SN

中华人民共和国出入境检验检疫行业标准

SN/T 1624—2009
代替 SN/T 1624—2005

进出口食品中嘧霉胺、嘧菌胺、腈菌唑、嘧菌酯残留量的检测方法 气相色谱-质谱法

Determination of pyrimethanil, mepanipyrim, myclobutanil and azoxystrobin residues in foods for import and export—GC-MS method

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前　言

本标准代替 SN/T 1624—2005《进出口蔬菜和水果中嘧霉胺、嘧菌胺、腈菌唑及嘧菌酯残留量检验方法》。

本标准与 SN/T 1624—2005 相比，主要变化如下：

——检测基质由 4 种增加到了 9 种；

——去掉了抽样部分；

——提取和净化方法根据基质的不同进行了相应的改变。

本标准的附录 A 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。

本标准由中华人民共和国河北出入境检验检疫局、中华人民共和国辽宁出入境检验检疫局、中华人民共和国天津出入境检验检疫局、中华人民共和国内蒙古出入境检验检疫局起草。

本标准主要起草人：郭春海、贾海涛、王凤池、陈瑞春、段文仲、卫锋、林安清、李刚。

本标准 2005 年首次发布，本次为第一次修订。

进出口食品中嘧霉胺、嘧菌胺、腈菌唑、嘧菌酯残留量的检测方法 气相色谱-质谱法

1 范围

本标准规定了试样的制备方法和保存条件以及粮谷、蔬菜、水果、坚果、茶叶、畜、禽、水产品、蜂产品中嘧霉胺、嘧菌胺、腈菌唑、嘧菌酯残留量检验的气相色谱-质谱测定方法。

本标准适用于大米、茄子、苹果、板栗、茶叶、牛肉、鸡肉、鱼、蜂蜜中嘧霉胺、嘧菌胺、腈菌唑、嘧菌酯残留量的测定。

2 方法提要

用丙酮或乙酸乙酯、丙酮和氯化钠水溶液提取试样中残留的嘧霉胺、嘧菌胺、腈菌唑、嘧菌酯，经液液萃取和石墨化炭黑柱/氨基柱组合柱净化，用气相色谱-质谱仪选择离子检测，外标法定量。

3 试剂和材料

除另有规定外，试剂均为分析纯，水为蒸馏水或去离子水。

3.1 丙酮：农残级。

3.2 乙酸乙酯。

3.3 乙腈：HPLC 级。

3.4 甲苯。

3.5 正己烷。

3.6 氯化钠。

3.7 无水硫酸钠：于 650 ℃灼烧 4 h，储于密封容器中备用。

3.8 氯化钠溶液(300 g/L)：称取 300 g 氯化钠用蒸馏水溶解并定容至 1 000 mL。

3.9 乙腈-甲苯(3+2，体积比)：取 30 mL 乙腈和 20 mL 甲苯，混合均匀。

3.10 乙腈-甲苯(3+1，体积比)：取 30 mL 乙腈和 10 mL 甲苯，混合均匀。

3.11 丙酮-正己烷(1+1，体积比)：取 50 mL 丙酮和 50 mL 正己烷，混合均匀。

3.12 乙腈饱和正己烷。

3.13 正己烷饱和乙腈。

3.14 石墨化炭黑柱/氨基柱组合柱：500 mg，6 mL；或者石墨化炭黑柱(500 mg，6 mL)与氨基柱(500 mg，3 mL)按照从上到下串联使用。

3.15 嘧霉胺标准品：分子式，$C_{12}H_{13}N_3$；CAS 编号 53112-28-0；纯度≥99%。

3.16 嘧菌胺标准品：分子式，$C_{14}H_{13}N_3$；CAS 编号 110235-47-7；纯度≥99%。

3.17 腈菌唑标准品：分子式，$C_{15}H_{17}ClN_4$；CAS 编号 88671-89-0；纯度≥99%。

3.18 嘧菌酯标准品：分子式，$C_{22}H_{17}N_3O_5$；CAS 编号 131860-33-8；纯度≥99%。

3.19 嘧霉胺、嘧菌胺、腈菌唑、嘧菌酯标准溶液：准确称取适量的嘧霉胺、嘧菌胺、腈菌唑、嘧菌酯标准品，用丙酮配制成 100 μg/mL 标准储备液，5 ℃以下贮存，6 个月以内使用。再用丙酮稀释成适当浓度的标准工作溶液，5 ℃以下贮存，3 个月以内使用。

4 仪器和设备

4.1 气相色谱-质谱联用仪:配置电子轰击离子源。
4.2 水平回旋式摇床。
4.3 涡旋混合器。
4.4 均质器。
4.5 离心机:4 000 r/min。
4.6 固相萃取装置。
4.7 旋转蒸发器。
4.8 氮气吹干仪。
4.9 离心管:玻璃,50 mL。
4.10 无水硫酸钠柱:150 mm×10 mm 玻璃层析柱,从下往上依次装入脱脂棉,5 cm 高的无水硫酸钠(3.7)。

5 试样的制备与保存

5.1 水果、蔬菜、坚果

水果、坚果去皮或核,取可食部分约 500 g,用粉碎机粉碎,装入洁净容器作为试样,密封并做好标识,于－18 ℃冰箱内保存。

5.2 动物源性食品

5.2.1 肉类及水产品:取样品中有代表性的可食部分约 500 g,用粉碎机粉碎,装入洁净容器作为试样,密封并做好标识,于－18 ℃冰箱内保存。

5.2.2 蜂蜜:取有代表性样品约 500 g,未结晶样品将其用力搅拌均匀,有结晶析出样品可将样品瓶盖塞紧后,置于不超过 60 ℃的水浴中,待样品全部溶化后搅匀,迅速冷却至室温。制备好的样品装入洁净容器内密封并做好标识,室温保存。

5.3 粮谷、茶叶

取样品约 500 g,用粉碎机粉碎至全部通过 20 目筛,装入洁净容器作为试样,密封并做好标识,室温保存。

制样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

6.1.1 水果、蔬菜

称取搅碎混匀的试样约 10 g(精确到 0.01 g)于 100 mL 锥形瓶中,加 25 mL 丙酮(3.1)和 15 g 氯化钠(3.6),在水平回旋式摇床上振摇 30 min。过滤到 250 mL 分液漏斗中,用 20 mL 丙酮(3.1)分两次洗涤锥形瓶及滤渣,合并滤液于分液漏斗中,加 25 mL 氯化钠溶液(3.8)和 30 mL 乙酸乙酯(3.2)于同一分液漏斗中,振摇 2 min,静置分层,收集有机相。下层水相再用 20 mL 乙酸乙酯(3.2)萃取一次,合并有机相,过无水硫酸钠柱(4.10)至心形瓶中。于 45 ℃水浴上抽真空旋转蒸发至干。

6.1.2 畜、禽、水产品

称取搅碎混匀的试样约 10 g(精确到 0.01 g)于 50 mL 离心管中,加 25 mL 乙酸乙酯(3.2),15 g 氯化钠(3.6),均质 1 min,以 4 000 r/min 离心 5 min。取上层有机相过无水硫酸钠柱(4.10)转入心形瓶。再每次用 20 mL 乙酸乙酯(3.2)分两次按上述步骤提取残渣,合并有机相于心形瓶中。于 45 ℃水浴上抽真空旋转蒸发至干。

6.1.3 粮谷、茶叶、板栗

称取搅碎混匀的试样约10 g(精确到0.01 g)于三角瓶中,加25 mL乙酸乙酯(3.2)(板栗样品加5 g氯化钠),在水平回旋式摇床上振摇30 min,过滤到250 mL分液漏斗中,用20 mL乙酸乙酯(3.2)分两次洗涤锥形瓶及滤渣,合并滤液于同一分液漏斗中并加入30 mL氯化钠溶液(3.8),振荡1 min,液液萃取,静置分层,乙酸乙酯层经无水硫酸钠柱(4.10)转入心形瓶中;水相再加入25 mL乙酸乙酯(3.2)液液萃取,静置分层,弃去水相,合并乙酸乙酯(3.2)层于上述同一心形瓶中。于45 ℃水浴上抽真空旋转蒸发至干。

6.1.4 蜂蜜

称取10 g(精确到0.01 g)蜂蜜样品于三角瓶中,加入20 mL氯化钠溶液(3.8)和5 mL丙酮(3.1)溶解,在水平回旋式摇床上振摇30 min,转入250 mL分液漏斗中,再分别用30 mL氯化钠溶液(3.8)分两次,50 mL乙酸乙酯(3.2)分两次洗原三角瓶,均转入同一分液漏斗,振摇2 min,静置分层(如果发生乳化,可将上层及乳化层在4 000 r/min离心5 min,取上层转入心形瓶),收集有机相到另一个分液漏斗。水相再用20 mL乙酸乙酯(3.2)提取两次,合并有机相到同一分液漏斗中,分液漏斗中加入40 mL氯化钠溶液(3.8)振摇1 min,静置(如果发生乳化,可将上层及乳化层在4 000r/min离心5 min),乙酸乙酯(3.2)层过无水硫酸钠柱(4.10)转入上述心形瓶,于45 ℃水浴上抽真空旋转蒸发至干。

6.2 净化

6.2.1 畜、禽、水产品、粮谷、茶叶、板栗

对6.1.2和6.1.3获得的试样残渣,加入40 mL乙腈饱和正己烷(3.12)分两次溶解,转入同一250 mL分液漏斗中,分别用50 mL正己烷饱和乙腈(3.13)分两次、乙腈饱和正己烷(3.12)10 mL洗心形瓶,均转入上述分液漏斗中。振荡分层,乙腈层过无水硫酸钠柱(4.10)转入原心形瓶,正己烷层每次再用正己烷饱和乙腈(3.13)15 mL洗两次,正己烷层弃去,乙腈层过无水硫酸钠柱合并入心形瓶,于45 ℃水浴上抽真空旋转蒸发至干。

6.2.2 石墨化炭黑柱/氨基柱组合柱净化

用1 mL乙腈-甲苯(3.10)溶解6.1.1、6.1.4、6.2.1得到的试样残渣,全部转入石墨化炭黑柱-氨基柱(3.14)。再用1 mL乙腈-甲苯(3.10)分两次洗心形瓶,并入上述石墨化炭黑柱-氨基柱(3.14),弃去全部流出液。用10 mL乙腈-甲苯(3.9)洗脱石墨化炭黑柱-氨基组合柱,接收全部洗脱液。于45 ℃水浴上氮气流吹干。用丙酮-正己烷(3.11)定容1.0 mL。供气相色谱-质谱分析。

6.3 测定

6.3.1 气相色谱-质谱条件

6.3.1.1 色谱条件

a) 色谱柱:DB-5MS, 30 m×0.25 mm(内径),0.25 μm,或相当者;

b) 色谱柱升温程序:210 ℃(2 min)$\xrightarrow{30\ ℃/min}$280 ℃$\xrightarrow{10\ ℃/min}$290 ℃(6 min);

c) 进样口温度:250 ℃;

d) 载气:氦气,纯度99.999%;

e) 载气流速:恒流模式1 mL/min;

f) 进样方式:不分流;

g) 进样量:2 μL;

h) 开阀时间:1 min。

6.3.1.2 质谱条件

a) 接口温度:280 ℃;

b) 离子源:电子轰击源(EI);

c) 电离电压:70 eV;

d) 离子源温度:230 ℃;

e) 检测方式:SIM;

f) 溶剂延迟时间:2.5 min;

g) 选择离子及相对丰度:见表1。

表1 选择离子及相对丰度表

被测组分	定量离子(相对丰度)/%	定性离子(相对丰度)/%		
嘧霉胺	198(100)	199(47)	118(3)	184(4)
嘧菌胺	222(100)	223(51)	208(5)	181(3)
腈菌唑	179(100)	150(53)	245(14)	288(13)
嘧菌酯	344(100)	388(28)	372(14)	403(13)

6.3.2 气相色谱-质谱测定

对样液及标准工作液等体积参差进样测定。实际应用的标准工作液和待测样液中,嘧霉胺、嘧菌胺、腈菌唑、嘧菌酯的响应值均应在仪器的线性范围之内。在上述气相色谱-质谱条件下,嘧霉胺、嘧菌胺、腈菌唑、嘧菌酯的保留时间分别为3.42 min、5.48 min、5.90 min和11.63 min。标准品气相色谱-质谱图参见附录A。

6.3.3 气相色谱-质谱确证试验

进行样品测定时,如果检出的质量色谱峰保留时间与标准样品一致,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,误差不超过表2规定的范围,则可判断样品中存在对应的被测物。

表2 定性确证时相对离子丰度的最大允许误差

相对离子丰度(基峰)/%	>50	>20~50	>10~20	≤10
允许的相对误差/%	±10	±15	±20	±50

6.3.4 空白试验

除不加样品外,按上述相同条件和步骤进行。

7 结果计算和表述

用色谱数据处理仪或按式(1)计算,计算结果需扣除空白值。

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

式中:

X_i——试样中嘧霉胺、嘧菌胺、腈菌唑、嘧菌酯含量,单位为毫克每千克(mg/kg);

A_i——样液中嘧霉胺、嘧菌胺、腈菌唑、嘧菌酯的峰面积;

c_i——标准溶液中嘧霉胺、嘧菌胺、腈菌唑、嘧菌酯的浓度,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}——标准溶液中嘧霉胺、嘧菌胺、腈菌唑、嘧菌酯的峰面积;

m——最终样液所代表的试样量,单位为克(g)。

8 方法的测定低限、回收率

8.1 测定低限

四种农药的测定低限分别为:嘧霉胺为0.01 mg/kg;嘧菌胺为0.01 mg/kg;腈菌唑为0.01 mg/kg;嘧菌酯为0.005 mg/kg。

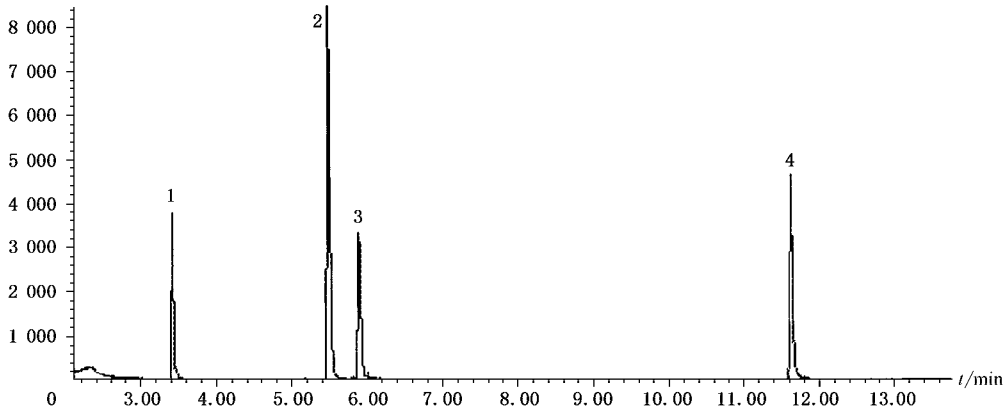
8.2 回收率

回收率数据见表3。

表3 样品的添加浓度及回收率数据

样品名称	添加浓度/(mg/kg)											
	嘧霉胺			嘧菌胺			腈菌唑			嘧菌酯		
	0.01	0.05	0.1	0.01	0.05	0.1	0.01	0.05	0.1	0.005	0.05	0.1
	回收率/%											
苹果	81.0～93.0	88.0～102.0	84.0～96.0	82.0～92.0	84.6～94.7	86.0～98.0	75.0～98.0	82.0～98.0	84.0～94.0	92.0～120.0	84～102.0	85.0～105
牛肉	81.0～92.0	82.0～92.0	82.0～96.0	75.0～97.0	84.0～110	84.0～91.0	82.0～95.0	82.0～102	83.0～97.0	82.0～102	82.0～98.0	88.0～105
鸡肉	84.0～110.0	82.0～98.0	81.0～95.0	83.0～104.0	82.0～104	84.0～96.0	84.0～105	82.0～94.0	82.0～104.0	82.0～102	82.0～108	84.0～96.0
茄子	81.0～95.0	84.0～102	84.0～96.0	80.0～95.0	80.0～96.0	84.0～97.0	81.0～116	82.0～96.0	82.0～95.0	84.0～110	84.0～104	87.0～97.0
大米	81.0～98.0	80～102	83.0～98.0	82.0～103	88.0～98.0	82.0～97.0	80.0～99.0	72.0～92.0	81.0～96.0	76.0～94.0	84.0～110	83.0～98.0
鱼肉	81.0～97.0	80.2～102	86.0～105	82.0～98.0	80.2～96.0	84.0～97.0	81.0～98.0	80.2～106	85.0～97.0	74.0～116	80.2～106	81.0～97.0
蜂蜜	83.0～97.0	80.2～102	84.0～98.0	81.0～96.0	80.2～106	81.0～99.0	73.0～106	80.6～110	82.0～96.0	80.0～120	86.0～94.0	81.0～105
板栗	80.0～95.0	80.0～102	85.0～104	82.0～115	82.0～102	80.0～95.0	84.0～107	82.0～100.0	81.0～98.0	82.0～108.0	84.0～110.0	84.0～98.0
茶	81.0～107.0	80.0～110	81.0～94.0	81.0～98.0	82.0～96.0	81.0～95.0	86.0～120	82.0～108.0	81.0～98.0	76.0～116	82.0～98.0	81.0～97.0

附 录 A
（资料性附录）
标准物质的气相色谱及气相色谱-质谱图



1——嘧霉胺；
2——嘧菌胺；
3——腈菌唑；
4——嘧菌酯。

图 A.1 标准混合物的总离子流图(TIC)(四种农药浓度均为 1 μg/mL)

Foreword

This standard is modified based on SN/T 1624—2005《Determination of Pyrimethanil，Mepanipyrim，Myclobutanil and Azoxystrobin residues in vegetable and fruit for import and export》.

This standard modified for SN/T 1624—2005 as follows：

—Sample matrix variety from four increase to nine；

—Take out sampling method；

—The method of extraction and clean up is corresponding changed based on matrix variety.

Annex A is an informative annex.

This standard was proposed by and is under the charge of State Authentication and Supervised Committee of the People's Republic of China.

This standard was drafted by Hebei Entry-Exit Inspection and Quarantine Bureau，Liaoning Entry-Exit Inspection and Quarantine Bureau，Tianjin Entry-Exit Inspection and Quarantine Bureau，Neimenggu Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Guochunhai，Jiahaitao，Wangfengchi，Chenruichun，Duanwenzhong，Weifeng，Linanqing and Ligang.

This standard is published for the first time in 2005. This standard is modified for the first time.

Determination of pyrimethanil, mepanipyrim, myclobutanil and azoxystrobin residues in foods for import and export—GC-MS method

1 Scope

This standard specifies the method of sample preparation and determination by gas chromatography-Mass spectrum of Pyrimethanil, Mepanipyrim, myclobutanil and Azoxystrobin residues in grain, vegetable, fruit, nut, tea, animal, birds, fish and bees.

This standard is applicable for the determination of Pyrimethanil, Mepanipyrim, Myclobutanil and Azoxystrobin residues in rice, egg, plant, apple, chestnut, tea, beef, chicken, fish and bee.

2 Principle

After the residues of pyrimethanil, Mepanipyrim, Myclobutanil and Azoxystrobin are extracted by acetone or ethyl acetate or sodium chloride solution from the sample. Clean up by liquid-liquid extraction and ENVI-Carb/NH_2. detected by GC-MS, quantitative analysis using external standard method.

3 Reagents and materials

Unless otherwise specified, all reagents are analytically pure "water" is distilled water.

3.1 Acetone: Pesticide grade.

3.2 Ethyl acetate.

3.3 Acetonitrile: HPLC-grade.

3.4 Toluene.

3.5 *n*-hexane.

3.6 Sodium chloride.

3.7 Anhydrous sodium sulfate: Ignite at 650 ℃ for 4 h, and store in air-tight container.

3.8 Sodium chloride solution(300 g/L): Weight 300 g sodium chloride, dissolve and exactly diluted to 1 000 mL.

3.9 Acetontrile-toluene (3+2, *V*/*V*).

3.10 Acetontrile-toluene (3+1, *V*/*V*).

3.11 Acetone-*N*-hexane (1+1, *V*/*V*).

3.12 *n*-hexane saturation with acetonitrile.

3.13 Acetonitrile saturation with *n*-hexane.

3.14 Column of ENVI-Carb + amido, 500 mg, 6 mL: Rinse the column of ENVI-Carb + amido with 3 mL Acetontrile-Toluene(3+1) before use.

3.15 Pyrimethanil standard: Molecular formula $C_{12}H_{13}N_3$; CAS No. 53112-28-0; Purity≥99%.

3.16 Mepanipyrim standard: Molecular formula $C_{14}H_{13}N_3$; CAS No. 110235-47-7; Purity≥99%.

3.17 Myclobutanil standard: Molecular formula $C_{15}H_{17}ClN_4$; CAS No. 88671-89-0; Purity≥99%.

3.18 Azoxystrobin standard: Molecular formula $C_{22}H_{17}N_3O_5$; CAS No. 131860-33-8; Purity≥99%.

3.19 Pyrimethanil, Mepanipyrim, Myclobutanil, Azoxystrobin standard solution: Accurately weigh an adequate amount of four standard, dissolve in acetone to make up four standard stock solutions of 100 μg/mL. The solution should be stored below 5 ℃, use within six months. According to the content of analyzed matter, make up mixture standard working solution by adding adequate each of the stock solution and diluting with acetone. The solution should be stored below 5 ℃. Use within three months.

4 Apparatus and equipment

4.1 GC-MS equipped with EI source.

4.2 Orbital shaker.

4.3 Vortex shaker.

4.4 Homogenizer.

4.5 Centrifuge, 4 000 r/min.

4.6 Solid phase extraction equipment.

4.7 Rotary evaporator.

4.8 Nitrogen evaporator.

4.9 Glass centrifuge tube,50 mL.

4.10 Column of anhydrous sodium sulfate: In the glass chromatographic column of 150 mm × 10 mm(i. d.),in turn adding absorbent cotton and 5 cm of anhydrous sodium sulfate(3.7).

5 Sample preparation and storage

5.1 Fruit, vegetable, nut

Remove pell or core, the edible portion is blended about 500 g, break to pieces by disintegrator, the sample is placed in a clear container as the test sample,airproof and marked. The test sample should be stored at −18 ℃.

5.2 Animal original food

5.2.1 Animal, birds, fish

The combined primary sample is reduced to 500 g,the edible portion is blended,break to pieces by disintegrator,the sample is placed in a clear container as the test sample,airproof and marked. The test sample should be stored at −18 ℃.

5.2.2 Honey

The combined primary sample is reduced to 500 g,not crystal sample beat up to equalityc. Crystal sample parked in water-bath in not exceed 60 ℃,after the sample unfreezed and beat up to equalityc,cooling to room temperature. The sample is placed in a clear container as the test sample,airproof and marked. The test sample should be stored at room temperature.

5.3 Grain,tea

The combined primary sample is reduced to 500 g,which is crushed with a grinder and let wholly pass through a 20 mesh sieve. The sample is placed in a clear container as the test sample,airproof and marked. The test sample should be stored at room temperature.

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

6 Procedure

6.1 Extraction

6.1.1 Fruit, vegetable

Weight ca 10 g(accurate to 0.01 g) of the test sample into 100 mL conical flask,adding 25 mL acetone(3.1) and 15 g sodium chloride(3.6),shake for 30 min on a orbital shaker,filtering with funnel, transfer the filtrate into separator funnel. Rinse the conical flask with 20 mL acetone(3.1) in twice portions and filter,combine the filtrates to the 250 mL separator funnel. Add 25 mL sodium chloride solution(3.8) and 30 mL ethyl acetate(3.2),shake vigorously 2 min,let stand for separating comperately,collect the organic phase. Water phase was extracted once with 20 mL ethyl acetate(3.2), combine organic phase and pass the organic phase through the cloumn of anhydrous sodium sulfatrate (4.10) into a heart-shape bottle. Evaporate the solution to dryness with a rotary evaporator under reduced at 45 ℃ water-bath.

6.1.2 Animal, birds,fish

Weight ca 10 g(accurate to 0.01 g) of the test sample into 50 mL centrifugal tube,adding 25 mL ethyl acetate(3.2) and 15 g sodium chloride(3.6),homogenized for 1 min,centrifuge for 5 min at 4 000 r/min. Collect the organic phase pass the organic phase through the column of anhydrous sodium sulfate (4.10) into a heart-shape bottle. heart-shape bottle was rinsed once with 20 mL ethyl acetate(3.2),combine organic phase and pass the organic phase through the column of anhydrous sodium sulfate (4.10) into a heart-shape bottle. Evaporate the solution to dryness with a rotary evaporator under reduced at 45 ℃ water-bath.

6.1.3 Gain,Tea,chestnut

Weight ca 10 g(accurate to 0.01 g) of the test sample into 100 mL conical flask(adding 5 g sodium chloride for chestnut sample),adding 25 mL ethyl acetate(3.2),shake for 30 min on a orbital shaker, filtering with funnel and transfer the filtrate into 250 mL separator funnel. The residue in the conical flask was rinsed with 20 mL ethyl acetate(3.2)in twice portions,combine the filtrates to the same separator funnel. Add 30 mL sodium chloride solution(3.8)in the same separator funnel,shake vigorously 2 min,let stand for separating comperately,Water phase was extracted once with 20 mL ethyl acetate(3.2),combine organic phase and pass the organic phase through the column of anhydrous sodium sulfate(4.10)and transfer into a heart-shape bottle. Evaporate the solution to dryness with a rotary evaporator under reduced at 45 ℃ water-bath.

6.1.4 Honey

Weight ca 10 g(accurate to 0.01 g) of the test sample into 100 mL conical flask,disslove the test sample with 20 mL sodium chloride solution(3.8)and 5 mL acetone(3.1),shake for 30 min on a or-

bital shaker and transfer into separator funnel. The residue in the conical flask was rinsed with 30 mL sodium chloride solution(3.8)in twice portions and 50 mL ethyl acetate(3.2) in twice portions,and transfer the all solution into the separator funnel. Shake lightly 1 min,let stand for separating comperately(if occur emulsificate,layer of emulsification is centrifuged for 5 min at 4 000 r/min),collect the organic phase to another separator funnel. Water phase was extracted once with 20 mL ethyl acetate(3.2)in twice portions,and combine the organic phase to the above separator funnel. Adding 40 mL sodium chloride solution(3.8)into the above separator funnel,Shake lightly 1 min,let stand for separating comperately(if occur emulsificate,layer of emulsification centrifuge for 5 min at 4 000 r/min). Pass the organic phase through the column of anhydrous sodium sulfate (4.10) and transfer into a heart-shape bottle. Evaporate the solution to dryness with a rotary evaporator under reduced at 45 ℃ water-bath.

6.2 Clean up

6.2.1 Animal, birds, fish, chestnut,gain and tea

For obtain the test sample residue from 6.1.2 and 6.1.3,adding 40 mL *n*-hexane saturation with acetonitrile(3.12) in twice portions dissloved and transfer into the separator funnel. Rinse the heart-shape bottle with 50 mL acetonitrile saturation with *n*-hexane(3.13) in twice portions and 10 mL *n*-hexane saturation with acetonitrile(3.12),and transfer into the same separator funnel. Shake vigorously 2 min,let stand for separating comperately,pass layer of acetonitrile through the cloumn of anhydrous sodium sulfate(4.10) and into a heart-shape bottle. The layer of *n*-hexane was extracted twice with 15 mL acetonitrile saturation with *n*-hexane(3.13). Combine the layer of acetonitrile and pass layer of acetonitrile through the cloumn of anhydrous sodium sulfate(4.10) and into same heart-shape bottle. Evaporate the solution to dryness with a rotary evaporator under reduced at 45 ℃ water-bath.

6.2.2 Clean up with column of ENVI-Carb/NH_2

Disslove the residue in 2 mL Acetontrile-toluene (3.10) for obtain the test sample from 6.1.1, 6.1.4 and 6.2.1,transfer the solution into column of ENVI-Carb/NH_2. Rinse the heart-shape bottle with 1 mL acetontrile-toluene(3.10), pouring the solution into the column,Discard the effluent. Then elute the column with 10 mL acetontrile-toluene(3.9). Collet all the elution and below nearly dry with nitrogen at 45 ℃ unit. Make up to 1.0 mL with acetone-*N*-hexane(3.11) promptly for GC-MS determination.

6.3 Determination

6.3.1 GC-MS operating conditions

6.3.1.1 GC operating conditions

a) Capilary column: DB-5MS or equals. 30 m×0.25 mm(i.d.)0.25 μm(film thickness);

b) Column oven temperature procedure: 210 ℃(2 min)$\xrightarrow{30\ ℃/min}$280 ℃$\xrightarrow{10\ ℃/min}$290 ℃(6 min);

c) Injection temperature: 250 ℃;

d) Carrier gas: Helium, purity 99.999%;

e) Carrier gas flowrate: Constant mode 1 mL/min;

f) Injection mode: Splitless;

g) Injection volume: 2 μL;

h) Split valve on: 1.0 min.

6.3.1.2 MS operating conditions

a) Interface temperature: 280 ℃;

b) Ion Source: Electron Impact Ion Source (EI);

c) Electron Energy: 70 eV;

d) Source temperature: 230 ℃;

e) Detection mode: SIM;

f) Solvent delay time:2.5 min;

g) Selected ions (m/z) and relative intensity (%): see Table 1.

Table 1—Selected ions and relative intensity

analyte	quantitive ion(Relative intensity)/%	qualative(Relative intensity)/%		
Pyrimethanil	198(100)	199(47)	118(3)	184(4)
Mepanipyrim	222(100)	223(51)	208(5)	181(3)
Myclobutanil	179(100)	150(53)	245(14)	288(13)
Azoxystrobin	344(100)	388(28)	372(14)	403(13)

6.3.2 GC-MS determination

The standard working solution should be randomly injected in-between the injections of the sample solution of equal volume. The respond of Pyrimethanil, Mepanipyrim, Myclobutanil and Azoxystrobin

residues in the standard working solution and the sample solution should be within the linear range of the instrument detection. Under the above GC-MS operating condition, the retention time of Pyrimethanil, Mepanipyrim, Myclobutanil and Azoxystrobin residues separately is ca 3.42 min, 5.48 min, 5.90 min and 11.63 min. For the chromatogram of the standard, see annex A.

6.3.3 GC-MS confirmation

If the retention times of sample chromatogram peaks are consistent with the standards, and after subtracted background noise, the relative intensity ratios of each qualitative ions are also consistent with similar concentration standards, within the tolerances (see table 2), we can confirm that there are corresponding analyte in the sample.

Table 2—Maximun permitted tolerances for relative ion abundance while confirmation

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

6.3.4 Blank test

The operation of the blank test is the same as that described in the method of determination, but without addition of sample.

7 Calculation and expression of result

The calculation of result is carried out by data processor or according to the formula (1) the blank value shall be subtracted from the result of calculation.

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

Where:

X_i—the residue content of the determined pesticides in the test sample, unit is mg/kg;

A_i—the peak area of the one of the determined pesticides in the sample solution;

c_i—the concentration of the one of determined pesticides in the standard working solution, unit is μg/mL;

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

A_{si}—the peak area of the one of the determined pesticides in the standard working solution;

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

8 Limit of determination and recovery

8.1 Limit of determination

The limit of determination of this method: Pyrimethanil is 0.01 mg/kg; Mepanipyrim is 0.01 mg/kg; Myclobutanil is 0.01 mg/kg; Azoxystrobin is 0.005 mg/kg.

8.2 Recovery

See table 3.

Table 3—Fortifying levels of Pyrimethanil, Mepanipyrim, Myclobutanil and Azoxystrobin in samples and its corresponding recoveries (GC-MS)

Sample name	Fortifying concentration/(mg/kg)											
	Pyrimethanil			Mepanipyrim			Myclobutanil			Azoxystrobin		
	0.01	0.05	0.1	0.01	0.05	0.1	0.01	0.05	0.1	0.005	0.05	0.1
	Recovery/%											
Apple	81.0～93.0	88.0～102.0	84.0～96.0	82.0～92.0	84.6～94.7	86.0～98.0	75.0～98.0	82.0～98.0	84.0～94.0	92.0～120.0	84～102.0	85.0～105
Beef	81.0～92.0	82.0～92.0	82.0～96.0	75.0～97.0	84.0～110	84.0～91.0	82.0～95.0	82.0～102	83.0～97.0	82.0～102	82.0～98.0	88.0～105
Chicken	84.0～110.0	82.0～98.0	81.0～95.0	83.0～104.0	82.0～104	84.0～96.0	84.0～105	82.0～94.0	82.0～104.0	82.0～102	82.0～108	84.0～96.0
Eggplant	81.0～95.0	84.0～102	84.0～96.0	80.0～95.0	80.0～96.0	84.0～97.0	81.0～116	82.0～96.0	82.0～95.0	84.0～110	84.0～104	87.0～97.0
Rice	81.0～98.0	80～102	83.0～98.0	82.0～103	88.0～98.0	82.0～97.0	80.0～99.0	72.0～92.0	81.0～96.0	76.0～94.0	84.0～110	83.0～98.0
Fish	81.0～97.0	80.2～102	86.0～105	82.0～98.0	80.2～96.0	84.0～97.0	81.0～98.0	80.2～106	85.0～97.0	74.0～116	80.2～106	81.0～97.0
Honey	83.0～97.0	80.2～102	84.0～98.0	81.0～96.0	80.2～106	81.0～99.0	73.0～106	80.6～110	82.0～96.0	80.0～120	86.0～94.0	81.0～105
Chestnut	80.0～95.0	80.0～102	85.0～104	82.0～115	82.0～102	80.0～95.0	84.0～107	82.0～100.0	81.0～98.0	82.0～108.0	84.0～110.0	84.0～98.0
Tea	81.0～107.0	80.0～110	81.0～94.0	81.0～98.0	82.0～96.0	81.0～95.0	86.0～120	82.0～108.0	81.0～98.0	76.0～116	82.0～98.0	81.0～97.0

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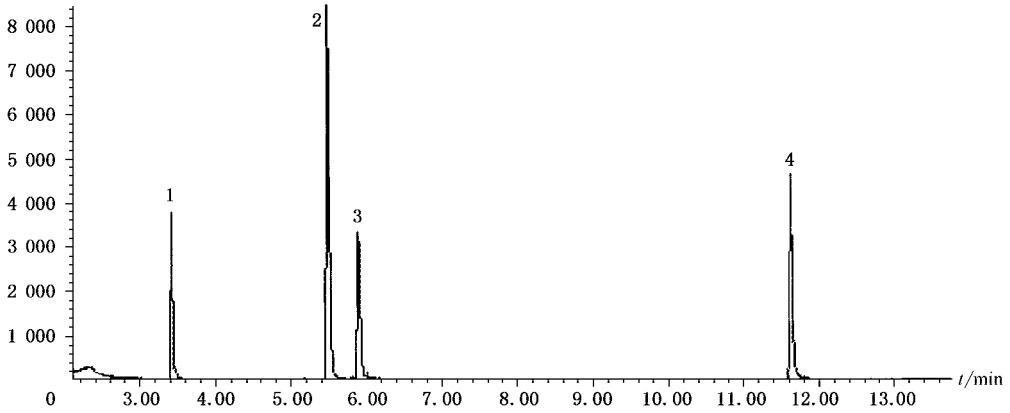
Annex A
（Information）
GC-MS chromatogram of the standard



1—Pyrimethanil；

2—Mepanipyrim；

3—Myclobutanil；

4—Azoxystrobin.

Figure A. 1—GC-MS chromatogram（TIC）of the mixture standard solution（1 μg/mL）

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