

# SN

## 中华人民共和国出入境检验检疫行业标准

SN/T 4655—2016

### 出口食品中草甘膦及其代谢物残留量 测定方法 液相色谱-质谱/质谱法

Determination of glyphosate and its metabolize residues  
in foodstuffs for export—HPLC-MS/MS method

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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：中华人民共和国厦门出入境检验检疫局检验检疫技术中心、宁波出入境检验检疫局、福建出入境检验检疫局。

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## 出口食品中草甘膦及其代谢物残留量 测定方法 液相色谱-质谱/质谱法

### 1 范围

本标准规定了出口食品中草甘膦及其代谢物(氨基磷酸)残留量的液相色谱-质谱/质谱测定方法。

本标准适用于茶叶、小麦、玉米、稻谷、甘蔗、大豆、柑橘、苹果、桃、葡萄、香蕉、西瓜、大麦茶、棉籽油中草甘膦及其代谢物残(氨基磷酸)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 方法提要

试样用水提取,经透析袋、RP柱及石墨化碳黑吸附剂净化,用液相色谱-质谱/质谱仪测定,外标法定量。

### 4 试剂材料

除非另有说明,所用试剂均为分析纯,水为GB/T 6682规定的一级水。

4.1 甲醇,色谱纯。

4.2 乙腈,色谱纯。

4.3 乙酸铵,色谱纯。

4.4 乙酸铵溶液(5 mmol/L, pH:10~11):准确称取 3.855 g(精确至 0.000 1 g)乙酸铵,用水定容至 50 mL,配制成 1 mol/L 乙酸铵溶液;再取其中 5 mL,用水定容至 1 000 mL,氨水调节 pH 至 10~11。

4.5 标准物质:草甘膦(glyphosate, GLY, CAS:1071-83-6),纯度 $\geq 98.0\%$ ;氨基磷酸(aminomethyl phosphonic acid, AMPA, CAS:1066-51-9),纯度 $\geq 98.0\%$ 。

4.6 透析袋:直径 22 mm,压平宽度(半周长)34 mm,截留分子量 3 500 Da。

4.7 标准品溶液的配制:准确称取 GLY 和 AMPA 标准品各 10 mg,用水定容至 10 mL,配置成浓度为 1 mg/mL 的标准储备溶液。

4.8 标准储备的配制:吸取标准品溶液,用水稀释,分别配制成浓度为 10  $\mu\text{g/mL}$  的标准储备液。

4.9 标准工作溶液的配制:根据需要,临时时吸取一定量的标准储备液,用水溶液稀释成适当浓度的混合标准工作溶液。

4.10 透析袋处理:用剪刀剪至 10 cm~20 cm 小段,用水煮沸 10 min,二次水洗净,保存至乙醇中;使用前均用二次水清洗干净。

4.11 OnGuard II RP 柱(1.0 cc 柱):使用前 5 mL 甲醇、5 mL 水活化,静置半小时;使用后甲醇清洗

保存。

4.12 石墨化碳黑吸附剂(GCB):40  $\mu\text{m}$ ~60  $\mu\text{m}$ ,ProElut 填料,或相当者。

4.13 微孔滤膜:0.22  $\mu\text{m}$ ,有机系,聚四氟乙烯(PTFE)膜。

## 5 仪器与设备

5.1 液相色谱-质谱/质谱仪:配有电喷雾离子源(ESI)。

5.2 电子天平:感量分别为 0.01 g,0.000 1 g。

5.3 组织捣碎机。

5.4 粉碎机。

5.5 均质器:15 000 r/min。

5.6 离心机:5 000 r/min 以上。

5.7 高速离心机:15 000 r/min 以上。

5.8 超声仪。

5.9 离心管:50 mL。

5.10 试管:50 mL

5.11 注射器。

5.12 涡漩混匀器。

5.13 氮吹仪。

## 6 试样制备与保存

### 6.1 试样制备

#### 6.1.1 液体样品

液体样品混匀备用。

#### 6.1.2 果蔬类

取代表性样品约 500 g,将其切碎后,用组织捣碎机将样品加工成浆状,混匀,装入洁净容器,密封,标明标记。

#### 6.1.3 粮谷类和茶叶类

取代表性样品约 500 g,用粉碎机粉碎,过 20 目筛,混匀,装入洁净容器,密封,标明标记。

### 6.2 试样保存

将试样于-5  $^{\circ}\text{C}$  以下避光保存。

注: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。

## 7 分析步骤

### 7.1 提取

液体样品、粮谷类、及果蔬样品称取试样 4 g(精确到 0.01 g),于 50 mL 离心管(5.9)中,加入 6 mL



水,于高速(15 000 r/min)均质器(5.5)上均质 3 min,以 15 000 r/min 离心(5.7)5 min,收集水相,用水定容至 10 mL。

茶叶类样品称取试样 2 g(精确到 0.01 g),于 50 mL 离心管(5.9)中,加入 10 mL 水,于高速(15 000 r/min)均质器(5.5)上均质 3 min,将离心管以 15 000 r/min 离心(5.7)5 min,收集上层液,用水定容至 10 mL。

7.2 净化

取样品提取液 10 mL 加入经活化的透析袋(4.10)中,并将透析袋置于已加入 10 mL 水的 50 mL 试管(5.10)中,超声(5.8)1 h。取出透析袋,从玻璃管中取出 5 mL 残液,用 5 mL 注射器(5.11)注入已活化的 RP 柱(4.11),弃去 RP 柱前 2 mL 流出液,用 5 mL 离心管收集后 3 mL 流出液;加入 20 mg GCB(4.12),旋窝震荡 2 min 后于 15 000 r/min 离心 5 min,取 2 mL 上清液至洁净的玻璃管中 60 ℃下氮吹(5.13)至干,残渣用 0.5 mL 的水溶液溶解,过聚四氟乙烯(PTFE)膜,供液相色谱-质谱/质谱仪(LC-MS/MS)分析。

7.3 测定

7.3.1 液相色谱-质谱/质谱条件

液相色谱-质谱/质谱条件如下:

- a) 色谱柱:NH2P-50 2D 柱,长 150 mm,内径 2.0 mm,粒径 5 μm 或相当者;
- b) 流动相:A:5 mmol/L 乙酸铵水溶液(氨水调 pH10~11),B:乙腈溶液;流速:0.25 mL/min,梯度洗脱程序见表 1;
- c) 柱温:35 ℃;
- d) 进样量:10 μL;
- e) 离子源:电喷雾(ESI);
- f) 扫描方式:负离子;
- g) 监测方式:多反应监测(MRM);
- h) 质谱条件参见附录 A。

表 1 流动相梯度洗脱程序

时间/min	流动相 A/%	流动相 B/%
0	20.0	80.0
2.00	20.0	80.0
2.01	80.0	20.0
8.00	80.0	20.0
8.01	20.0	80.0
12.00	20.0	80.0

7.3.2 色谱测定

7.3.2.1 定性测定

按照液相色谱-质谱/质谱条件测定样品和标准工作溶液,样品的质量色谱峰保留时间与标准品中

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对应的保留时间一致;且样品中各组分定性离子的相对丰度与接近浓度的标准工作溶液中相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%

7.3.2.2 定量测定

在仪器最佳工作条件下,对标准工作溶液进样。用标准工作曲线按外标法定量,样品溶液中被测物的响应值均应在仪器测定的线性范围内。根据试样中被测样液的含量情况,选取响应值相近的标准工作液进行色谱分析。标准工作液和样液中待测物的响应值均应在仪器线性响应范围内,如果待测物含量超过线性范围,则重新取样分析,用水稀释到线性范围内后分析。在上述色谱条件下 GLY 和 AMPA 的参考保留时间约为 4.68 min 和 4.20 min,标准溶液的多反应监测(MRM)色谱图参见附录 B。

7.4 空白试验

除不加试样外,均按上述操作步骤进行。

8 结果计算和表达

结果用色谱数据处理机或按式(1)计算试样中草甘膦和氨甲基磷酸的残留量:

$$X = \frac{c \times V \times 10 \times 1\,000}{m \times 1\,000} \dots\dots\dots (1)$$

式中:

- X —— 试样中草甘膦或氨甲基磷酸残留含量,单位为毫克每千克(mg/kg);
- c —— 标准工作溶液中草甘膦或氨甲基磷酸的浓度,单位为微克每毫升(μg/mL);
- V —— 样液最终定容体积,单位为毫升(mL);
- m —— 试样称取质量,单位为克(g)。

注: 计算结果应扣除空白值。

9 测定低限和回收率

9.1 测定低限

本方法对草甘膦和氨甲基磷酸残留量的测定低限均为 50 μg/kg。

9.2 回收率

不同添加浓度范围内回收率的实验数据,见表 3。

表 3 本方法添加浓度及回收率范围( $n=6$ )

基质	添加 浓度 mg/kg	回收率范围		基质	添加 浓度 mg/kg	回收率范围	
		草甘膦	氨甲基膦酸			草甘膦	氨甲基膦酸
茶叶	0.05	76.4%~88.3%	79.2%~88.5%	柑橘	0.05	77.3%~84.9%	82.4%~90.2%
	0.1	83.2%~90.6%	85.7%~96.8%		0.1	89.4%~99.2%	85.9%~94.7%
	1	82.9%~97.0%	84.5%~96.3%		1	90.4%~97.3%	91.3%~96.3%
小麦	0.05	78.9%~89.2%	79.9%~90.1%	苹果	0.05	83.6%~93.2%	84.8%~94.2%
	0.1	86.2%~93.5%	89.9%~99.1%		0.1	90.3%~97.2%	91.2%~97.4%
	1	82.6%~89.5%	86.1%~95.7%		0.5	91.8%~97.6%	89.8%~96.6%
	5	94.9%~104.1%	90.6%~98.2%		1	93.8%~99.7%	91.0%~98.7%
玉米	0.05	80.8%~96.2%	83.1%~95.2%	桃	0.05	78.8%~86.3%	79.3%~90.2%
	0.1	89.3%~94.2%	89.4%~97.2%		0.1	88.3%~95.8%	89.9%~97.0%
	1	95.4%~101.4%	89.3%~94.5%		1	86.9%~99.0%	90.3%~103.4%
稻谷	0.05	76.3%~89.3%	80.2%~92.8%	葡萄	0.05	80.2%~93.2%	76.6%~83.2%
	0.1	83.9%~95.1%	87.4%~94.9%		0.1	89.5%~101.7%	85.3%~92.7%
	1	90.6%~99.3%	97.2%~102.3%		1	85.3%~97.8%	90.2%~96.7%
西瓜	0.05	78.3%~89.4%	77.8%~90.3%	香蕉	0.05	78.3%~89.2%	80.5%~92.7%
	0.1	86.9%~97.3%	86.9%~94.2%		0.1	87.0%~93.5%	84.6%~92.6%
	1	97.3%~105.4%	89.4%~99.2%		1	84.9%~97.6%	81.0%~89.5%
大豆	0.05	79.5%~89.9%	82.7%~92.7%	甘蔗	0.05	82.5%~92.8%	83.2%~92.8%
	0.1	80.8%~94.0%	87.4%~93.0%		0.1	81.3%~90.1%	84.9%~90.4%
	1	89.4%~93.7%	86.3%~90.4%		1	84.7%~94.7%	86.3%~92.9%
	2	85.9%~96.3%	85.1%~91.4%		2	84.2%~97.8%	86.3%~97.8%
大麦 茶	0.05	77.2%~89.8%	80.9%~93.8%	棉籽 油	0.05	77.2%~92.9%	79.2%~93.1%
	0.1	89.0%~97.9%	87.3%~94.6%		0.1	79.8%~90.4%	78.4%~87.4%
	2	90.7%~98.2%	89.9%~97.2%		1	88.2%~96.0%	85.2%~89.9%



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附录 A  
(资料性附录)  
参考质谱条件

A.1 参考条件 1

- a) 电离源方式:电喷雾电离;
- b) 扫描方式:负离子模式;
- c) 监测方式:多反应监测(MRM);
- d) 干燥气温度(DGT):200 ℃;
- e) 干燥气流速(DGF):14 L/mm;
- f) 雾化器压力(GS1):20 PSI(氮气);
- g) 鞘流气温度(SGT):250 ℃(氮气);
- h) 鞘流气流速:11 L/min;
- i) 离子源喷雾电压 IS:−2 500 V;
- j) 喷嘴电压:−2 000 V;
- k) 定性离子对( $m/z$ )、定量离子对( $m/z$ ),碎裂电压(V),碰撞能量(eV),保留时间见表 A.1。

表 A.1 多反应监测参数表<sup>1)</sup>

组分名称	定性离子对 $m/z$	定量离子对 $m/z$	驻留时间 ms	碎裂电压 (Frag)/V	碰撞能量 (CE)/eV	保留时间 min
草甘膦	167.8/63.0	167.8/63.0	100	380	25	4.68
	167.8/81.0		100	380	15	
	167.8/124.0		100	380	8	
氨甲基磷酸	110.0/63.0	110.0/63.0	100	380	20	4.20
	110.0/79.0		100	380	35	
	110.0/81.0		100	380	10	

A.2 参考条件 2

- a) 电离源方式:电喷雾电离;
- b) 扫描方式:负离子模式;
- c) 监测方式:多反应监测(MRM);
- d) 碰撞气流速比(CAD):4;
- e) 气帘气压力(CUR):35;
- f) 雾化器压力(GS1):60 Psi(氮气);

1) 非商业性声明:表 A.1 所列参考质谱条件是在 Agilent 6490 QQQ MS/MS 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。



- g) 加热辅助气(GS2):60 Psi(氮气);
- h) 雾化温度:550 ℃;
- i) 离子源喷雾电压 IS:−4 500.0;
- j) 定性离子对( $m/z$ )、定量离子对( $m/z$ ),去簇电压(V),入口电压(V),碰撞能量(eV),碰撞池出口电压(V),保留时间见表 A.2。

表 A.2 多反应监测参数表<sup>2)</sup>

组分名称	定性离子对 $m/z$	定量离子对 $m/z$	驻留时间 ms	去簇电压 (DP)/V	入口电压 (EP)/V	碰撞能量 (CE)/eV	碰撞池 出口电压 (CXP)/V
草甘膦	167.8/63.0	167.8/63.0	100	−48.02	−10	−33.04	−15
	167.8/124.0		100	−53.27	−10	−16.99	−15
	167.8/150.0		100	−49.95	−10	−12.50	−15
	167.8/81.0		100	−45.88	−10	−20.270	−15
氨甲基 膦酸	110.0/63.0	110.0/63.0	100	−57.55	−10	−23.97	−15
	110.0/79.0		100	−61.08	−10	−32.97	−15
	110.0/81.0		100	−55.91	−10	−17.79	−15

2) 非商业性声明:表 A.2 所列参考质谱条件是在 API 4000+LC/MS/MS 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)

草甘膦和氨甲基磷酸标准品多反应监测(MRM)色谱图

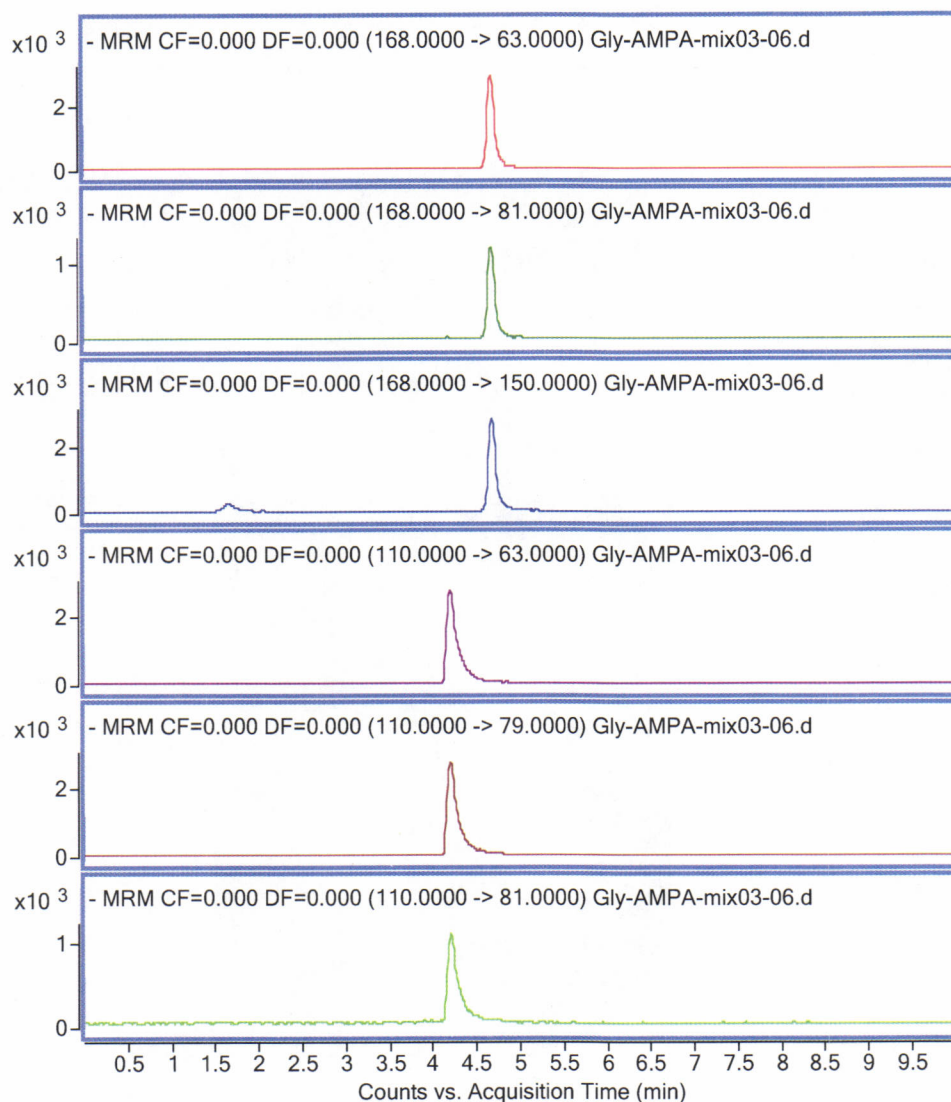


图 B.1 标准品多反应检测(MRM)色谱图(Agilent 6 490 QQQ)

从上至下依次为: 167.8/63.0, 167.8/81.0, 167.8/150.0;  
110.0/63.0, 110.0/79.0, 110.0/81.0

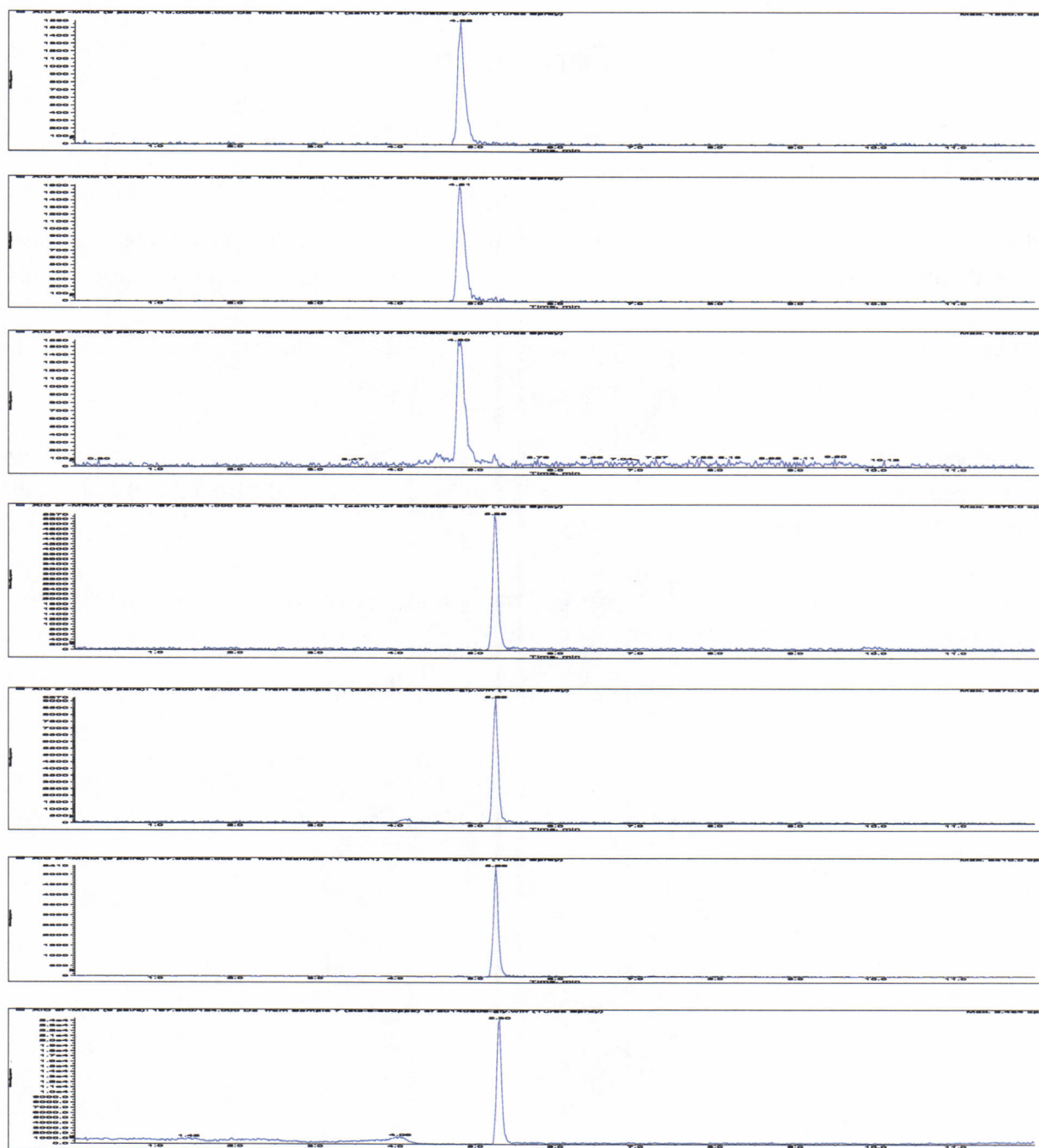


图 B.2 标准品多反应检测(MRM)色谱图(API 4 000+ QQQ)

从上至下依次为: 110.0/63.0, 110.0/78.0, 110.0/81.0;  
167.8/81.0, 167.8/150.0, 167.8/63.0, 167.8/124.0

## Foreword

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

Please pay attention to some of the content of this document may be involved in the patent, the issuing authority of this document does not assume responsibility for the identification of 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 the Xiamen Entry-Exit Inspection and Quarantine Bureau, Ningbo Entry-Exit Inspection and Quarantine Bureau and Fujian Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

This main drafter of this standard is Xu Dunming, Chen Shubing, Wu Ming, Du Fengjun, Yu Yucheng, Liu Zhengcai, Zhang Jing, Zhang Zhigang.



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**Note:** This English version, a translation from the Chinese text, is solely for guidance.



# Determination of glyphosate and its metabolite residues in foodstuffs for export—HPLC-MS/MS method

## 1 Scope

This standard specifies the determination and confirmation of residues of glyphosate and its metabolite residues (aminomethyl phosphonic acid) by HPLC-MS/MS in foodstuffs for export.

This standard is applicable to the determination and confirmation of glyphosate and its metabolite residues in tea, wheat, corn, rice, sugarcane, soybean, citrus, apple, peach, grape, banana, watermelon, pomegranate and cottonseed oil for export.

## 2 References

The following documents were indispensable for the application of this standard. For dated references, only the dated edition was applied to this document. For undated references, the last edition of normative document (including all modification list) referred to applies.

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

## 3 Principle

The sample was extracted with water and purified by dialysis bag, RP column and Graphitized carbon black, then detected by HPLC-MS/MS and quantified by external standard method.

## 4 Reagents and Materials

Unless otherwise specified, all the reagents used should be analytically pure, “water” is I grade water stipulated by GB/T 6682.

4.1 Methanol: HPLC grade.

4.2 Acetonitrile: HPLC grade.

4.3 Ammonium acetate: HPLC grade.

4.4 Ammonium acetate solution (5 mmol/L, pH: 10-11): Accurately weight 3.855 g (accurating to 0.000 1 g) ammonium acetate and dissolve with water to a volume of 50 mL, formulated as a ammonium acetate solution of 1 mol/L; Then take 5 mL and dissolve with water to a volume of 1 000 mL with the pH adjusted to 10-11.

4.5 Standard: glyphosate (GLY, CAS: 1071-83-6), Purity  $\geq 98\%$ ; aminomethyl phosphonic acid (AMPA, CAS: 1066-51-9), Purity  $\geq 98\%$ .

4.6 Dialysis bag: diameter as 22 mm, semi-perimeter as 34 mm and molecular cut off as 3 500 Da.

4.7 Standard solution: Accurately weight GLY and AMPA 10 mg each and dissolve with water to a volume of 10 mL, formulated as a standard solution of 1 mol/L.

4.8 Standard store solution: Accurately take any standard solution, dissolve with water to standard store solution of 10  $\mu\text{g/mL}$  of each.

4.9 Standard working solution: According to the concentration required, take a certain amount volume of standard store solution and dissolve with water to appropriate concentration as standard working solution before use.

4.10 Treatment for dialysis bag: 10 cm-20 cm small fragments was cut by scissor, boiling water for 10 minutes, then cleaning by water and stored in ethanol; Cleaning is necessary before and after use.

4.11 OnGuard II RP column (1.0 cc column): It was activated by 5 mL methanol and 5 mL water and let stand for half an hour before use; Clean with methanol after use.

4.12 Graphitized carbon black (GCB): 40  $\mu\text{m}$ —60  $\mu\text{m}$ , padding as the ProElut or equivalent.

4.13 Membrane filter: 0.22  $\mu\text{m}$ , It is organic system and PTFE membrane.

## 5 Apparatus and equipment

5.1 High Performance Liquid Chromatography with triple quadrupole tandem mass spectrometer (HPLC-MS/MS) equipped with ESI source.

5.2 Balance: sensibility reciprocal is 0.01 g and 0.000 1 g respectively.

5.3 Tissues homogenizer.

5.4 Grinder.

- 5.5 Homogenizer: 15 000 r/min.
- 5.6 Centrifuge: 5 000 r/min above.
- 5.7 High speed centrifuge: 15 000 r/min above.
- 5.8 Ultrasonic water bath.
- 5.9 Centrifuge tube: 50 mL.
- 5.10 Test tube: 50 mL.
- 5.11 Injection syringe.
- 5.12 Vortex mixer.
- 5.13 Pressured gas blowing concentrator.

## 6 Sample preparation and storage

### 6.1 Sample preparation

#### 6.1.1 Liquid samples

Liquid samples were blended as backup.

#### 6.1.2 Fruit and vegetable samples

The original sample is mixed from which 500 g is taken for analysis. The samples were placed in a clean container to be used as the test sample after grinded thoroughly and the container having the test sample is well sealed and labeled.

#### 6.1.3 Food grains and tea samples

The original samples were mixed from which a 500 g was taken for analysis. The sample is divided to two portions equally after grinded thoroughly and passed through a 20 mesh sieve. Then the sample is placed in a clean container to be used as the test sample. The container having the test sample is well sealed and labeled.

### 6.2 Storage of samples

All the samples should be stored at  $-5^{\circ}\text{C}$ .



Note: In the sample operation and preparation process, it should be prevent the pollution of samples and content changes of residues.

## 7 Method of Determination

### 7.1 Extraction

Weigh 4 g (Accurate to 0.01 g) of liquid samples, fruit and vegetable samples and food grains samples into 50 mL centrifuge tube (5.9) and add 6 mL water. Mix well for 3 min by homogenizer (5.5) of 15 000 r/min and then centrifuge (5.7) for 5 min by 15 000 r/min either. Water phase was collected and dissolve with water to a volume of 10 mL.

Weigh 2 g (Accurate to 0.01 g) of tea samples into 50 mL centrifuge tube (5.9) and add 10 mL water. Mix well for 3 min by homogenizer (5.5) of 15 000 r/min and then centrifuge (5.7) for 5 min by 15 000 r/min either. Water phase was collected and dissolve with water to a volume of 10 mL.

### 7.2 Clean-up

10 mL extracting solution was injected into dialysis bag (4.10) which had been activated and then put the dialysis bag into 50 mL test tube, the tube was added 10 mL water before. Take out the dialysis bag after it was balanced for 1 h in ultrasonic water bath, 5 mL raffinate from tube was injected into RP column by injector, refuse the first 2 mL solution wash-out from RP column(4.11)and collect the remaining 3 mL. Adding 20 mg GCB (4.12) and vortex oscillation for 2 minutes, then the mixed solution centrifuged for 5 minutes under 15 000 r/min. Take 2 mL supernatant into a clean tube and dry nitrogen (5.13) at 60 °C. The residue dissolved with water to 0.5 mL, pass through PTFE membrane and ready for LC-MS/MS determination.

### 7.3 Determination

#### 7.3.1 HPLC-MS/MS operation conditions

HPLC-MS/MS operation conditions:

- a) Column: NH<sub>2</sub>P-50 2D column, 150 mm × 2.0 mm (i.d.), 5 μm or equivalent;
- b) Mobile phase: A: 5 mmol/L ammonium acetate aqueous solution (ammonium hydroxide adjust pH to 10-11), B: acetonitrile; flow rate: 0.25 mL/min, gradient elution program were showed in table 1;
- c) Column temperature: 35 °C ;



- d) Injection volume: 10  $\mu$ L;
- e) Source: ESI;
- f) Scanning mode: Negative;
- g) Monitoring mode: MRM;
- h) Other MS parameters: see Annex A.

Table 1 Mobile phase gradient elution program

Time/min	Mobile phase A/%	Mobile phase B/%
0	20.0	80.0
2.00	20.0	80.0
2.01	80.0	20.0
8.00	80.0	20.0
8.01	20.0	80.0
12.00	20.0	80.0

### 7.3.2 HPLC-MS/MS determination

#### 7.3.2.1 Qualitative determination

Under the above HPLC-MS/MS operating conditions, the ratio of the chromatographic retention time of analyte shall correspond to that of the calibration solution. The relative intensities of the detected ions of each analyte shall correspond to those of the calibration standard solution at comparable concentrations, the allowed relative deviation of relative intensity is less than within the table 2 tolerance, the same compound in sample must be confirmed.

Table 2 Maximum permitted tolerances for relative ion intensities while confirmation

Relative ion intensities/%	>50%	>20%~50%	>10%~20%	≤10%
Permitted relative tolerances/%	±20%	±25%	±30%	±50%

#### 7.3.2.2 Quantitative determination

According to the estimated approximate concentration of target compounds in the sample solution,

select the standard working solution of similar concentration to that of sample solution. The responses of target compounds in the standard working solution and the 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 the sample solution of equal volume. Under the above HPLC-MS/MS operating condition, the retention time of GLY and AMPA is about 4.68 min and 4.20 min each. HPLC-MS/MS multiple reaction monitoring chromatogram of GLY and AMPA standard are shown respectively in the Annex B.

#### 7.4 Reagent blank test

The reagent blank test is taken the same complete analytical procedure applied without the test portion or using an equivalent amount of suitable solvent in place of the test portion.

### 8 Calculation and expression of the result

Calculate the concentration of GLY and AMPA in samples according to the following equation or data-processing software:

$$X = \frac{c \times V \times 10 \times 1\,000}{m \times 1\,000} \dots\dots\dots (1)$$

Where

$X$  = content in the test sample, in mg/kg.

$c$  = concentration of GLY or AMPA in the standard solution, in  $\mu\text{g/mL}$ .

$V$  = final volume of sample extract, in mL.

$m$  = mass of sample, in g.

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

### 9 Limit of determination and recovery

#### 9.1 Limit of determination

The limits of determination of GLY and AMPA are 50  $\mu\text{g/kg}$  both.

## 9.2 Recovery

The recovery data of determination of GLY and AMPA with different spike levels were showed in table 3.

Table 3 Recovery data of GLY and APMA with different spike levels ( $n=6$ )

Matrix	Spike levels mg/kg	Recovery		Matrix	Spike levels mg/kg	Recovery	
		GLY	AMPA			GLY	AMPA
Tea	0.05	76.4% ~ 88.3%	79.2% ~ 88.5%	citrus	0.05	77.3% ~ 84.9%	82.4% ~ 90.2%
	0.1	83.2% ~ 90.6%	85.7% ~ 96.8%		0.1	89.4% ~ 99.2%	85.9% ~ 94.7%
	1	82.9% ~ 97.0%	84.5% ~ 96.3%		1	90.4% ~ 97.3%	91.3% ~ 96.3%
wheat	0.05	78.9% ~ 89.2%	79.9% ~ 90.1%	Appale	0.05	83.6% ~ 93.2%	84.8% ~ 94.2%
	0.1	86.2% ~ 93.5%	89.9% ~ 99.1%		0.1	90.3% ~ 97.2%	91.2% ~ 97.4%
	1	82.6% ~ 89.5%	86.1% ~ 95.7%		0.5	91.8% ~ 97.6%	89.8% ~ 96.6%
	5	94.9% ~ 104.1%	90.6% ~ 98.2%		1	93.8% ~ 99.7%	91.0% ~ 98.7%
corn	0.05	80.8% ~ 96.2%	83.1% ~ 95.2%	peach	0.05	78.8% ~ 86.3%	79.3% ~ 90.2%
	0.1	89.3% ~ 94.2%	89.4% ~ 97.2%		0.1	88.3% ~ 95.8%	89.9% ~ 97.0%
	1	95.4% ~ 101.4%	89.3% ~ 94.5%		1	86.9% ~ 99.0%	90.3% ~ 103.4%
rice	0.05	76.3% ~ 89.3%	80.2% ~ 92.8%	grape	0.05	80.2% ~ 93.2%	76.6% ~ 83.2%
	0.1	83.9% ~ 95.1%	87.4% ~ 94.9%		0.1	89.5% ~ 101.7%	85.3% ~ 92.7%
	1	90.6% ~ 99.3%	97.2% ~ 102.3%		1	85.3% ~ 97.8%	90.2% ~ 96.7%
watermelon	0.05	78.3% ~ 89.4%	77.8% ~ 90.3%	banana	0.05	78.3% ~ 89.2%	80.5% ~ 92.7%
	0.1	86.9% ~ 97.3%	86.9% ~ 94.2%		0.1	87.0% ~ 93.5%	84.6% ~ 92.6%
	1	97.3% ~ 105.4%	89.4% ~ 99.2%		1	84.9% ~ 97.6%	81.0% ~ 89.5%
soybean	0.05	79.5% ~ 89.9%	82.7% ~ 92.7%	sugarcane	0.05	82.5% ~ 92.8%	83.2% ~ 92.8%
	0.1	80.8% ~ 94.0%	87.4% ~ 93.0%		0.1	81.3% ~ 90.1%	84.9% ~ 90.4%
	1	89.4% ~ 93.7%	86.3% ~ 90.4%		1	84.7% ~ 94.7%	86.3% ~ 92.9%
	2	85.9% ~ 96.3%	85.1% ~ 91.4%		2	84.2% ~ 97.8%	86.3% ~ 97.8%
ptisan	0.05	77.2% ~ 89.8%	80.9% ~ 93.8%	cottonseed oil	0.05	77.2% ~ 92.9%	79.2% ~ 93.1%
	0.1	89.0% ~ 97.9%	87.3% ~ 94.6%		0.1	79.8% ~ 90.4%	78.4% ~ 87.4%
	2	90.7% ~ 98.2%	89.9% ~ 97.2%		1	88.2% ~ 96.0%	85.2% ~ 89.9%



## Annex A (Informative)

### Referenced mass spectrometry conditions

#### A.1 Agilent 6490

- a) Ionization source way: ESI;
- b) Scanning mode: Negative;
- c) Monitoring mode: MRM;
- d) Dry Gas temperature: 200 °C ;
- e) Dry gas flow rate: 14 L/min;
- f) Gas pressure: 20 PSI;
- g) Sheath gas temperature: 250 °C ;
- h) Sheath gas flow rate: 11 L/min;
- i) Ion source spray voltage: - 2 500 V ;
- j) Nozzle voltage: - 2 000 V ;
- k) Qualitative ion, Quantitative ion, Fragmentor, Collision Energy and Retention time are shown in table A.1.

Table A.1 MRM analytical parameters for GLY and AMPA<sup>1)</sup>

Compounds	Quantitative ion $m/z$	Quantitative ion $m/z$	Dwell time ms	Fragmentor V	Collision Energy/eV	Retention time/min
GLY	167.8/63.0	167.8/63.0	100	380	25	4.68
	167.8/81.0		100	380	15	
	167.8/124.0		100	380	8	
AMPA	110.0/63.0	110.0/63.0	100	380	20	4.20
	110.0/79.0		100	380	35	
	110.0/81.0		100	380	10	

1) Non-commercial statement: the equipments Agilent 6490 QQQ MS/MS involved in this standard method are not related to commercial aims, and the analysts are encouraged to use equipments of different corporation or different type.



## A.2 ABI 4000 +

- a) Ionization source way: ESI;
- b) Scanning mode: Negative;
- c) Monitoring mode: MRM;
- d) Collision airflow speed ratio: 4;
- e) Curtain gas: 35;
- f) Gas pressure: 60 PSI;
- g) Auxiliary heating gas: 60 PSI;
- h) Atomization temperature: 550 °C;
- i) Ion source spray source voltage: -4 500 V;
- j) Qualitative ion, Quantitative ion, Fragmentor, Collision Energy and Retention time are shown in table A.2.

Table A.2 MRM analytical parameters for GLY and AMPA<sup>2)</sup>

Compounds	Qualitative ion $m/z$	Quantitative ion $m/z$	Dwell time ms	Declustering Potential (DP)/V	Entrance Potential (EP)/V	Collision Energy (CE)/eV	Collision pool export Potential (CXP)/V
GLY	167.8/63.0	167.8/63.0	100	-48.02	-10	-33.04	-15
	167.8/124.0		100	-53.27	-10	-16.99	-15
	167.8/150.0		100	-49.95	-10	-12.50	-15
	167.8/81.0		100	-45.88	-10	-20.270	-15
AMPA	110.0/63.0	110.0/63.0	100	-57.55	-10	-23.97	-15
	110.0/79.0		100	-61.08	-10	-32.97	-15
	110.0/81.0		100	-55.91	-10	-17.79	-15

2) Non-commercial statement: the equipments ABI 4000 + QQQ MS/MS involved in this 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)

## Referenced mass spectrometry conditions Chromatograms of standards of GLY and AMPA

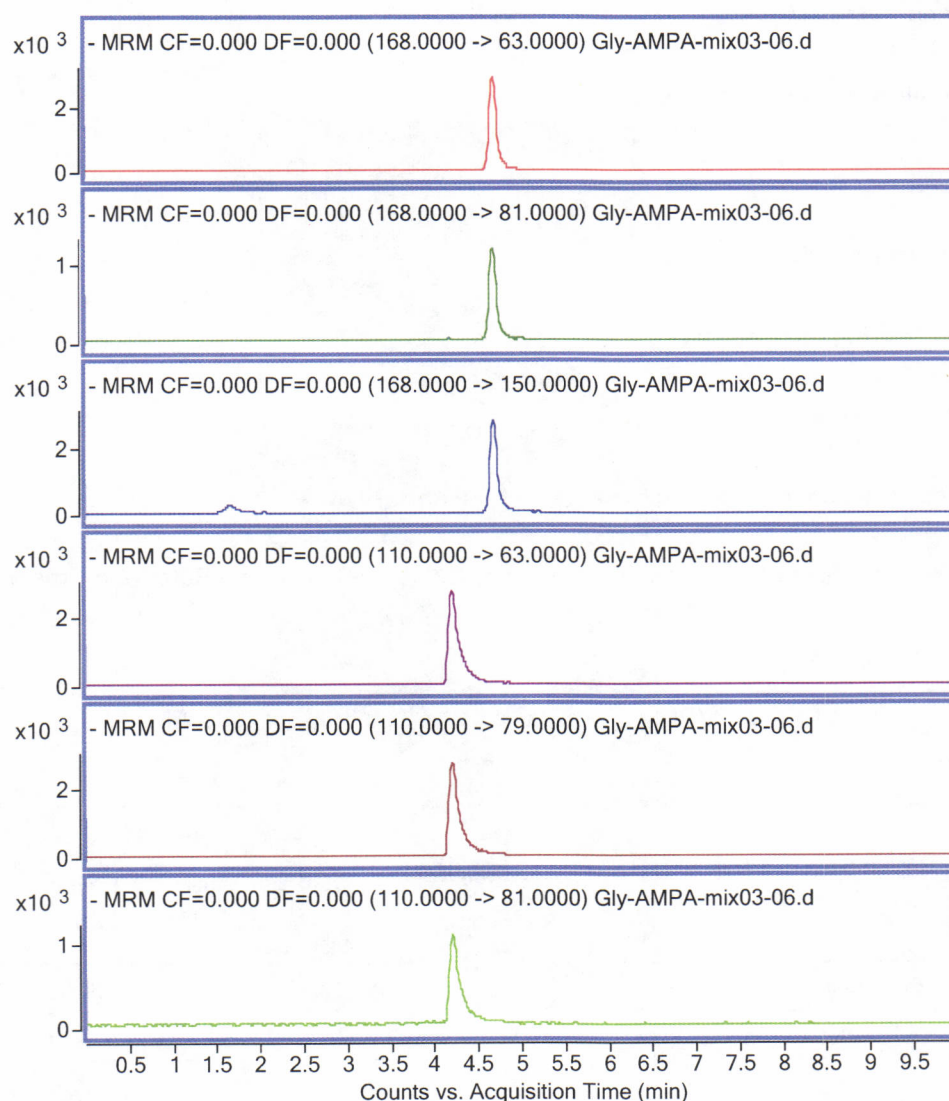


Figure B.1 Chromatograms of standard of GLY and AMPA(Agilent 6 490 QQQ)  
From up to down: 167.8/63.0, 167.8/81.0, 167.8/150.0;  
110.0/63.0, 110.0/79.0, 110.0/81.0

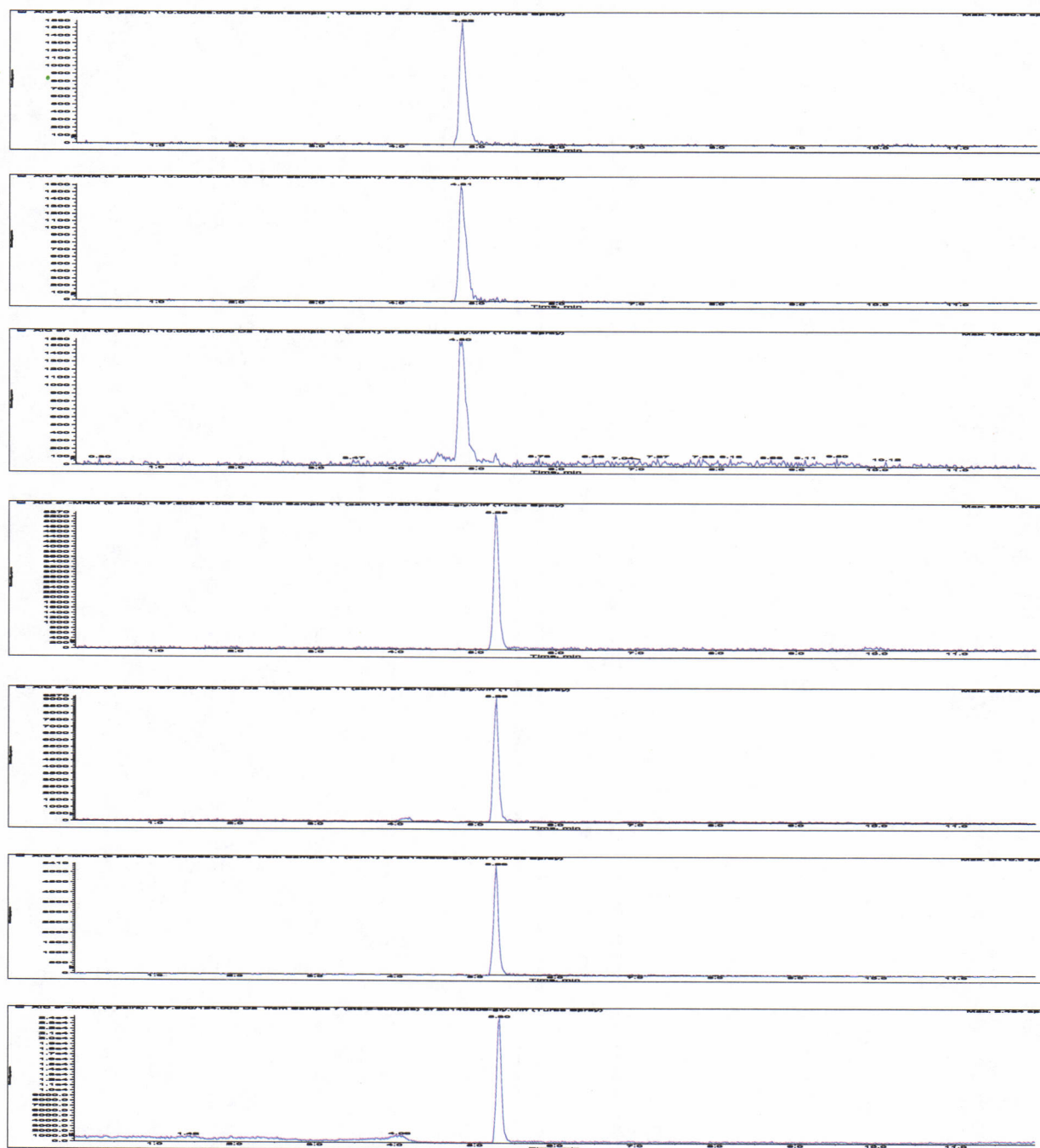


Figure B.2 Chromatograms of standard of GLY and AMPA(API 4 000+ QQQ)

From up to down: 110.0/63.0, 110.0/78.0, 110.0/81.0;

167.8/81.0, 167.8/150.0, 167.8/63.0, 167.8/124.0



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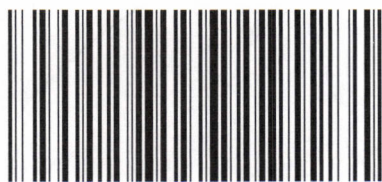
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