL，10 mL。

11.6 玻璃试管：20 mL。

11.7 尖嘴吸管。

11.8 微量可调移液器：10 μL，200 μL，1 000 μL。

11.9 微量注射器：10 μL。

12 试样制备与保存

12.1 试样制备

12.1.1 水果或蔬菜

取有代表性样品500 g，将其可食用部分切碎后不可用水洗涤，用捣碎机将样品加工成浆状。混匀，装入洁净的盛样容器内，密封并标明标记。

12.1.2 茶叶及粮谷

取有代表性样品500 g，用粉碎机粉碎并通过2.0 mm圆孔筛。混匀，装入洁净的盛样容器内，密封并标明标记。

12.1.3 肉及肉制品

取代表性样品500 g，将其切碎后，用捣碎机将样品加工成浆状，混匀，装入洁净的盛样容器内，密封并标明标记。

12.2 试样保存

茶叶、蜂产品、调味品及粮谷类等试样于0℃～4℃保存；水果蔬菜类和肉及肉制品类等试样于－18℃以下冷冻保存。

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。

13 测定步骤

13.1 提取与净化

13.1.1 对于大米、板栗、蜂蜜样品，称取 2 g(精确至 0.001 g)均匀试样于 10 mL 离心试管中，加入 2 mL 水，加入无水硫酸钠使之饱和，加入 2 mL 丙酮＋正己烷混合液(10.4)振荡提取两次，每次 2 min，然后离心 3 min(离心速度为 2 000 r/min)，吸取上层提取液于刻度离心管；残渣再用 2 mL 正己烷提取两次，合并提取液于刻度离心管中，在多功能微量化样品处理仪或其他相当的仪器上，于 40℃下用氮气流吹至 1.0 mL，供进样分析。

13.1.2 对于菠菜、苹果、食醋样品，称取 2 g(精确至 0.001 g)均匀试样于 10 mL 离心试管中。对于茶叶样品，称取 0.5 g(精确至 0.001 g)均匀试样于 10 mL 离心试管中，加入 2 mL 水，加入无水硫酸钠使之饱和，用 2 mL 丙酮＋正己烷混合液(10.4)振荡提取两次，每次 2 min，然后离心 3 min(离心速度为 2 000 r/min)，吸取上层提取液于另一离心试管中。残渣再用 2 mL 正己烷提取两次，合并上层提取液，待净化。在石墨化碳黑固相萃取柱上端装入 1 cm 高的无水硫酸钠，先用 4 mL 丙酮＋正己烷混合液(10.4)预淋洗固相萃取柱，弃去全部预淋洗液，然后将上述提取液倾入固相萃取柱中，待提取液全部流出固相萃取柱后，再用 4 mL 丙酮＋正己烷混合液(10.4)洗脱，收集全部流出液于刻度离心管中，最后在多功能微量化样品处理仪或其他相当的仪器上，于 40℃下用氮气流吹至 1.0 mL，供进样分析。

13.1.3 对于牛肉、鸡肉、鱼肉样品，称取 2 g(精确至 0.001 g)均匀试样于 10 mL 离心试管中，加入无水硫酸钠使之饱和，用 6 mL 丙酮＋正己烷混合液(10.4)振荡提取一次，时间 2 min，然后离心 3 min(离心速度为 2 000 r/min)，收集上层提取液于另一试管中。残渣再用 2 mL 正己烷提取两次，合并上层提取液，待净化。在中性氧化铝固相萃取柱上端装入 1 cm 高的无水硫酸钠，先用 4 mL 丙酮＋正己烷混合液(10.4)预淋洗固相萃取柱，弃去全部预淋洗液，然后将上述提取液倾入固相萃取柱中，待提取液全部流出固相萃取柱后，再用 4 mL 丙酮＋正己烷混合液(10.4)洗脱，收集全部流出液于刻度离心管中，最后在多功能微量化样品处理仪或其他相当的仪器上，于 40℃下用氮气流吹至 1.0 mL，供进样分析。

13.2 测定

13.2.1 色谱条件

a) 色谱柱：EQUITY™-1701 毛细管柱，30 m×0.32 mm(内径)×1.0 μm 或相当者；
b) 升温程序：初始温度 100℃，以 10℃/min 升至 220℃，保持 10 min；
c) 进样口温度：250℃；
d) 检测器温度：250℃；
e) 载气：氮气，纯度大于等于 99.99%，流量 5.0 mL/min；
f) 氢气：75 mL/min；
g) 空气：100 mL/min；
h) 尾吹气：20 mL/min；
i) 进样方式：无分流进样，1.0 min 后开阀；
j) 进样量：2 μL。

13.2.2 色谱测定

根据样液中异稻瘟净含量情况，选定与样液浓度相近的标准工作溶液。异稻瘟净标准工作溶液和样液中异稻瘟净响应值均应在仪器检测线性范围内。标准工作溶液和样液等体积穿插进样测定，以保留时间定性，测量峰面积与标准工作液比较进行定量。在上述色谱条件下，异稻瘟净标准物质的色谱图参见附录 B 中图 B.1。

13.3 空白试验

除不加试样外，按上述测定步骤进行。

13.4 结果计算和表述

用色谱数据处理机或按式(2)计算试样中异稻瘟净残留量，计算结果需扣除空白值。

$$X = \frac{A \times c \times V}{A_S \times m} \quad \cdots\cdots(2)$$

式中：

X——样品中异稻瘟净含量，毫克每千克(mg/kg)；

A——样液中异稻瘟净的峰面积；

A_S——标准工作溶液中异稻瘟净的峰面积；

c——标准工作溶液中异稻瘟净浓度，微克每毫升(μg/mL)；

V——样液最终定容体积，毫升(mL)；

m——称取的试样质量，克(g)。

14 测定低限、回收率

14.1 测定低限

本方法在大米、菠菜、苹果、牛肉、鸡肉、鱼肉、蜂蜜、板栗和食醋中测定低限为 0.005 mg/kg；在茶叶中测定低限为 0.01 mg/kg。

14.2 回收率

添加浓度和回收率见表 3。

表 3 回收率数据

样品名称	添加浓度/(mg/kg)	回收率范围/%
大米	0.005	75.0～94.0
	0.01	81.0～106
	0.2	88.0～92.5
菠菜	0.005	92.2～102
	0.01	82.3～108
	0.02	82.0～98.5
苹果	0.005	74.8～91.2
	0.01	78.5～98.1
	0.02	80.5～105
牛肉	0.005	93.8～106
	0.01	86.5～104
	0.02	87.5～112
鸡肉	0.005	84.0～101
	0.01	85.0～102
	0.02	81.0～103
鱼肉	0.005	94.6～100
	0.01	86.0～117
	0.02	86.0～108

表 3(续)

样品名称	添加浓度/(mg/kg)	回收率范围/%
蜂蜜	0.005	82.6～91.6
	0.01	86.6～105
	0.02	77.0～92.0
板栗	0.005	72.6～88.8
	0.01	72.5～88.8
	0.02	78.5～85.5
茶叶	0.005	80.2～107
	0.01	84.0～106
	0.02	86.8～107
食醋	0.005	93.6～100
	0.01	89.5～105
	0.02	94.5～102

附 录 A
（资料性附录）
异稻瘟净标准物质总离子流色谱图和质谱图

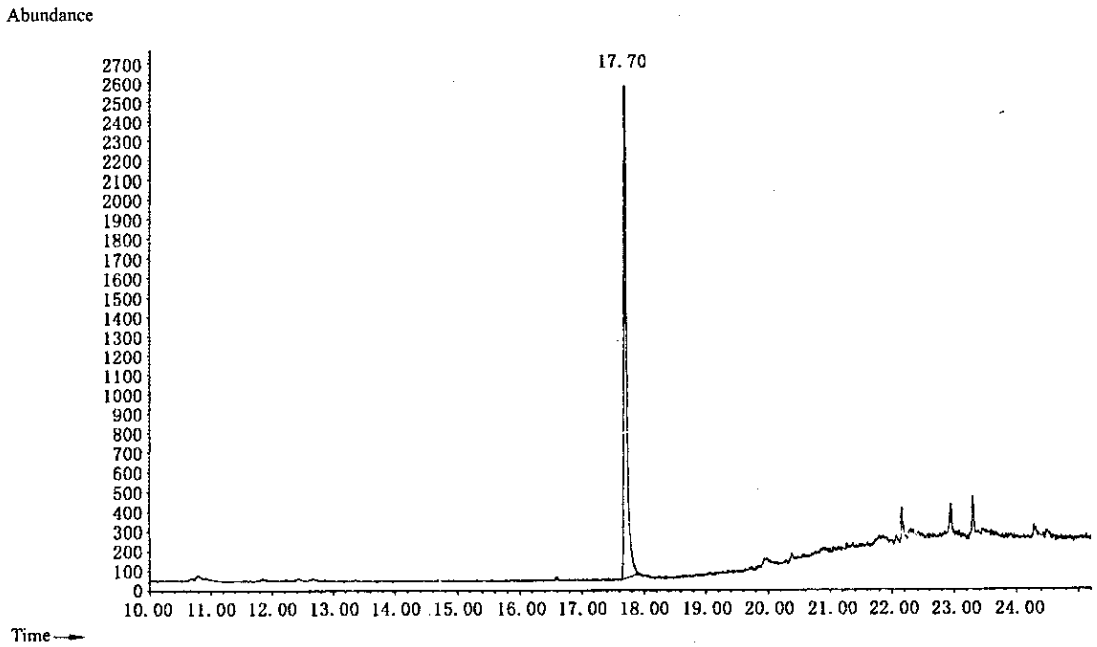

图 A.1 异稻瘟净标准物质的总离子流色谱图

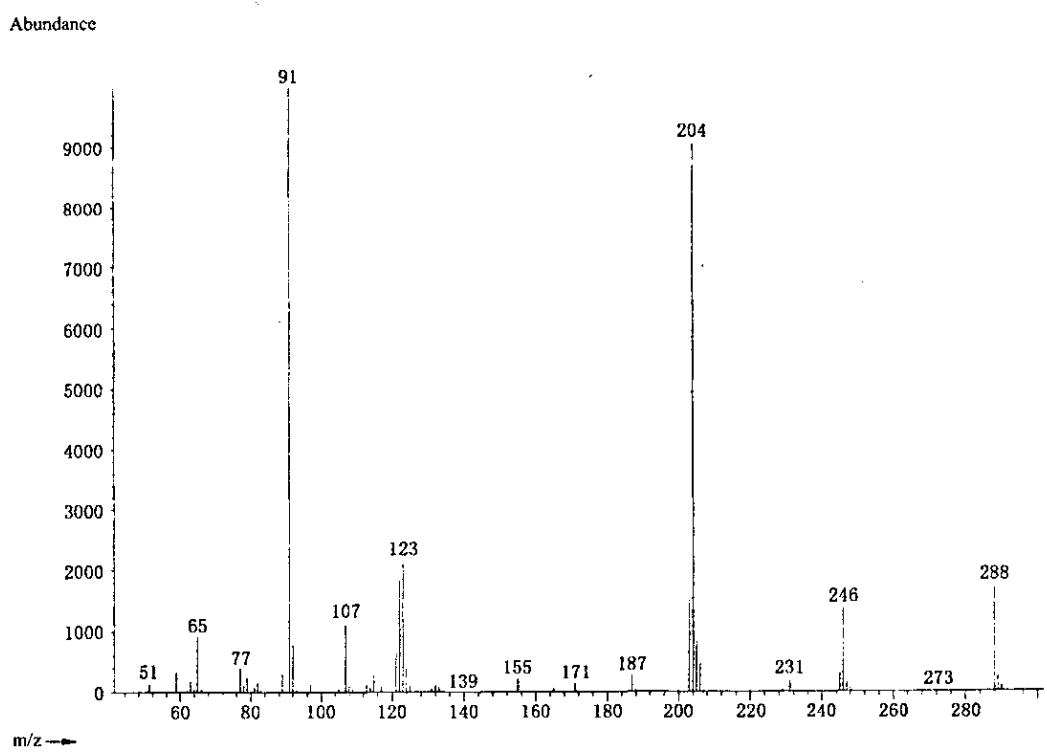

图 A.2 异稻瘟净标准物质的质谱图

附 录 B
（资料性附录）
异稻瘟净标准物质的色谱图

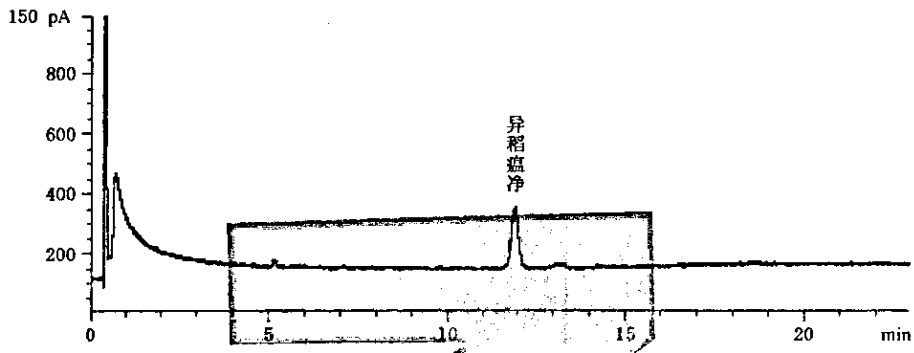

图 B.1 异稻瘟净标准物质的色谱图

Foreword

Annex A and B of this standard are informative annexes.

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 Hunan Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Tnner Mongolian Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Heilongjiang Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Fujian Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Li Yongjun, Yan Hongfei, ZhangYing, Huang Zhiqiang, Wang Meiling, Ligang, Yang Changzhi, Yangfang.

This standard is a professional standard for Entry-Exit Inspection and Quarantine promulgated for the first time.

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

Determination of iprobenfos residues in food for import and export

The first method Gas chromatography-mass spectrometry method

1 Scope

This standard specifies the determination and confirmation of iprobenfos residues by gas chromatography-mass spectrometry in food.

This standard is applicable to the determination and confirmation of iprobenfos residue content in tea, leek, Alliumchinese, apple, chestnut, bee honey, vinegar, rice, beef, chicken, fish.

2 Principle

The iprobenfos residues in the test sample are extracted with acetone-*n*-hexane (1+2). Cleaned up by passing through a graphitized carbon black cartridge or neutral aluminum oxide cartridge. The elutes solution is evaporated and made up to a definite volume. Determination and confirmation is made by means of gas chromatograph equipped with mass selective detector, using external standard method.

3 Reagents and materials

Unless otherwise specified, the reagents should be analytically pure, "Water" is redistilled water.

3.1 Acetone.

3.2 *n*-Hexane.

3.3 Sodium chloride.

3.4 Anhydrous sodium sulfate: Ignite at 650℃ for 4 h, and keep in a desiccator.

3.5 Acetone-*n*-hexane solution: Acetone + *n*-hexane(1+2).

3.6 Acetone-*n*-hexane solution: Acetone + *n*-hexane(1+1).

3.7 Iprobenfos standard ($C_{13}H_{21}O_3PS$, CAS: 26087-47-8): Purity≥99%.

3.8 Iprobenfos standard stock solution: Accurately weight an adequate amount of iprobenfos standard, dissolve in a small volume of acetone. Dilute with acetone to form a standard stock solution of 1 000 μg/mL in concerntration. Then dilute the standard stock solution with *n*-hexane to the required concerntration as the standard working solution. The standard solution should be stored below 4℃ and keep in dark place.

3.9 Graphitized carbon black cartridge: 3 mL, 125 mg, or equivalent.

3.10 Neutral aluminum oxide cartridge: 3 mL, 125 mg, or equivalent.

4 Apparatus and equipment

4.1 Gas chromatograph, equipped with mass detector.

4.2 Tissue triturator.

4.3 Grinder.

4.4 Vortex mixer.

4.5 Solid phase extraction with mechanical vacuum pump.

4.6 Multifunctional sample treatment unit for micro-chemical method, or equivalent.

4.7 Centrifuge: 3 000 r/min.

4.8 Centrifuge tube: 15 mL.

4.9 Graduated tube: 15 mL.

4.10 Micro-syringe: 10 μL.

5 Preparation and storage of test sample

5.1 Preparation of test samples

5.1.1 Fruits and vegetables

The representative samples is reduced to ca 500 g, which has been removed shell, seed, peel, stem,

root, coronal(do not wash by water), then cut up the edible portions are blended and then homogenized thoroughly in a high speed blender, and then are placed in a clean container as the test sample, which is sealed and labeled.

5.1.2 Tea and grains or cereals

The representative samples is reduced to ca 500 g, which is crushed with a grinder and let wholly pass through 2.0 mm sieve, and then are placed in a clean container as the test sample, which is sealed and labeled.

5.1.3 Meats and meat products

The representative samples is reduced to ca 500 g, The eatable potions are thoroughly ground and homogenized in a meat grinder. And then are placed in a clean container as the test sample, which is sealed and labeled.

5.2 Storage of test samples

The test samples of tea, bee products, grains or cereals should be stored below 0℃ ~4℃. The test samples of fresh fruits, vegetables, meat and meat products should be stored below −18℃. In the course of sampling and sample preparation, precaution must be taken to avoid contamination or any factors which may cause the change of residue content.

6 Procedure

6.1 Extraction

For tea, chestnut, bee honey, rice, weigh ca 1 g (accurate to 0.001 g) of the test sample into a 15 mL centrifuge tube. For leek, Alliumchinense, apple, vinegar, beef, chicken, fish, weigh ca 2 g (accurate to 0.001 g) of the test sample into a 15 mL centrifuge tube. Add 1 g sodium chloride, then add 2 mL redistilled water, blend for 30 s, stand for 15 min. Add 3 mL of acetone-*n*-hexane (3.5), blend for 2 min in vortex mixer, centrifuge for 1 min under 2 500 r/min. Transfer the upper acetone-*n*-hexane extract into another 15 mL graduated tube, Repeat the procedure with 3 mL of acetone-*n*-hexane twice, Combine acetone-*n*-hexane extracts.

6.2 Clean up

6.2.1 Tea, leek, Allium chinense, apple, chestnut, bee honey: Set up the solid phase extraction vaccum manifold and mechanical pump (about 1 cm thickness anhydrous sodium sulfate was put into the graphitized carbon black cartridge). First wash the graphitized carbon black cartridge with 1 mL × 3 acetone, then wash it with 1 mL × 3 *n*-hexane, keep flow speed at 1.5 mL/min. Discard the eluent. Transfer the extracts into the cartridge. Wash the test tube with 3 mL acetone + *n*-hexane solution

(3.6) and add into the cartridge. Collect the total eluent and blow nearly dry with nitrogen at 45℃ unit, Dissolve the residue and dilute exactly to 0.5 mL with *n*-hexane for GC/MS.

6.2.2 Vinegar, rice, beef, chicken, fish: Set up the solid phase extraction vaccum manifold and mechanical pump (about 1 cm thickness anhydrous sodium sulfate was put into the neutral aluminum oxide cartridge). First wash neutral aluminum oxide cartridge with 3 mL acetone, then wash it with 3 mL *n*-hexane, keep flow speed at 1.5 mL/min. Discard the eluent. Transfer the extracts into the cartridge. Wash the test tube with 3 mL acetone + *n*-hexane solution (3.6) and add into the cartridge. Collect the total eluent and blow nearly dry with nitrogen at 45℃ unit, Dissolve the residue and dilute exactly to 0.5 mL with *n*-hexane for GC/MS.

6.3 Determination

6.3.1 GC/MS operating conditions

a) Column: HP-5MS fused quartz capillary column, 30 m × 0.25 mm (i.d), film thickness 0.25 μm or the equivalent;

b) Column temperature: Initial temperature 80℃, ramp at 7℃/min to 205℃, ramp at 25℃/min to 280℃, hold for 5 min;

c) Injection port temperature: 280℃;

d) GC/MS interface temperature: 270℃;

e) Carrier gas: Helium, purity≥99.995%, 0.8 mL/min;

f) Injection volume: 1 μL;

g) Injection mode: Splitless, purge after 1 min;

h) Ionization mode: EI;

i) Ionization energy: 70 eV;

j) Acquistion mode: SIM;

k) Monitor ion (m/z): 203, 204, 246, 288; quantitative ion: 204;

l) Sovlent delay: 10 min.

6.3.2 GC/MS determination and GC/MS confirmation

According to the approximate concentration of iprobenfos in the test sample solution, select the

standard working solution with similar peak area to that of sample solution. The standard working solution should be injected randomly in between the injections of sample solution of equal volume. The responses of iprobenfos in the standard working solution and sample solution should be in the linear range of the instrumental detection.

According to the GC/MS operating conditions (6.3.1), if the retention time of samplechromatogram peaks are consistent with the standards, and subtracted from backgroud compensation, selected ions are all present and the relative ion abundence of the selected ionsaccording with that of the calibration standard, at comparable concentrations, within the tolerances (seen table 1). Under the above GC/MSD operating conditions, the retention time of iprobenfos is 17.70 min, and the ratio of the monitoring ions (m/z) is 203 : 204 : 246 : 288 = 17 : 100 : 15 : 19. See Annex A.

Table 1—Maximum permitted tolerances for relative ion abundance while confirmation

Relative bundance (base peak)/%	>50	>20~50	>10~20	≤10
Permitted tolerances/%	±10	±15	±20	±50

6.4 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.

6.5 Calculation and expression of result

The calculation of iprobenfos content in the sample is carried out by GC/MS data processor or according to the following formula (1). The blank value should be subtracted from the above result of calculation.

$$X = \frac{A \cdot c_S \cdot V}{A_S \cdot m} \qquad \cdots\cdots (1)$$

where

X—Iprobenfos content in the sample, mg/kg;

A—Peak area of iprobenfos in the sample solution;

c_S—Peak area of iprobenfos in the standard working solution, μg/mL;

A_S—Concentration of iprobenfos in the standard working solution;

V—Final volume of sample solution, mL;

m—Mass of test sample, g.

7 Limit of determination and recovery

7.1 Limit of determination

The limit of determination of this method is 0.005 mg/kg.

7.2 Recovery

According to the experimental data, the fortifying concentration of isoprofos for each sample and its corresponding recoveries see table 2.

Table 2—The recovery of the method

Sample	Spiked concentration/(mg/kg)	Range of recovery/%
rice	0.005	91.9～113.1
	0.01	91.6～108.1
	0.20	97.5～119.0
tea	0.005	74.9～93.3
	0.01	90.8～107.9
	0.02	83.5～108.1
leek	0.005	86.8～110.1
	0.01	91.9～110.5
	0.02	107.1～118.9
apple	0.005	75.3～86.5
	0.01	92.8～119.9
	0.02	95.6～113.1
honey	0.005	96.8～114.4
	0.01	97.7～115.8
	0.02	91.2～112.8
chestnut	0.005	109.9～120.9
	0.01	90.2～108.8
	0.02	88.8～119.5
vinegar	0.005	93.4～104.2
	0.01	89.9～100.6
	0.02	95.2～107.2
allium chinense	0.005	87.6～98.5
	0.01	93.7～100.6
	0.02	82.1～94.3
fish	0.005	81.8～104.9
	0.01	80.9～94.0
	0.02	90.0～106.8

Table 2 (continued)

Sample	Spiked concentration/(mg/kg)	Range of recovery/%
beef	0.005	86.4～99.4
	0.01	92.4～111.0
	0.02	94.2～111.9
chicken	0.005	70.9～103.0
	0.01	70.2～93.1
	0.02	73.3～92.7

The second method Gas chromatography method

8 Scope

This standard specifies the determination and confirmation of iprobenfos residues by gas chromatography-mass spectrometry in food.

This standard is applicable for determination of Isoprofos residue in rice, spinach, apple, beef, chicken, fish, bee honey, chestnut, tea, vinegar.

9 Principle

Isoprofos residues is extracted from the test sample with with acetone-hexane (1+2) and hexane. Then, the extraction solution is cleaned up and determinated by Gas chromatograph with FPD. External standard method is used for quantitative measurement.

10 Reagents and materials

Unless otherwise specified, all reagents used should be of analytical grade, "water" is distilled water or corresponding de-ionized water.

10.1 Acetone.

10.2 *n*-Hexane.

10.3 Anhydrous sodium sulfate: Ignite at 650℃ for 4 h, and keep in a desiccator.

10.4 Acetone-*n*-hexane solution: Acetone + *n*-hexane (1+2).

10.5 Iprobenfos standard ($C_{13}H_{21}O_3PS$, CAS: 26087-47-8): Purity≥99%.

10.6 Iprobenfos standard solution: Accurately weight an adequate amount of iprobenfos standard, dissolve in a small volume of acetone. Dilute with acetone to form a standard stock solution of 1 000 μg/mL in concerntration. Then dilute the standard stock solution with *n*-hexane to the required concerntration as the standard working solution. The standard solution should be stored below 4℃ and keep in dark place.

10.7 Graphitized carbon black cartridge: 3 mL, 125 mg, or equivalent.

10.8 Neutral aluminum oxide cartridge: 3 mL, 125 mg, or equivalent.

11 Apparatus and equipment

11.1 Gas chromatograph (equipped with detector FPD).

11.2 Vortex mixer.

11.3 Centrifuge: 3 000 r/min.

11.4 Multifunction sample treatment unit for microchemical method or equivalent.

11.5 Centrifuge tube with ground stopper: 5 mL, 10 mL.

11.6 Test-tube: 20 mL.

11.7 Glass capillary-tip pipettes.

11.8 Micro-adjustable volumne pipettes: 10 μL, 200 μL, 1 000 μL.

11.9 Micro-syringe: 10 μL.

12 Preparation and storage of test sample

12.1 Preparation of test samples

12.1.1 Fruits and vegetables

The representative samples is reduced to ca 500 g, which has been removed shell, seed, peel, stem, root, coronal (do not wash by water), then cut up the edible portions are blended and then homogenized thoroughly in a high speed blender, and then are placed in a clean container as the test sample,

which is sealed and labeled.

12.1.2 Tea and grains or cereals

The representative samples is reduced to ca 500 g, which is crushed with a grinder and let wholly pass through 2.0 mm sieve, and then are placed in a clean container as the test sample, which is sealed and labeled.

12.1.3 Meats and meat products

The representative samples is reduced to ca 500 g. The eatable potions are thoroughly ground and homogenized in a meat grinder. And then are placed in a clean container as the test sample, which is sealed and labeled.

12.2 Storage of test samples

The test samples of tea, bee products, grains or cereals should be stored below 0℃ ~4℃. The test samples of fresh fruits, vegetables, meat and meat products should be stored below −18℃.

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

13 Procedure

13.1 Extraction and cleanup

13.1.1 For rice, chestnut, bee honey, weigh 2 g of the test sample (accurate to 0.001 g) in 10 mL centrifuge tube. Add 2 mL water, then saturate the sample with anhydrous sodium sulfate. Add 2 mL acetone + hexane (10.4), blend for 2 min in vortex mixer and centrifuge for 3 min. Repeat the procedure with 2 mL acetone + hexane (10.4) twice, Then, the residue are extracted with 2 mL hexane, blend for 2 min in vortex mixer, and centrifuge for 3 min. Repeat the procedure with 2 mL hexane twice, Combine the upper layer to obtain the extracted solution. Transfer all the extracts to centrifuge tube with ground stopper and concentrate to 1.0 mL with nitrogen at 40℃. The concentrated solution is ready for gas chromatographic determination. While emulsified during extracting, increase the extract solution volume.

13.1.2 For Spinach, apple, vinegar, weigh 2 g of the test sample (accurate to 0.001 g) in 10 mL centrifuge tube. For tea, weigh 0.5 g of the test sample (accurate to 0.001 g) in 10 mL centrifuge tube, add 2 mL water, then saturate the sample with anhydrous sodium sulfate. Add 2 mL acetone + hexane (10.4), blend for 2 min in vortex mixer and centrifuge for 3 min. Repeat the procedure with 2 mL acetone + hexane (10.4) twice, Then, the residue are extracted with 2 mL hexane, blend for 2 min in vortex mixer, and centrifuge for 3 min. Repeat the procedure with 2 mL hexane twice. Combine the upper layer to obtain the extracted solution. Elute the graphitized carbon black cartridge with

4 mL acetone + hexane (10.4). Discarded the eluent. Transfer all extracts to the graphitized carbon black cartridge, then elute the cartridge with 4 mL acetone + hexane (10.4). Collect all the eluent in centrifuge tube with ground stopper and concentrate to 1.0 mL with nitrogen at 40℃. The concentrated solution is ready for gas chromatography determination.

13.1.3 For beef, chicken, fish, weigh 2 g of the test sample (accurate to 0.001 g) in 10 mL centrifuge tube. Add 2 mL water, then saturate the sample with anhydrous sodium sulfate. Add 6 mL of acetone + hexane (10.4), blend for 2 min in vortex mixer and centrifuge for 3 min. Then, the residue are extracted with 2 mL hexane, blend for 2 min in vortex mixer, and centrifuge for 3 min. Repeat the procedure with 2 mL hexane twice, Combine the upper layer to obtain the extracted solution. Elute the neutral aluminum oxide cartridge with 4 mL acetone + hexane (10.4). Discarded the eluent. Transfer all extracts to the neutral aluminum oxide cartridge, then elute the cartridge with 4 mL acetone + hexane (10.4). Collect all the eluent in centrifuge tube with ground stopper and concentrate to 1.0 mL with nitrogen at 40℃. The concentrated solution is ready for gas chromatography determination.

13.2 Determination

13.2.1 GC operating conditions

a) Column: EQUITY™-1701 used quartz capillary column, 30 m × 0.32 mm × 1.0 μm (i.d), or equivalent;

b) Column temperature: Initial temperature 100℃, ramp at 10℃/min to 220℃, hold for 10 min;

c) Injection port temperature: 250℃;

d) Detector temperature: 250℃;

e) Nitrogen: Purity≥99.99%, carrier gas 5.0 mL/min;

f) Hydrogen: 75 mL/min;

g) Oxygen: 100 mL/min;

h) Make-up gas: 20 mL/min;

i) Injection mode: splitless purge after 1 min;

j) Injection volume: 2 μL.

13.2.2 GC determination

According to the approximate concentration of isoprofos pesticide in the test sample solution, select the standard working solution with similar peak area to that of sample solution. The responses of isoprofos pesticide in the standard working solution and sample solution should be within the linear range of the instrumental detection. The standard working solution should be injected randomly in between the injections of sample solution of equal volume. Identify by the RT and quantify with peak area. Under the above GC opening condition, the Gas Chromatogram of isoprofos standard is seen figure B.1 in Annex B.

13.3 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.

13.4 Calculation and expression of result

The calculation of isoprofos pesticide content in the sample is carried out by GC data processor or according to the formula (2). The blank value should be subtracted from the above result of calculation.

$$X = \frac{A \cdot c \cdot V}{A_s \cdot m} \qquad \cdots\cdots (2)$$

where

X—the residue content of isoprofos in the test sample, mg/kg;

A—the peak area of isoprofos in the sample solution;

A_s—the peak area of isoprofos in the standard working solution;

c—the concentration of isoprofos in the standard working solution, μg/mL;

V—the final volume of sample solution, mL;

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

14 Limit of determination and recovery

14.1 Limit of determination

The limit of determination in rice, spinach, apple, cattle muscle, chicken muscle, fish musucle, bee honey, chestnut and vinegar by this method is 0.005 mg/kg; tea is 0.01 mg/kg.

14.2 Recovery

According to the experimental data, the fortifying concentration of isoprofos for each sample and its

corresponding recoveries see table 3:

Table 3—The recovery of the method

Sample	Spiked concentration/(mg/kg)	Range of the recovery/%
rice	0.005	75.0～94.0
	0.01	81.0～106
	0.2	88.0～92.5
leek	0.005	92.2～102
	0.01	82.3～108
	0.02	82.0～98.5
apple	0.005	74.8～91.2
	0.01	78.5～98.1
	0.02	80.5～105
beef	0.005	93.8～106
	0.01	86.5～104
	0.02	87.5～112
chicken	0.005	84.0～101
	0.01	85.0～102
	0.02	81.0～103
fish	0.005	94.6～100
	0.01	86.0～117
	0.02	86.0～108
Bee honey	0.005	82.6～91.6
	0.01	86.6～105
	0.02	77.0～92.0
chestnut	0.005	81.8～104.9
	0.01	80.9～94.0
	0.02	90.0～106.8
tea	0.005	86.4～99.4
	0.01	92.4～111.0
	0.02	94.2～111.9
vinegar	0.005	70.9～103.0
	0.01	70.2～93.1
	0.02	73.3～92.7

Annex A
(Informative)
Total ion chromatogram and Mass spectrum of the iprobenfos standard

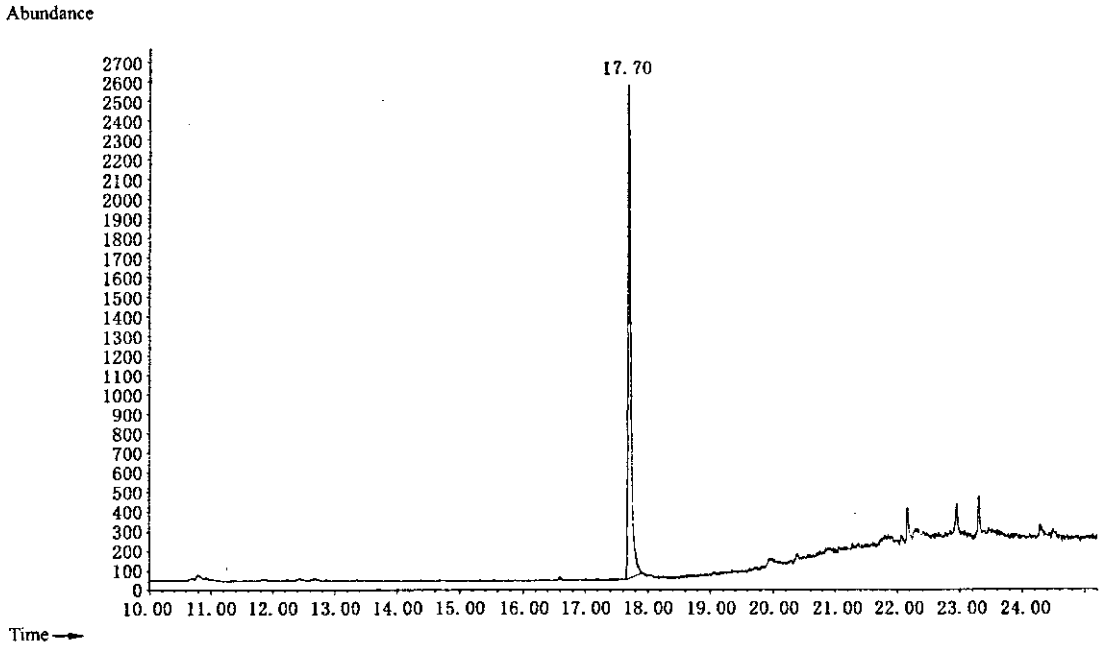

Figure A. 1—Total ion chromatogram of the iprobenfos standard

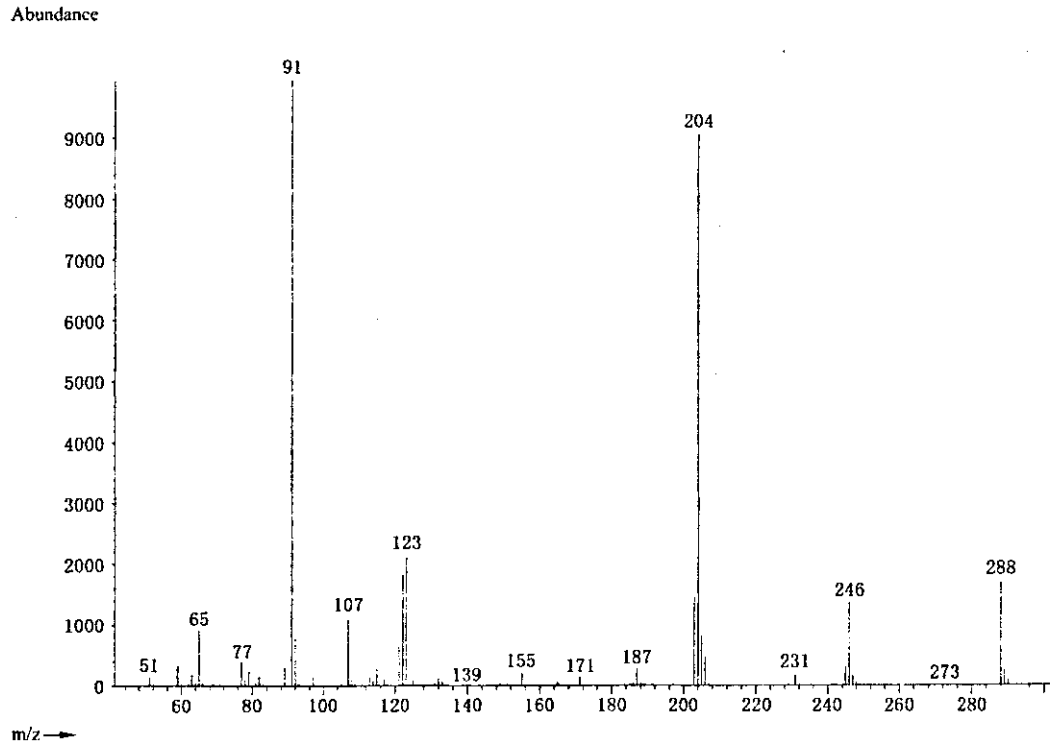

Figure A. 2—Mass spectrum of the iprobenfos standard

Annex B
(Informative)
Chromatogram of the standard

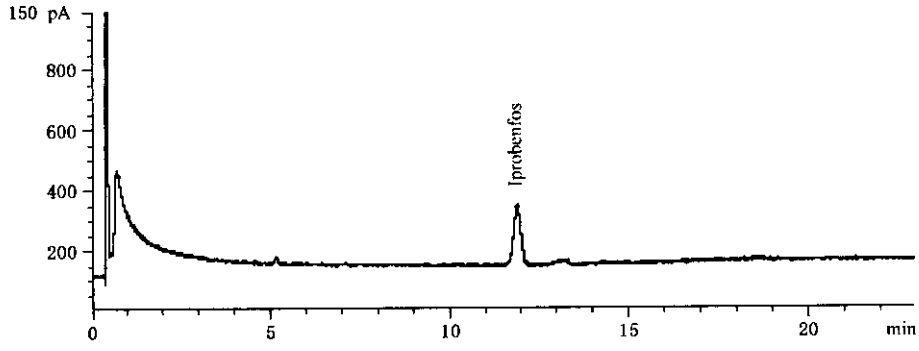

Figure B. 1—Chromatogram of the standard

中华人民共和国出入境检验检疫
行 业 标 准
进出口食品中异稻瘟净残留量的检测方法
SN/T 1967—2007
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