

SN

中华人民共和国出入境检验检疫行业标准

SN/T 1923—2007

进出口食品中草甘膦残留量的检测方法 液相色谱-质谱/质谱法

Determination of glyphosate residues in food for import and export—
HPLC-MS/MS method

2007-05-23 发布

2007-12-01 实施

中华人民共和国
国家质量监督检验检疫总局 发布

前 言

本标准的附录 A 和附录 B 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：中华人民共和国上海出入境检验检疫局、中华人民共和国黑龙江出入境检验检疫局、中华人民共和国吉林出入境检验检疫局、中华人民共和国江苏出入境检验检疫局、中华人民共和国辽宁出入境检验检疫局、中华人民共和国河北出入境检验检疫局。

本标准主要起草人：李波、郭德华、邓晓军、杨长志、王明泰、丁涛、卫锋、郭春海、金淑萍。

本标准系首次发布的出入境检验检疫行业标准。

进出口食品中草甘膦残留量的检测方法

液相色谱-质谱/质谱法

1 范围

本标准规定了食品中草甘膦残留量检验的制样和液相色谱-串联质谱测定方法。

本标准适用于大豆、小麦、大米、玉米、甘蔗、柑橙、紫苏、板栗、茶叶、虾、鱼、畜禽肉、蜂蜜、香料、人参、草甘膦(PMG)及其代谢产物氨基磷酸(AMPA)残留量的检测和确证。

2 方法提要

试样用水提取,经阳离子交换柱(CAX)净化,与9-芴基甲基三氯甲烷[9-Fluorenylmethylchloroformate(FMOC-Cl)]衍生化反应后,用液相色谱-质谱/质谱测定,内标法定量。

3 试剂和材料

除另有规定外,所用试剂均为分析纯,水为二次超纯水。

- 3.1 甲醇:液相色谱纯。
- 3.2 丙酮:液相色谱纯。
- 3.3 二氯甲烷:液相色谱纯。
- 3.4 盐酸。
- 3.5 氢氧化钾。
- 3.6 磷酸二氢钾。
- 3.7 硼酸钠($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$)。
- 3.8 甲酸。
- 3.9 无水乙酸铵。
- 3.10 乙腈:液相色谱纯。
- 3.11 20%氢氧化钾溶液:称取20 g氢氧化钾溶于适量水,并用水稀释至100 mL。
- 3.12 3 mol/L 盐酸溶液、0.3 mol/L 盐酸溶液:量取270 mL 盐酸,加适量水并稀释至1 000 mL,此溶液浓度为3 mol/L;再用水稀释10倍即为0.3 mol/L。
- 3.13 流动相A:0.1%甲酸的乙腈溶液,移取1 mL 甲酸于1 000 mL 乙腈中,混匀。
- 3.14 流动相B:称取0.154 g 无水乙酸铵溶解于适量水中,加入1 mL 甲酸,用水定容至1 000 mL。
- 3.15 酸度调节剂:称取16 g 磷酸二氢钾溶于160 mL 水中,加入13.4 mL 盐酸和40 mL 甲醇,混匀。
- 3.16 CAX 洗脱液:分别量取160 mL 水、2.7 mL 盐酸和40 mL 甲醇,混匀。
- 3.17 5%(体积分数)硼酸盐缓冲溶液(pH=9):称取5 g 硼酸钠($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$),用水溶解并定容至100 mL。
- 3.18 9-芴基甲基三氯甲烷[FMOC-Cl]:纯度不低于99.0%,低于5℃保存。
- 3.19 1.0 g/L FMOC-Cl 丙酮溶液:称取100 mg FMOC-Cl,用丙酮溶解并定容至100 mL。
- 3.20 草甘膦(PMG)标准物质(Glyphosate, CAS号:1071-83-6,分子式: $\text{C}_3\text{H}_8\text{NO}_5\text{P}$):纯度不低于98.0%。
- 3.21 氨基磷酸(AMPA)标准物质(aminomethylphosphonic acid, CAS号:1066-51-9,分子式: $\text{C}_1\text{H}_6\text{NO}_3\text{P}$):纯度不低于98.0%。

3.22 同位素内标 1,2- $C^{13}N^{15}$ 草甘膦:100 $\mu\text{g}/\text{mL}$ 水溶液。

3.23 草甘膦(PMG)、氨基磷酸(AMPA)标准储备溶液(1.0 mg/mL):分别精确称取 50 mg ($\pm 0.1 \text{ mg}$) 的草甘膦(PMG)、氨基磷酸(AMPA)标准品于聚乙烯塑料瓶中,分别加入一定量的水按式(1)计算,加 2 滴盐酸,充分振摇,确保其全部溶解,低于 5 $^{\circ}\text{C}$ 保存,有效期为 1 年。

$$m_w = \frac{m_s \times P_s \times D_w}{C_s} \dots\dots\dots(1)$$

式中:

- m_w ——所需加水的质量,单位为克(g);
- m_s ——标准品的称样量,单位为毫克(mg);
- P_s ——标准品的纯度(100%=1.00);
- D_w ——水的密度(1.00 g/mL);
- C_s ——标准储备液的浓度(1.0 mg/mL)。

3.24 草甘膦(PMG)、氨基磷酸(AMPA)混合标准中间溶液:用水分别稀释成 1.0 $\mu\text{g}/\text{mL}$ 、10.0 $\mu\text{g}/\text{mL}$, 低于 5 $^{\circ}\text{C}$ 保存,有效期为 6 个月。

3.25 同位素内标 1,2- $C^{13}N^{15}$ 草甘膦工作溶液:用水分别稀释成 1.0 $\mu\text{g}/\text{mL}$ 、10.0 $\mu\text{g}/\text{mL}$, 低于 5 $^{\circ}\text{C}$ 保存,有效期为 6 个月。

3.26 草甘膦(PMG)、氨基磷酸(AMPA)混合标准工作溶液:根据需要,临用时吸取一定量的混合标准中间溶液(3.24)和同位素内标工作溶液(3.25),用水稀释配制适当浓度的混合标准工作溶液(参考线性浓度范围为 0 ng/mL 至 10 ng/mL),每毫升该混合标准工作溶液含有同位素内标 1,2- $C^{13}N^{15}$ 草甘膦 6 ng 。

3.27 CAX 阳离子交换柱:AG 50W-X8(200 目~400 目), H^+ ,0.8 $\text{cm} \times 4 \text{ cm}$ 。使用时不采用真空泵抽气,且不得使其干涸。

注:可采用商品化的 CAX 小柱[Bio-Rad Poly-Prep No. 731-6214 CA 94547,USA],或同等性能的其他小柱。

3.28 水相滤膜:0.45 μm 。

4 仪器和设备

- 4.1 液相色谱串联质谱仪:配有电喷雾(ESI)离子源。
- 4.2 旋转蒸发器。
- 4.3 振荡器。
- 4.4 混匀器。
- 4.5 固相萃取装置。
- 4.6 离心机:水平转子,转速不低于 4 000 r/min ,配有 250 mL 塑料离心瓶。
- 4.7 氮气吹干仪。

5 试样制备与保存

5.1 试样制备

5.1.1 茶叶、大豆、小麦、大米、玉米、香料、草药

取代表性样品约 200 g ,粉碎,通过孔径为 2.0 mm 的筛,装入洁净的容器内,密封,标明标记。

5.1.2 甘蔗、柑橙、紫苏、板栗

甘蔗:去皮、切成小段,称取约 200 g ,速冻后取出切成细末,混匀,装入洁净的容器内,密封,标明标记。

柑橙:取可食部分约 200 g ,匀浆,装入洁净的容器内,密封,标明标记。

紫苏:取可食部分(不可水洗)约 200 g ,捣碎均匀,装入洁净容器内,密封,标明标记。

板栗:取可食部分 200 g,粉碎,装入洁净的容器内,密封,标明标记。

5.1.3 虾、鱼、畜禽肉

从所取全部虾、鱼或畜禽肉样品中取出有代表性的可食部分约 200 g,捣碎并均质,装入洁净容器内,密封,标明标记。

5.1.4 蜂蜜

取蜂蜜代表性样品 200 g,未结晶的样品将其用力搅拌均匀,有结晶析出的样品可将样品瓶盖塞紧后,置于不超过 60℃的水浴中温热,待样品全部溶化后搅匀,迅速冷却至室温。在溶化时应注意防止水分挥发。制备好的试样装入洁净容器内,密封,标明标记。

5.2 试样保存

虾、鱼、畜禽肉试样于-18℃冷冻保存,其余试样于 0℃~4℃保存。在制样的操作过程中,应防止样品受到污染或发生目标化合物残留量的变化。

6 测定步骤

6.1 提取

称取约 10 g 均匀试样(茶叶试样约 5 g,精确到 0.01 g),置于 250 mL 塑料离心瓶中,加 100 μ L 同位素内标工作溶液 10 μ g/mL(3.25),加 100 mL 水(茶叶样品浸泡 0.5 h)、50 mL 二氯甲烷,振荡 20 min,于 4 000 r/min 离心 10 min。将上层水溶液转移至另一塑料离心瓶中,残渣再加入 50 mL 水重复提取一次,合并上层水溶液,充分混匀后,取出 4.5 mL 至 10 mL 塑料具塞试管中,加 0.5 mL 酸度调节剂(3.15),混匀。

6.2 净化

CAX 小柱(3.27)经 10 mL 水活化后,加入 1.0 mL 提取液,用 0.7 mL CAX 洗脱液(3.16)淋洗两次,再用 11 mL CAX 洗脱液(3.16)洗脱并收集,洗脱液于 45℃减压旋转蒸发至干,加 1 mL 5%硼酸盐缓冲溶液(3.17)溶解残渣,此时 pH 约为 9 左右,需要时用 20%氢氧化钾溶液(3.11)和 3 mol/L 盐酸溶液、0.3 mol/L 盐酸溶液(3.12)调节 pH 至 9。

6.3 衍生化

取混合标准工作溶液(3.26)各 1.0 mL 加入 200 μ L 5%硼酸盐缓冲溶液(3.17),混匀。此标准系列溶液与净化后样液分别加入 200 μ L 1.0 g/L FMOC-Cl 丙酮溶液(3.19),混匀,室温下进行衍生化反应,放置过夜。将衍生化后溶液通过 0.45 μ m 滤膜(3.28),供液相色谱-串联质谱测定。

6.4 测定

6.4.1 液相色谱条件

- 色谱柱: C₁₈柱, 150 mm×2.1 mm(内径), 粒度 5 μ m 或相当者;
- 流动相梯度洗脱程序见表 1。

表 1 梯度洗脱程序

梯度时间/min	流动相比例/(%)	
	流动相 A	流动相 B
0	20	80
5	70	30
8	95	5
12	95	5
13	20	80
20	20	80

- c) 流速:200 μL/min;
- d) 进样量:30 μL。

6.4.2 质谱条件

- a) 离子源:电喷雾离子源;
- b) 扫描方式:正离子模式;
- c) 检测方式:多反应监测 MRM;
- d) 雾化气、帘帘气、辅助加热气、碰撞气均为高纯氮气;使用前应调节各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(IS)、雾化气压力(GS1)、气帘气压力(CUR)、辅助气流速(GS2)、去集簇电压(DP)、碰撞能等电压(CE)值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。

6.4.3 液相色谱-质谱/质谱检测及确证

按照确定的液相色谱-串联质谱条件测定样品和混合标准工作溶液(3.2.6),响应值均应在仪器检测的线性范围内,以色谱峰面积按内标法定量。在上述色谱条件下草甘膦(PMG)、氨甲基膦酸(AM-PA)的参考保留时间分别为 9.2 min、9.5 min,标准溶液的选择性离子流图参见附录 B 中图 B.1。

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中对应的保留时间偏差在±2.5%之内;且试样谱图中各组分定性离子的相对丰度与标准工作溶液中定性离子的相对丰度,其允许偏差不超过表 2 规定的范围时,则可确定为样品中存在这种农药残留。

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 至 50	>10 至 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,均按上述操作步骤进行。

6.6 结果计算和表述

用色谱数据处理机或按式(2)计算样品中草甘膦(PMG)、氨甲基膦酸(AMPA)残留量。计算结果需扣除空白值。

$$X = \frac{C \times C_i \times A \times A_{st} \times V}{C_{st} \times A_i \times A_s \times m \times 1\,000} \dots\dots\dots(2)$$

式中:

- X——样品中待测组分残留量,单位为毫克每千克(mg/kg);
- C——草甘膦(PMG)或氨甲基膦酸(AMPA)标准工作溶液的浓度,单位为纳克每毫升(ng/mL);
- C_i——样液中内标物的浓度,单位为纳克每毫升(ng/mL);
- A——样液中草甘膦(PMG)或氨甲基膦酸(AMPA)的峰面积;
- A_{st}——标准工作溶液中内标物的峰面积;
- V——样品溶液最终定容体积,单位为毫升(mL);
- C_{st}——标准工作溶液中内标物的浓度,单位为纳克每毫升(ng/mL);
- A_i——样液中内标物的峰面积;
- A_s——草甘膦(PMG)或氨甲基膦酸(AMPA)标准工作溶液的峰面积;
- m——最终样液代表的试样质量,单位为克(g)。

本方法草甘膦的残留量测定结果系指草甘膦和其代谢产物氨甲基膦酸(AMPA)残留量之和。

7 测定低限、回收率

7.1 测定低限

本方法测定低限:茶叶的测定低限为 0.10 mg/kg,其他样品的测定低限为 0.05 mg/kg。

7.2 回收率

本方法添加浓度及回收率试验数据见表3。

表3 添加浓度及回收率试验数据

基质名称	化合物	添加浓度/ (mg/kg)	回收率范围/ (%)
大豆	草甘膦 (PMG)	0.05	74.0~104.0
		0.10	69.3~106.0
		0.50	78.0~108.0
	氨基甲酸 (AMPA)	0.05	66.2~94.8
		0.10	82.1~112.0
		0.50	80.0~112.0
大米	草甘膦 (PMG)	0.05	74.2~102.4
		0.10	70.6~108.0
		0.50	91.0~106.8
	氨基甲酸 (AMPA)	0.05	70.0~94.8
		0.10	73.1~110.0
		0.50	70.4~102.6
甘蔗	草甘膦 (PMG)	0.05	69.2~96.0
		0.10	79.2~110.0
		0.50	82.0~110.0
	氨基甲酸 (AMPA)	0.05	67.2~98.0
		0.10	74.1~115.0
		0.50	78.0~106.0
紫苏	草甘膦 (PMG)	0.05	70.6~96.0
		0.10	78.4~109.0
		0.50	74.4~98.8
	氨基甲酸 (AMPA)	0.05	72.8~101.2
		0.10	71.5~97.5
		0.50	70.4~96.8
猪肉	草甘膦 (PMG)	0.05	76.0~100.2
		0.10	92.0~110.0
		0.50	76.4~116.0
	氨基甲酸 (AMPA)	0.05	78.0~96.8
		0.10	82.4~108.0
		0.50	74.0~94.8
鱼	草甘膦 (PMG)	0.05	76.0~100.4
		0.10	86.1~108.0
		0.50	78.0~110.0
	氨基甲酸 (AMPA)	0.05	72.0~96.4
		0.10	73.1~105.0
		0.50	70.0~96.6

表 3(续)

基质名称	化合物	添加浓度/ (mg/kg)	回收率范围/ (%)
茶叶	草甘膦 (PMG)	0.10	72.0~112.0
		0.20	70.5~106.0
		1.0	75.1~105.0
	氨基甲酸 (AMPA)	0.10	77.5~118.0
		0.20	66.0~96.5
		1.0	71.2~104.0
香料	草甘膦 (PMG)	0.05	68.0~108.0
		0.10	69.4~113.0
		0.50	64.0~106.0
	氨基甲酸 (AMPA)	0.05	73.6~102.0
		0.10	78.4~119.0
		0.50	68.0~98.6
小麦	草甘膦 (PMG)	0.05	76.0~106.8
		0.10	76.2~105.0
		0.50	77.2~112.0
	氨基甲酸 (AMPA)	0.05	68.0~98.8
		0.10	74.3~110.0
		0.50	70.0~108.0
玉米	草甘膦 (PMG)	0.05	72.0~112.0
		0.10	82.1~105.0
		0.50	78.0~110.0
	氨基甲酸 (AMPA)	0.05	68.2~104.4
		0.10	75.4~112.0
		0.50	68.2~106.0
橙	草甘膦 (PMG)	0.05	66.4~98.0
		0.10	78.6~110.0
		0.50	70.2~106.0
	氨基甲酸 (AMPA)	0.05	67.2~96.2
		0.10	70.2~108.0
		0.50	78.6~108.0
板栗	草甘膦 (PMG)	0.05	82.8~106.0
		0.10	81.2~104.0
		0.50	85.2~107.6
	氨基甲酸 (AMPA)	0.05	74.0~110.0
		0.10	74.4~102.0
		0.50	84.2~107.6

表 3(续)

基质名称	化合物	添加浓度/ (mg/kg)	回收率范围/ (%)
鸡肉	草甘膦 (PMG)	0.05	74.0~92.4
		0.10	79.1~105.0
		0.50	72.8~110.0
	氨基甲酸 (AMPA)	0.05	76.4~94.8
		0.10	76.4~107.0
		0.50	70.0~93.0
虾	草甘膦 (PMG)	0.05	73.8~94.0
		0.10	78.4~108.0
		0.50	72.0~104.0
	氨基甲酸 (AMPA)	0.05	66.4~94.4
		0.10	75.3~112.0
		0.50	68.0~96.4
蜂蜜	草甘膦 (PMG)	0.05	70.8~101.0
		0.10	79.4~110.0
		0.50	82.0~104.0
	氨基甲酸 (AMPA)	0.05	72.0~107.0
		0.10	74.8~102.0
		0.50	72.2~109.6
人参	草甘膦 (PMG)	0.05	70.2~108.0
		0.10	86.5~121.0
		0.50	80.2~110.0
	氨基甲酸 (AMPA)	0.05	68.0~96.8
		0.10	68.5~114.0
		0.50	70.0~93.0

附录 A¹⁾

(资料性附录)

HPLC-MS/MS 仪器参数参考条件

- a) 电喷雾电压(IS):3 500 V;
 b) 雾化气压力(GS1):50 Pa;
 c) 气帘气压力(CUR):20 Pa
 d) 辅助气流速(GS2):25 Pa;
 e) 离子源温度(TEM):250℃;
 f) 定性离子对、定量离子对、去簇电压(DP)、碰撞气能量(CE)、碰撞室出口电压(CXP)、碰撞室入口电压(EP)见表 A. 1。

表 A. 1 草甘膦(PMG)、氨基甲酸(AMPA)的定性离子对、定量离子对、DP、CE、CXP

名 称	定性离子对 m/z	定量离子对 m/z	DP/V	CE/V	CXP/V	EP/V
草甘膦(PMG)	392.0/88.0	392.0/88.0	63	30	16	8
	392.0/214.0		54	15	14	8
氨基甲酸(AMPA)	334.0/179.1	334.0/179.1	60	28	10	6
	334.0/112.0		48	17	10	11
1,2-C ¹³ N ¹⁵ 草甘膦		395.0/91.0	65	29	14	6

1) 所列参数是在 API4000 质谱仪上完成的,此处列出试验用仪器型号仅是为了提供参考,并不涉及商业目的,鼓励标准使用者尝试采用不同厂家或型号的仪器。

附录 B
(资料性附录)

草甘膦(PMG)、1,2- $C^{13}N^{15}$ 草甘膦(IS)、氨基膦酸(AMPA)标准品选择性离子图谱

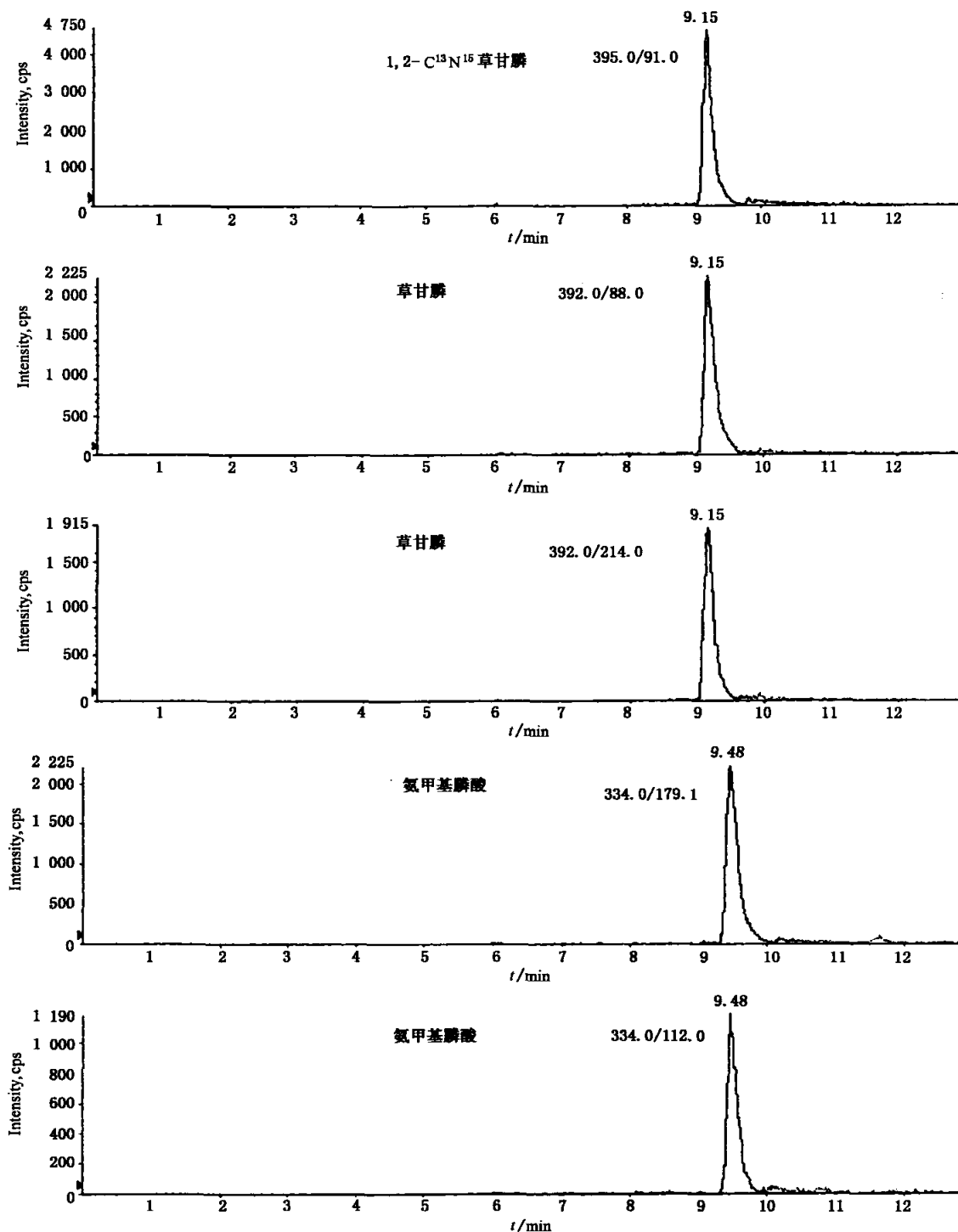


图 B.1 草甘膦(PMG)、1,2- $C^{13}N^{15}$ 草甘膦(IS)、氨基膦酸(AMPA)标准品衍生物的选择离子图谱

Foreword

Annex A and annex B of this standard is an informative annex.

This standard was proposed by and is under the charged of certification and accreditation administration of the People's Republic of China.

This standard was drafted by Shanghai Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Heilongjiang Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Jilin Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Jiangsu Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Liaoning Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Hebei Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The standard was mainly drafted by Li Bo, Guo Dehua, Deng Xiaojun, Yang Changzhi, Wang Mingtai, Ding 'Tao, Wei Feng, Guo Chunhai, Jin Shuping.

This standard is a professional standard for entry-exit inspection and quarantine promulgated for the first time.

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

Determination of glyphosate residues in food for import and export—HPLC-MS/MS method

1 Scope

The standard specifies the method of sample preparation and determination of glyphosate and its metabolite, AMPA residues in foods for export and import by HPLC-MS/MS.

This standard is applicable to the determination and confirmation of glyphosate and AMPA residues in foodstuff of plant origin (wheat, barley, soybean, corn, rice, tea, vegetable, fruit, chestnut etc.) and animal origin (shrimp, fish, poultry meat and honey samples) for import and export.

2 Principle

Glyphosate and AMPA residues are extracted from the sample with water and cleaned up with cation-exchange (CAX) column followed by derivation with 9-fluorenylmethyl chloroformate (FMOC-Cl). The residual content is determined by HPLC-MS/MS, and quantified by internal standard method.

3 Reagents and materials

Unless otherwise specified, all the reagents used should be analytical grade, “water” is HPLC-grade water.

3.1 Methanol: HPLC grade.

3.2 Acetone: HPLC grade.

3.3 Dichloromethane: HPLC grade.

3.4 Hydrochloric acid.

3.5 Potassium hydroxide.

3.6 Potassium dihydrogen phosphate.

3.7 Disodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$).

3.8 Formic acid.

3.9 Ammonium acetate.

3.10 Acetonitrile; HPLC grade.

3.11 20% (m/v) potassium hydroxide; dissolve 20 g of potassium hydroxide to final volume of 100 mL with water.

3.12 3 mol/L HCl, 0.3 mol/L HCl.

3.13 Mobile phase A: dissolve 1 mL formic acid to final volume of 1 000 mL with acetonitrile.

3.14 Mobile phase B: dissolve 0.154 g of ammonium acetate and 1 mL formic acid to final volume of 1 000 mL with water.

3.15 Acidic modifier solution: dissolve 16 g of KH_2PO_4 in 160 mL water with the addition of 40 mL of methanol and 13.4 mL of HCl.

3.16 CAX mobile phase solution: combine 160 mL water, 2.7 mL HCl, and 40 mL methanol.

3.17 5% (V/V) borate buffer (pH=9): dissolve 5 g of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ to final volume of 100 mL with water.

3.18 9-fluorenylmethyl chloroformate [FMOC-Cl]; purity $\geq 99\%$, stored at $< 5^\circ\text{C}$.

3.19 1.0 g/L FMOC-Cl; dissolve 100 mg of FMOC-Cl to final volume of 100 mL with acetone.

3.20 Glyphosate (PMG) [CAS No. 1071-83-6, molecular formula: $\text{C}_3\text{H}_8\text{NO}_5\text{P}$]; purity $> 98\%$.

3.21 AMPA [CAS No. 1066-51-9, molecular formula: $\text{C}_1\text{H}_6\text{NO}_3\text{P}$]; purity $> 98\%$.

3.22 Glyphosate 1,2- $\text{C}^{13}\text{N}^{15}$: 100 $\mu\text{g}/\text{mL}$.

3.23 Stock standard solution of PMG and AMPA (1.0 mg/mL); accurately weigh 50 mg (± 0.1 mg) PMG and AMPA standard in two polyethylene or polypropylene bottles, add appropriate volume of water into the bottles to make a solution containing PMG and AMPA all of 1.0 mg/mL. Calculate m_w , weight of water needed (g), as the formula (1), add 2 drops of HCl and mix thoroughly, these solutions can be used one year when stored at $< 5^\circ\text{C}$.

$$m_w = \frac{m_s \times P_s \times D_w}{C_s} \dots\dots\dots (1)$$

Where

- m_w —weight of water needed, g;
- m_s —weight of reference standard, mg;
- P_s —purity of the reference standard, (100% = 1.00);
- D_w —density of water (as sume 1.00 g/mL);
- C_s —concentration of analyte in the final solution (1.0 mg/mL) .

3.24 Mixture intermediate standard solutions: dilute stock standard solution of PMG and AMPA (1.0 mg/mL) to 1.0 $\mu\text{g/mL}$ and 10.0 $\mu\text{g/mL}$ with water, add 2 d HCl and store at $<5^\circ\text{C}$, assign a shelflife of 6 months.

3.25 Working standard solutions of Glyphosate 1,2- $\text{C}^{13}\text{N}^{15}$: dilute glyphosate 1,2- $\text{C}^{13}\text{N}^{15}$: (100 $\mu\text{g/mL}$) to 1.0 $\mu\text{g/mL}$ and 10.0 $\mu\text{g/mL}$ with in water, store at $<5^\circ\text{C}$, assign a shelflife of 6 months.

3.26 Working standard solutions: dilute the intermediate standard solutions(3.24) and glyphosate 1,2- $\text{C}^{13}\text{N}^{15}$ working standard solutions(3.25) with water according to practical need (The reference calibration range is 0 ng/mL~10 ng/mL). The concentration of glyphosate 1,2- $\text{C}^{13}\text{N}^{15}$ in per working standard solution is 6 ng/mL.

3.27 CAX columns: prepacked, disposable analyte preparation columns filled with 2 mL bed volume of strongly cationic exchanger, 8% crosslinked styrene divinylbenzene resin (AG 50W-X8 (200 mesh~400 mesh), H^+ , 0.8 cm \times 4 cm [Bio-Rad Poly-Prep No. 731-6214 CA 94547, USA].

3.28 0.45 μm film.

4 Apparatus and equipment

4.1 Liquid chromatography equipped with electrospray ionization mass spectrometry.

4.2 Rotary vacuum evaporator.

4.3 Vibrator.

4.4 Vortex mixer.

4.5 Apparatus of SPE.

4.6 Centrifuge; 4 000 r/min equipped with 250 mL polyethylene or polypropylene bottles.

4.7 Nitrogen evaporator.

5 Preparation and storage of test sample

5.1 Preparation of sample

5.1.1 Tea, grain, rice, corn, spices, herbs

Take about 200 g of representative sample, pass through a 2.0 mm sieve, mix thoroughly and place into a clean container as test sample, seal and label.

5.1.2 Sugarcane, orange, vegetables, chestnut

Sugarcane: peel off and cut into little slices, take about 200 g of the treated sample for quick-freeze and pestle into powder, mix thoroughly and place in a clean container as the test sample, seal and label.

Orange: take about 200 g eatable part of the sample, homogenize and place in a clean container as the test sample, seal and label.

Vegetable: take the representative portions from the whole primary sample. It is about 200 g, and homogenize and place into a clean container as the test sample, seal and label.

Chestnut: take about 200 g edible part of the sample, smash and place into a clean container as the test sample, seal and label.

5.1.3 Shrimp, fish, poultry and pork

Take about 200 g edible part of representative portions from the whole primary shrimp or fish or poultry meat, homogenize and place into a clean container as the test sample, seal and label.

5.1.4 Honey

Take about 200 g of representative sample. The non-crystallized sample should be stirred well to make homogeneous while the crystallized sample must be warmed under a water-bath at no more than 60°C with the sample bottle covered tightly for prevention of loss of water, mix thoroughly when all sample has melted, then cool immediately to room temperature. Take the prepared sample into two sample bottles, seal and label.

5.2 Storage of sample

Shrimp, fish, poultry and pork samples are stored at -18°C . Other test samples should be stored at temperature ranged from 0°C to 4°C . In course of sampling and sample preparation, it must be taken to avoid contamination or any factors which may cause the change of residue content.

6 Procedure

6.1 Extraction

Weigh ca 10 g of the test sample (accurate to 0.01 g) (but weigh the tea sample about 5 g) into a 250 mL polyethylene or polypropylene bottles, add 100 μL of working standard solutions of glyphosate 1,2- $\text{C}^{13}\text{N}^{15}$ 10 $\mu\text{g}/\text{mL}$ (3.25), 100 mL of water, 50 mL of dichloromethane, vibrate for 20 minutes, centrifuge for 10 min at 4 000 r/min. The supernatant is decanted to another poly bottle. Repeat the extraction above with another addition of 50 mL water. And combined the supernatant solution, mix thoroughly, 4.5 mL of extraction solution is transferred to a 10 mL polypropylene tube, add 0.5 mL acidic modifier solution (3.15) to the tube.

6.2 Cleaning up

Transfer 1.0 mL of solution above into the CAX column (3.27) which has been preconditioned with 10 mL water. Resin of column must be saturated with solution and vacuum is not needed. Wash the column with 0.7 mL of CAX mobile phase solution (3.16) twice and discard the elutes. Elute the analytes with 11 mL CAX mobile phase solution (3.16), collect the eluate with a 50 mL evaporate bottle. Evaporate to nearly dryness at $\leq 45^\circ\text{C}$. Add 1 mL 5% (V/V) borate buffer (pH=9) (3.17) to the bottle and dissolve the residue. If necessary, adjust pH to 9 with 20% KOH (3.11), 3 mol/L HCl or 0.3 mol/L HCl (3.12).

6.3 Derivatization

Withdraw 1.0 mL of working standard solutions (3.26) to the tube, add 200 μL of 5% (V/V) borate buffer (pH=9) (3.17). Add 200 μL of 1.0 g/L FMOC-Cl (3.19) to all extract solution and the working standard solution above, vortex mixture thoroughly, and derivatize over night at room temperature. After filtered with 0.45 μm film, the solutions are ready for determination by HPLC-MS/MS.

6.4 Determination

6.4.1 HPLC operating conditions

- a) Column: C_{18} , 5 μm , 150 mm \times 2.1 mm (i. d.), or the equivalent.
- b) Mobile phase: table 1.
- c) Flow rate: 200 $\mu\text{L}/\text{min}$.
- d) Injection volume: 30 μL .

Table 1—Gradient program of mobile phase

Time/min	Ratio/(%)	
	Mobile phase A	Mobile phase B
0	20	80
5	70	30
8	95	5
12	95	5
13	20	80
20	20	80

6.4.2 Mass spectral acquisition

- a) Ionic source: electrospray;
- b) Ionic polarity: positive mode;
- c) Monitor mode: multiple reaction monitoring;
- d) Nebulizer gas, Curtain gas, Auxiliary warm gas, collision gas were nitrogen 99.995%, before use all gas flow must be optimized, the reference conditions is listed as annex A;
- e) The pressure of IS, GS1, CUR, GS2, DP, CE also must be optimized, the reference parameters and transitions for confirmation and quantitation is listed as annex A.

6.4.3 Determination and confirmation by HPLC-MS/MS

According to operating parameters of HPLC-MS/MS above, sample solution and the standard working solution (3.26) are determined simultaneously. The responses of the analyte in the standard working solution and the sample solution all should be within the linear range of the instrument detection and quantified by internal standard. The reference retention time of PMG and AMPA is about 9.2 min and 9.5 min respectively, MRM chromatograms of the standards are listed as figure B. 1 in annex B.

Use the established LC/MS-MS parameters above for determination, and calculate the abundance ratio of two selected ion pairs of the sample solution and the standard working solution. If the retention times of sample chromatogram peaks are consistent with that of working solution, and relative abundance ratio tolerance is listed in table 2, it is positive to conclude that this pesticide do exist in the sample.

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

Relative intensity/(%)	>50	>20 to 50	>10 to 20	≤10
Permitted tolerances/(%)	± 20	± 25	± 30	± 50

6.5 Blank test

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

6.6 Calculation and expression of result

Calculate the content of PMG or AMPA residue in the test sample by HPLC-MS/MS data processor or according to the formula (2). The blank value should be subtracted from result of calculation above.

$$X = \frac{C \times C_i \times A \times A_{si} \times V}{C_{si} \times A_i \times A_s \times m \times 1\,000} \dots\dots\dots(2)$$

Where

- X—the residue content of PMG or AMPA in the test sample, mg/kg;
- C—the concentration of PMG or AMPA in standard working solution, ng/mL;
- C_i —the concentration of Glyphosate 1,2- $C^{13}N^{15}$ in sample solution, ng/mL;
- A—the peak area of PMG or AMPA in sample solution;
- A_{si} —the peak area of Glyphosate 1,2- $C^{13}N^{15}$ in standard working solution;
- V—the final volume of the sample solution, mL;
- C_{si} —the concentration of Glyphosate 1,2- $C^{13}N^{15}$ in standard working solution, ng/mL
- A_i —the peak area of Glyphosate 1,2- $C^{13}N^{15}$ in sample solution;
- A_s —the peak area of PMG or AMPA in standard working solution;
- m—mass of test sample of final sample solution, g.

The result means the sum of PMG and AMPA.

7 Limit of quantitation(LOQ) and recovery

7.1 Limit of quantitation

The limit of quantitation (LOQ) of the method for tea is 0.10 mg/kg, others are all 0.05 mg/kg.

7.2 Recovery

According to the experimental data, the fortified concentration of glyphosate and AMPA in all kinds of matrix are listed in table 3.

Table 3—Recovery range of PMG and AMPA

matrix	Chemicals	Concentration/ (mg/kg)	Recovery range/ (%)
soya bean	PMG	0.05	74.0~104.0
		0.10	69.3~106.0
		0.50	78.0~108.0
	AMPA	0.05	66.2~94.8
		0.10	82.1~112.0
		0.50	80.0~112.0
rice	PMG	0.05	74.2~102.4
		0.10	70.6~108.0
		0.50	91.0~106.8
	AMPA	0.05	70.0~94.8
		0.10	73.1~110.0
		0.50	70.4~102.6
sugarc ane	PMG	0.05	69.2~96.0
		0.10	79.2~110.0
		0.50	82.0~110.0
	AMPA	0.05	67.2~98.0
		0.10	74.1~115.0
		0.50	78.0~106.0
sweet basil	PMG	0.05	70.6~96.0
		0.10	78.4~109.0
		0.50	74.4~98.8
	AMPA	0.05	72.8~101.2
		0.10	71.5~97.5
		0.50	70.4~96.8
pig muscle	PMG	0.05	76.0~100.2
		0.10	92.0~110.0
		0.50	76.4~116.0
	AMPA	0.05	78.0~96.8
		0.10	82.4~108.0
		0.50	74.0~94.8
fish	PMG	0.05	76.0~100.4
		0.10	86.1~108.0
		0.50	78.0~110.0
	AMPA	0.05	72.0~96.4
		0.10	73.1~105.0
		0.50	70.0~96.6

Table 3 (Continued)

matrix	Chemicals	Concentration/ (mg/kg)	Recovery range/ (%)
tea	PMG	0.10	72.0~112.0
		0.20	70.5~106.0
		1.0	75.1~105.0
	AMPA	0.10	77.5~118.0
		0.20	66.0~96.5
		1.0	71.2~104.0
spices	PMG	0.05	68.0~108.0
		0.10	69.4~113.0
		0.50	64.0~106.0
	AMPA	0.05	73.6~102.0
		0.10	78.4~119.0
		0.50	68.0~98.6
wheat	PMG	0.05	76.0~106.8
		0.10	76.2~105.0
		0.50	77.2~112.0
	AMPA	0.05	68.0~98.8
		0.10	74.3~110.0
		0.50	70.0~108.0
maize	PMG	0.05	72.0~112.0
		0.10	82.1~105.0
		0.50	78.0~110.0
	AMPA	0.05	68.2~104.4
		0.10	75.4~112.0
		0.50	68.2~106.0
orange	PMG	0.05	66.4~98.0
		0.10	78.6~110.0
		0.50	70.2~106.0
	AMPA	0.05	67.2~96.2
		0.10	70.2~108.0
		0.50	78.6~108.0
chestnut	PMG	0.05	82.8~106.0
		0.10	81.2~104.0
		0.50	85.2~107.6
	AMPA	0.05	74.0~110.0
		0.10	74.4~102.0
		0.50	84.2~107.6

Table 3 (Continued)

matrix	Chemicals	Concentration/ (mg/kg)	Recovery range/ (%)
chicken muscle	PMG	0.05	74.0~92.4
		0.10	79.1~105.0
		0.50	72.8~110.0
	AMPA	0.05	76.4~94.8
		0.10	76.4~107.0
		0.50	70.0~93.0
shrimp	PMG	0.05	73.8~94.0
		0.10	78.4~108.0
		0.50	72.0~104.0
	AMPA	0.05	66.4~94.4
		0.10	75.3~112.0
		0.50	68.0~96.4
honey	PMG	0.05	70.8~101.0
		0.10	79.4~110.0
		0.50	82.0~104.0
	AMPA	0.05	72.0~107.0
		0.10	74.8~102.0
		0.50	72.2~109.6
ginseng	PMG	0.05	70.2~108.0
		0.10	86.5~121.0
		0.50	80.2~110.0
	AMPA	0.05	68.0~96.8
		0.10	68.5~114.0
		0.50	70.0~93.0

Annex A¹⁾
(Informative annex)
API 4000 HPLC-MS/MS Operating condions

- a) Electrospray capillary voltage(IS) : 3 500 V ;
- b) GS1 ;50 Pa ;
- c) CUR ;20 Pa ;
- d) GS2 ;25 Pa ;
- e) Ion source tempeture: 250℃ ;
- f) Transitions for confirmation and quantitation, DP, CE, CXP, EP are listed as table A. 1.

Table A . 1—Transitions for confirmation and quantitation, DP, CE and CXP

Compound	Transitions for confirmation m/z	Transitions for quantitation m/z	DP/V	CE/V	CXP/V	EP/V
PMG	392.0/88.0	392.0/88.0	63	30	16	8
	392.0/214.0		54	15	14	8
AMPA	334.0/179.1	334.0/179.1	60	28	10	6
	334.0/112.0		48	17	10	11
Glyphosate 1,2-C ¹³ N ¹⁵		395.0/91.0	65	29	14	6

1) the equipments and their types involved in the standard method are not related to commercial aims, and the analysts are encouraged to use equipments of different corporation or different type.

Annex B
(Informative annex)

Selected ion chromatograms of PMG, Glyphosate 1,2- $C^{13}N^{15}$, AMPA standards

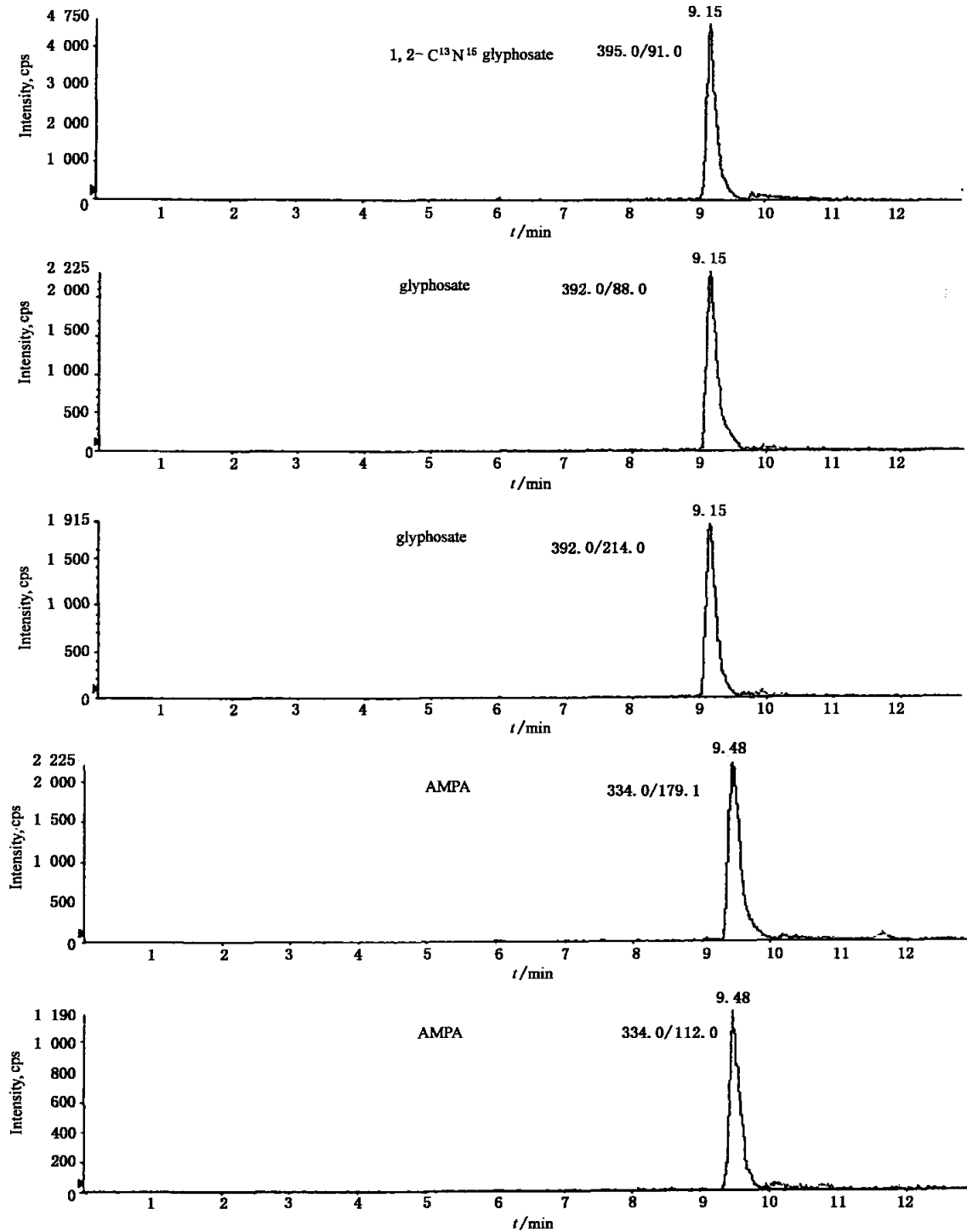


Figure B. 1—Selected ion chromatograms of PMG, Glyphosate, 1,2- $C^{13}N^{15}$ and AMPA standards