SN

中华人民共和国出入境检验检疫行业标准

SN/T 0278—2009
代替 SN 0278—1993

进出口食品中甲胺磷残留量检测方法

Determination of methamidophos residues in foods for import and export

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中华人民共和国
国家质量监督检验检疫总局　发布

前　言

本标准代替 SN 0278—1993《出口蔬菜中甲胺磷残留量检测方法》。

本标准与 SN 0278—1993 相比，主要变化如下：

——扩大了使用范围；

——增加了液相色谱-质谱/质谱确证方法；

——取消了 SN 0278—1993 的“2　抽样和制样”，增加了“试样制备与保存”；

——改进了样品前处理技术路线。

本标准附录 A 和附录 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：中华人民共和国广东出入境检验检疫局、中华人民共和国湖南出入境检验检疫局、中华人民共和国浙江出入境检验检疫局、中华人民共和国江苏出入境检验检疫局。

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本标准于 1993 年首次发布，本次为第一次修订。

进出口食品中甲胺磷残留量检测方法

1 范围

本标准规定了进出口食品中甲胺磷残留量检测的气相色谱测定和液相色谱-质谱/质谱确证方法。

本标准适用于进出口大米、绿豆、菠菜、荷兰豆、柑橘、葡萄、甘蓝、板栗、茶叶、猪肉、鸡肉、猪肝、罗非鱼、蜂蜜中甲胺磷残留量的测定和确证。

2 方法提要

样品经乙腈或乙酸乙酯提取后，通过固相萃取小柱净化，采用气相色谱（火焰光度检测器）测定，外标法定量。液相色谱-质谱/质谱确证。

3 试剂和材料

除另有规定外，试剂均为分析纯，水为去离子水。

3.1 丙酮。

3.2 乙酸乙酯。

3.3 正己烷。

3.4 乙腈：色谱纯。

3.5 甲醇。

3.6 无水硫酸钠：650 ℃灼烧 4 h，在干燥器内冷却至室温，储于密闭干燥器中备用。

3.7 氯化钠。

3.8 正己烷-丙酮(2+1，体积比)：取正己烷 100 mL，加入 50 mL 丙酮，混匀。

3.9 甲胺磷标准物质：纯度大于等于 97.0%。

3.10 甲胺磷标准储备液：准确称取适量甲胺磷，用乙酸乙酯配制成浓度为 1.00 mg/mL 的标准储备液。该溶液于－18 ℃保存。

3.11 甲胺磷标准中间溶液：准确吸取适量标准储备液，用乙酸乙酯稀释至浓度为 100.0 μg/mL 的标准中间溶液。该溶液在－18 ℃保存。

3.12 甲胺磷标准工作液：使用前根据需要将标准中间溶液用乙酸乙酯稀释成适当浓度的标准工作液。

3.13 石墨化碳黑固相萃取柱：250 mg，3 mL，或相当者。

3.14 弗罗里硅土固相萃取柱：1 000 mg，2 mL，或相当者。

3.15 氨基固相萃取柱：200 mg，3 mL，或相当者。

3.16 CHROMABOND XTR 固相萃取柱：3 000 mg，15 mL，或相当者。

3.17 微孔滤膜：0.45 μm，有机系。

4 仪器和设备

4.1 气相色谱仪：配有火焰光度(FPD-P)检测器。

4.2 液相色谱-质谱/质谱联用仪：配有电喷雾离子源。

4.3 天平：感量为 0.1 mg 和 0.01 g。

4.4 食品捣碎机。

4.5 高速均质机。

4.6 离心机：6 000 r/min。

4.7 旋转蒸发仪。

4.8 氮气吹干仪。

4.9 旋涡振荡器。

4.10 振荡器。

4.11 固相萃取装置。

4.12 具塞塑料离心管：50 mL，聚丙烯。

4.13 具塞玻璃刻度试管：5 mL。

5 样品制备与保存

5.1 样品制备

5.1.1 菠菜、荷兰豆、柑橘、葡萄、甘蓝

取有代表性样品约500 g(不可水洗)，将其可食用部分切碎后，用捣碎机加工成浆状。混匀，装入洁净容器，密闭，标明标记。

5.1.2 大米、绿豆、板栗、茶叶

取有代表性样品约500 g，用粉碎机粉碎并通过孔径2.0 mm圆孔筛。混匀，装入洁净容器，密闭，标明标记。

5.1.3 猪肉、鸡肉、猪肝、罗非鱼

取有代表性样品约500 g，剔骨去皮，用绞肉机绞碎，混匀，装入洁净容器，密闭，标明标记。

5.1.4 蜂蜜

取代表性样品约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。装入洁净容器，密封，标明标记。

5.2 试样保存

茶叶、蜂蜜、粮谷及坚果类等试样于0 ℃～4 ℃保存；水果蔬菜类和动物源性食品等试样于－18 ℃以下冷冻保存。

在抽样及制样的操作过程中，应防止样品受到污染或发生残留物含量的变化。

6 测定步骤

6.1 提取

6.1.1 大米、绿豆、板栗

称取5 g试样(精确至0.01 g)于50 mL离心管中，加3 g无水硫酸钠(3.6)，15 mL乙酸乙酯(3.2)，匀质提取1 min，振荡提取20 min，4 000 r/min离心5 min，移取上清液于试管中，再分别用10 mL乙酸乙酯洗涤残渣两次，合并提取液，45 ℃以下氮吹至约2 mL，待净化。

6.1.2 菠菜、青豆、柑橘、葡萄、甘蓝

准确称取10 g试样(精确至0.01 g)于50 mL离心管中，加3 g无水硫酸钠，15 mL乙酸乙酯，匀质提取1 min，4 000 r/min离心5 min，移取上清液于25 mL容量瓶中，再用10 mL乙酸乙酯洗涤残渣一次，涡旋振荡2 min，4 000 r/min离心5 min，合并提取液于容量瓶中，用乙酸乙酯定容至刻度。取上述的样本提取液12.5 mL待净化。

6.1.3 猪肉、鸡肉、猪肝、罗非鱼肉

称取5 g试样(精确至0.01 g)于50 mL离心管中，加入10 g无水硫酸钠，15 mL乙腈，匀质提取1 min，6 000 r/min离心5 min，移取上清液于25 mL容量瓶中，再用10 mL乙腈萃取残渣一次，合并提

取液于容量瓶中，用乙腈定容至刻度，移取 10 mL 提取液待净化。

6.1.4 茶叶

称取 0.5 g 试样(精确至 0.01 g)于 10 mL 离心管中，加 1.5 mL 水，浸泡 20 min，加 0.2 g 无水硫酸钠，振荡混匀，再分别用 2 mL 乙酸乙酯提取 3 次，旋涡振荡提取，4 000 r/min 离心 3 min，合并提取液，待净化。

6.1.5 蜂蜜

称取 2 g 试样(精确至 0.01 g)于 50 mL 离心管中，加 3 mL 水，0.5 g 氯化钠(3.7)，振荡混匀，过固相萃取柱(3.16)，保持 5 min 后，用 35 mL 乙酸乙酯淋洗，流速控制为 1 mL/min，滤液过无水硫酸钠收集于 50 mL 浓缩瓶中，在 45 ℃以下旋转浓缩至约 1 mL，待净化。

6.2 净化

6.2.1 大米、绿豆、板栗

石墨化碳黑固相萃取柱(3.13)用 2×2 mL 乙酸乙酯活化，弃去流出液。将待净化溶液过石墨化碳黑固相萃取柱，再用 2×2 mL 乙酸乙酯洗脱，流速控制为 1 mL/min，收集全部流出液于试管中，在 45 ℃以下氮吹至近干，用乙酸乙酯定容至 1.0 mL，待测。

6.2.2 菠菜、荷兰豆、柑橘、葡萄、甘蓝

石墨化碳黑固相萃取柱用 2×2 mL 乙酸乙酯活化，弃去流出液。将待净化溶液过石墨化碳黑固相萃取柱，再用 2×2 mL 乙酸乙酯洗脱，流速控制为 1 mL/min，收集全部过柱溶液于 25 mL 浓缩瓶中，在 45 ℃下旋转浓缩至约 0.5 mL，用乙酸乙酯定容至 1.0 mL，待测。

6.2.3 猪肉、鸡肉、猪肝、罗非鱼、板栗

在氟罗里硅土固相萃取柱(3.14)上填装 0.5 g 无水硫酸钠，用 2×2 mL 乙腈(3.4)活化，弃去流出液。取 10 mL 待净化液过柱，用 3 mL 乙腈洗脱，收集全部流出液于 25 mL 浓缩瓶，在 45℃以下旋转浓缩至约 2mL；再过石墨化碳黑固相萃取柱，在柱上填装 0.5 g 无水硫酸钠，用 2×2 mL 乙腈活化。将浓缩液过石墨化碳黑固相萃取柱，再用 3×1 mL 正己烷-丙酮(3.8)洗脱，流速控制为 1 mL/min，收集流出液于试管中，在 45 ℃以下氮吹至约 0.5 mL，用乙酸乙酯定容至 2.0 mL，过 0.45 μm 滤膜(3.17)后，待测。

6.2.4 茶叶

在石墨化碳黑固相萃取柱上填装 0.5 g 无水硫酸钠，用 2×2 mL 乙酸乙酯活化，弃去流出液。将待净化液过柱，用 2×2 mL 乙酸乙酯洗脱，流速控制为 1 mL/min，收集全部过柱溶液，在 45 ℃下吹氮浓缩至约 1 mL。氨基柱(3.15)先用 2×2 mL 正己烷-丙酮活化，将浓缩液过氨基柱后，3×1 mL 正己烷-丙酮洗脱，流速控制为 1 mL/min，收集全部流出液于试管中，在 45 ℃以下氮吹至约 0.5 mL，用乙酸乙酯定容至 1.0 mL，待测。

6.2.5 蜂蜜

在石墨化碳黑固相萃取柱上填装 0.5 g 无水硫酸钠，用 2×2 mL 乙酸乙酯活化，弃去流出液。将待净化浓缩液过石墨化碳黑固相萃取柱，2×2 mL 乙酸乙酯洗脱，流速控制为 1 mL/min，收集洗脱液于吹氮管中，在 45 ℃下吹氮浓缩至约 0.5 mL，用乙酸乙酯定容至 2.0 mL，待测。

6.3 测定

6.3.1 气相色谱条件

a) 色谱柱：HP-INNOWAX 毛细管柱，30 m×0.25 mm(内径)×0.25 μm，或性能相当者；

b) 升温程序：120 ℃(1 min)$\xrightarrow{15\ ℃/min}$200 ℃(8 min)$\xrightarrow{25\ ℃/min}$250 ℃(4 min)；

c) 进样口温度：230 ℃；

d) 检测器温度：245 ℃；

e) 载气：氦气(纯度 99.999%)，流量 4.0 mL/min；

f) 进样模式:无分流进样;

g) 进样量:1 μL。

6.3.2 气相色谱测定

根据样液中甲胺磷含量情况,选定峰面积相近的标准工作溶液。标准工作溶液和样液中甲胺磷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参插进样测定。在上述色谱条件下,甲胺磷的保留时间约为 11.32 min。标准品的色谱图参见附录 A 中图 A.1。

6.4 定性确证

6.4.1 LC/MS-MS 质谱条件

6.4.1.1 液相色谱参考条件

a) 色谱柱:Shiseido C_{18}柱, 150 mm×2.0 mm(内径),5 μm,或相当者;

b) 柱温:40 ℃;

c) 流动相:甲醇-水(80+20,体积比);

d) 流速:0.30 mL/min;

e) 进样量:10 μL。

6.4.1.2 质谱参考条件

a) 离子源:电喷雾离子源(ESI);

b) 扫描方式:负离子扫描;

c) 检测方式:多反应选择离子检测(MRM);

d) 电喷雾电压(IS): 4 500 V;

e) 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;

f) 辅助气温度(TEM):350 ℃;

g) 定性离子对、定量离子对、采集时间、去簇电压及碰撞能量见表 1。

注:非商业性声明,质谱条件是在 API 3000 液相色谱-质谱/质谱联用仪上完成,此处列出试验用仪器型号仅为提供参考,并不涉及商业目的,鼓励标准使用者尝试不同厂家或型号的仪器。

表 1 甲胺磷定性离子对、定量离子对、去簇电压及碰撞能量

被测物名称	定性离子对 (m/z)	采集时间/ ms	去簇电压/ V	碰撞能量/ V
甲胺磷	142.2/94.3	200	70	22
	142.2/112.3			18

6.4.2 液相色谱-质谱/质谱法定性确证

当进行 GC 样品测定时,检出试样中甲胺磷的量大于方法检测限时,取 0.5 mL 气相色谱上机测定溶液,用氮气吹干,用甲醇稀释到适当浓度,以 LC-MS/MS 法定性确证。被测组分选择 1 个母离子,2 个以上子离子,在相同实验条件下,如果样品中待检测物质与标准溶液中对应的保留时间偏差在±2.5%之内;且样品谱图中各组分定性离子的相对丰度与浓度接近的标准溶液谱图中对应的定性离子的相对丰度进行比较,偏差不超过表 2 规定的范围,被确证的样品可判定为甲胺磷阳性检出。标准品的子离子全扫描质谱图和多反应监测(MRM)色谱图参见附录 B 中图 B.1、图 B.2。

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

6.5 空白试验

除不加试样外，按上述测定步骤进行。

7 结果计算和表述

用色谱数据处理机或按式(1)计算试样中甲胺磷的残留含量，计算结果需扣除空白值。

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

式中：

X——试样中甲胺磷含量，单位为微克每千克(μg/kg)；

A——样液中甲胺磷的峰面积；

c——标准工作溶液中甲胺磷浓度，单位为微克每升(μg/L)；

V——最终样液的定容体积，单位为毫升(mL)；

A_s——标准工作溶液中甲胺磷的峰面积；

m——最终样液所代表试样量，单位为克(g)。

注：计算结果应表示到小数点后两位。

8 测定低限、回收率

8.1 测定低限

本方法茶叶的测定低限为 50 μg/kg，大米、绿豆、菠菜、荷兰豆、柑橘、葡萄、甘蓝、板栗、猪肉、鸡肉、猪肝、罗非鱼、蜂蜜中甲胺磷的测定低限均为 10 μg/kg。

8.2 回收率

甲胺磷添加浓度及回收率的实验数据见表 3。

表 3 甲胺磷的回收率

样 品 名 称	添加浓度/(μg/kg)	回收率/%
大米	10	82.0～92.0
	50	82.8～95.2
	200	93.0～102
绿豆	10	80.0～88.0
	50	91.6～103
	200	99.2～101
菠菜	10	80.0～107
	50	81.2～88.2
	200	80.2～89.8
荷兰豆	10	80.0～85.0
	50	80.0～89.8
	200	80.7～81.7
甘蓝	10	88.0～97.0
	50	80.0～81.2
	200	80.0～80.9

表 3（续）

样 品 名 称	添加浓度/(μg/kg)	回收率/%
柑橘	10	80.0～84.0
	50	80.0～87.4
	200	79.5～86.3
葡萄	10	81.0～86.0
	50	80.2～90.6
	200	80.6～89.4
板栗	10	84.0～99.0
	50	80.4～93.2
	200	80.1～81.0
茶叶	50	87.2～93.8
	200	98.3～111
	500	81.0～86.8
猪肉	10	93.0～105
	50	84.8～102
	200	85.4～101
鸡肉	10	84.0～111
	50	88.4～101
	200	101～107
猪肝	10	79.6～96.1
	50	77.6～91.0
	200	79.0～86.5
罗非鱼	10	84.4～108
	50	78.6～97.8
	200	83.5～102
蜂蜜	10	93.8～105
	50	84.8～102
	200	85.0～104

附　录　A
（资料性附录）
甲胺磷标准物质气相色谱图

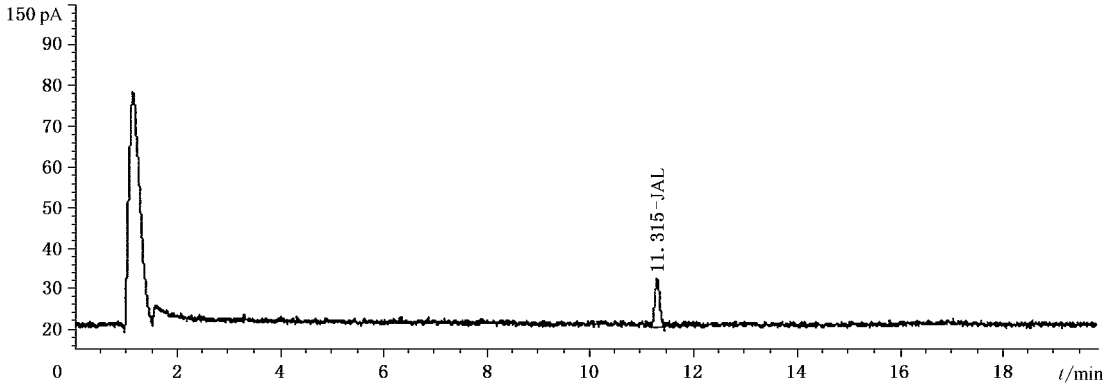

图 A.1　甲胺磷标准物质（10 μg/L）气相色谱图（GC-FPD）

附 录 B
（资料性附录）
甲胺磷标准品 LC-MS/MS 质谱图和色谱图

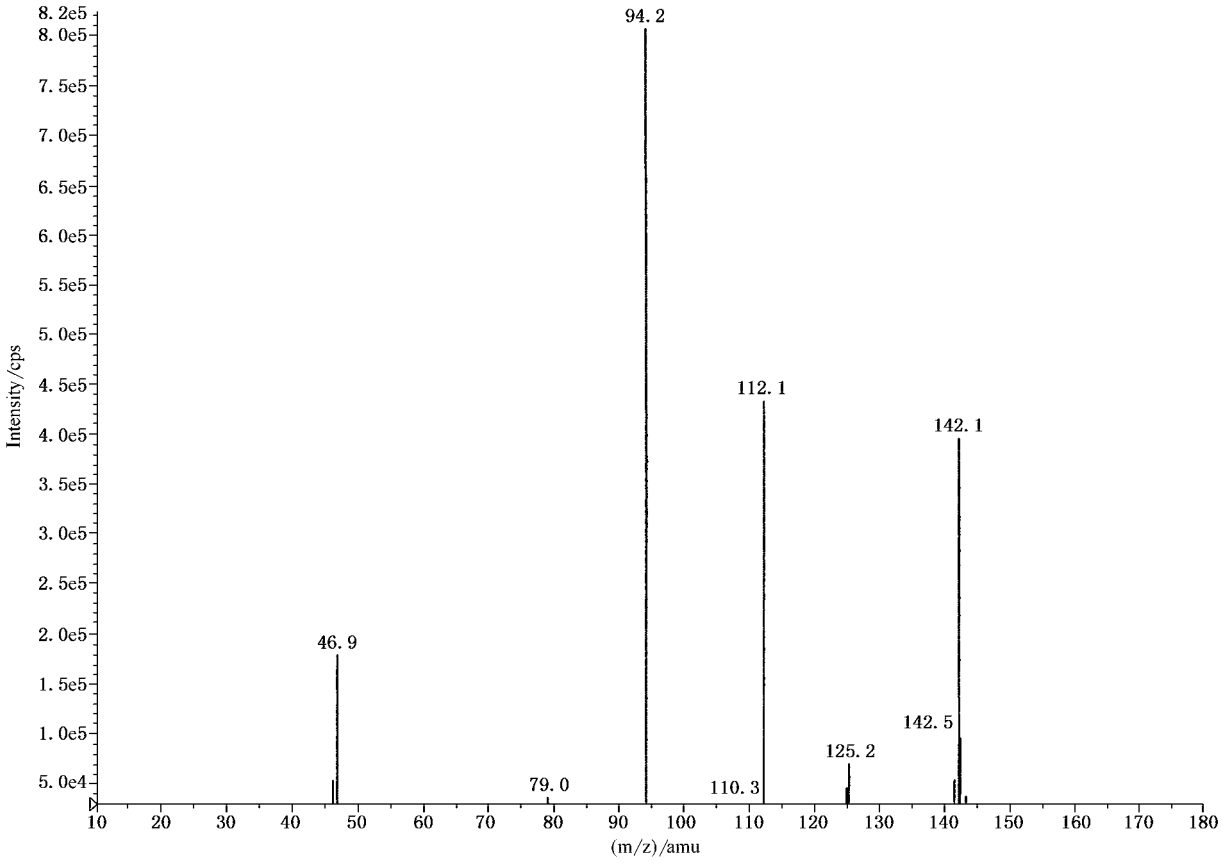

图 B.1 甲胺磷标准品子离子全扫描质谱图

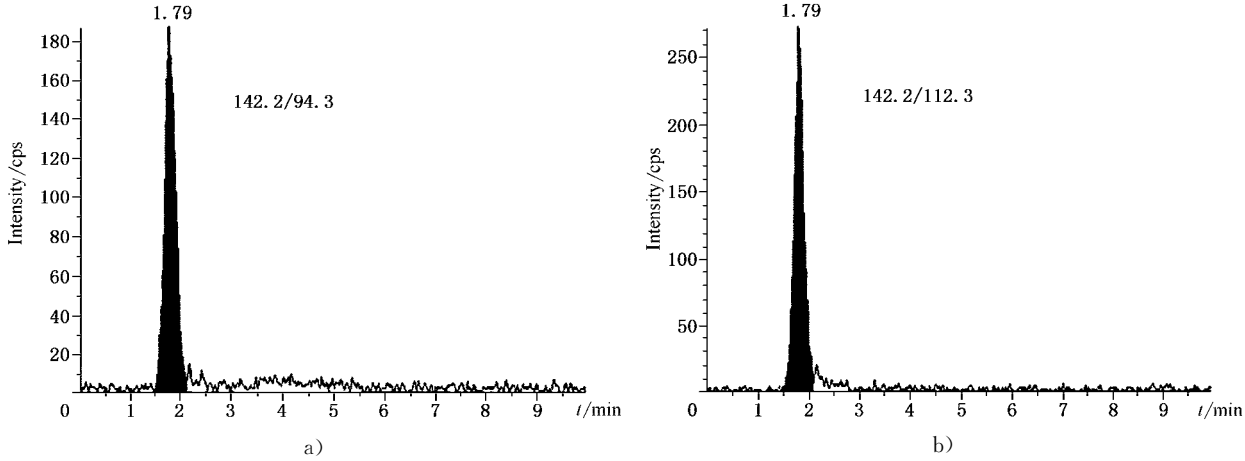

图 B.2 甲胺磷标准品多反应监测(MRM)色谱图(10 μg/L)

Foreword

This standard substituted SN 0273—1993《method for determination of methamidophos residues in vegetables for export》.

The differences between this standard and SN 0273—1993 are as follows:

——Enlarged scope about applied.

——Added method of confirmation by liquid mass spectrometry.

——Removed"2 Sampling and Sample Preparation" in SN 0351—1995, added "Preparation and storage of test sample".

——Improved on technical line about extraction of sample.

Annex A and B of this standard are informative annexs.

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

This standard was drafted by Guangdong Entry-Exit Inspection and Quarantine Bureau of the People's Republic of Hunan Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Zhejiang Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China and Jiangsu Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Chen Jie, Wang Lan, Xie Jianjun, Lin Haidan, Wu Yingxuan, Zhang Ying, Ding Huiying, Shen Chongyu.

This standard is published for the first time in 1993. The standard is modified for the first time.

Determination of methamidophos residues in foods for import and export

1 Scope

The standard specifies the determination and confirmation of methamidophos residues in foods by Gas chromatography and liquid mass spectrometry.

This standard is applicable to the determination and confirmation of methamidophos residues in rice, mung bean, spinage, vegetable pea, orange, grape, cabbage, chestnut, tea, pork, chicken, pork liver, tilapia and honey.

2 Principle

The residues in test samples are extracted with acetonitrile or ethyl acetate. The extracts are cleaned up by neutral alumina SPE cartridge or florisil SPE cartridge or active carbon SPE cartridge. Determination and confirmation made by GC (FPD) and LC-MS/MS using external standard method.

3 Reagents and materials

Unless otherwise specified, all reagents used should be analytical grade, "water"is distilled water.

3.1 Acetone.

3.2 Ethyl acetate.

3.3 *n*-Hexane.

3.4 Acetonitrile: Chromatogram grade.

3.5 Methanol.

3.6 Anhydrous sodium sulfate: Ignite at 650 ℃ for 4 h, and keep in a desicator after cooling.

3.7 Sodium chloride.

3.8 *n*-Hexane : Acetone (2+1, *V*/*V*): Mix 50 mL acetone with 100 mL hexane.

3.9 Methamidophos: Purity≥97.0%.

3.10 Standard stock solution: Accurately weigh adequate amount of methamidophos standards, dissolve with ethyl acetate and prepare a solution of 1.00 mg/mL as standard stock solution. Standard stock solution stored at −18 ℃ in refrigerator.

3.11 Standard middle solution: pipette adequate amount of standard stock solution, dilute with ethyl acetate to prepare a solution of 100.0 μg/mL as standard working solution. Stored in a refrigerator at −18 ℃.

3.12 Standard working solutions: Pipette adequate amount of standard middle solution, dilute with ethyl acetate to prepare appropriate concentration standard working solutions. Stored in a refrigerator at −18 ℃.

3.13 Active carbon SPE tubes: 250 mg, 3 mL, ENVI-Carb, or equivalent.

3.14 Florisil SPE cartridge: 1 000 mg, 2 mL, or equivalent.

3.15 NH_2 SPE cartridge: 200 mg, 3 mL, or equivalent.

3.16 CHROMABOND XTR SPE cartridge: 3 000 mg, 15 mL, or equivalent.

3.17 Syringe drieven filter: 0.45 μm (FH).

4 Apparatus and equipment

4.1 Gas chromatograph, equipped with flame photometric detector.

4.2 LC-MS/MS spectrometry.

4.3 Analytical balance: 0.1 mg and 0.01 g.

4.4 Food triturator.

4.5 High speed homogenizer.

4.6 Centrifuge: 6 000 r/min.

4.7 Rotary vacuum evaporator.

4.8 Nitrogen evaporator.

4.9 Vortex shaker.

4.10 Shaker.

4.11 Solid phase extraction vacuum manifold.

4.12 Centrifuge tube, polytetrafluoroethylene, 50 mL.

4.13 Glass tube with scale, 5 mL with stopper.

5 Samples preparation and storage

5.1 Preparation of test samples

5.1.1 Spinage, vegetable pea, orange, grape, cabbage

Representative sample about 500 g (no using water wash). Edible parts is minced and prepared to be starchy with a blender. The sample is mixed and placed in clean containers, then sealed and labeled.

5.1.2 Rice, mung bean, chestnut, tea

Representative samples about 500 g, which is crushed and passed through a 2.0 mm mesh sieve respectively. The sample is mixed and placed in clean containers, then sealed and labeled.

5.1.3 Pork, chicken, pork liver, tilapia

Representative sample about 500 g, the bone and tegument should be discarded. Sample is blended with a blender, mixed and placed in a clean container, then sealed and labeled.

5.1.4 Honey

Representative sample about 500 g. The sample which is not crystallized shall be stirred well to make homogeneous. If the sample is crystallized, it must 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 samle melting, precautions must be taken to avoid evaporation of water from the sample. Place in a clean container, which is labeled and sealed.

5.2 Storage of sample

Tea, honey, grain and nut should be stored at 0 ℃～4 ℃. Vegetable and fruit should be stored below －18 ℃.

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

6 Procedure

6.1 Extraction

6.1.1 Rice, mung bean, chestnut

Weigh 5 g (accurate to 0.01 g) of test sample into a 50 mL centrifuge tube, then add 15 mL of ethyl acetate(3.2) and 3 g of anhydrous sodium sulfate(3.6). Homogenize the sample for 1 min. Shake sample for 20 min using shaker, then centrifuged it for 5 min at 4 000 r/min. The supernatant layer was transfered into glass tube. Residue was rinsed twice with 10 mL of ethyl acetate washing toolholder bits and another ethyl acetate. Transfer the supernatant solvent and mix 2 times solvent extracted. Evaporate the organic solution to 2 mL with nitrogen evaporator at 45 ℃. The solution will be cleaned up.

6.1.2 Spinage, vegetable pea, orange, grape, cabbage

Weigh accurately 10 g (accurate to 0.01 g) of tested sample into a 50 mL centrifuge tube, add 3 g of anhydrous sodium sulfate and 15 mL of ethyl acetate, then mixed 1 min. Centrifuged the sample for 5 min at 4 000 r/min, the supernatant layer was transfered into 25 mL colorimetric tube with stopper. Residue was rinsed with 10 mL ethyl acetate washed toolholder bits and swirled for 2 min, centrifuged for 5 min at 4 000 r/min. Combined the all organic solution in the 25 mL colorimetric tube. Mix well and transfer 12.5 mL of extract solution for cleaning up.

6.1.3 Pork, chicken, pork liver, tilapia

Weigh 5 g of test sample into 50 mL centrifuge tube, add 10 g of anhydrous sodium sulfate, 15 mL of acetonitrile. Homogenize the sample for 1 min, then use 10 mL of acetonitrile wash the toolholder bits and transfer it to the former centrifuge tube. After centrifuged for 5 min at 6 000 r/min, transfer 10 mL of the supernatant layer into a 20 mL tube for cleaning up.

6.1.4 Tea

Weigh 0.5 g (accurate to 0.01 g) of test sample into a 10 mL centrifuge tube, add 1.5 mL water in it and immerse for 20 min. Add 0.2 g of s anhydrous sodium sulfate, swirled to mix. And add 3 × 2 mL of ethyl acetate to extract, swirl for 2 min each time, centrifuge at 4 000 r/min for 3 min, and combined the 3 times supernatants for cleaning up.

6.1.5 Honey

Weigh 2 g(accurate to 0.01 g) of test sample into a 50 mL centrifuge tube, add 3 mL water, 0.5 g of sodium chloride (3.7), swirl to mix. The diluted solution was passed through a CHROMABOND RTX SPE cartridge (3.16) under which connect with a cartridge filled in 1 g anhydrous sodium sulfate, keep for 5 min, then rinse the cartridge with 35 mL of ethyl acetate, at a flow rate of 1 mL/min.

Collect the eluents into a 50 mL heart-shaped flask. Evaporate nearly to 1 mL with rotary evaporator at 45 ℃ for cleaning up.

6.2 Clean-up

6.2.1 Rice, mung bean, chestnut

Setting SPE vacuum manifold. Condition the ENVI-Carb SPE cartridge (3.13) with 2×2 mL of ethyl acetate before use, then discard the solvent. Pass the sample extraction solution through the cartridge and rinse the cartridge with 2×2 mL of ethyl acetate at a flow rate of 1 mL/min. Collect the eluents in a 10 mL glass tube and evaporate under nitrogen to approximately 0.5 mL at 45 ℃. Dissolve the residue and dilute to 1.0 mL with ethyl acetate for GC analysis.

6.2.2 Spinage, vegetable pea, orange, grape, cabbage

Setting SPE vacuum manifold. Condition the ENVI-Carb SPE cartridge(3.9) with 2×2 mL of ethyl acetate before use, then discard the solvent. Pass the sample extraction solution through the cartridge and rinse the column with 2×2 mL of ethyl acetate at a flow rate of 1 mL/min. Collect the eluents in a 50 mL glass tube and evaporate under nitrogen to nearly dryness at 45 ℃. Dissolve the residue and dilute to 1.0 mL with ethyl acetate for GC analysis.

6.2.3 Pork, chicken, pork liver, tilapia

Setting SPE vacuum manifold, add 0.5 g anhydrous sodium sulfate into a florisil SPE cartridge (3.14). Condition the florisil cartridge with 4 mL of acetonitrile before use, then discard the solvent. Pass the sample extraction solution through the cartridge and rinse the column with 3.0 mL of acetonitrile at a flow rate of 1.0 mL/min. Collect the eluents in a 25 mL heart-shaped flask and evaporate to nearly 2.0 mL at 45 ℃. And then pass through the ENVI-Carb SPE cartridge (filled in 0.5 g anhydrous sodium sulfate on the top of the cartridge bed). Rinse the cartridge with 3×1 mL of *n*-hexane-acetone (3.8) at a flow rate of 1 mL/min. Collect the eluents in a 10 mL glass tube and evaporate under nitrogen to 0.5 mL at 45 ℃. Dissolve the residue and dilute to 2.0 mL with ethyl acetate, filtered with 0.45 μm membrane (3.17) for GC analysis.

6.2.4 Tea

Setting SPE manifold. Condition the ENVI-Carb column with 2×2 mL of ethyl acetate before use (filled in 0.5 g anhydrous sodium sulfate on the top of the cartridge bed), then discard the solvent. Pass the sample extraction solution through the cartridge and rinse the column with 2×2 mL of ethyl acetate at a flow rate of 1 mL/min. Collect the eluents and evaporate under nitrogen to nearly 1 mL at 45 ℃, then pass through the NH_2 cartridge(3.15). Rinse the column with 3×1 mL of *n*-hexane-acetone at a flow rate of 1 mL/min. Collect the eluents in a 10 mL glass tube and evaporate under nitrogen to 0.5 mL at 45 ℃. Dissolve the residue and dilute to 1.0 mL with ethyl acetate for GC analysis.

6.2.5 Honey

Setting SPE vacuum manifold. Condition the ENVI-Carb SPE cartridge with 2×2 mL of ethyl acetate before use(filled in 0.5 g anhydrous sodium sulfate on the top of the cartridge bed), then discard the solvent. Pass the sample extraction solution through the cartridge and rinse the cartridge with 2×2 mL of ethyl acetate at a flow rate of 1 mL/min. Collect the eluents in a 10 mL glass tube and evaporate under nitrogen to approximately 0.5 mL at 45 ℃. Dissolve the residue and dilute to 2.0 mL with ethyl acetate for GC analysis.

6.3 Determination

6.3.1 GC operating conditions

a) GC column: Capillary column, HP-INNOWAX, 30 m×0.25 mm(i.d.)×0.25 μm or equivalent;

b) Temperature increasing programme: 120 ℃/(1.0 min) $\xrightarrow{15\ ℃/min}$ 200 ℃ (8.0 min) $\xrightarrow{25\ ℃/min}$ 250 ℃ (4 min);

c) Injection port temperature: 230 ℃;

d) Detection temperature: 245 ℃;

e) Carrier gas: Nitrogen, purity≥99.999%; Fow rate: 4.0 mL/min;

f) Injection mode: Splitless;

g) Injection volumn: 1 μL.

6.3.2 GC determination

According to approximate concentration of analyte, select the standard working solution with similar response to that of sample solution. The responses of methamidophos in the standard working solution and sample solution should be in 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. At the above GC conditions, the retention time is 11.32 min, and the GC chromatogram of the methamidophos standard see annex A.

6.4 Qualitative confirmation

6.4.1 LC-MS/MS operating conditions

6.4.1.1 HPLC

a) Column: Shiseido C_{18}, 5 μm, 150 mm × 2.0 mm(i.d.) or equivalent;

b) Column temperature:40 ℃;

c) Mobile phase: Methanol-water(80 + 20, *V*/*V*);

d) Flow rate: 0.30 mL/min;

e) Injection volumn: 10 μL.

6.4.1.2 MS/MS

a) Ion source: ESI;

b) Scan mode: negative mode;

c) Detection mode:Multiple reaction monitoring(MRM);

d) Ionspray voltage (IS):4 500 V;

e) 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 sensitivity of mass spectrometer;

f) TEM: 350 ℃;

g) Quality ions, quantity ions,declustering potential(DP) and collision energy(CE) are shown in table 1.

Non-commercial statement: The equipments and their types API 3 000 involved in the standardmethod are not related to commercial aims, and the analysts are encouraged to use equipments of different corporation or different type.

Table 1—Monitor ions and Quantitative ions

Analyte	Quality ions (m/z)	Dwell time/ ms	DP/ V	CE/ V
Methamidophos	142.2/94.3	200	70	22
	142.2/112.3			18

6.4.2 LC-MS/MS confirmation

Transfer 0.5 mL test sample solution with GC analysis evaporate under nitrogen to dryness at 45 ℃.

Dissolve the residue and dilute with methanol for LC-MS/MS analysis. LC-MS/MS should be applied for confirmation the quality of the methamidophos when the quantity of methamidophos in sample is higher than the detection limit. The standard solution and sample solution were analyzed according to the operating condition assigned in 6.4.1. The qualitative ions for each analyst include one precursor ion and two product ions at least. Under the same determination conditions, the retention time of the analyte in the sample shall match that of the calibration standard within the tolerances 2.5%. The relative intensities of the detected ions of each analyst, shall correspond to those of the calibration standard at comparable concentrations, within the tolerances shown in table 2, then the corresponding analyte must be present in the sample. For LC-MS/MS chromatogram (MRM) of methamidophos standards, see figure B.1 and figure B.2 in annex B.

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

6.5 Blank test

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

7 Calculation and expression of result

Calculation the content of methamidophos residues in the test sample by GC data processor or according to the formula (1). The blank value should be substracted from the above result of calculation:

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

Where:

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

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

c—the concentration of methamidophos in the standard working solution, μg/L;

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

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

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

8 Limit of determination and recovery

8.1 Limit of determination

The limit determination of this method for methamidophos in tea is 50 μg/kg, and the limit determination of this method for methamidophos in the other foods is 10 μg/kg.

8.2 Recovery

According to the experimental data, the fortifying concentrations of methamidophos and their corresponding recoveries are shown in table 3.

Table 3—The recovery of methamidophos

Sample	Fortified concentration/(μg/kg)	Recovery/%
Rice	10	82.0～92.0
	50	82.8～95.2
	200	93.0～102
Mung bean	10	80.0～88.0
	50	91.6～103
	200	99.2～101
Spinage	10	81.2～88.0
	50	89.0～102
	200	80.2～89.8
Vegetable pea	10	80.0～85.0
	50	80.0～89.8
	200	80.7～81.7
Cabbage	10	88.0～97.0
	50	80.0～81.2
	200	80.0～80.9
Orange	10	80.0～84.0
	50	80.0～87.4
	200	79.5～86.3
Grape	10	81.0～86.0
	50	80.2～90.6
	200	80.6～90.4
Chestnut	10	84.0～99.0
	50	80.4～93.2
	200	80.1～81.0

Table 3（Continued）

Sample	Fortified concentration/（μg/kg）	Recovery/%
Tea	50	87.2～93.8
	200	98.3～110.8
	500	81.0～86.8
Pork	10	93.0～105
	50	84.8～102
	200	85.4～101
Chicken	10	84.0～111
	50	88.4～101
	200	101～107
Pig liver	10	79.6～96.1
	50	77.6～91.0
	200	79.0～86.5
Tilapia	10	84.4～108
	50	78.6～97.8
	200	83.5～102
Honey	10	93.8～105
	50	84.8～102
	200	85.0～104

Annex A
（Informative）
GC chromatogram of the methamidophos standard

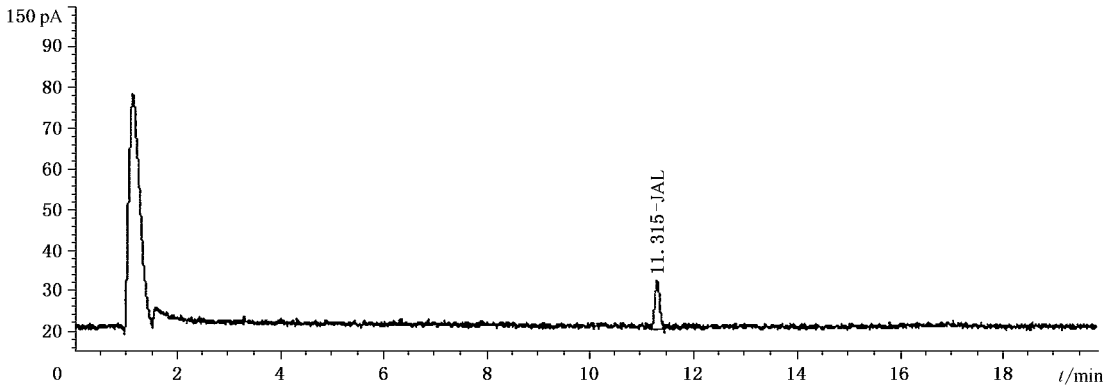

Figure A.1—GC-FPD chromatogram of the methamidophos standard（10 μg/L）

Annex B
（Informative）
LC-MS/MS chromatogram of methamidophos standard

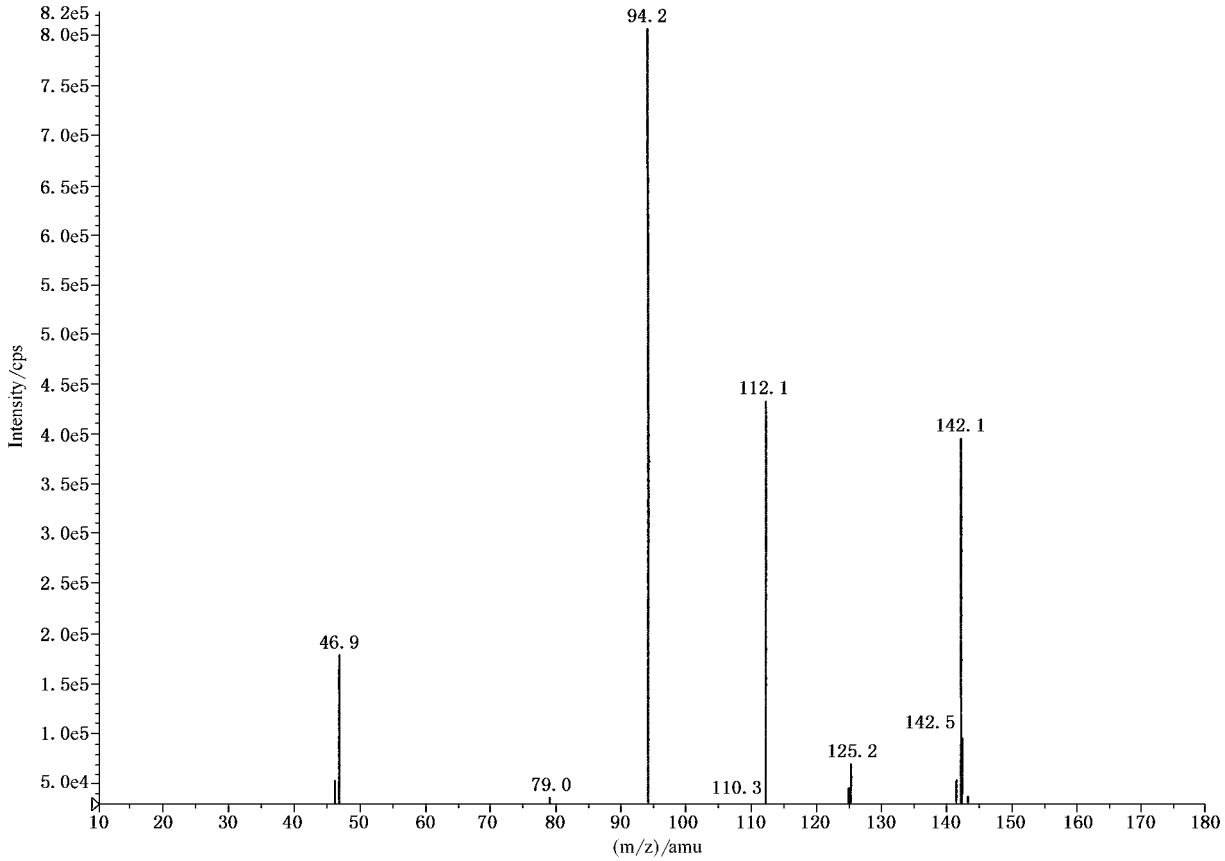

Figure B. 1—LC-MS/MS chromatogram of methamidophos standard

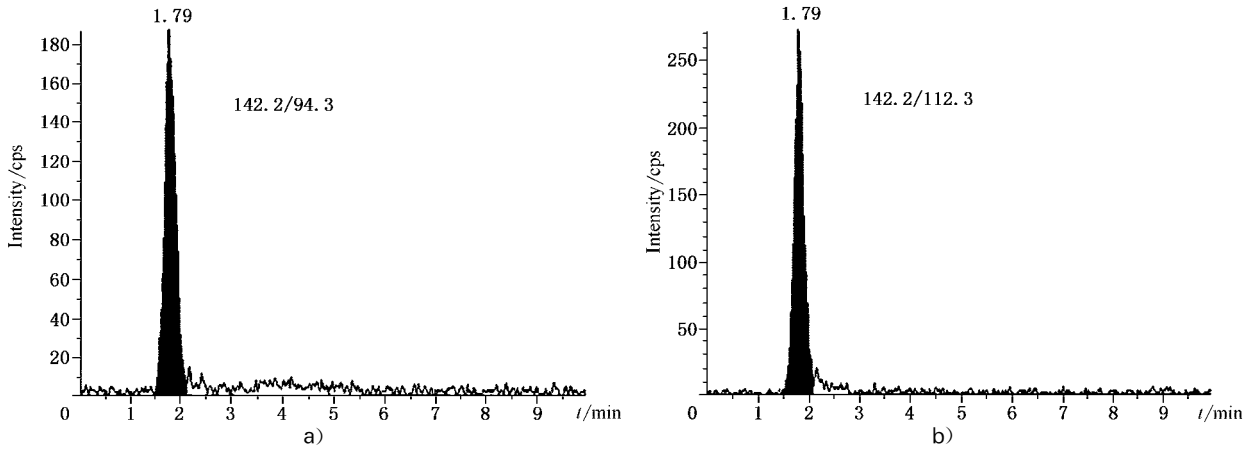

Figure B. 2—MRM chromatogram of methamidophos standard（10 μg/L）

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