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中华人民共和国出入境检验检疫行业标准

SN/T 2917—2011

出口食品中烯酰吗啉残留量检测方法

Determination of dimethomorph residues in foodstuffs for export

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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：中华人民共和国河南出入境检验检疫局、中华人民共和国云南出入境检验检疫局、中国检验检疫科学研究院、中华人民共和国山东出入境检验检疫局。

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出口食品中烯酰吗啉残留量检测方法

1 范围

本标准规定了出口食品中烯酰吗啉残留量的液相色谱-质谱/质谱测定方法和气相色谱-质谱测定方法。

本标准第一法适用于葱、大蒜、菠菜、豌豆、番茄、马铃薯、苹果、柑橘、猪肉、猪肝、牛肾和牛奶中烯酰吗啉残留量的检测和确证，第二法适用于荷兰豆、白菜、鲜山葵、萝卜、脱水洋葱、干姜、大米、芸豆、核桃、普洱茶、猪肉、蜂蜜中烯酰吗啉残留量的检测和确证。

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 第一法 液相色谱-质谱/质谱法

3.1 方法提要

样品经乙腈提取，经分散固相萃取净化，液相色谱-质谱/质谱法测定，外标法定量。

3.2 试剂和材料

除特别规定外，所用试剂均为分析纯，水为 GB/T 6682 规定的一级水。

3.2.1 乙腈：液相色谱纯。

3.2.2 正己烷：液相色谱纯，使用前先用乙腈饱和。

3.2.3 甲酸：液相色谱纯。

3.2.4 氯化钠。

3.2.5 无水硫酸镁：研磨后在 650 ℃下烘 4 h，干燥器中冷却后备用。

3.2.6 固相萃取吸附剂：PSA(primary secondary amine，伯仲胺)。

3.2.7 固相萃取吸附剂：C_{18} 型。

3.2.8 固相萃取吸附剂：石墨碳吸附剂 GCB(graphitized carbon black)。

3.2.9 甲酸溶液：0.1%(体积分数)。将 1 mL 甲酸(3.2.3)用水稀释至 1 000 mL。

3.2.10 0.1%甲酸-乙腈溶液：(6+4，体积比)。将 6 体积的 0.1%甲酸溶液(3.2.9)与 4 体积的乙腈混匀后备用。

3.2.11 烯酰吗啉标准物质(dimethomorph，CAS 编号：110488-70-5，分子式：$C_{21}H_{22}ClNO_4$)：纯度大于等于 98%。

3.2.12 烯酰吗啉标准储备液：准确称取烯酰吗啉标准品 10.0 mg，用甲醇溶解并定容至 100 mL，浓度为 100 mg/L。0～4 ℃中保存。

3.2.13 烯酰吗啉基质标准工作液：根据需要吸取一定量的标准储备液(3.2.12)用空白样品基质溶液稀释成适当浓度的标准工作液，使用前配制。

3.2.14 微孔滤膜：0.22 μm，有机相。

3.3 仪器和设备

3.3.1 液相色谱-质谱/质谱联用仪，配有电喷雾(ESI)源。
3.3.2 组织捣碎机。
3.3.3 天平：感量分别为 0.1 mg 和 0.01 g。
3.3.4 均质器。
3.3.5 涡旋振荡器。
3.3.6 离心机。
3.3.7 氮吹仪。

3.4 试样制备与保存

3.4.1 葱、大蒜、菠菜、豌豆、番茄、马铃薯、苹果、柑橘

从所取全部样品中取出有代表性可食部分约 500 g，充分捣碎均质，均分成两份，分别装入洁净容器内。密封作为试样，标明标记。于－18 ℃以下保存。

3.4.2 猪肉、猪肝、牛肾

从所取全部样品中取出有代表性可食部分约 500 g，充分捣碎均质，均分成两份，分别装入洁净容器内。密封作为试样，标明标记。于－18 ℃以下保存。

3.4.3 牛奶

从所取全部样品中取出有代表性样品约 500 g，充分均匀，均分成两份，分别装入洁净容器内。密封作为试样，标明标记。于－18 ℃以下保存。

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。

3.5 测定步骤

3.5.1 提取

称取 5 g 试样(准确至 0.01 g)于 50 mL 离心管中，加入 10 mL 水和 10 mL 乙腈，均质 2 min，加入 1.5 g氯化钠(3.2.4)和 2 g 无水硫酸镁(3.2.5)，涡旋振荡器上剧烈振荡 2 min，4 000 r/min 离心 3 min，上清液转移至 25 mL 容量瓶中。残渣用 10 mL 乙腈重复提取一次，合并上清液，用乙腈定容至刻度，待净化。

3.5.2 净化

3.5.2.1 猪肉、猪肝、牛肾、牛奶

准确移取 5.0 mL 提取液至 10 mL 试管中，加入 2 mL 乙腈饱和的正己烷(3.2.2)，振荡 1 min，4 000 r/min 离心 1 min，弃去正己烷层。加入 300 mg 无水硫酸镁、250 mg PSA(3.2.6)和 100 mg C_{18}(3.2.7)，剧烈振荡 1 min，4 000 r/min 离心 3 min，取 2.5 mL 上清液在低于 50 ℃下氮气吹至近干，用 0.1%甲酸-乙腈溶液(3.2.10)溶解并定容至 1 mL，0.22 μm 滤膜(3.2.14)过滤，供液相色谱-质谱/质谱测定。

3.5.2.2 葱、大蒜、菠菜、豌豆、番茄、马铃薯、苹果、柑橘

准确移取 5.0 mL 提取液至 10 mL 试管中，加入 300 mg 无水硫酸镁、100 mg PSA、200 mg C_{18}，剧

烈振荡 1 min,4 000 r/min 离心 3 min,若已经无色澄清,则无需再加入石墨炭吸附剂,若仍有明显色泽,则需要加入 50 mg 石墨炭吸附剂(3.2.8),剧烈振荡 1 min,4 000 r/min 离心 2 min,取 0.5 mL 上清液用 0.1%甲酸溶液定容至 1 mL,0.22 μm 滤膜过滤,供液相色谱-质谱/质谱测定。

3.5.3 测定

3.5.3.1 液相色谱条件

3.5.3.1.1 色谱柱:C_{18},150 mm×2.0 mm(内径),5 μm,或性能相当者。

3.5.3.1.2 柱温:室温。

3.5.3.1.3 流动相:0.1%甲酸-乙腈(6+4,体积比)。

3.5.3.1.4 流速:0.25 mL/min。

3.5.3.1.5 进样量:10 μL。

3.5.3.2 质谱条件

3.5.3.2.1 离子源:电喷雾离子源。

3.5.3.2.2 扫描方式:正离子扫描。

3.5.3.2.3 检测方式:多反应监测模式(MRM)。

3.5.3.2.4 雾化气、气帘气、辅助加热气、碰撞气均为高纯氮气及其他合适气体;使用前应调节各气体流量以使质谱灵敏度达到检测要求,喷雾电压、去集簇电压、碰撞能等电压值应优化至最佳灵敏度,参考质谱参数见表 A.1。

3.5.3.2.5 监测离子对、定量离子对见表 1。

表 1 烯酰吗啉的监测离子对和定量离子对

被 测 物	监测离子对(m/z)	定量离子对(m/z)
烯酰吗啉	388.0/301.1 388.0/165.2	388.0/301.1

3.5.3.3 液相色谱-质谱/质谱测定

3.5.3.3.1 定性测定

按照液相色谱-质谱/质谱条件测定样品和基质标准工作溶液,如果检测的质量色谱峰保留时间与标准品保留时间相差不超过±5%,定性离子对的相对丰度(是用相对于最强离子丰度的强度百分比表示)与浓度相当基质标准工作溶液的相对丰度一致,相对丰度允许偏差不超过表 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

3.5.3.3.2 定量测定

根据试样中被测物的含量情况,选取响应值相近的基质标准工作液一起进行色谱分析。基质标准

工作液和待测液中烯酰吗啉的响应值应在仪器线性响应范围内。在上述色谱条件下烯酰吗啉两种异构体的参考保留时间分别为 8.6 min、9.5 min。采用两种异构体峰面积的加和进行定量。烯酰吗啉的标准溶液的多反应监测(MRM)色谱图参见图 B.1。

3.5.4 空白试验

除不加试样外,均按上述步骤进行。

3.6 结果计算

样品中烯酰吗啉残留量按式(1)计算。

$$X = c \times \frac{V}{m} \times \frac{1\,000}{1\,000} \quad \cdots\cdots(1)$$

式中:

X ——试样中被测组分含量(以两种异构体峰面积之和计),单位为微克每千克(μg/kg);

c ——从标准工作曲线得到的被测组分溶液浓度,单位为纳克每毫升(ng/mL);

V ——试样溶液定容体积,单位为毫升(mL);

m ——最终定容体积试样溶液所代表试样的质量,单位为克(g)。

注:计算结果应扣除空白值。

3.7 测定低限和回收率

3.7.1 测定低限

对于蔬菜和水果,本方法的测定低限为 10.0 μg/kg;

对于动物肌肉、肝脏、肾脏和奶,本方法的测定低限为 2.0 μg/kg。

3.7.2 回收率

不同基质在不同添加水平下的回收率见表 3 和表 4。

表 3 蔬菜和水果在不同添加水平下的回收率 %

样品	添加水平		
	10 μg/kg	50 μg/kg	100 μg/kg
葱	75.2～88.1	77.6～87.8	75.6～83.6
大蒜	86.1～95.4	84.2～94.0	83.6～94.3
菠菜	73.8～81.6	73.8～83.4	73.6～82.6
豌豆	85.6～95.7	85.0～96.6	84.6～95.3
番茄	85.2～93.2	90.0～98.6	87.6～98.1
马铃薯	85.9～96.6	90.2～99.0	87.3～96.2
苹果	90.8～97.7	89.6～99.2	86.2～98.3
柑橘	83.8～100.4	90.8～98.0	90.0～98.9

表 4　动物肌肉、肝脏、肾脏、奶在不同添加水平下的回收率　%

样　品	添加水平		
	2 μg/kg	10 μg/kg	50 μg/kg
猪肉	80.0～97.5	83.8～95.2	86.6～96.4
猪肝	79.5～94.5	82.6～93.1	85.2～96.0
牛肾	82.5～95.5	83.1～94.0	84.0～94.4
牛奶	85.0～96.0	88.6～97.5	88.6～97.2

4　第二法　气相色谱-质谱法

4.1　方法提要

试样中的烯酰吗啉经丙酮-正己烷-乙酸乙酯提取，经活性炭柱/氟罗里硅土柱固相萃取净化，气相色谱-负化学离子源质谱法测定与确证，外标法定量。

4.2　试剂和材料

除另有规定外，所用试剂均为分析纯，水为去离子水。

4.2.1　乙酸乙酯：农残级。

4.2.2　正己烷：农残级。

4.2.3　丙酮：农残级。

4.2.4　甲苯：农残级。

4.2.5　乙腈：农残级。

4.2.6　甲苯-乙腈(1＋3，体积比)。

4.2.7　丙酮-正己烷(1＋1，体积比)。

4.2.8　提取液：移取 100 mL 乙酸乙酯加入 900 mL 丙酮-正己烷(4.2.7)中，混匀后备用。

4.2.9　氟罗里硅土固相萃取柱：Florisil，1 000 mg，6 mL，或相当者。

4.2.10　石墨化炭固相萃取柱：ENVI-Carb，500 mg，6 mL，或相当者。

4.2.11　中性氧化铝固相萃取柱：N-Al_2O_3，1 000 mg，6 mL，或相当者。

4.2.12　烯酰吗啉标准物质(dimethomorph，$C_{21}H_{22}ClNO_4$，CAS 编号：110488-70-5)：纯度大于等于 98.0%。

4.2.13　烯酰吗啉标准储备溶液：准确称取适量的烯酰吗啉标准品，用丙酮稀释配制成 100 μg/mL 的标准储备液，4 ℃下保存。

4.2.14　烯酰吗啉标准工作液：根据需要用丙酮稀释成适当浓度的标准工作溶液，临用时配制。

4.2.15　微孔滤膜：0.22 μm，有机相。

4.3　仪器和设备

4.3.1　气相色谱-质谱仪：配有负化学离子源(NCI)。

4.3.2　组织捣碎机。

4.3.3　粉碎机。

4.3.4　均质器。

4.3.5　氮吹仪。

4.3.6 旋转蒸发仪。

4.4 试样制备与保存

4.4.1 蔬菜或水果类

取代表性样品500 g，或去壳、去籽、去皮、去茎、去根、去冠(不可用水洗涤)，将其可食用部分切碎后，依次用捣碎机将样品加工成浆状。混匀，均分成两份作为试样，分装入洁净的容器内，密闭并标明标记，于－18 ℃以下保存。

4.4.2 茶叶、坚果及粮谷类

取代表性样品500 g，用粉碎机粉碎并通过2.0 mm圆孔筛。混匀，分装入洁净的容器内，密闭并标明标记，于4 ℃以下保存。

4.4.3 肉及肉制品

取代表性样品500 g，将其切碎后，依次用捣碎机将样品加工成浆状，混匀，分装入洁净的盛样袋内，密封并标明标记，于－18 ℃以下保存。

4.4.4 蜂产品

取有代表性样品500 g，对无结晶的蜂蜜样品将其搅拌均匀；对有结晶析出的蜂蜜样品，在密闭情况下，将样品瓶置于不超过60 ℃的水浴中温热，振荡，待样品全部融化后搅匀，迅速冷却至室温，在融化时应注意防止水分挥发。制备好的试样均分成两份，分别装入样品瓶中，密封，并标明标记，室温保存。

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。

4.5 测定步骤

4.5.1 提取

对于含水量较低的或油脂含量较高的样品(坚果、肉及肉制品、蜂产品等)，准确称取5 g均匀试样(精确至0.01 g)。对于含水量较高的试样(蔬菜、水果等)，准确称取10 g均匀试样(精确至0.01 g)。对于脱水蔬菜、茶叶和蜂蜜样品，准确称取5 g均匀试样(精确至0.01 g)，并加入10 mL水，混匀，浸泡半小时或溶解。

将称取的样品置于250 mL的具塞锥形瓶中，加入50 mL提取溶剂(4.2.8)，放置于振荡器中振荡60 min，过滤于150 mL浓缩瓶中。再加入30 mL提取溶剂重复提取一次，合并提取液，60 ℃下氮吹仪浓缩至干。用2 mL甲苯-乙腈(1＋3)溶解，待净化。

4.5.2 净化

4.5.2.1 蔬菜、茶叶等植源性食品样品

自上而下将石墨化炭固相萃取柱(4.2.10)与氟罗里硅土固相萃取柱(4.2.9)串联连接，使用前用10 mL甲苯-乙腈(4.2.6)预淋洗，弃去流出液。将样品提取液倾入上述串联柱中，用20 mL甲苯-乙腈进行洗脱。收集洗脱液于250 mL浓缩瓶中，于40 ℃水浴中浓缩至近干。用丙酮溶解并定容至1.0 mL，供气相色谱-质谱测定和确证。

4.5.2.2 肉类等动源性产品

自上而下将中性氧化铝固相萃取柱(4.2.11)与氟罗里硅土固相萃取柱串联连接，使用前用10 mL

甲苯-乙腈预淋洗，弃去流出液。将样品提取液倾入柱中，用 20 mL 甲苯-乙腈进行洗脱。收集全部洗脱液于 250 mL 浓缩瓶中，于 40 ℃水浴中浓缩至近干。用丙酮溶解并定容至 1.0 mL，供气相色谱-质谱测定和确证。

4.5.3 测定

4.5.3.1 气相色谱-质谱条件

4.5.3.1.1 色谱柱：石英弹性毛细管柱 DB-5 ms，30 m×0.25 mm(内径)，膜厚 0.25 μm，或相当者。

4.5.3.1.2 色谱柱温度：100 ℃ $\xrightarrow{20\ ℃/min}$ 280 ℃(2 min)。

4.5.3.1.3 进样口温度：280 ℃。

4.5.3.1.4 色谱-质谱接口温度：250 ℃。

4.5.3.1.5 载气：氦气，纯度大于等于 99.999%；流速，1.0 mL/min。

4.5.3.1.6 进样量：1 μL。

4.5.3.1.7 进样方式：不分流进样，1.5 min 后开阀。

4.5.3.1.8 电离方式：NCI。

4.5.3.1.9 电离能量：125 eV。

4.5.3.1.10 离子源温度：150 ℃。

4.5.3.1.11 四极杆温度：106 ℃。

4.5.3.1.12 反应气：甲烷，纯度大于等于 99.99%，反应气流速：2 mL/min。

4.5.3.1.13 检测方式：选择离子监测方式(SIM)。

4.5.3.1.14 选择监测离子(m/z)：定量离子 387；定性离子 388，389，390。

4.5.3.1.15 溶剂延迟时间：10 min。

4.5.3.2 气相色谱-质谱检测及确证

根据样液中烯酰吗啉含量的情况，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。标准工作溶液和样液中烯酰吗啉的相应值均应在仪器的线性范围内。

如果样液与标准工作溶液的选择离子色谱图中，在不大于±5%保留时间处有色谱峰出现，并且在扣除背景后的样品质量色谱图中，所选离子均出现，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 5)。在 4.5.3.1 条件下，烯酰吗啉的 Z 体和 E 体保留时间是 18.31 min 和 19.16 min，其监测离子(m/z)丰度比为 387∶388∶389∶390=100∶64∶70∶58 对其进行确证；根据定量离子 m/z 387 对其进行外标法定量。4.5.3.1 条件下，烯酰吗啉标准物的气相色谱-质谱选择离子流色谱图和全扫描质谱图参见图 C.1 和图 D.1。

表 5 使用定性气相色谱-质谱时相对离子丰度最大允许误差

相对丰度(基峰)/%	>50	>20～50	>10～20	≤10
GC-MS/NCI 相对离子丰度最大允许误差/%	±20	±25	±30	±50

4.5.4 空白试验

除不加试样外，均按上述步骤进行。

4.6 结果计算和表述

用色谱数据处理机或按式(2)计算试样中烯酰吗啉残留量：

$$c_x = \frac{A_x \times c_s \times V_x}{A_s \times m} \quad \cdots\cdots(2)$$

式中：

c_x ——试样中烯酰吗啉残留量，单位为微克每千克(μg/kg)；

A_x——样液中烯酰吗啉定量离子的峰面积；

c_s ——标准工作液中烯酰吗啉的浓度，单位为微克每毫升(μg/mL)；

V_x——样液最后定容体积，单位为毫升(mL)；

A_s——标准工作液中烯酰吗啉定量离子的峰面积；

m ——最终样液所代表的试样质量，单位为克(g)。

4.7 测定低限、回收率

4.7.1 测定低限

本方法的测定低限荷兰豆、白菜、萝卜、鲜山葵等为 2 μg/kg，干姜、普洱茶、芸豆、猪肉、大米、核桃仁、蜂蜜等为 4 μg/kg。

4.7.2 回收率

见表 6、表 7。

表 6 食品中烯酰吗啉的回收率数据(一) %

样品名称	添加水平		
	2 μg/kg	10 μg/kg	20 μg/kg
荷兰豆	85.2～105.6	78.8～95.7	77.3～108.0
白菜	63.4～89.4	66.5～75.9	68.2～75.0
鲜山葵	67.4～78.8	75.6～93.7	81.1～94.1
萝卜	71.6～83.0	75.6～99.6	89.1～115.2

表 7 食品中烯酰吗啉的回收率数据(二) %

样品名称	添加水平		
	4 μg/kg	10 μg/kg	20 μg/kg
脱水洋葱	76.2～94.4	67.2～84.2	62.8～74.9
干姜	94.6～109.2	87.6～114.8	89.5～115.6
大米	65.4～81.8	66.8～88.4	84.4～99.0
芸豆	90.2～102.6	72.4～94.3	72.6～84.3
核桃仁	85.4～104.8	74.5～91.4	73.6～81.4
普洱茶	60.6～84.2	62.6～81.6	71.4～89.4
猪肉	93.4～108.2	79.0～91.4	66.4～76.1
蜂蜜	84.2～99.5	72.4～94.3	72.6～84.2

附 录 A
（资料性附录）
液相色谱-质谱/质谱法参考质谱参数[1)]

表 A.1 液相色谱-质谱/质谱法参考质谱参数

质 谱 参 数	参 数 值
雾化气/MPa	0.21
气帘气/MPa	0.14
辅助加热气/MPa	0.28
碰撞气	8
辅助加热气温度/℃	600
喷雾电压/V	5 500
解簇电压/V	70
碰撞能/eV	27(m/z 388.0/301.1) 42(m/z 388.0/165.2)

1) 非商业性声明：附录 A 所列参数是用 API 4000 质谱仪完成的，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 B
（资料性附录）
烯酰吗啉标准溶液的多反应监测(MRM)色谱图(液相色谱-质谱/质谱法)

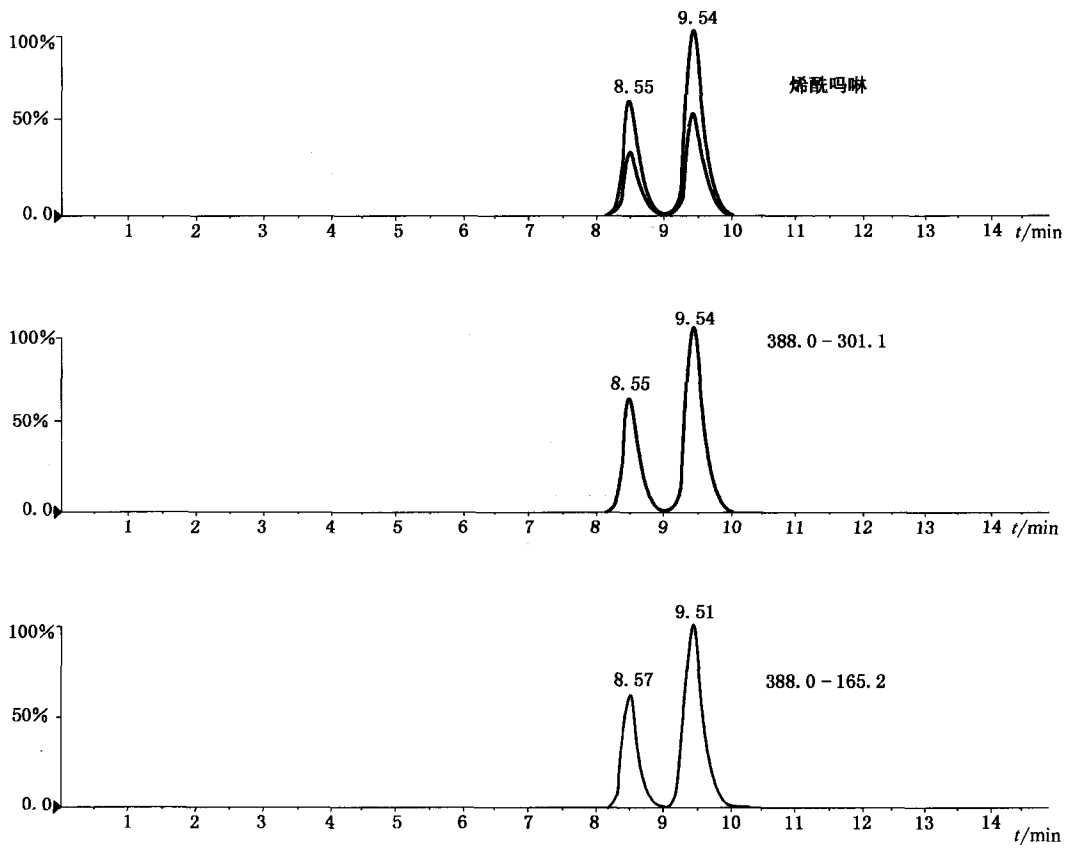

图 B.1 烯酰吗啉标准溶液的多反应监测(MRM)色谱图

附 录 C
（资料性附录）
烯酰吗啉标准溶液 GC-MS/NCI 选择离子流色谱图

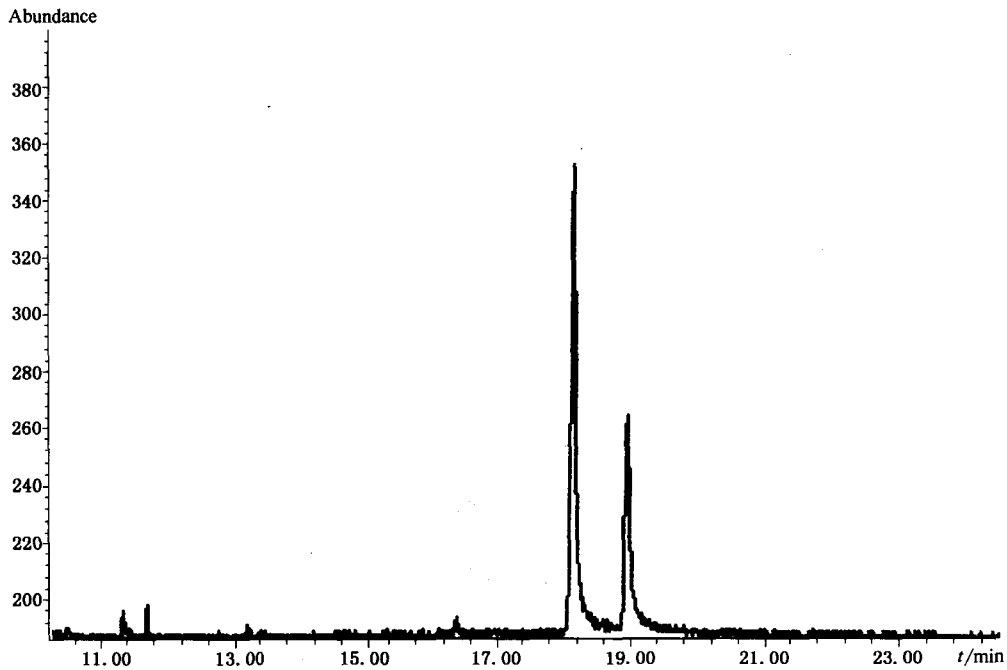

图 C.1 烯酰吗啉标准溶液 GC-MS/NCI 选择离子流色谱图

附 录 D
（资料性附录）
烯酰吗啉标准溶液 GC-MS/NCI 全扫描质谱图

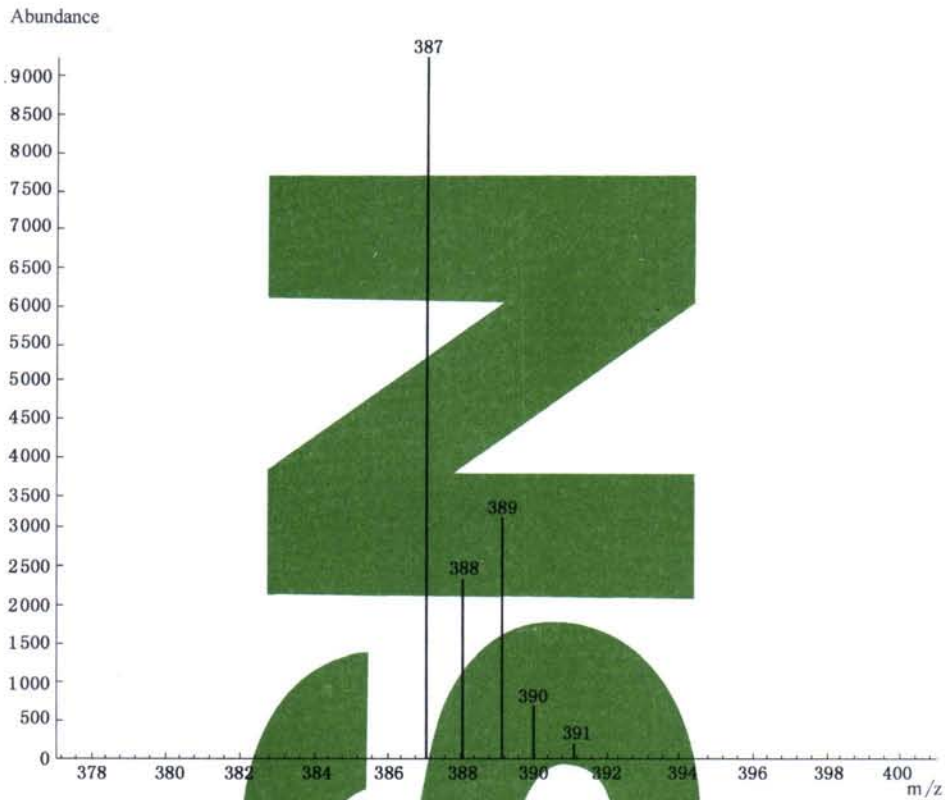

图 D.1 烯酰吗啉标准溶液（0.2 μg/mL）GC-MS/NCI 全扫描质谱图

Foreword

This standard was drafted according to the principle of GB/T 1. 1—2009.

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

This standard was drafted by Henan Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Yunnan Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Chinese Academy of Inspection and Quarantine, Shandong Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of the first method are Guo Junfeng, Yang Jizhou, Wang Sufang, Peng Tao, Wei Wei, Zhang Shoujie.

The main drafters of the second method are Peng Yunxia, Ma Xiaogang, Wang Jianhua.

Determination of dimethomorph residues in foodstuffs for export

1 Scope

This standard specifies the method of determination of dimethomorph residue in foodstuffs by liquid chromatography-tandem mass spectrometry(LC-MS/MS)and gas chromatography-negative chemical ionization mass spectrometry(GC-MS/NCI).

The method Ⅰ is applicable for the determination and confirmation of dimethomorph residues in shallot,garlic,spinach,pea,tomato,potato,apple,orange,animal muscle,liver,kidney and milk.

The method Ⅱ is applicable for the determination and confirmation of dimethomorph residues in sweet broad pea, cabbage, horseradish, radish, dehydrate onion, dry ginger, rice, bean, walnut kenel oolong tea,pork,honey,whitebait.

2 Normative references

The following documents are 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(including subsequent amendments)referred to applies.

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

3 Method Ⅰ:LC-MS/MS method

3.1 Principle

Dimethomorph residues in sample are extracted with acetonitrile. The extraction solution is cleaned with solid-phase dispersion method and determined by LC-MS/MS,quantified by external standard method.

3.2 Reagents and materials

Unless otherwise specified,all reagents used are A. R. and "water" is grade 1 st water specified in GB/T 6682.

3.2.1 Acetonitrile: HPLC grade.

3.2.2 *n*-Hexane: HPLC grade. Saturated by acetonitrile(3.2.1) before using.

3.2.3 Formic acid: HPLC grade.

3.2.4 Sodium chloride.

3.2.5 Magnesium sulfate: Be grinded and dried for 4h at 650 ℃, then ready for use after cooling in desiccator.

3.2.6 Primary secondary amine, PSA sorbent.

3.2.7 C_{18} sorbent.

3.2.8 Graphitized carbon black(GCB).

3.2.9 0.1% formic acid solution: Dilute 1 mL formic acid(3.2.3) to 1 000 mL with water.

3.2.10 0.1% formic acid − acetonitrile solution(6+4, *V* + *V*).

3.2.11 Dimethomorph standard(CAS No. 110488-70-5, molecular formula: $C_{21}H_{22}ClNO_4$): Purity≥98%.

3.2.12 Standard stock solution: 100 mg/L. Accurately weigh 10.0 mg of dimethomorph, dissolve with methanol and dilute to 100 mL. The solution should be stored at the temperature 0～4 ℃.

3.2.13 Matrix standard working solution: According to the requirement, accurately pipet an adequate volume of 100 mg/L standard stock solution of dimethomorph(3.2.12), dilute with blank matrix solution to prepare a appropriate standard working solution. The solution is made only when used.

3.2.14 0.22 μm membrane filter for organic.

3.3 Apparatus and equipment

3.3.1 Liquid chromatography-tandem mass spectrometry, equipped with electrospray ion source.

3.3.2 Tissue triturator.

3.3.3 Balance, 0.1 mg and 0.01 g sensitivity.

3.3.4 Homogenizer.

3.3.5 Vortex mixer.

3.3.6 Centrifuge.

3.3.7 Nitrogen evaporator.

3.4 Preparation and storage of test sample

3.4.1 Shallot, garlic, spinach, pea, tomato, potato, apple, orange

About 500 g representative esculent samples shall be taken from all samples, then grinded and blended to produce homogenous samples, divided into two equal portions and put in suitable clean containers, sealed and labeled. The prepared samples shall be stored in −18 ℃ refrigerator.

3.4.2 Muscle, liver, kidney

About 500 g representative esculent samples shall be taken from all samples, then grinded and blended to produce homogenous samples, divided into two equal portions and put in suitable clean containers, sealed and labeled. The prepared samples shall be stored in −18 ℃ refrigerator.

3.4.3 Milk

About 500 g representative samples shall be taken from all samples, and blended to produce homogenous samples, divided into two equal portions and put in suitable clean containers, sealed and labeled. The prepared samples shall be stored in −18 ℃ refrigerator.

In the process of sample preparation, precaution shall be taken to avoid contamination or any factors that may cause change of the residue content.

3.5 Procedure

3.5.1 Extraction

Accurately weigh 5 g of the test sample(accurate to 0.01 g)into a 50 mL centrifuge tube. Add 10 mL water, 10 mL acetonitrile, homogeneous for 2 min. Add 2 g $MgSO_4$ (3.2.5)and 1.5 g NaCl(3.2.4), vortex vigorously for 2 min. Then centrifuge 3 min at 4 000 r/min. Transfer the supernatant into a 25 mL flask. Add 10 mL acetonitrile and extract once more. Combine the supernatant and dilute to scale with acetonitrile, then ready for cleaning up.

3.5.2 Clean up

3.5.2.1 Muscle, liver, kidney and milk

Transfer 5 mL sample extract solution accuratly to 10 mL tube. Add 2 mL *n*-hexane(3.2.2)and vortex vigorously for 1 min. Discard the *n*-hexane. Add 300 mg $MgSO_4$, 250 mg PSA(3.2.6), 100 mg C_{18}

(3.2.7) to the tube and vortex vigorously for 1 min. Then centrifuge 3 min at 4 000 r/min. Transfer 2.5 mL supernatant accuratly and evaporate to dryness in a water bath below 50 ℃. Add 0.1% formic acid-acetonitrile solution(3.2.10)to dissolve the residue and dilute to 1 mL. Then filtered with 0.22 μm membrane(3.2.14)and ready for LC-MS/MS determination.

3.5.2.2 Shallot, garlic, spinach, pea, tomato, potato, apple, orange

Transfer 5 mL sample extract solution accuratly to 10 mL tube. Add 300 mg $MgSO_4$, 100 mg PSA, 200 mg C_{18} to the tube and vortex vigorously for 1 min. Then centrifuge 3 min at 4 000 r/min. If the supernatant is not clear, 50 mgGCB(3.2.8)is necessary and vortex vigorously for 1 min. Then centrifuge 2 min at 4 000 r/min. Transfer 0.5 mL supernatant accuratly and add 0.1% formic acid solution to 1 mL. Then filtered with 0.22 μm membrane and ready for LC-MS/MS determination.

3.5.3 Determination

3.5.3.1 LC operating conditions

3.5.3.1.1 Column: C_{18} column, 5 μm, 150 mm × 2.1 mm(i.d.)or equivalent.

3.5.3.1.2 Column temperature: Room temperature.

3.5.3.1.3 Mobile phase: 0.1% formic acid-acetonitrile solution(6+4, *V*/*V*).

3.5.3.1.4 Flow rate: 0.25 mL/min.

3.5.3.1.5 Injection volume: 10 μL.

3.5.3.2 MS operating conditions

3.5.3.2.1 Ion source: ESI.

3.5.3.2.2 Scan mode: Positive mode.

3.5.3.2.3 Monitor mode: Multiple reaction monitoring.

3.5.3.2.4 Nebulizer gas, curtain gas, heater gas and collision gas are high purity nitrogen or equivalent, optimize the flow rate of each gas to reach the requirement of the senitivity of mass spectrometry. DP, EP, CE shall be optimized to the most best sensitivity. Reference MS parameters see table A.1.

3.5.3.2.5 Monitor ions and quantity ions are listed in table 1.

Table 1—Monitor ions and quantity ions of dimethomorph

Analyte	Monitor ions(m/z)	Quantity ions(m/z)
Dimethomorph	388.0/301.1 388.0/165.2	388.0/301.1

3.5.3.3 LC-MS/MS determination

3.5.3.3.1 Qualification determination

Under the same determination conditions, the variation range of the retention time for the peak of analyte in unknown sample and in the matrix standard working solution can not be out of range of ±5%. The variation range of the ion ratio between the two daughter ions for the unknown sample and the matrix standard working solution at the similar concentration can not be out of range of table 2. Then the corresponding analyte must be present in the sample.

Table 2—Maximum permitted tolerances for relative ion intensities while confirmation

Relative intensity/%	>50	>20~50	>10~20	≤10
Permitted tolerances/%	±20	±25	±30	±50

3.5.3.3.2 Quantitation determination

According to the approximate concentration of analyte in sample solution, select the matrix standard working solution with similar responses to that of sample solution. The responses of the analyte in the matrix standard working solution and the sample solution shall be within the linear range of the instrument detection. Under the above LC-MS/MS operating condition, the retention time of Z dimethomorph and E dimethomorph are 8.6 min and 9.5 min respectively, multi chromatograms of the standards see figure B.1.

3.5.4 Blank test

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

3.6 Calculation and expression of result

Calculate the content of dimethomorph in the test sample according to formula (1).

$$X = c \times \frac{V}{m} \times \frac{1\,000}{1\,000} \qquad \cdots\cdots(1)$$

Where

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

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

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

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

Note: The blank value shall be subtracted from the above result of calculation.

3.7 Limit of quantification and recovery

3.7.1 Limit of quantification

For vegetables and fruits, the limit of quantification for dimethomorph is 10.0 μg/kg.

For animal muscle, liver, kidney and milk, the limit of quantification for dimethomorph is 2.0 μg/kg.

3.7.2 Recovery

See table 3 and table 4.

Table 3—Recovery of vegetables and fruits in three levels %

Samples	Recovery		
	10 μg/kg	50 μg/kg	100 μg/kg
Shallot	75.2~88.1	77.6~87.8	75.6~83.6
Garlic	86.1~95.4	84.2~94.0	83.6~94.3
Spinach	73.8~81.6	73.8~83.4	73.6~82.6
Pea	85.6~95.7	85.0~96.6	84.6~95.3
Tomato	85.2~93.2	90.0~98.6	87.6~98.1
Potato	85.9~96.6	90.2~99.0	87.3~96.2
Apple	90.8~97.7	89.6~99.2	86.2~98.3
Orange	83.8~100.4	90.8~98.0	90.0~98.9

Table 4—Recovery of animal muscle, liver, kidney and milk in three levels %

Samples	Recovery		
	2 μg/kg	10 μg/kg	50 μg/kg
Pork	80.0~97.5	83.8~95.2	86.6~96.4
Pig liver	79.5~94.5	82.6~93.1	85.2~96.0
Cattle kidney	82.5~95.5	83.1~94.0	84.0~94.4
Milk	85.0~96.0	88.6~97.5	88.6~97.2

4 Method Ⅱ: GC-MS method

4.1 Principle

Dimethomorph residue is extracted with acetone-hexane-ethyl acetate. Primary clean-up procedure is based on an active carbon ENVI-Carb column or one florsil solid phase extraction(SPE)column. The elute is condensed and dissolved for determined and confirmed by gas chromatography-negative chemical ionization mass spectrometry(GC-MS/NCI)using external standard method.

4.2 Reagents and materials

Unless otherwise specified, all the reagents used shall be analytical grade. "Water" is redistilled water.

4.2.1 Ethyl acetate: Residue grade.

4.2.2 Hexane: Residue grade.

4.2.3 Acetone: Residue grade.

4.2.4 Toluene: Residue grade.

4.2.5 Acetonitrile: Residue grade.

4.2.6 Toluene-acetonitrile(1+3, *V*/*V*).

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

4.2.8 Extration solution: 100 mL ethyl acetate in 900 mL acetone-hexane(4.2.7), mixed for use.

4.2.9 Florisil SPE tube: 1 000 mg, 6 mL, or equivalent.

4.2.10 Active carbon SPE tube: ENVI-Carb 500 mg, 6 mL, or equivalent.

4.2.11 Neutral aluminum oxide SPE tube: *N*-Al_2O_3, 1 000 mg, 6 mL, or equivalent.

4.2.12 Dimethomorph ($C_{21}H_{22}ClNO_4$, CAS No. 110488-70-5), purity≥98.0%.

4.2.13 Dimethomorph standard stock solution: Accurately weight an adequate amount of dimethomorph standard, dissolve in a small volume of acetone. Dilute with acetone to form a standard stock solution of 100 μg/mL in concentration(Be stored below 4 ℃).

4.2.14 Dimethomorph standard working solution: Then dilute the standard stock solution with acetone to the required concentration as the standard working solution. The solution is made only when used.

4.2.15 0.22 μm membrane filter for organic.

4.3 Apparatus and equipment

4.3.1 Gas chromotograph-mass spectrometry(MSD): Equipped with negative chemical ionization (NCI).

4.3.2 Tissue triturator.

4.3.3 Grinding machine.

4.3.4 Homogenizer.

4.3.5 Nitrogen evaporator.

4.3.6 Rotatory evaporator.

4.4 Sample preparation and storage

4.4.1 Vegetables or fruits

About 500 g representative samples shall be taken from all samples, the edible parts are selected, cut into mince and homogenized thoroughly into pulp by a high speed tissue triturator. Then divide the pulp into two equal portions. Each portion is put in a clean container which is sealed, labled and stored below −18 ℃.

4.4.2 Tea, nuts or cereals

About 500 g representative samples shall be taken from all samples, and grounded into powder and then passed through a mesh with 2.0 mm round holes. The passed powder is mixed and divided into two portions. Each portion is put into one clean sample bottle which is sealed, labled and stored below 4 ℃.

4.4.3 Meats and meat products

About 500 g representative samples shall be taken from all samples, the edible parts are cut into mince and homogenized by a high speed tissue triturator. The mixed primary sample is divided into two equal portion. Each portion is put into one clean sample bottle which is sealed, labled and stored below −18 ℃.

4.4.4 Bee products

About 500 g representative samples shall be taken from all samples, and the sample that is not crystallized shall be stirred well to make homogeneous. If the sample is crystallized, it shall be warmed in a water-bath below 60 ℃ with the sample bottle covered tightly, mix thoroughly when all sample has melted, then cool immediately to room temperature. In the course of melting the sample, precautions shall be taken to avoid evaporation of water from the sample. Then divide the pulp into two equal portions. Each portion is put in a clean container which is sealed, labled and stored at room temperature.

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

4.5 Procedure

4.5.1 Extraction

For low water content or high oil content sample, such as nuts, meats and meat products, and bee products, weigh 5 g(accurate to 0.01 g) of the test sample. For high water content sample, such as vegetables and fruits, weigh 10 g(accurate to 0.01 g) of the test sample. For dehydrated vegetable, tea and bee products, weigh 5 g(accurate to 0.01 g) of the test sample, mixed with 10 mL water for half an hour or dissolved.

Put the sample into 250 mL stoppered conical flask. Add 50 mL etraction solution(4.2.8) vibrating for 60 min in the oscillator. Filter the extract into a 150 mL condensor. The residue is extracted with 30 mL etraction solution again, filter and combine the extracts into the same condensor. Evaporate the extract to dry by nitrogen evaporator with a water bathing temperature of 60 ℃. Add 2 mL toluene-acetonitrile(1+3) to dissolve the residue and waiting for cleanup operation.

4.5.2 Cleanup

4.5.2.1 High pigments content sample

Couple the active carbon SPE tube(4.2.10) and florisil SPE tube(4.2.9) up to down. Rinse the two columns with 10 mL toluene-acetonitrile(4.2.6) in advance. Discard the washing. Transfer the sample solution into the column, then elute with 20 mL toluene-acetonitrile. Collection eluates into 250 mL concentrate bottle. Eaporate to nearly dry in 40 ℃ water bath. Dissolve the residue and dilute exactly to 1.0 mL with acetone for the GC-MS determination and confirmation.

4.5.2.2 High oil content sample

Couple the neutral aluminum oxide SPE tube(4.2.11) and florisil SPE tube up to down. Rinse the two columns with 10 mL toluene－acetonitrile in advance. Discard the washing. Transfer the sample solu-

tion into the column, then elute with 20 mL toluene-acetonitrile. Collection eluates into 250 mL concentrate bottle. Eaporate to nearly dry in 40 ℃ water bath. Dissolve the residue and dilute exactly to 1.0 mL with acetone for the GC-MS determination and confirmation.

4.5.3 Determination

4.5.3.1 GC-MS operation conditions

4.5.3.1.1 Column: DB-5 ms fused quartz capillary column, 30 m × 0.25 mm(i.d.), film thickness 0.25 μm, or the equivalent.

4.5.3.1.2 Column temperature: 100 ℃ $\xrightarrow{20\ ℃/min}$ 280 ℃ (2 min).

4.5.3.1.3 Inlet temperature: 280 ℃.

4.5.3.1.4 Interface temperature: 250 ℃.

4.5.3.1.5 Carrier gas: Helium, purity≥99.999%, flow rate = 1.0 mL/min.

4.5.3.1.6 Injection volumn: 1 μL.

4.5.3.1.7 Injection mode: Splitless, purge after 1.5 min.

4.5.3.1.8 Ionization mode: NCI.

4.5.3.1.9 Ionization energe: 125 eV.

4.5.3.1.10 Ionization source temperature: 150 ℃.

4.5.3.1.11 Quadropole temperature: 106 ℃.

4.5.3.1.12 Reagent gas: Methane, purity≥99.99%.

4.5.3.1.13 Acquisition mode: Selected ion monitoring(SIM)mode.

4.5.3.1.14 Selected monitoring ions(m/z): Quantified by 387; Confirmed by 388, 389 and 390.

4.5.3.1.15 Solvent delay time: 10 min.

4.5.3.2 GC-MS determination and confirmation

According to the approximate concentration of the pesticide in the sample solution, select the standard working solution with similar concentration of the sample solution. The standard working solution

shall be injected in-between the injections of the sample solution with one common volume. The response of dimethomorph in the standard working solution and sample solution shall be within the linear range of the instrument detection.

If there is a peak appeared at the retention time in range of ±5% for both of sample solution and standard working solution, and the qualification ions for every compound must be found, and the variation range of the ion ratio between the two daughter ions for the unknown sample and the standard working solution at the similar concentration cannot be out of range of table 5. Under 4. 5. 3. 1 operating conditions, the retention time of Z dimethomorph and E dimethomorph are 18. 31 min and 19. 16 min, and the ratio of monitoring ions(m/z) is 387 : 388 : 389 : 390 = 100 : 64 : 70 : 58, quantified by external method according to quantitative ion m/z 387. The GC-MS SIM chromatogram and its mass spectrum of dimethomorph standard solution are shown respectively by figure C. 1 and figure D. 1.

Table 5—Maximum permitted tolerances for relative ion intensities while confirmation

Relative intensity/%	>50	>20~50	>10~20	≤10
Permitted tolerances/%	±20	±25	±30	±50

4. 5. 4 Blank test

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

4. 6 Calculation and expression of result

Calculate the content of dimethomorph residue in the test sample by GC-MSD data processor or using formula(1).

$$c_x = \frac{A_x \times c_s \times V_x}{A_s \times m} \qquad \text{.....(2)}$$

Where

c_x —the residue content of dimethomorph in the test sample, μg/kg;

A_x —the area of quantitative ion for dimethomorph in the sample solution;

c_s —the concentration of dimethomorph in the standard working solution, μg/mL;

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

A_s —the area of quantitative ion for dimethomorph in the standard working solution;

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

4.7 Limit of determination and recovery

4.7.1 Limit of determination

For sweet broad pea, cabbage, radish and horse radish, the limit of determination of this method is 2 μg/kg. For dry ginger, puer tea, bean, pork, rice, walnutmeat and honey, the limit of determination is 4 μg/kg.

4.7.2 Recovery

See table 6 and table 7.

Table 6—Recovery of dimethomorph at three spiked levels in foods(Ⅰ) %

Samples	Recovery		
	2 μg/kg	10 μg/kg	20 μg/kg
Sweet broad pea	85.2~105.6	78.8~95.7	77.3~108.0
Cabbage	63.4~89.4	66.5~75.9	68.2~75.0
Horseradish	67.4~78.8	75.6~93.7	81.1~94.1
Radish	71.6~83.0	75.6~99.6	89.1~115.2

Table 7—Recovery of dimethomorph at three spiked levels in foods(Ⅱ) %

Samples	Recovery		
	4 μg/kg	10 μg/kg	20 μg/kg
Dehydrate onion	76.2~94.4	67.2~84.2	62.8~74.9
Dry ginger	94.6~109.2	87.6~114.8	89.5~115.6
Rice	65.4~81.8	66.8~88.4	84.4~99.0
Bean	90.2~102.6	72.4~94.3	72.6~84.3
Walnutmeat	85.4~104.8	74.5~91.4	73.6~81.4
Puer tea	60.6~84.2	62.6~81.6	71.4~89.4
Pork	93.4~108.2	79.0~91.4	66.4~76.1
Honey	84.2~99.5	72.4~94.3	72.6~84.2

Annex A
(Informative)
LC-MS/MS method reference MS parameters[1)]

Table A. 1—LC-MS/MS method reference MS parameters

MS parameters	Parameter values
GS1/MPa	0.21
CUR/MPa	0.14
GAS2/MPa	0.28
CAD	8
Temperature/℃	600
IS/V	5500
DP/V	70
CE/eV	27(m/z 388.0/301.1) 42(m/z 388.0/165.2)

1) Non-commercial statement: Results are acquired by API 4000 LC-MS/MS, but the equipments and their types involved in the standard method are not related to commercial aims, and the analysts are encouraged to use equipments of different corporation or different type.

Annex B
(Informative)
Multiple reaction monitoring(MRM) chromatogram of dimethomorph
(LC-MS/MS method)

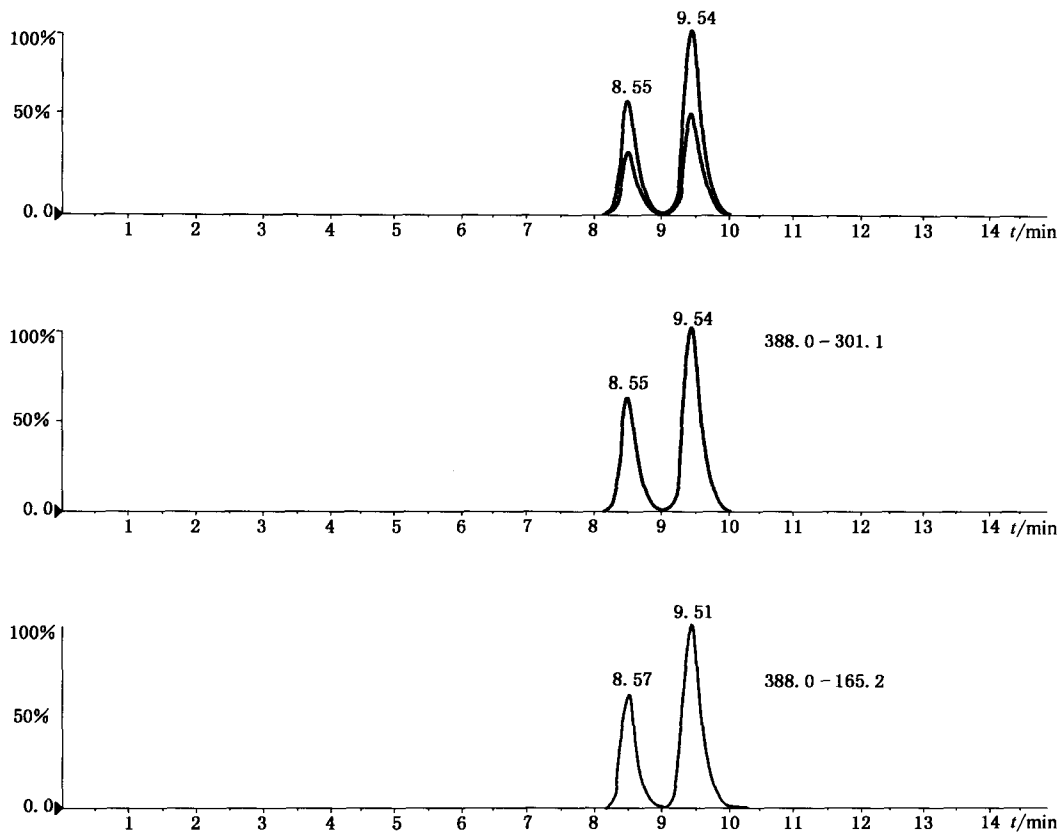

Figure B. 1—Multiple reaction monitoring(MRM) chromatogram of dimethomorph

Annex C
(Informative)
GC-MS/NCI selected ion chromatogram of the dimethomorph standard solution

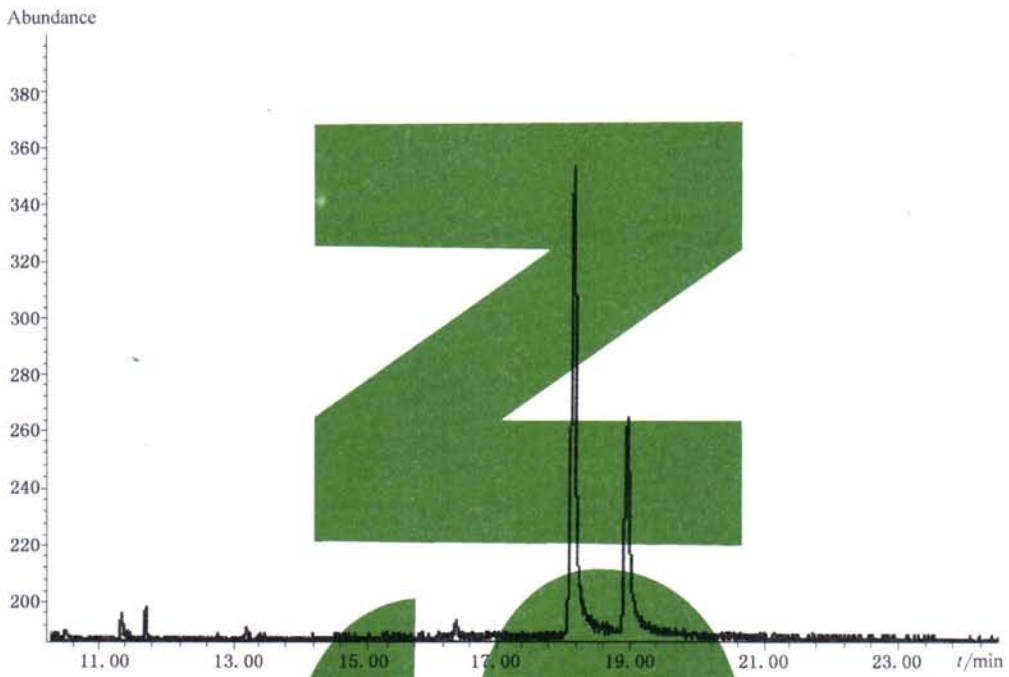

Figure C. 1—GC-MS/NCI selected ion chromatogram of the dimethomorph standard solution

Annex D
(Informative)
Mass spectrum of dimethomorph standard solution gained from GC-MS/NCI

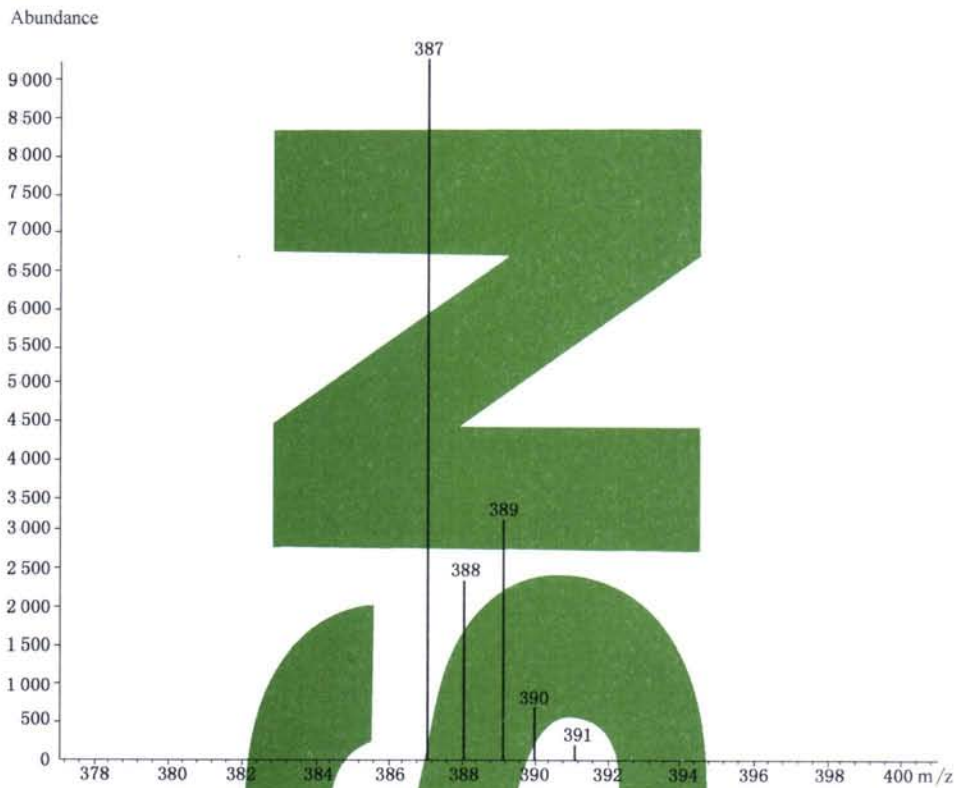

Figure D. 1—Mass spectrum of dimethomorph standard solution (0. 2 μg/mL) gained from GC-MS/NCI