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中华人民共和国出入境检验检疫行业标准

SN/T 4850—2017

出口食品中草铵膦及其代谢物残留量的测定 液相色谱-质谱/质谱法

Determination of glufosinate-ammonium and metabolites residues in export food—Liquid chromatography-tandem mass spectrometry method

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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：中华人民共和国泉州出入境检验检疫局综合技术服务中心、中华人民共和国福建出入境检验检疫局、中华人民共和国江西出入境检验检疫局、福建省高建发茶叶有限公司、泉州市洛江泉岩茶业有限公司、福建农林大学。

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出口食品中草铵膦及其代谢物残留量的测定 液相色谱-质谱/质谱法

1 范围

本标准规定了出口食品中草铵膦及其代谢物 N-乙酰基草铵膦、3-(甲基膦基)丙酸残留量的液相色谱-质谱/质谱检测方法。

本标准适用于茶叶、稻谷、大豆、玉米、柑橘、苹果、桃、葡萄、香蕉、木瓜、番茄、胡萝卜、马铃薯、洋葱、开心果、菜籽油中草铵膦及其代谢物 N-乙酰基草铵膦、3-(甲基膦基)丙酸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 原理

样品中的草铵膦及其代谢物 N-乙酰基草铵膦、3-(甲基膦基)丙酸用水提取，经二氯甲烷和 C_{18} 固相萃取小柱净化，用液相色谱-质谱/质谱法，电喷雾负离子模式电离(ESI-)，基质外标法定量。

4 试剂和材料

除另有规定外，所有试剂均为分析纯，水为二次超纯水。

4.1 乙腈：色谱纯。

4.2 无水乙酸铵：色谱纯。

4.3 氨水：25%，色谱纯。

4.4 二氯甲烷，色谱纯。

4.5 乙酸铵水溶液(1 mmol/L，pH=11)：准确称取 0.077 08 g 的无水乙酸铵溶解于适量水中，用氨水调节 pH=11，用水定容至 1 000 mL。

4.6 草铵膦标准物质(Glufosinate ammonium，GLU，CAS 号：77182-82-2，分子式：$C_5H_{15}N_2O_4P$)：纯度≥97.5%。

4.7 N-乙酰基草铵膦标准物质(N-Acetyl-glufosinate，NAG，CAS 号：73634-73-8，分子式：$C_7H_{14}NO_5P$)：纯度≥98.5%。

4.8 3-(甲基膦基)丙酸标准物质(3-methylphosphinicopropionic acid，MPP，CAS 号：15090-23-0，分子式：$C_4H_9O_4P$)：纯度≥98.0%。

4.9 草铵膦、N-乙酰基草铵膦(NAG)、3-(甲基膦基)丙酸(MPP)标准储备溶液(1.0 mg/mL)：精确称取 50 mg 的草铵膦和 NAG、MPP 标准品，用水溶解并定容至 50 mL，存放于聚乙烯或聚丙烯塑料瓶中。低于 5 ℃保存。

4.10 草铵膦、N-乙酰基草铵膦(NAG)、3-(甲基膦基)丙酸(MPP)标准中间溶液(1.0 μg/mL)：取一定

量的草铵膦标准储备溶液(4.9),用水稀释成 1.0 μg/mL,存放于聚乙烯或聚丙烯塑料瓶中,低于 5 ℃保存。

4.11 C_{18}固相萃取柱:500 mg,6 mL 柱。使用前用 5 mL 甲醇、5 mL 水活化。

4.12 滤膜:0.22 μm,水相。

5 仪器和设备

5.1 液相色谱串联质谱仪:配有电喷雾(ESI)离子源。

5.2 粉碎机。

5.3 组织捣碎机。

5.4 涡旋振荡器。

5.5 高速离心机,转速不低于 12 000 r/min。

5.6 固相萃取装置。

5.7 超声清洗器。

5.8 分析天平:感量 0.1 mg 和 0.01 g。

5.9 pH 计。

5.10 移液器:1 mL,5 mL。

5.11 螺旋盖聚丙烯离心管:50 mL。

5.12 圆孔筛:2.0 mm。

6 试样制备与保存

6.1 试样制备

6.1.1 茶叶和粮谷类

取有代表性样品约 200 g,用粉碎机粉碎并通过 2.0 mm 圆孔筛,混匀,装入洁净容器内,密封并标识。

6.1.2 水果蔬菜类

取有代表性样品 500 g,将可食部分切碎后(不可水洗),用组织捣碎机将样品加工成浆状,混匀,装入洁净容器内,密封并标识。

6.1.3 植物油类

植物油类液体样品混匀备用。

6.2 试样保存

水果、蔬菜类试样在−18 ℃保存。茶叶、谷物类试样、植物油类液体样品常温保存。

7 测定步骤

7.1 待测样品溶液的制备

7.1.1 样品提取

称取均质好的试样茶叶 1 g(精确至 0.01 g),植物油、谷物、水果蔬菜 5 g(精确至 0.01 g),置于

50 mL具塞聚丙烯离心管中，移液器准确加入 10 mL 水，10 mL 二氯甲烷，充分涡旋振荡 10 min，超声提取 10 min，以 12 000 r/min 离心 5 min，收集上清液，待净化。

7.1.2 样品净化

取样品提取液 5 mL 加入已经活化了的 C_{18} 固相萃取柱中，弃去前 3 mL 流出液，收集后 2 mL 流出液，过 0.22 μm 水相滤膜，滤液供液相色谱-质谱/质谱检测。

7.2 基质混合标准工作溶液的制备

称取 5 个均质好的阴性样品茶叶 1 g(精确至 0.01 g)，植物油、谷物、水果蔬菜 5 g(精确至 0.01 g)，分别置于 50 mL 具塞聚丙烯离心管中，加入适量混合标准溶液，其余按照 7.1 步骤操作完成，制成草铵膦、N-乙酰基草铵膦、3-(甲基膦基)丙酸含量均为 0.05 μg/mL、0.1 μg/mL、0.2 μg/mL、0.5 μg/mL、1.0 μg/mL 的基质标准溶液。

7.3 测定

7.3.1 液相色谱参考条件

液相色谱参考条件如下：

a) 色谱柱：NH2P-50 2D 柱，150 mm×2.0 mm，粒度 5 μm 或相当者；
b) 柱温：35 ℃；
c) 流速：0.25 mL/min；
d) 进样量：5 μL；
e) 流动相：流动相 A：1 mmol/L 乙酸铵水溶液(pH=11)(4.5)，流动相 B：乙腈。推荐梯度洗脱程序见表 1，不同仪器洗脱条件可能不同。

表 1 流动相梯度洗脱程序表

步骤	梯度时间/min	流动相 A/%	流动相 B(乙腈)/%
1	0	25	75
2	2	25	75
3	2.01	80	20
4	6	80	20
5	6.01	25	75
6	10	25	75

7.3.2 质谱/质谱参考条件

质谱/质谱参考条件如下：

a) 离子源：电喷雾离子源(ESI)；
b) 扫描方式：负离子模式扫描；
c) 监测模式：多反应监测(MRM)；
d) 雾化气、干燥气、碰撞气均为高纯氮气；使用前应调节气体流量以使质谱灵敏度达到检测要求，详细条件参见附录 A；
e) 喷雾电压、雾化气压力、干燥气温度、干燥气流速、源内碎裂电压、碰撞能量应优化至最优灵敏度，参考条件和定性离子对、定量离子参见附录 A。

7.3.3 定量测定

按照确定的液相色谱-质谱/质谱条件测定样品和基质标准工作溶液，响应值应在仪器检测的线性范围内，以色谱峰面积外标法定量。在上述色谱条件下草铵膦及其代谢物 N-乙酰基草铵膦、3-(甲基膦基)丙酸的参考保留时间分别为 6.06 min、6.18 min 和 6.23 min，标准溶液的多反应监测(MRM)色谱图参见附录 B 中图 B.1。

7.3.4 定性测定

在相同实验条件下，试样中待测物质的保留时间与标准工作溶液的保留时间偏差在±2.5%之内；且在扣除背景后的样液图谱中，所选择的离子对均出现，各定性离子对的相对丰度与浓度接近的标准溶液谱图中离子的相对丰度相比，其偏差不超过表 2 规定的范围，则可确定为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
允许的相对偏差/%	±20	±25	±30	±50

7.4 空白试验

除不加试样外，均按上述操作步骤进行。

7.5 结果计算和表述

用色谱数据处理软件或按式(1)计算样品中草铵膦及代谢物残留量。计算结果需扣除空白值。

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

式中：

X ——样品中被测组分的残留量，单位为毫克每千克(mg/kg)；

c ——从基质标准工作曲线上得到的上机溶液中被测组分的浓度，单位为纳克每毫升(ng/mL)；

V ——样品提取溶液体积，单位为毫升(mL)；

m ——试样称取质量，单位为克(g)。

8 测定低限和回收率

8.1 测定低限

本方法草铵膦及具代谢物 N-乙酰基草铵膦、3-(甲基膦基)丙酸的测定低限：茶叶为 0.1 mg/kg，稻谷、大豆、玉米、柑橘、苹果、桃、葡萄、香蕉、木瓜、番茄、胡萝卜、马铃薯、洋葱、开心果、菜籽油为 0.05 mg/kg。

8.2 回收率

本方法样品的添加浓度及回收率试验数据参见附录 C 中表 C.1。

附 录 A
（资料性附录）
质谱参考参数[1)]

质谱参考参数如下：

a） 干燥气温度：300 ℃；
b） 干燥气流速：10 L/min；
c） 雾化气压力：0.31 MPa(45 psi)；
d） 电喷雾电压：−4 500 V；
e） 鞘流气温度：350 ℃；
f） 鞘流气流速：12 L/min；
g） 喷嘴电压：−1 000 V；
h） 定性离子对、定量离子对、源内碎裂电压、碰撞能量见表 A.1。

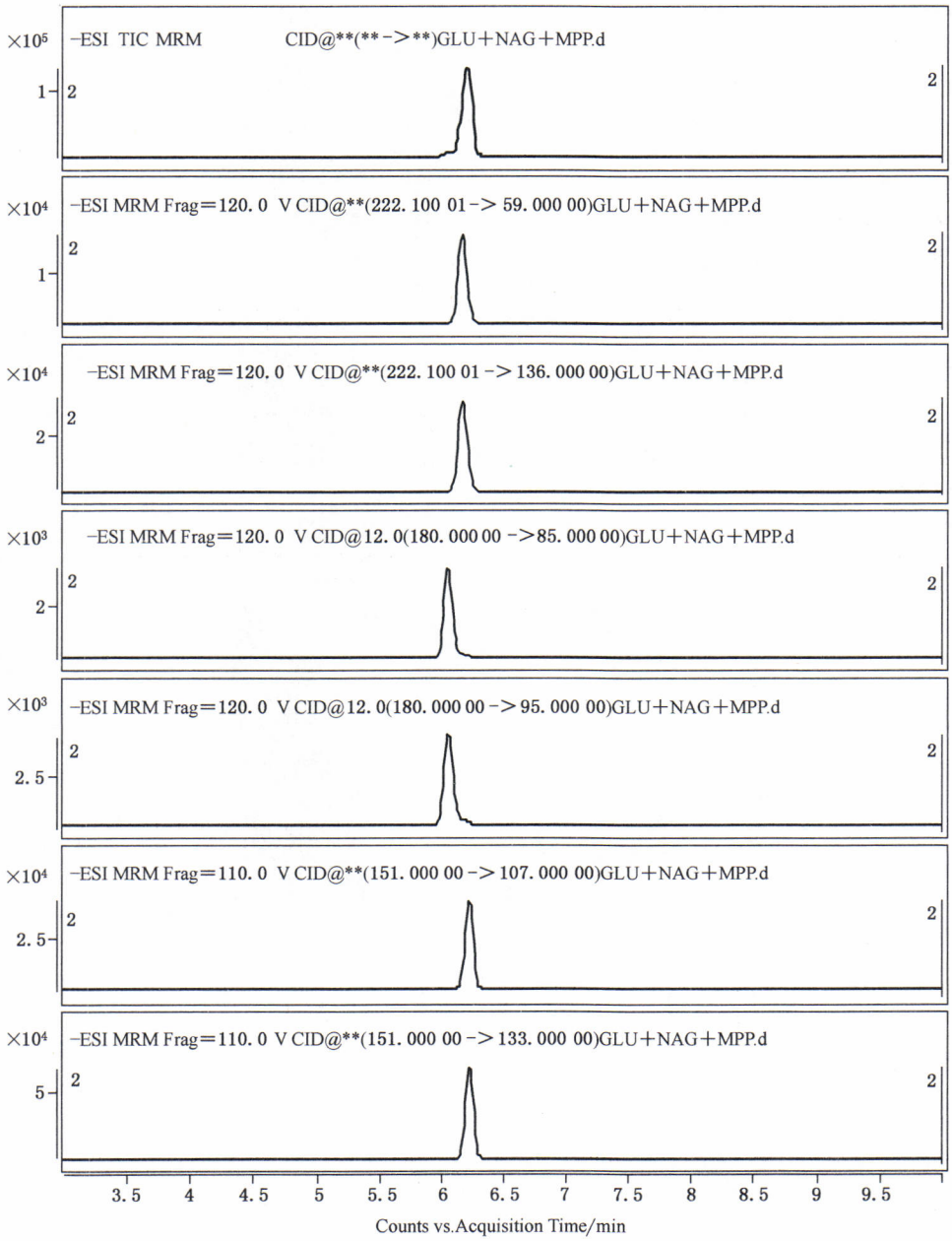
表 A.1 草铵膦及代谢物的定性离子对、定量离子对、源内碎裂电压、碰撞能量

名称	定量离子对 m/z	定性离子对 m/z	源内碎裂电压/V	碰撞能量/V
草铵膦	180/95	180/95;180/85	120	12;12
N-乙酰基草铵膦	222/136	222/136;222/59	120	20;10
3-(甲基膦基)丙酸	151/133	151/133;151/107	110	6;10

1） 非商业性声明：附录 A 所列参考质谱条件是在 Agilent HPLC-QQQ 6460 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 B
（资料性附录）
草铵膦、N-乙酰基草铵膦和 3-（甲基膦基）丙酸标准品的多反应监测（MRM）色谱图

草铵膦、N-乙酰基草铵膦和 3-（甲基膦基）丙酸标准品的多反应监测（MRM）色谱图，见图 B.1。



说明：

从上至下依次为：TIC 总离子流图；N-乙酰基草铵膦：222/59，222/136；草铵膦：180/85，180/95；3-（甲基膦基）丙酸：151/107，151/133 离子对的 MRM 图。

图 B.1 草铵膦、N-乙酰基草铵膦和 3-（甲基膦基）丙酸标准品的多反应监测（MRM）色谱图

附 录 C
（资料性附录）
草铵膦及代谢物 N-乙酰基草铵膦和 3-(甲基膦基)丙酸样品加标回收率

草铵膦及代谢物 N-乙酰基草铵膦和 3-(甲基膦基)丙酸的添加浓度和回收率试验数据，见表 C.1。

表 C.1 样品添加草铵膦及代谢物 N-乙酰基草铵膦和 3-(甲基膦基)丙酸的浓度及回收率试验数据($n=6$)

样品基质	添加物质	添加水平 mg/kg	回收率范围 %	样品基质	添加物质	添加水平 mg/kg	回收率范围 %
茶叶	草铵膦	0.1	72.5～115	大豆	草铵膦	0.05	72.6～95.3
		0.2	73.0～106			0.1	78.4～111
		1	75.8～105			1	75.7～105
	N-乙酰基草铵膦	0.1	67.5～99.6		N-乙酰基草铵膦	0.05	73.0～95.6
		0.2	77.4～113			0.1	79.7～115
		1	75.7～106			1	78.2～109
	3-(甲基膦基)丙酸	0.1	65.5～88.6		3-(甲基膦基)丙酸	0.05	72.5～93.6
		0.2	78.9～108			0.1	78.4～108
		1	80.7～105.3			1	78.7～105
稻谷	草铵膦	0.05	66.5～98.6	柑橘	草铵膦	0.05	73.5～115
		0.1	78.4～111			0.1	75.0～107
		1	70.7～106			1	76.8～116
	N-乙酰基草铵膦	0.05	69.7～95.6		N-乙酰基草铵膦	0.05	65.5～99.8
		0.1	77.8～107			0.1	77.4～115
		1	80.7～104			1	75.8～105
	3-(甲基膦基)丙酸	0.05	77.5～99.6		3-(甲基膦基)丙酸	0.05	65.7～89.6
		0.1	83.4～109			0.1	73.9～106
		1	80.7～107			1	82.7～106
玉米	草铵膦	0.05	76.5～104	苹果	草铵膦	0.05	66.8～98.1
		0.1	78.9～110			0.1	78.0～110
		1	72.0～106			1	78.7～104
	N-乙酰基草铵膦	0.05	65.5～88.6		N-乙酰基草铵膦	0.05	69.2～98.6
		0.1	78.9～108			0.1	77.4～109
		1	80.7～105			1	80.7～104
	3-(甲基膦基)丙酸	0.05	68.5～96.6		3-(甲基膦基)丙酸	0.05	77.3～99.2
		0.1	74.4～107			0.1	83.5～110
		1	77.5～107			1	83.7～108

表 C.1（续）

样品基质	添加物质	添加水平 mg/kg	回收率范围 %	样品基质	添加物质	添加水平 mg/kg	回收率范围 %
桃	草铵膦	0.05	78.5～105	木瓜	3-(甲基膦基)丙酸	0.05	69.5～99.3
		0.1	78.0～111			0.1	78.4～111
		1	72.7～104			1	78.7～108
	N-乙酰基草铵膦	0.05	67.5～89.9	番茄	草铵膦	0.05	76.5～104
		0.1	79.9～106			0.1	78.9～110
		1	84.7～107			1	72.0～106
	3-(甲基膦基)丙酸	0.05	69.5～96.9		N-乙酰基草铵膦	0.05	65.5～88.6
		0.1	74.9～107			0.1	78.9～108
		1	79.5～109			1	80.7～105
葡萄	草铵膦	0.05	68.5～98.6		3-(甲基膦基)丙酸	0.05	68.5～96.6
		0.1	78.9～110			0.1	74.4～107
		1	75.7～104			1	77.5～107
	N-乙酰基草铵膦	0.05	69.1～97.6		草铵膦	0.05	72.9～98.7
		0.1	79.8～106			0.1	77.5～106
		1	80.9～106			1	75.9～107
	3-(甲基膦基)丙酸	0.05	79.5～99.1	开心果	N-乙酰基草铵膦	0.05	72.3～96.6
		0.1	85.4～109			0.1	79.4～108
		1	85.7～103			1	80.1～106
香蕉	草铵膦	0.05	74.5～111		3-(甲基膦基)丙酸	0.05	73.9～98.7
		0.1	78.4～110			0.1	78.4～111
		1	72.6～106			1	75.7～105
	N-乙酰基草铵膦	0.05	65.4～88.9	胡萝卜	草铵膦	0.05	72.5～115
		0.1	78.2～106			0.1	73.0～106
		1	83.7～107			1	75.8～106
	3-(甲基膦基)丙酸	0.05	68.9～97.6		N-乙酰基草铵膦	0.05	67.5～99.6
		0.1	74.4～107			0.1	77.4～113
		1	77.5～107			1	75.7～106
木瓜	草铵膦	0.05	72.4～95.8		3-(甲基膦基)丙酸	0.05	65.5～88.6
		0.1	78.6～111			0.1	78.9～108
		1	75.7～105			1	80.7～105
	N-乙酰基草铵膦	0.05	72.5～95.7	马铃薯	草铵膦	0.05	66.5～98.6
		0.1	78.4～115			0.1	78.4～111
		1	75.3～106			1	70.7～106

表 C.1（续）

样品基质	添加物质	添加水平 mg/kg	回收率范围 %	样品基质	添加物质	添加水平 mg/kg	回收率范围 %
马铃薯	N-乙酰基草铵膦	0.05	69.7～95.6	洋葱	3-(甲基膦基)丙酸	0.05	66.5～89.6
		0.1	77.8～107			0.1	68.9～112
		1	80.7～104			1	82.7～106
	3-(甲基膦基)丙酸	0.05	77.5～99.6	菜籽油	草铵膦	0.05	77.5～95.9
		0.1	83.4～109			0.1	78.5～110
		1	80.7～107			1	79.7～115
洋葱	草铵膦	0.05	70.5～114		N-乙酰基草铵膦	0.05	77.5～98.3
		0.1	73.6～107			0.1	78.1～109
		1	74.8～102			1	75.4～115
	N-乙酰基草铵膦	0.05	69.5～99.3		3-(甲基膦基)丙酸	0.05	79.5～95.8
		0.1	78.4～110			0.1	78.9～106
		1	78.7～108			1	75.9～115

Foreword

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

Some parts of the standard may have relationship with some patents. The release dapartment have no responsibility to recognize these patents.

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

This standard was drafted by Comprehensive Technology Service Centre of Quanzhou Entry-Exit Inspection and Quanratine Bureau of the Peole's Republic of China, Fujian Entry-Exit Inspection and Quanratine Bureau of the Peole's Republic of China, Jiangxi Entry-Exit Inspection and Quanratine Bureau of the Peole's Republic of China, Fujian province KOH KION HUA's tea co., LTD, Quanzhou Luojiang Quanyan tea industry co., LTD, Fujian Agriculture and Forestry University.

The main drafters of this standard are Huang Huoshui, Wei Hang, Weng Chengwu, Zou Qiang, Lin Yonghui, Liu Zhengcai, Lin Chunying, Rong Jiefeng, Li Yijun, Sun Weijiang, Yang Fang.

Determination of glufosinate-ammonium and metabolites residues in export food—Liquid chromatography-tandem mass spectrometry method

1 Scope

This standard specifies the determination method of glufosinate-ammonium and its metabolites N-acetyl-glufosinate, 3-methylphosphinicopropionic acid residues in export food by liquid chromatography-tandem mass spectrometry.

This standard is applicable to the determination and confirmation of glufosinate-armnonium and its metabolites N-acetyl-glufosinate, 3-methylphosphinicopropionic acid residues in tea, rice, soybean, corn, orange, apple, peach, grape, banana, papaya, tomato, carrot, potato, onion, pistachio and rapeseed oil.

2 Normative referenoes

The following documents are necessary for this standard. For dated reference, only dated editins shall apply to this standard. For undatated references, the latest edition of the normative document (including subsequent amendments) is referred for application.

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

3 Principle

Glufosinate-armnonium and its metabolites N-acetyl-glufosinate, 3-methylphosphinicopropionic acid residues in the test sample are extracted with water and cleaned up by dichloromethane and C_{18} solid phase extraction column. The residual contents are determined by liquid chromatography- tandem mass spectrometry using negative ion mode electrospray ionization (ESI-) and quantified by external standard method.

4 Reagents and materials

Unless otherwise specified, all the reagents used should be analytical pure, and "water" is ultrapure water.

4.1 Acetonitrile: HPLC grade.

4.2 Anhydrous ammonium acetate: HPLC grade.

4.3 Ammonium hydroxide: 25%, HPLC grade.

4.4 Dichloromethane: HPLC grade.

4.5 Ammonium acetate solution (1 mmol/L, pH=11): Accurately weigh 0.077 08 g anhydrous ammonium acetate, dissolved with appropriate water, adjust pH=11 with ammonium hydroxide and diluted to 1 000 mL using water.

4.6 Glufosinate ammonium standard (GLU): CAS number: 77182-82-2, molecular formula: $C_5H_{15}N_2O_4P$, purity≥97.5%.

4.7 N-Acetyl-glufosinate standard (NAG): CAS number: 73634-73-8, molecular formula: $C_7H_{14}NO_5P$, purity≥98.5%.

4.8 3-methylphosphinicopropionic acid standard (MPP): CAS number: 15090-23-0, molecular formula: $C_4H_9O_4P$, purity≥98.0%.

4.9 Standard stock solution (1.0 mg/mL): Accurately weigh 50 mg GLU, NAG and MPP standards into 50 mL volumetric flask and dissovle to volume with water, and mix to homogeneity. The solution can be stored in polyethylene or polypropylene bottles at <5 ℃.

4.10 Standard intermediate solution (1.0 μg/mL): Dilute stock standard solution (4.9) to 1.0 μg/mL with water and stored in polyethylene or polypropylene bottles at <5 ℃.

4.11 C_{18} solid phase extraction (SPE) column: 500 mg, 6mL. Condition C_{18} SPE column with 5 mL methanol and 5 mL water before using.

4.12 Membrane filter: 0.22 μm, aqueous phase.

5 Apparatus and equipment

5.1 Liquid chromatography tandem mass spectrometry: Equipped with electrospray ionization ion source.

5.2 Grinder.

5.3 Tissue blender.

5.4 Whirlpool mixer.

5.5 Centrifuge: >12 000 r/min.

5.6 Solid phase extraction apparatus.

5.7 Ultrasonic extractor.

5.8 Analytical balance: sensitive quality 0.1 mg and 0.01 g.

5.9 pH meter.

5.10 Pipette: 1 mL, 5 mL.

5.11 Polypropylene centrifugal tube with screw top: 50 mL.

5.12 Sieve: 2.0 mm.

6 Sample preparation and storage

6.1 Preparation of test samples

6.1.1 Tea and cereals

Take about 200 g of representative sameple, to grind with a grinder into powder and through 2.0 mm sieve, mix thoroughly. Put in clean containers, to seal and laber them.

6.1.2 Fruit and vegetables

Take about 500 g of representative sameple, cut up the eatable part (do not wash), to smash with a tissue blender into pulp, mix thoroughly. Put in clean containers, to seal and laber them.

6.1.3 Plant oils

Mix the plant oil liquid samples thoroughly to be weighed.

6.2 Srorage of test samples

Fruit and vegetable samples should be frozen and stored below −18 ℃. Tea, cereal and plant oil smples should be stored at room temperature.

7 Procedure

7.1 Preparation of testing sample solution

7.1.1 Sample extraction

Weigh homogeneous tea sample 1 g (accurate to 0.01 g), plant oil, cereal, fruit and vegetable sample 5 g (accurate to 0.01 g) into a 50 mL polypropylene centrifuge tube. Add 10 mL water and 10 mL dichloromethane, vortex shake for 10 min and then ultrasonic extract 10 min, centrifuge for 5 min at 12 000 r/min. Collect the supernatant, waiting for purification.

7.1.2 Cleaning up

Transfer 5 mL extration solution into activated C_{18} SPE column, abandon to first 3 mL effluent, collect the latter 2 mL effluent. After filtered with 0.22 μm water film, the solutions are ready for determination by liquid chromatography- tandem mass spectrometry.

7.2 Preparation of matrix mixed standard working solution

Weigh homogeneous five negative samples, tea sample 1 g (accurate to 0.01 g), plant oil, cereal, fruit and vegetable sample 5 g (accurate to 0.01 g) into 50 mL polypropylene centrifuge tubes, add suitable amount of mixed standard solution (4.10), the rest step is according to 7.1 to make the matrix standard solution, the content of GLU, NAG and MPP in solution are 0.05 μg/mL, 0.1 μg/mL, 0.2 μg/mL, 0.5 μg/mL, 1.0 μg/mL.

7.3 Determination

7.3.1 HPLC reference operating conditions

HPLC reference operating conditions are as follows:

a) Column: NH2P-50 2D column, 150 mm × 2.0 mm, 5 μm or equivalent;

b) Column temperature: 35 ℃;

c) Flow rate: 0.25 mL/min;

d) Injector volume: 5 μL;

e) The mobile phase: A: 1 mmol/L ammonium acetate solution (pH = 11) (4.5), B: acetonitrile. Recommended gradient elute condition see table 1. Different instrument elution conditions may be different.

Table 1—Gradient elute condition of mobile phase

step	Time/min	Mobile phase A/%	Mobile phase B/%
1	0	25	75
2	2	25	75
3	2.01	80	20
4	6	80	20
5	6.01	25	75
6	10	25	75

7.3.2 MS/MS reference operating conditions

MS/MS reference operating conditions are as follows:

a) Ion source:ESI;

b) Scan mode:Negative;

c) Monitoring model:Multiple reaction monitoring (MRM);

d) Sheath gas, dry gas, collision gas were nitrogen (purity >99.999%); adjust all gas flow to optimize performance before used. The reference conditions are listed as Annex A;

e) Capillary voltage, nebulizer pressure, dry gas temperature, dry gas flow, fragmentor and collision Energy also must be optimized; the reference parameters and ion pair for confirmation and quantitation are listed as Annex A.

7.3.3 Quantitation determination

According to operating parameters of LC-MS/MS above, sample solution and the matrix standard working solution are determined simultaneously. The responses of the analyte in the solutions all should be within the linear range of the instrument detection and quantified by external standard method using chromatographic peak areas. The reference retention time of GLU, NAG and MPP is about 6.06 min, 6.18 min and 6.23 min repectively. MRM chromatograms of the standards are listed as figure B. 1 in Annex B.

7.3.4 Qualitative determination

Under the same determination condition, the ratio of the chromatographic retention time of the analysis shall correspond to that of the calibration solution at a tolenrance of ±2.5%. And in the sample solution after deducting the background, the relative intensities of the detected ions of each analysts shall compound to those of the calibration standard at comparable concentrations, within the tolerances shown in table 2, and then the cooresponding for relative ion intensities while confirmation.

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

7.4 Blank test

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

7.5 Calculation and expression of result

Calculate the content of glufosinate-ammonium and its metabolites residue in the test sample using the software of chromatography data or the followed foamula (1). The blank value should be subtraced from the above result of calculation.

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

where:

X—the residue content in the test samples, mg/kg;

c—the concentration of sample solution by matrix standard working curve, ng/mL;

V—the volume of sample extration solution, mL;

m—the mass of the test sample, g.

8 Limit of determination and recovery

8.1 Limit of determination

The determination limit of glufosinate-ammonium and its metabolites N-acetyl-glufosinate, 3-methylphosphinicopropionic acid residues in this method: tea is 0.1 mg/kg; rice, soybean, corn, orange, apple, peach, grape, banana, papaya, tomato, carrot, potato, onion, pistachio and rapeseed oil are 0.05 mg/kg.

8.2 Recovery

The fortified concentration and recoveries of glufosinate-ammonium and its metabolites in all kinds of matric are listed as table C. 1 in Annex C.

Annex A
(Informative)
MS/MS reference operating conditions[1)]

MS/MS reference operating conditions are as follows:

a) Dry gas temperature:300 ℃;

b) Dry gas flow rate:10 L/min;

c) Nebulizer pressure:0.31 MPa(45 psi);

d) Capillary voltage: −4 500 V;

e) Sheath gas temperature:350 ℃;

f) Sheath gas flow rate:12 L/min;

g) Nozzle voltage: −1 000 V;

h) Quantitative ion pair, qualitative ion pair, fragmentor and collision energy see table A.1.

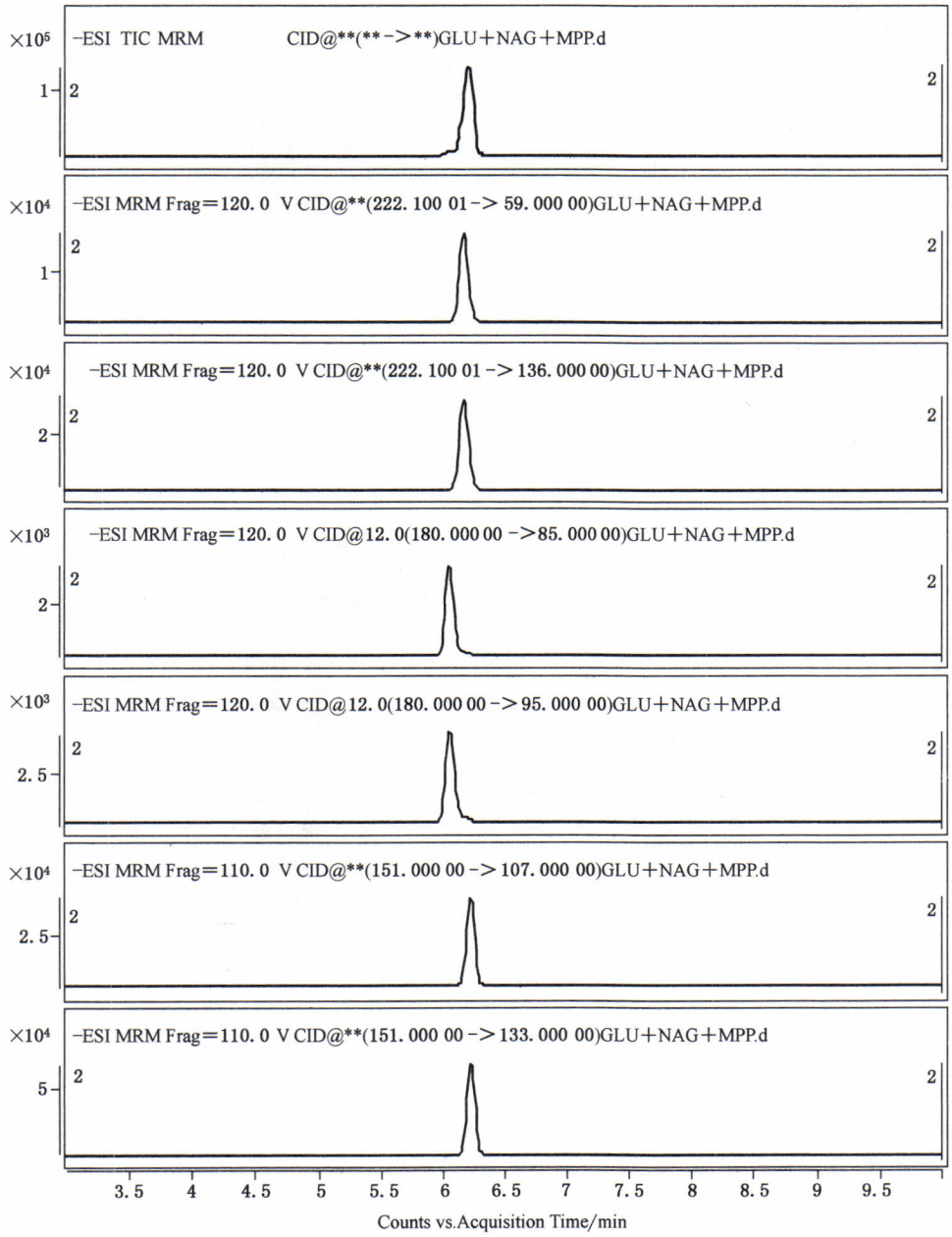
Table A. 1—Optimal operation conditions of glufosinate-ammonium and its metabolites

Compound	Quantitative ion pair *m/z*	qualitative ion pair *m/z*	fragmentor V	collision energy V
glufosinate-ammonium	180/95	180/95;180/85	120	12;12
N-acetyl-glufosinate	222/136	222/136;222/59	120	20;10
3-methylphosphinicopropionic acid	151/133	151/133;151/107	110	6;10

1) Non-commercial statement: The equipment and their types Agilent HPLC-QQQ 6460 LC-MS/MS involved in the standard method are not related to the commercial aims, and the analysts are encouraged to use equipments of different corporation or different type.

Annex B
(Informative)
MRM chromatograms of the GLU,NAG and MPP standards

MRM chromatograms of the GLU,NAG and MPP standards see figure B.1.



key

Up to bottom: TIC chromatogram; MRM chromatogram of NAG: 222/59, 222/136; GLU: 180/85, 180/95; MPP: 151/107, 151/133

Figure B.1—MRM chromatograms of the GLU,NAG and MPP standards

Annex C
(Informative)
Recovery range of GLU, NAG and MPP

Recovery date of GLU, NAG and MPP($n=6$) see Table C.1.

Table C.1 Recovery date of GLU, NAG and MPP($n=6$)

Matrix	Chemicals	Concentration mg/kg	Recovery range %	Matrix	Chemicals	Concentration mg/kg	Recovery range %
Tea	GLU	0.1	72.5～115	Soybean	GLU	0.05	72.6～95.3
		0.2	73.0～106			0.1	78.4～111
		1	75.8～105			1	75.7～105
	NAG	0.1	67.5～99.6		NAG	0.05	73.0～95.6
		0.2	77.4～113			0.1	79.7～115
		1	75.7～106			1	78.2～109
	MPP	0.1	65.5～88.6		MPP	0.05	72.5～93.6
		0.2	78.9～108			0.1	78.4～108
		1	80.7～105.3			1	78.7～105
Rice	GLU	0.05	66.5～98.6	Orange	GLU	0.05	73.5～115
		0.1	78.4～111			0.1	75.0～107
		1	70.7～106			1	76.8～116
	NAG	0.05	69.7～95.6		NAG	0.05	65.5～99.8
		0.1	77.8～107			0.1	77.4～115
		1	80.7～104			1	75.8～105
	MPP	0.05	77.5～99.6		MPP	0.05	65.7～89.6
		0.1	83.4～109			0.1	73.9～106
		1	80.7～107			1	82.7～106
Corn	GLU	0.05	76.5～104	Apple	GLU	0.05	66.8～98.1
		0.1	78.9～110			0.1	78.0～110
		1	72.0～106			1	78.7～104
	NAG	0.05	65.5～88.6		NAG	0.05	69.2～98.6
		0.1	78.9～108			0.1	77.4～109
		1	80.7～105			1	80.7～104
	MPP	0.05	68.5～96.6		MPP	0.05	77.3～99.2
		0.1	74.4～107			0.1	83.5～110
		1	77.5～107			1	83.7～108

Table C.1 (continued)

Matrix	Chemicals	Concentration mg/kg	Recovery range %	Matrix	Chemicals	Concentration mg/kg	Recovery range %
Peach	GLU	0.05	78.5～105	Papaya	MPP	0.05	69.5～99.3
		0.1	78.0～111			0.1	78.4～111
		1	72.7～104			1	78.7～108
	NAG	0.05	67.5～89.9	tomato	GLU	0.05	76.5～104
		0.1	79.9～106			0.1	78.9～110
		1	84.7～107			1	72.0～106
	MPP	0.05	69.5～96.9		NAG	0.05	65.5～88.6
		0.1	74.9～107			0.1	78.9～108
		1	79.5～109			1	80.7～105
Grape	GLU	0.05	68.5～98.6		MPP	0.05	68.5～96.6
		0.1	78.9～110			0.1	74.4～107
		1	75.7～104			1	77.5～107
	NAG	0.05	69.1～97.6	Pistachio	GLU	0.05	72.9～98.7
		0.1	79.8～106			0.1	77.5～106
		1	80.9～106			1	75.9～107
	MPP	0.05	79.5～99.1		NAG	0.05	72.3～96.6
		0.1	85.4～109			0.1	79.4～108
		1	85.7～103			1	80.1～106
Banana	GLU	0.05	74.5～111		MPP	0.05	73.9～98.7
		0.1	78.4～110			0.1	78.4～111
		1	72.6～106			1	75.7～105
	NAG	0.05	65.4～88.9	Garrot	GLU	0.05	72.5～115
		0.1	78.2～106			0.1	73.0～106
		1	83.7～107			1	75.8～106
	MPP	0.05	68.9～97.6		NAG	0.05	67.5～99.6
		0.1	74.4～107			0.1	77.4～113
		1	77.5～107			1	75.7～106
Papaya	GLU	0.05	72.4～95.8		MPP	0.05	65.5～88.6
		0.1	78.6～111			0.1	78.9～108
		1	75.7～105			1	80.7～105
	NAG	0.05	72.5～95.7	Potato	GLU	0.05	66.5～98.6
		0.1	78.4～115			0.1	78.4～111
		1	75.3～106			1	70.7～106

Table C.1 (continued)

Matrix	Chemicals	Concentration mg/kg	Recovery range %	Matrix	Chemicals	Concentration mg/kg	Recovery range %
Potato	NAG	0.05	69.7～95.6	Onion	MPP	0.05	66.5～89.6
		0.1	77.8～107			0.1	68.9～112
		1	80.7～104			1	82.7～106
	MPP	0.05	77.5～99.6	Rapeseed oil	GLU	0.05	77.5～95.9
		0.1	83.4～109			0.1	78.5～110
		1	80.7～107			1	79.7～115
Onion	GLU	0.05	70.5～114		NAG	0.05	77.5～98.3
		0.1	73.6～107			0.1	78.1～109
		1	74.8～102			1	75.4～115
	NAG	0.05	69.5～99.3		MPP	0.05	79.5～95.8
		0.1	78.4～110			0.1	78.9～106
		1	78.7～108			1	75.9～115

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