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中华人民共和国出入境检验检疫行业标准

SN/T 3983—2014

出口食品中氨基酸类有机磷除草剂残留量的测定　液相色谱-质谱/质谱法

Determination of phosphonic and amino acid group-containing herbicides residues in foodstuffs for export—LC-MS/MS method

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中华人民共和国
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前　言

本标准按照 GB/T 1.1—2009 给出的规则起草。

请注意本文件的某些内容可能涉及专利。本文件的发布机构不承担识别这些专利的责任。

本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：中华人民共和国深圳出入境检验检疫局、深圳市检验检疫科学研究院。

本标准主要起草人：张建莹、胡晓苑、吴凤琪、张毅、罗耀、吴卫东、岳振峰。

出口食品中氨基酸类有机磷除草剂残留量的测定　液相色谱-质谱/质谱法

1　范围

本标准规定了大米、小麦、大豆、玉米、奶白菜、葡萄、橙子、马铃薯、大蒜、茶叶、虾肉、鱼肉和蜂蜜等食品中草甘膦(glyphosate)及其代谢产物氨甲基膦酸(aminomethyl phosphonic acid，AMPA)和草铵膦(glufosinate)等氨基酸类有机膦除草剂残留量的液相色谱-质谱/质谱测定方法。

本标准适用于大米、小麦、大豆、玉米、奶白菜、葡萄、橙子、马铃薯、大蒜、茶叶、虾肉、鱼肉和蜂蜜中草甘膦、氨甲基膦酸(AMPA)和草铵膦残留量的测定与确证。

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　方法提要

采用水提取试样中(大米、奶白菜、葡萄、橙子)残留的氨基酸类有机膦除草剂类药物，经二氯甲烷净化，与9-芴基甲基氯甲酸酯衍生化反应后，液相色谱-质谱/质谱检测和确证，内标法定量。

采用水和二氯甲烷混合溶液提取试样中(小麦、大豆、玉米、大蒜和马铃薯)残留的氨基酸类有机膦除草剂类药物，提取液经三氯乙酸沉淀蛋白，二氯甲烷净化，与9-芴基甲基氯甲酸酯衍生化反应后，液相色谱-质谱/质谱检测和确证，内标法定量。

虾肉、鱼肉、蜂蜜和茶叶中的氨基酸类有机膦除草剂类药物残留采用水和二氯甲烷混合溶液提取，提取液经阳离子交换柱(CAX)净化，与9-芴基甲基氯甲酸酯衍生化反应后，液相色谱-质谱/质谱检测和确证，内标法定量。

4　试剂和材料

除另有说明外，所用试剂均为分析纯，试验用水为符合GB/T 6682规定的一级水。

4.1　二氯甲烷：高效液相色谱纯。

4.2　丙酮：色谱纯。

4.3　甲醇：色谱纯。

4.4　乙腈：色谱纯。

4.5　三氯乙酸。

4.6　盐酸。

4.7　硼酸钠($Na_2B_4O_7 \cdot 10H_2O$)。

4.8　乙酸铵：色谱纯。

4.9　甲酸：色谱纯。

4.10 9-芴基甲基氯甲酸酯[9-fluorenylmethylchloroformate(FMOC-CL)]:纯度不低于 99.0%,4 ℃保存。

4.11 氨水。

4.12 CAX 洗脱液:分别量取 160 mL 水、2.7 mL 盐酸(4.6)和 40 mL 甲醇(4.3),混匀。

4.13 5%硼酸盐缓冲溶液(pH 值 9.0):称取 5 g 硼酸钠(4.7),用水溶解并定容至 100 mL,混匀。

4.14 衍生化试剂:称取 200 mg FMOC-CL,用丙酮(4.2)溶解并定容至 100 mL,混匀。

4.15 20%三氯乙酸溶液:称取 20 g 三氯乙酸(4.5)溶于适量水中,稀释定容至 100 mL。

4.16 5 mmoL 乙酸铵水溶液(含 0.1%甲酸):称取 0.385 4 g 乙酸铵溶解于适量的水中,加入 1 mL 甲酸(4.9),用水定容至 1 000 mL,混匀。

4.17 乙腈(含 0.1%甲酸):吸取 1 mL 甲酸于 1 000 mL 乙腈(4.4)中,混匀。

4.18 标准物质:草甘膦(glyphosate,CAS 号:1071-83-6),纯度 99.0%;氨甲基膦酸(AMPA,CAS 号:1066-51-9),纯度 99.0%;草铵膦(glufosinate,CAS 号:51276-47-2),纯度 99.0%;草甘膦同位素内标(1,2-$C^{13}N^{15}$草甘膦):100 μg/mL(相关化学信息参见附录 A 中表 A.1)。

4.19 标准储备液的配制:分别准确称取一定量的草甘膦、氨甲基膦酸和草铵膦标准物质,用水溶解(另加 2 滴盐酸)后配制成浓度为 1 000 mg/L 的标准储备溶液,5 ℃以下避光保存,可使用 6 个月。

4.20 混合标准中间溶液的配制:准确移取适量体积的标准储备液(4.19),用水稀释成浓度为 10.0 mg/L 的混合标准工作液。5 ℃以下避光保存,可使用 3 个月。

4.21 同位素内标标准工作溶液的配制:准确移取适量体积的标准母液(4.18),用水稀释成浓度为 10.0 mg/L的内标标准工作液。5 ℃以下避光保存,可使用 3 个月。

4.22 混合标准工作溶液:吸取适量的混合标准中间溶液(4.20)和同位素内标工作溶液(4.21),用水配制成外标浓度为 5.00 μg/L、10.0 μg/L、25.0 μg/L、50.0 μg/L 和 100 μg/L 的混合标准工作溶液(同位素内标物浓度均为 20 μg/L),当天配制。

4.23 阳离子交换固相萃取柱:AG 50W-X8 树脂,200 目～400 目,H^+型,0.8 cm×4 cm(可采用美国 Bio-Rad 公司同等性能的 CAX 商品化小柱[1)],或同等性能的其他小柱)。

4.24 微孔滤膜:0.45 μm,水相型。

5 仪器和设备

5.1 液相色谱-质谱/质谱仪:配有电喷雾离子源(ESI)。

5.2 固相萃取装置。

5.3 涡旋混匀器。

5.4 旋转蒸发仪。

5.5 聚丙烯离心管:50 mL,15 mL,具塞,带刻度。

5.6 离心机:转速可达 9 500 r/min。

5.7 均质器。

6 试样制备与保存

6.1 一般要求

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。

1) 非商业性声明:此处列出色谱柱型号仅是为了提供参考,不涉及商业目的,鼓励标准使用者尝试采用不同厂家或规格的色谱柱。

6.2 大米、奶白菜、小麦、茶叶、大豆、葡萄、马铃薯、大蒜和橙子

从所取全部样品中取出有代表性样品约 500 g，装入洁净容器中，密封，并标明标记，于－18 ℃冷藏存放。

6.3 玉米、虾和鱼

从所取全部样品中取出有代表性样品可食部分约 500 g，充分搅碎混匀，试样装入洁净容器中，密封，并标明标记，于－18 ℃以下冷冻存放。

6.4 蜂蜜

取未开封蜂蜜样品置于 50 ℃～60 ℃的水浴中温热，待样品全部溶化后搅拌均匀，取试样约 500 g 均分为两份，装入洁净容器，密封，并标明标记。常温保存。

7 分析步骤

7.1 试样提取

7.1.1 大米、奶白菜、葡萄和橙子

称取 5 g(精确至 0.01 g)试样于 50 mL 具塞离心管中，加入草甘膦内标(4.21)50 μL，涡旋振荡 30 s，再加入 25 mL 水和 10 mL 二氯甲烷，涡旋振荡提取 10 min，9 500 r/min 离心 5 min。吸取上清液 4 mL 到 15 mL 离心管中，使用氨水(4.11)调节 pH 值至 7，加入 5 mL 二氯甲烷，高速涡旋 30 s，9 500 r/min离心 5 min。吸取上清液 1.0 mL 到 15 mL 离心管中，待衍生化。

7.1.2 小麦、大豆、玉米、大蒜和马铃薯

称取 5 g(精确至 0.01 g)试样于 50 mL 具塞离心管中，加入草甘膦内标(4.21)50 μL，涡旋振荡 30 s，再加入 25 mL 水和 10 mL 二氯甲烷，涡旋振荡提取 10 min，9 500 r/min 离心 5 min。吸取上清液 5 mL 到 15 mL 离心管中，加入 0.2 mL 20 %三氯乙酸(4.15)溶液，涡旋振荡提取 1 min，于 9 500 r/min 离心 5 min。取上清液 4 mL 到 15 mL 离心管中，使用氨水调节 pH 值至 7，加入 5 mL 二氯甲烷，高速涡旋 30 s，9 500 r/min 离心 5 min。吸取上清液 1.0 mL 到 15 mL 离心管中，待衍生化。

7.1.3 虾肉、鱼肉、蜂蜜和茶叶

称取 5 g(精确至 0.01 g)试样于 50 mL 具塞离心管中，加入草甘膦内标(4.21)50 μL，涡旋振荡 30 s，再加入 25 mL 水和 10 mL 二氯甲烷，高速匀浆提取 10 min，于 9 500 r/min 离心 5 min。取上清液 6 mL 到 15 mL 离心管中，再加入 4 mL 二氯甲烷，高速涡旋 30 s，9 500 r/min 离心 5 min，取上清液 4 mL到 15 mL 离心管中，使用盐酸调节 pH 值至 2，待净化。

7.2 净化

吸取 1.0 mL 提取液(7.1.3)于活化好的 CAX 固相萃取柱(4.23)中，以约 1.5 mL/min 的流速使样液全部通过固相萃取柱，再吸取 1.0 mL CAX 淋洗液淋洗，弃去流出液，用 12 mL CAX 淋洗液洗脱，收集洗脱液于干净的 50 mL 磨口瓶中，50 ℃减压旋蒸浓缩至近干，加入 1.0 mL 5%硼酸盐缓冲溶液(4.13)，涡旋溶解残渣，待衍生化。

7.3 衍生化

吸取 300 μL 硼酸盐缓冲溶液(4.13)到净化液(7.2)中，再加入 200 μL 衍生化试剂(4.14)，涡旋振荡

1 min，室温静置衍生过夜。将衍生化后溶液涡旋振荡 1 min，收集水层过 0.45 μm 滤膜(4.24)，供液相色谱-串联质谱测定。

7.4 测定

7.4.1 液相色谱条件

液相色谱条件如下：

a) 色谱柱：C_{18}色谱柱，150 mm×2.1 mm(内径)，2.7 μm，或相当者；

b) 柱温：30 ℃；

c) 进样量：20 μL；

d) 流动相、流速及梯度洗脱条件见表 1。

表 1 流动相、流速及梯度洗脱条件

时间 min	流速 mL/min	5 mmol 乙酸铵水溶液 (含 0.1%甲酸) %	乙腈 (含 0.1 %甲酸) %
0	0.300	85	15
9.00	0.300	85	15
12.0	0.300	15	85
14.0	0.300	15	85
15.0	0.300	85	15
20.0	0.300	85	15

7.4.2 质谱条件

质谱条件如下：

a) 离子化模式：电喷雾离子源(ESI)，正离子扫描；

b) 质谱扫描方式：多反应监测(MRM)；

c) 分辨率：单位分辨率；

d) 其他参考质谱条件参见附录 B 中表 B.1。

7.4.3 液相色谱-质谱/质谱测定

7.4.3.1 定性测定

在相同实验条件下进行处理，样品中待测物质的保留时间，与混合标准溶液的保留时间偏差在±2.5 %之内；每种化合物的质谱定性离子至少应包括一个母离子和两个子离子，且样品中各组分定性离子的相对丰度与浓度接近的基质混合标准工作溶液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，则可判定为样品中存在对应的待测物。

表 2 定性确证时相对离子丰度的最大允许偏差

相对离子丰度/%	>50	>20～50	>10～20	≤10
允许的最大偏差/%	±20	±25	±30	±50

7.4.3.2 定量测定

在仪器最佳工作条件下，对混合标准工作溶液进样，样品溶液中待测物的响应值均应在仪器测定的线性范围内，以色谱峰面积按内标法定量。氨基酸类有机膦除草剂标准物质的多反应监测（MRM）色谱图参见附录 C 中的图 C.1。

7.4.4 空白实验

除不加入标准物质外，按上述测定步骤进行。

8 结果计算和表述

用液相色谱-质谱/质谱数据处理软件或者按照式(1)计算试样中检测目标物残留量，计算结果应扣除空白值：

$$X_i = \frac{c \times c_i \times A \times A_{si} \times V}{c_{si} \times A_s \times A_i \times m} \times \frac{1\,000}{1\,000} \qquad \cdots\cdots(1)$$

式中：

X_i ——样品中氨基酸类除草剂残留量，单位为毫克每千克（mg/kg）；

c ——样液中氨基酸类有机膦除草剂标准工作溶液的浓度，单位为毫克每升（mg/L）；

c_i ——样液中内标物的浓度，单位为毫克每升（mg/L）；

A ——样液中氨基酸类有机膦除草剂的峰面积；

A_{si} ——标准工作溶液中内标物的峰面积；

V ——样液最终定容体积，单位为毫升（mL）；

c_{si} ——标准工作溶液中内标物的浓度，单位为毫克每升（mg/L）；

A_s ——标准工作溶液中氨基酸类有机膦除草剂峰的面积；

A_i ——样液中内标物的峰面积；

m ——最终样液代表的试样质量，单位为克（g）。

9 测定低限和回收率

9.1 测定低限

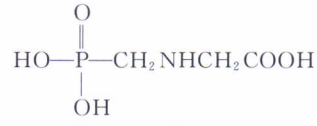
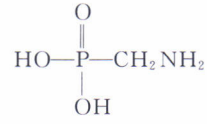
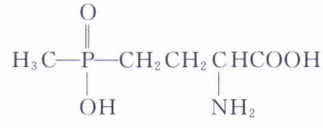
本方法对 3 种氨基酸类有机膦除草剂的测定低限均为 0.050 0 mg/kg。

9.2 回收率

在四个添加水平下，大米、小麦、大豆、玉米、奶白菜、葡萄、橙子、马铃薯、大蒜、茶叶、虾肉、鱼肉和蜂蜜中草甘膦、氨甲基膦酸和草铵膦的回收率数据参见附录 D 中表 D.1。

附　录　A
（资料性附录）
氨基酸有机膦类除草剂的相关信息

表 A.1　氨基酸有机膦类除草剂的相关信息

化合物名称	英文名	CAS 号	分子式	相对分子质量	分子结构
草甘膦	Glyphosate	1071-83-6	$C_3H_8NO_5P$	169.08	
氨甲基膦酸	AMPA	1066-51-9	$C_1H_6NO_3P$	111.04	
草铵膦	Glufosinate	51276-47-2	$C_5H_{15}N_2O_4P$	181.12	

附 录 B
（资料性附录）
参考质谱条件[2)]

参考质谱条件如下：

a） 电喷雾电压：5 500V；

b） 雾化气压力(GS1)：275.8 kPa(40 psi)；

c） 辅助气流速(GS2)：413.7 kPa(60 psi)；

d） 幕帘气流速(CUR)：172.4 kPa(25 psi)；

e） 离子源温度：500 ℃；

f） 碰撞气 CAD：68.95 kPa(10 psi)；

g） 其他质谱参数见表 B.1。

表 B.1 氨基酸有机膦类除草剂测定的质谱参数

化合物名称	定性离子对 (m/z)	定最离子对 (m/z)	去簇电压 DP V	碰撞能量 CE eV	碰撞室入口电压 CXP V	碰撞室出口电压 EP V
草甘膦-FMOC	392.0/88.0	392.0/88.0	63	30	16	8
	392.0/214.0		54	15	14	8
氨甲基膦酸-FMOC	334.0/179.0	334.0/179.0	60	28	10	6
	334.0/112.0		48	17	10	11
草铵膦-FMOC	404.0/136.1	404.0/136.1	60	29	14	8
	404.0/208.2		60	15	14	8
草甘膦同位素内标-FMOC	395.0/91.1	395.0/91.1	65	29	14	6

注：对于不同质谱仪器，仪器参数可能存在差异，测定前应将质谱参数优化到最佳。

2） 非商业性声明：附录 B 所列参考质谱条件是在 API 5000 型液质联用仪上完成的，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。

附　录　C
（资料性附录）
标准溶液的多反应监测（MRM）色谱图

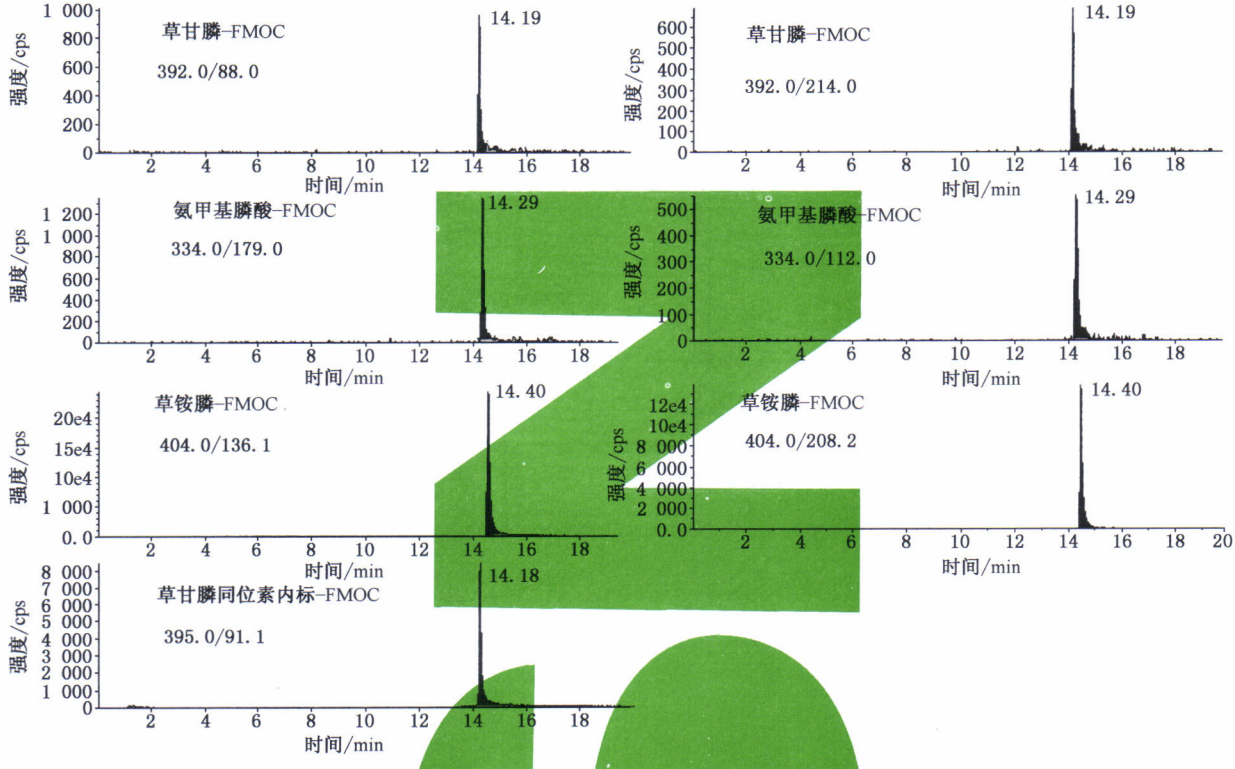


图 C.1　氨基酸类有机膦除草剂标准溶液的多反应监测（MRM）色谱图

附　录　D
（资料性附录）
回收率

表 D.1　不同基质中不同添加水平下的回收率数据

化合物名称	添加水平 mg/kg	大米回收率范围 %	玉米回收率范围 %	大豆回收率范围 %	奶白菜回收率范围 %	大蒜回收率范围 %	马铃薯回收率范围 %	橙子回收率范围 %	葡萄回收率范围 %	小麦回收率范围 %	茶叶回收率范围 %	虾肉回收率范围 %	鱼肉回收率范围 %	蜂蜜回收率范围 %
草甘膦	0.050 0	83.8～104	84.8～94.8	82.6～96.4	84.1～103	72.9～82.7	88.3～103	77.3～93.8	82.0～91.0	82.0～91.0	95.3～113	70.0～77.4	79.5～86.2	80.9～87.4
	0.100	80.0～89.9	86.0～97.7	75.8～89.7	84.6～101	71.5～77.7	85.2～94.8	79.2～94.9	70.4～84.4	70.4～84.4	79.3～92.2	72.9～97.4	83.2～89.4	79.5～86.2
	0.500	78.5～87.0	77.5～89.0	82.0～91.0	95.3～113	70.0～77.4	80.9～87.4	79.5～86.2	75.8～86.2	75.8～86.2	70.0～96.1	79.3～88.4	78.1～112	79.4～88.1
	5.00	79.3～112	80.6～90.7	75.8～86.2	70.0～96.1	79.3～88.4	79.4～88.1	78.1～112	82.0～91.0	82.0～91.0	95.3～113	70.0～77.4	79.5～86.2	80.9～87.4
氨甲基膦酸	0.050 0	86.6～107	87.8～97.6	87.8～99.8	70.0～84.0	75.5～82.9	84.0～90.1	92.4～110	75.0～84.0	75.0～84.0	83.0～94.2	79.0～95.0	70.3～78.6	87.2～97.2
	0.100	84.9～93.8	83.2～93.4	84.7～93.8	81.2～95.6	68.4～73.8	80.4～87.5	105～116	70.7～97.4	70.7～97.4	71.7～86.0	79.8～90.0	77.1～91.5	87.0～109
	0.500	79.5～89.0	75.0～84.0	83.0～94.2	79.0～95.0	70.3～78.6	74.3～80.6	87.2～97.2	78.6～90.2	78.6～90.2	78.5～86.2	70.2～82.3	73.1～96.5	79.6～106
	5.00	87.8～114	78.6～90.2	78.5～86.2	70.2～82.3	73.1～96.5	76.6～88.8	79.6～106	75.0～84.0	75.0～84.0	83.0～94.2	79.0～95.0	70.3～78.6	87.2～97.2
草铵膦	0.050 0	70.0～81.2	70.0～77.8	70.0～74.8	70.0～82.9	70.2～82.6	88.1～92.6	70.3～82.7	70.0～75.5	70.0～75.5	70.0～76.0	72.8～81.3	72.8～79.4	77.6～80.0
	0.100	72.4～76.6	70.0～75.5	70.0～76.0	72.8～81.3	72.8～79.4	83.6～94.3	77.6～80.0	70.0～78.0	70.0～78.0	70.0～76.5	70.0～78.7	86.1～92.9	70.4～74.7
	0.500	70.0～75.5	70.0～78.0	70.0～76.5	70.0～78.7	86.1～92.9	78.8～89.0	70.4～74.7	72.4～89.9	72.4～89.9	70.0～82.3	70.3～86.6	72.2～87.4	84.7～89.3
	5.00	72.1～82.1	74.3～80.9	70.0～82.8	74.8～81.2	70.0～86.0	76.4～85.8	80.8～90.2	74.3～80.9	74.3～80.9	70.0～82.8	74.8～81.2	70.0～86.0	80.8～90.2

Foreword

This standard is drafted according to GB/T 1.1—2009.

Please note that some of the content of this standard may be involved in the parent, the publisher of this standard does not assume the responsibility to identify these patents.

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

The standard was drafted by Shenzhen Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Shenzhen Academy of Inspection and Quarantine.

This standard was mainly drafted by Zhang Jianying, Hu Xiaoyuan, Wu Fengqi, Zhang Yi, Luo Yao, Wu Weidong, Yue Zhenfeng.

Determination of phosphonic and amino acid group-containing herbicides residues in foodstuffs for export—LC-MS/MS method

1 Scope

This standard specifies the methods of determination by liquid chromatography-tandem mass spectrometry of glyphosate, Amino methyl phosphoric acid (AMPA) and glufosinate in rice, wheat soybean, corn, milk cabbage, potato, garlic, grapes, orange, tea, shrimp, fish and honey.

This standard is applicable to the determination and conformation of glyphosate, AMPA and glufosinate in rice, wheat, soybean, corn, milk cabbage, potato, garlic, grapes, orange, tea, shrimp, fish and honey.

2 Quoted normative documents

The following documents for the application of this document is essential. For dated references, only the dated version applies to this document. For undated references, the latest version (including all amendments) applies to this document.

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

3 Abstract

The residues of phosphorus-containing amino-acid herbicides residues in the test sample (rice, milk cabbage, grapes, orange) were extracted with water, defatted using an extraction step with dichloromethane. After that, the aqueous extract were derived using fluorenylmethychloroformate (FMOC-C1) in borate buffer for subsequent HPLC-MS/MS analysis. Isotope-labed glyphosate (1, 2-$^{13}C^{15}N$) was used as the internal standard for the quantitative analysis of the three residues.

The residues of phosphorus-containing amino-acid herbicides residues in the test sample (wheat, soybean, corn, garlic and potato) were extracted with water, defatted using an extraction step with dichloromethane. Then the extraction of protein were precipitated using trichloroacetic acid. After that, the aqueous were derived using fluorenylmethychloroformate (FMOC-C1) in borate buffer for subsequent HPLC-MS/MS analysis, using internal standard method.

The residues of phosphorus-containing amino-acid herbicides residues in the test sample (shrimp,

fish and honey) were extracted with water, defatted using an extraction step with dichloromethane. Then the extracted soulution was purified using a cation-exchange(CAX) solid phase extraction cartridge. After that, the aqueous extract were derived using fluorenylmethychloroformate(FMOC-C1) in borate buffer for subsequent HPLC-MS/MS analysis, using internal standard method.

4 Reagents and materials

Unless otherwise specified, all reagents shall be of analytical grade; "water" is the first grade water prescribed by GB/T 6682.

4.1 Dichloromethane, HPLC grade.

4.2 Acetone, HPLC grade.

4.3 Methanol, HPLC grade.

4.4 Acetonitrile, HPLC grade.

4.5 Trichloroacetic acid.

4.6 Hydrochloric acid.

4.7 Sodium borate ($Na_2B_4O_7 \cdot 10H_2O$).

4.8 Ammonium acetate.

4.9 Formic acid, HPLC grade.

4.10 9-Fluorenylmethylchloroformate(FMOC-CL): 99.0% purity, 4 ℃ preservation.

4.11 Ammonium hydroxide.

4.12 Washing solution and eluent: accurately measure 160 mL water, 2.7 mL hydrochloric acid (4.6) and 40 mL methanol (4.3), mixed.

4.13 5% Sodium borate buffer solution (pH 9.0): weigh 5 g sodium borate (4.7), dissolved with water to 100 mL, mixed.

4.14 Derivatization reagent: accurately weighed 200 mg FMOC-CL, dissolved with acetone (4.2) to 100 mL, mixed.

4.15 20% Trichloroacetic acid solution: weighed 20 g trichloroacetic acid (4.5) to 100 mL volumetric flask, diluted with water.

4.16 5 mmoL Ammonium acetate aqueous (contained 0.1% formic acid): accurately weighed 0.385 4 g ammonium acetate to 1 000 mL volumetric flask, and added 1 mL formic acid (4.9), diluted with water.

4.17 Acetonitrile (contained 0.1% formic acid): accurately measured 1 mL formic acid to 1 000 mL volumetric flask, diluted with acetonitrile.

4.18 Standards: glyphosate, CAS No. 1071-83-6, 99.0% purity; AMPA, CAS No. 1066-51-9, 99.0% purity; glufosinate, CAS No. 51276-47-2, 99.0% purity; isotope-labeled glyphosate($1,2\text{-}^{13}C^{15}N$) standard solution, 100 μg/mL (Chemical information contained in Table A.1 in Annex A).

4.19 Standard stock solution: accurately weighed certain amount of glyphosate, AMPA and glufosinate, dissolved with water and made 1 000 mg/L standard stock solutions. These solutions can be preserved for 6 months under 5 ℃ avoiding sunlight.

4.20 Mixed standard solution: accurately measured certain volume standard stock solutions (4.19) respectively, make a 10.0 mg/L mixed standard solution with water. The solution can be preserved for 3 months under 5 ℃ avoiding sunlight.

4.21 Isotope-labeled glyphosate ($1,2\text{-}^{13}C^{15}N$) standard solution: Accurately measured certain volume Isotope – labeled glyphosate ($1,2\text{-}^{13}C^{15}N$) standard stock solution (4.18), dissolved with water and maked 10.0 mg/L standard stock solution. The solution can be preserved for 3 months under 5 ℃ avoiding sunlight.

4.22 Mixed standard working solution: Accurately measured a certain volume standard stock solution (4.20) and isotope-labeled standard solution (4.21), make 5 mixed standard solutions with water: 5.00 μg/L, 10.0 μg/L, 25.0 μg/L, 50.0 μg/L and 100 μg/L (the concentration of the internal standard are all 20 μg/L). The solution shall be prepared daily.

4.23 Cation exchange solid-phase extraction column: AG 50W-X8 resin, 200 mesh～400 mesh, H^+ type, 0.8 cm × 4 cm (Also can use the same performance of the United States of America Bio-Rad company of commercial CAX cartridges[1], or equivalent).

4.24 Filter: 0.45 μm membrane filters for aqueous.

5 Apparatus and equipments

5.1 LC-MS/MS: Equipped with electrospray ion source.

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1) Non-commercial statement: the column and its type involved in the standard method is only for reference and not related to any commercial aim, and the analysts are encouraged to use columns of different corporation or different type.

5.2 SPE equipment.

5.3 Horizontal reciprocating oscillator.

5.4 Vortex mixer.

5.5 Polypropylene centrifuge tube: 50 mL and 15 mL, with screw cap.

5.6 Centrifuge: Rotate speed can reach 9 500 r/min.

5.7 Homogenizer.

6 Sample preparation and storage

6.1 Requirement

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

6.2 Rice, milk cabbage, wheat, tea, soybean, grapes, potato, garlic and orange

Take approximately 500 g of representative sample, smash thoroughly by a homogenizer, mix thoroughly. The prepared sample shall be devided into two parts, and put into clean containers, seal and label them. Sample shall be kept at −18 ℃.

6.3 Corn, shrimp and fish

Take approximately 500 g of representative sample, collect the edible parts (do not wash with water) and cut into minces. Crush with a crusher into pulp and mix thoroughly. The prepared sample shall be devided into two part, and put into a clean containers. Seal and label them. The test sample shall be stored at the temperature below −18 ℃.

6.4 Honey

Take approximately 500 g of packaging honey sample heating in 50 ℃ ~ 60 ℃ water bath. Then mix the sample thoroughly till completely dissolved. The prepared sample shall be devided into two part, and put into clean containers, seal and label them. Sample shall be kept at room temperature.

7 Procedure

7.1 Extraction

7.1.1 Rice, milk cabbage, grapes and orange

Weigh 5 g of test sample (accurate to 0.01 g) into 50 mL polypropylene centrifuge tube with cap, add

50 μL internal standard solution (4.21), vortex for 30 s, then add 25 mL water and 10 mL dichloromethane, vortex for 10 min, centrifuged 5 min at 9 500 r/min. Transfer 4 mL supernatant to another 15 mL centrifuge tube, and use ammonium hydroxide to ajust the pH value of the solution to 7. Measure 5 mL dichloromethane to solution, vortex mix 30 s, centrifuged 5 min at 9 500 r/min. After that, measure 1.0 mL solution to a 15 mL centrifuge tube for the next derivatization process.

7.1.2 Wheat, soybean, corn, garlic and potato

Weigh 5 g of test sample (accurate to 0.01 g) into 50 mL polypropylene centrifuge tube with cap, add 50 μL internal standard solution (4.21), vortex for 30 s, then add 25 mL water and 10 mL dichloromethane, vortex for 10 min, centrifuged 5 min at 9 500 r/min. Measure 5 mL supernatant to a 15 mL centrifuge tube, add 0.2 mL 20% trichloroacetic acid solution (4.15), vortex for 1 min, centrifuged 5 min at 9 500 r/min. Transfer 4 mL supernatant to another 15 mL centrifuge tube, and use ammonium hydroxide to adjust the pH to 7. Measure 5 mL dichloromethane to solution, vortex mix 30 s, centrifuged 5 min at 9 500 r/min. After that, measure 1.0 mL solution to a 15 mL centrifuge tube for the further derivatization process.

7.1.3 Shrimp, fish, honey and tea

Weigh 5 g of test sample (accurate to 0.01 g) into 50 mL polypropylene centrifuge tube with cap, add 50 μL internal standard solution (4.21), vortex for 30 s, then add 25 mL water and 10 mL dichloromethane, homogenize for 10 min, centrifuged 5 min at 9 500 r/min. Transfer 6 mL supernatant to another 15 mL centrifuge tube, measure 4 mL dichloromethane to solution, vortex for 30 s, centrifuged 5 min at 9 500 r/min. Measure 4 mL supernatant to another 15 mL centrifuge tube, and use hydrochloric acid to adjust the pH to 2 for the further cleanup process.

7.2 Cleanup

Transfer 1.0 mL of the extraction (7.1.3) on the actived silica CAX SPE column (4.23), kept the flow rate 1.5 mL/min, add 1.0 mL washing solution, discard the eluate, add 12 mL eluent, collect the eluate to rotary evaporation vacuum concentration in 50 ℃ water bath to dry. Add 1.0 mL sodium borate buffer solution (4.13), vortex mix for the next derivatization process.

7.3 Derivatization

Accurately measured 300 μL sodium borate buffer solution (4.13) to the purified liquid (7.2), then added in 200 μL derivative solution (4.14), vortex mixed 1 min, then standing at room temperature overnight derivatization. After that, vortex mixed 1 min, the filter with 0.45 μm aqueous membrane (4.24) for LC-MS/MS detection.

7.4 Determination

7.4.1 LC operating conditions

LC operating conditions are as following.

a) Column: C_{18} column, 150 mm × 2.1 mm (i.d.), 2.7 μm, or equivalent;

b) Column temperature: 30 ℃;

c) Injection volume: 20 μL;

d) Mobile phase, flow speed and the elution gradient are listed in Table 1.

Table 1—Mobile phase, flow speed and the elution gradient

Time min	Flow speed mL/min	5 mmol ammonium acetate aqueous (contained 0.1% formic acid) %	Acetonitrile (contain 0.1% formic acid) %
0	0.300	85	15
9.00	0.300	85	15
12.0	0.300	15	85
14.0	0.300	15	85
15.0	0.300	85	15
20.0	0.300	85	15

7.4.2 Mass operation conditions

Mass operation conditions are as following:

a) Ionization mode: ESI+;

b) Scan mode: MRM;

c) Resolution: Unit;

d) Other operating conditions are listed in Table B.1 of Annex B.

7.4.3 LC-MS/MS determination

7.4.3.1 Qualitative determination

Select one parent ion and two or more than two daughter ions for each analyst. If the deviation of retention time of analyst between test sample and standard solution is within ±2.5% under the same experiment conditions, and the difference of relative ion ratio of analyst between test sample and standard solution is also within the error allowed (the max deviation allowed for relative ion ratio are listed in Table 2, corresponding analyst would be considered to be in the sample.

Table 2—Max deviation allowed for relative ion ratio in qualitative determination

Relative ion ratio/%	>50	>20~50	>10~20	≤10
Max deviation allowed/%	±20	±25	±30	±50

7.4.3.2 Quantitative determination

Under the optimized instrument working condition, different mixed calibration solutions are injected into the instrument in a separate run. The quantitative determination of the residues is according to the peak areas with internal standard method. The response of residues in the standard working solution and sample solution shall be in the linear range of the instrumental detection. The multiple reaction monitoring (MRM) chromatograms of glyphosate, AMPA and glufosinate residues are shown as Figure C.1 in Annex C.

7.4.4 Blank test

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

8 Calculation and expression of result

The calculation of glyphosate, AMPA and glufosinate residues in the sample is according to foumula (1) or using the LC-MS/MS data process software, the blank value shall be subtracted from the result:

$$X_i = \frac{c \times c_i \times A \times A_{si} \times V}{c_{si} \times A_s \times A_i \times m} \times \frac{1\,000}{1\,000} \qquad \cdots\cdots (1)$$

Where:

X_i —the residue content of analyte in the test sample, mg/kg;

c —the concentration of analyte in the standard working solution, mg/L;

c_i —the residue content of internal standard in the test sample, mg/L;

A —peak area of analyte in the sample solution;

A_{si} —peak area of internal standard in the standard working solution;

V —the final volume of sample solution, mL;

c_{si} —the concentration of internal standard in the standard working solution, mg/L;

A_s —Peak area of analyte in the standard working solution;

A_i —Peak area of internal standard in the sample solution;

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

9 Limit of determination and recovery

9.1 Limit of determination

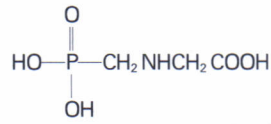
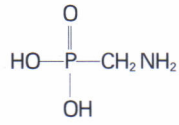
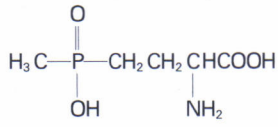
The limit determination of glyphosate, AMPA and glufosinate in rice, wheat, soybean, corn, milk cabbage, potato, garlic, grapes, orange, tea, shrimp, fish and honey is 0.050 0 mg/kg.

9.2 Recovery

The recovery of glyphosate, AMPA and glufosinate in rice, wheat, soybean, corn, milk cabbage, potato, garlic, grapes, orange, tea, shrimp, fish and honey are list in Table D.1 of Annex D.

Annex A
(Informative)
Relevant information of phosphonic and amino acid group-containing herbicides

Table A.1—Relevant information of phosphonic and amino acid group-containing herbicides

Compound	CAS No.	Molecular formula	Molecular weight	Structural formula
Glyphosate	1071-83-6	$C_3H_8NO_5P$	169.08	
AMPA	1066-51-9	$C_1H_6NO_3P$	111.04	
Glufosinate	51276-47-2	$C_5H_{15}N_2O_4P$	181.12	

Annex B
(Informative)
Operating conditions of mass spectrometer[2)]

Operating conditions of mass spectrometer are as following:

a) Ionspray voltage(IS):5 500 V;

b) Nebulize gas(GS1):275.8 kPa(40 psi);

c) Desolution gas(GS2):413.7 kPa(60 psi);

d) Curtain gas(CUR):172.4 kPa(25 psi);

e) Auxiliary heating gas temperature(TEM):500 ℃;

f) Collision gas(CAD):68.95 kPa(10 psi);

g) Other mass operating conditions are listed in Table B.1.

Table B.1—Mass parameters for phosphonic and amino acid group-containing herbicides detection

Compound name	Qualitative ion (m/z)	Quantitative ion (m/z)	DP V	CE eV	CXP V	EP V
Glyphosate -FMOC	392.0/88.0	392.0/88.0	63	30	16	8
	392.0/214.0		54	15	14	8
AMPA -FMOC	334.0/179.0	334.0/179.0	60	28	10	6
	334.0/112.0		48	17	10	11
Glufosinate -FMOC	404.0/136.1	404.0/136.1	60	29	14	8
	404.0/208.2		60	15	14	8
Glyphosate(IS) -FMOC	395.0/91.1	395.0/91.1	65	29	14	6

Note: For the different MS equipment, the parameters may be different, and the MS parameters shall be optimized to the best before analysis.

2) Non-commercial statement: the mass parameters in Annex B are accomplished by API 5000 LC-MS/MS, the equipment and its type involved in the standard method is only for reference and not related to any commercial aim, and the analysts are encouraged to use equipments of different corporation or different type.

Annex C
(Informative)
MRM chromatogram of standard solution

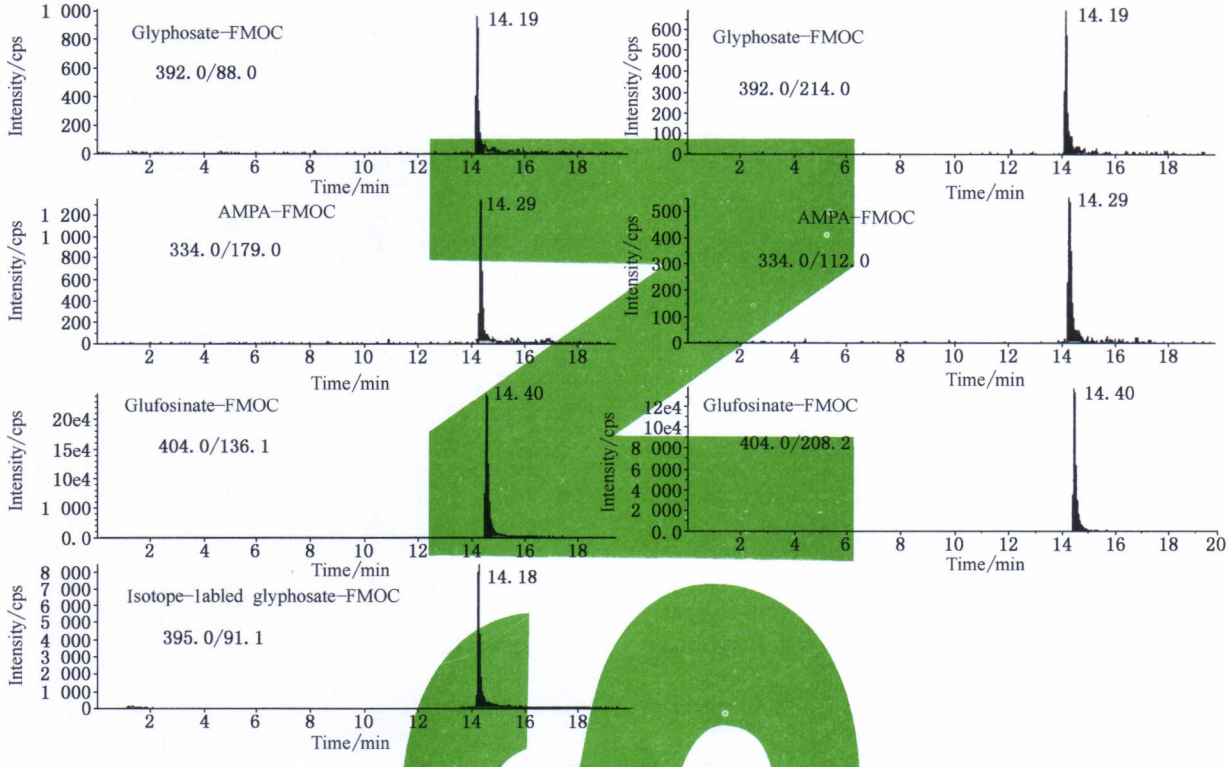


Figure C.1—MRM chromatogram of phosphonic and amino acid group-containing herbicides standard solution

Annex D
(Informative)
Recovery

Table D.1—Recovery data of different spiked level in different matrix

Compound name	Spiked level mg/kg	Rice recovery %	Corn Recovery %	Soybean recovery %	Milk Chinese cabbage recovery %	Garlic recovery %	Potato Recovery %	Orange recovery %	Grapes recovery %	Wheat recovery %	Tea recovery %	Shrimp recovery %	Fish recovery %	Honey recovery %
Glyphosate	0.050 0	83.8～104	84.8～94.8	82.6～96.4	84.1～103	72.9～82.7	88.3～103	77.3～93.8	82.0～91.0	82.0～91.0	95.3～113	70.0～77.4	79.5～86.2	80.9～87.4
	0.100	80.0～89.9	86.0～97.7	75.8～89.7	84.6～101	71.5～77.7	85.2～94.8	79.2～94.9	70.4～84.4	70.4～84.4	79.3～92.2	72.9～97.4	83.2～89.4	79.5～86.2
	0.500	78.5～87.0	77.5～89.0	82.0～91.0	95.3～113	70.0～77.4	80.9～87.4	79.5～86.2	75.8～86.2	75.8～86.2	70.0～96.1	79.3～88.4	78.1～112	79.4～88.1
	5.00	79.3～112	80.6～90.7	75.8～86.2	70.0～96.1	79.3～88.4	79.4～88.1	78.1～112	82.0～91.0	82.0～91.0	95.3～113	70.0～77.4	79.5～86.2	80.9～87.4
AMPA	0.050 0	86.6～107	87.8～97.6	87.8～99.8	70.0～84.0	75.5～82.9	84.0～90.1	92.4～110	75.0～84.0	75.0～84.0	83.0～94.2	79.0～95.0	70.3～78.6	87.2～97.2
	0.100	84.9～93.8	83.2～93.4	84.7～93.8	81.2～95.6	68.4～73.8	80.4～87.5	105～116	70.7～97.4	70.7～97.4	71.7～86.0	79.8～90.0	77.1～91.5	87.0～109
	0.500	79.5～89.0	75.0～84.0	83.0～94.2	79.0～95.0	70.3～78.6	74.3～80.6	87.2～97.2	78.6～90.2	78.6～90.2	78.5～86.2	70.2～82.3	73.1～96.5	79.6～106
	5.00	87.8～114	78.6～90.2	78.5～86.2	70.2～82.3	73.1～96.5	76.6～88.8	79.6～106	75.0～84.0	75.0～84.0	83.0～94.2	79.0～95.0	70.3～78.6	87.2～97.2
Glufosinate	0.050 0	70.0～81.2	70.0～77.8	70.0～74.8	70.0～82.9	70.2～82.6	88.1～92.6	70.3～82.7	70.0～75.5	70.0～75.5	70.0～76.0	72.8～81.3	72.8～79.4	77.6～80.0
	0.100	72.4～76.6	70.0～75.5	70.0～76.0	72.8～81.3	72.8～79.4	83.6～94.3	77.6～80.0	70.0～78.0	70.0～78.0	70.0～76.5	70.0～78.7	86.1～92.9	70.4～74.7
	0.500	70.0～75.5	70.0～78.0	70.0～76.5	70.0～78.7	86.1～92.9	78.8～89.0	70.4～74.7	72.4～89.9	72.4～89.9	70.0～82.3	70.3～86.6	72.2～87.4	84.7～89.3
	5.00	72.1～82.1	74.3～80.9	70.0～82.8	74.8～81.2	70.0～86.0	76.4～85.8	80.8～90.2	74.3～80.9	74.3～80.9	70.0～82.8	74.8～81.2	70.0～86.0	80.8～90.2