



# 中华人民共和国出入境检验检疫行业标准

SN/T 4891—2017

## 出口食品中螺虫乙酯残留量的测定 高效液相色谱和液相色谱-质谱/质谱法

Determination of Spirotetramat residue in foodstuffs for export—HPLC and  
LC-MS/MS 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：中华人民共和国重庆出入境检验检疫局、中华人民共和国万州出入境检验检疫局。

本标准主要起草人：唐柏彬、夏明星、李光满、郝存显、曹淑瑞、郑小玲、张雷、李贤良、王国民。

# 出口食品中螺虫乙酯残留量的测定

## 高效液相色谱和液相色谱-质谱/质谱法

### 1 范围

本标准规定了出口食品中螺虫乙酯及其代谢物残留量的液相色谱-质谱/质谱测定方法和出口食品中螺虫乙酯残留量的高效液相色谱测定方法。

本标准液相色谱-质谱/质谱法适用于猪肉、猪肝、猪脂肪、牛奶、鸡蛋、蜂蜜、大豆、小麦、白菜、荔枝和葡萄干等出口食品中螺虫乙酯及其代谢物残留量的定量测定和确证；高效液相色谱法适用于大豆、小麦、白菜、荔枝和葡萄干等出口食品中螺虫乙酯残留量的定量测定。

### 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

#### 第一法 液相色谱-质谱/质谱法（LC-MS/MS 法）

### 3 方法提要

试样用乙腈提取，提取液经浓缩、定容后用正己烷液液萃取净化，用液相色谱-质谱/质谱测定，外标法定量和确证。

### 4 试剂和材料

除另有规定外，所用试剂均为分析纯，水为符合 GB/T 6682 规定的一级水。

4.1 乙腈：色谱纯。

4.2 正己烷：色谱纯。

4.3 甲酸：色谱纯。

4.4 乙酸铵：色谱纯。

4.5 无水硫酸镁：分析纯。

4.6 无水乙酸钠：分析纯。

4.7 50%乙腈：乙腈-水（50+50，体积比）。

4.8 2 mmol/L 乙酸铵（含 0.1%甲酸）：称取 0.154 g 乙酸铵（4.4），用 950 mL 水溶解，加入 1.0 mL 甲酸（4.3），用水稀释至 1 000 mL。

4.9 标准品：螺虫乙酯及其 4 种代谢物，纯度均大于 90%，化合物详细信息参见附录 A 中表 A.1。

4.10 混合标准储备液（100 mg/L）：分别准确称取含螺虫乙酯及其代谢物 5 mg 的标准品，用乙腈（4.1）溶解并定容至 50 mL，混匀。-18℃以下避光保存。

4.11 混合标准中间液（1.0 μg/mL）：临用前取适量 100 mg/L 混合标准储备液（4.10），用 50%乙腈

(4.7)稀释成 1.0  $\mu\text{g/mL}$  标准中间液。

4.12 混合标准工作液:临用前取适量 1.0  $\mu\text{g/mL}$  标准中间液(4.11),用 50%乙腈稀释至 0.5  $\text{ng/mL}$ 、1.0  $\text{ng/mL}$ 、2.0  $\text{ng/mL}$ 、5.0  $\text{ng/mL}$ 、10  $\text{ng/mL}$ 、20  $\text{ng/mL}$  和 50  $\text{ng/mL}$  标准工作液。

4.13 微孔滤膜:尼龙 66 或相当者,0.22  $\mu\text{m}$ 。

## 5 仪器和设备

5.1 液相色谱-质谱/质谱仪:带电喷雾离子源(ESI)。

5.2 分析天平:感量为 0.01 g 和 0.1 mg。

5.3 均质器,20 000 r/min。

5.4 旋涡混合器。

5.5 离心机:10 000 r/min。

5.6 组织捣碎机。

5.7 氮吹浓缩仪。

5.8 超声波清洗器。

## 6 试样的制备与保存

### 6.1 荔枝、白菜、猪肉、猪肝、鸡蛋

取有代表性样品约 500 g,荔枝去皮去核,猪肉、猪肝去脂肪,鸡蛋去壳后,用高速匀浆机绞碎,装入洁净容器,密闭并标明标记,于 $-18\text{ }^{\circ}\text{C}$ 保存。

### 6.2 大豆、小麦

取整粒有代表性样品约 500 g,用高速粉碎机粉碎,装入洁净容器,密闭并标明标记,于阴凉干燥处保存。

### 6.3 葡萄干

取有代表性样品约 500 g,加入等量水,密封浸泡过夜,用高速匀浆机绞碎,装入洁净容器,密闭并标明标记,于 $-18\text{ }^{\circ}\text{C}$ 保存。

### 6.4 牛奶、蜂蜜

取有代表性样品约 500 mL,混合均匀,装入洁净容器,密闭并标明标记,于 $4\text{ }^{\circ}\text{C}$ 保存。

### 6.5 猪脂肪

取有代表性样品约 500 g,加热熬制,弃去油渣,冷却后装入洁净容器,密闭并标明标记,于 $4\text{ }^{\circ}\text{C}$ 保存。  
在制样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 样品处理

### 7.1 提取

#### 7.1.1 荔枝、白菜、葡萄干

准确称取 10 g(精确至 0.01 g,葡萄干样品相当于 5 g 样品)样品,置于 50 mL 离心管中,加入

20 mL乙腈(4.1), 20 000 r/min 均质提取 3 min, 加入 6.0 g 无水硫酸镁(4.5), 1.0 g 无水乙酸钠(4.6), 混匀, 静置 2 min, 7 000 r/min 离心 3 min, 将上清液转移至 50 mL 比色管中。用 20 mL 乙腈清洗均质器刀头, 将清洗液转移至残渣中, 并将残渣捣碎, 置于超声波中超声提取 30 min(每隔 10 min 取出振摇一次), 7 000 r/min 离心 3 min, 合并提取液至 50 mL 比色管中并用乙腈定容至刻度, 混匀后待净化。

### 7.1.2 大豆、小麦

准确称取 5 g(精确至 0.01 g)样品, 置于 50 mL 离心管中, 加入 8 mL 水, 混匀, 静置 30 min, 接下来按照 7.1.1 中“加入 20 mL 乙腈……”的步骤进行。

### 7.1.3 鸡蛋、牛奶

准确称取 10 g(精确至 0.01 g)样品, 置于 50 mL 离心管中, 加入 20 mL 乙腈, 置于超声波中超声提取 30 min(每隔 10 min 取出振摇一次), 取出, 接下来按照 7.1.1 中“加入 6.0 g 无水硫酸镁……”的步骤进行。

### 7.1.4 蜂蜜

准确称取 10 g(精确至 0.01 g)样品, 置于 50 mL 离心管中, 加入 10 mL 水, 混匀, 加入 20 mL 乙腈, 涡旋混合提取 2 min, 7 000 r/min 离心 3 min, 将上清液转移至 50 mL 比色管中, 样品再用 20 mL 乙腈涡旋提取一次, 合并提取液至 50 mL 比色管中并用乙腈定容至刻度, 混匀后待净化。

### 7.1.5 猪脂肪

准确称取 10 g(精确至 0.01 g)样品, 置于 50 mL 离心管中, 加入 10 mL 正己烷(4.2), 混匀, 加入 20 mL 乙腈, 涡旋混合提取 2 min, 7 000 r/min 离心 3 min, 将下层溶液转移至 50 mL 比色管中, 样品再用 20 mL 乙腈涡旋提取一次, 合并提取液至 50 mL 比色管中并用乙腈定容至刻度, 混匀后待净化。

## 7.2 净化

荔枝、白菜、鸡蛋、牛奶和蜂蜜样液移取 5.00 mL 待净化液, 葡萄干、大豆和小麦样液移取 10.00 mL 待净化液于试管中, 于 40 °C 水浴氮气吹干, 加入 1.00 mL 50% 乙腈(4.7)溶解残渣, 加入 1 mL 正己烷, 涡旋混合 30 s, 4 000 r/min 离心 3 min, 取下层溶液过微孔滤膜(4.13), 用液相色谱-质谱/质谱仪测定。

## 7.3 基质标准工作溶液的制备

取空白样品, 按 7.1 和 7.2 操作至“于 40 °C 水浴氮气吹干”, 加入 1.00 mL 标准工作液(4.12)溶解残渣, 加入 1 mL 正己烷, 涡旋混合 30 s, 4 000 r/min 离心 3 min, 取下层溶液过微孔滤膜, 用液相色谱-质谱/质谱仪测定。

## 7.4 测定

### 7.4.1 液相色谱-质谱/质谱条件

液相色谱-质谱/质谱条件如下:

- 色谱柱:  $C_{18}$  柱, 100 mm(柱长)  $\times$  2.1 mm(内径), 粒度 1.7  $\mu$ m, 或相当者;
- 流动相: 乙腈(A)-2 mmol/L 乙酸铵(含 0.1% 甲酸)(4.8), 梯度洗脱程序见表 1;
- 流速: 0.3 mL/min;
- 柱温: 40 °C;
- 进样量: 5  $\mu$ L;

- f) 离子源:电喷雾离子源;
- g) 扫描方式:正离子;
- h) 检测方式:多反应监测(MRM);
- i) 质谱/质谱参考条件参见附录 B。

表 1 流动相梯度洗脱程序

| 梯度时间/min      | 流动相 A/% | 流动相 B/% |
|---------------|---------|---------|
| 0.0           | 10      | 90      |
| 0.5           | 10      | 90      |
| 2.0           | 80      | 20      |
| 4.0           | 80      | 20      |
| 4.5           | 10      | 90      |
| 5             | 10      | 90      |
| 注:柱前平衡 2 min。 |         |         |

7.4.2 定性测定

按照 7.4.1 仪器条件测定基质标准工作溶液和样液,在相同实验条件下,样品中待测物质的保留时间与标准溶液的保留时间偏差在±2.5%之内;且样品中各组分定性离子的相对丰度与浓度接近的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 |
|-----------|-----|--------|--------|-----|
| 允许的相对偏差/% | ±20 | ±25    | ±30    | ±50 |

7.4.3 定量测定

在仪器最佳工作条件下,对基质标准工作溶和样液进行测定,外标法定量,样液中待分析物的响应值均应在仪器测定的线性范围内,若超出线性范围应适当稀释,同时用相同稀释倍数的基质标准工作溶进行定量。在上述色谱条件下,螺虫乙酯及其代谢物的色谱峰保留时间分别为 BYI08330-enol-glucoside;2.50 min,BYI08330-mono-hydroxy;2.93 min,BYI08330-cis-enol;3.05 min,BYI0-8330-cis-keto-hydroxy;3.12 min,螺虫乙酯;3.43 min。标准溶液多反应监测(MRM)色谱图参见附录 C 中图 C.1。

8 空白试验

除不加试样外,均按上述操作步骤进行。

9 结果计算与表述

9.1 用色谱数据处理机或按照式(1)计算样品中螺虫乙酯及其代谢物的残留量。

$$X = \frac{(c - c_0) \times V_2}{m \times \frac{V_1}{50}} \times \frac{1\,000}{1\,000} \dots\dots\dots (1)$$

式中:

- $X$  ——试样中待测物的含量,单位为微克每千克( $\mu\text{g}/\text{kg}$ );  
 $c$  ——试样溶液中待测物的浓度,单位为纳克每毫升( $\text{ng}/\text{mL}$ );  
 $c_0$  ——空白试验溶液中待测物的浓度,单位为纳克每毫升( $\text{ng}/\text{mL}$ );  
 $V_1$  ——移取提取液体积,单位为毫升( $\text{mL}$ );  
 $V_2$  ——测定用样液定容体积,单位为毫升( $\text{mL}$ );  
 $m$  ——试样质量,单位为克( $\text{g}$ ).

9.2 按照式(2)计算样品中螺虫乙酯及其代谢物总量(以螺虫乙酯计)的残留量。

$$X_{\text{总}} = X_{\text{BYI}} + X_{\text{glu}} \times 0.805\,7 + X_{\text{mono}} \times 1.231 + X_{\text{enol}} \times 1.239 + X_{\text{keto}} \times 1.177 \dots\dots\dots (2)$$

式中:

- $X_{\text{总}}$  ——试样中螺虫乙酯及其代谢物总量(以螺虫乙酯计)的含量,单位为微克每千克( $\mu\text{g}/\text{kg}$ );  
 $X_{\text{BYI}}$  ——试样中螺虫乙酯的含量,单位为微克每千克( $\mu\text{g}/\text{kg}$ );  
 $X_{\text{glu}}$  ——试样中 BYI08330-enol-glucoside 的含量,单位为微克每千克( $\mu\text{g}/\text{kg}$ );  
 $X_{\text{mono}}$  ——试样中 BYI08330-mono-hydroxy 的含量,单位为微克每千克( $\mu\text{g}/\text{kg}$ );  
 $X_{\text{enol}}$  ——试样中 BYI08330-cis-enol 的含量,单位为微克每千克( $\mu\text{g}/\text{kg}$ );  
 $X_{\text{keto}}$  ——试样中 BYI0-8330-cis-keto-hydroxy 的含量,单位为微克每千克( $\mu\text{g}/\text{kg}$ ).

## 10 测定低限(LOQ)

猪肉、猪肝、猪脂肪、牛奶、鸡蛋、蜂蜜、大豆、小麦、白菜、荔枝和葡萄干样品中螺虫乙酯及其代谢物的测定低限均为  $1.0\,\mu\text{g}/\text{kg}$ 。

## 11 回收率

不同样品基质中螺虫乙酯及其代谢物的回收率试验数据参见附录 D 中表 D.1。

### 第二法 高效液相色谱法(HPLC)

## 12 方法提要

试样用乙腈提取,提取液经浓缩,残渣再用乙酸乙酯溶解后用碱性氧化铝柱净化,净化洗脱液浓缩定容,用高效液相色谱测定,外标法定量。

## 13 试剂和材料

除特殊注明外,所有试剂和材料同 4.1,4.2,4.5~4.7,4.13。

13.1 乙酸乙酯:色谱纯。

13.2 60%乙腈:乙腈-水(60+40,体积比)。

13.3 标准品:螺虫乙酯标准品,纯度大于 90%,化合物详细信息参见附录 A 中表 A.1。

13.4 标准储备液(500 mg/L):准确称取含 10 mg 螺虫乙酯的标准品,用乙腈溶解并定容至 20 mL,混匀。—18 ℃以下避光保存。

13.5 标准工作液:临用前取适量 500 mg/L 标准储备液(13.4),用 50%乙腈(4.7)稀释至 0.21 μg/mL、0.5 μg/mL、1.0 μg/mL、2.0 μg/mL、5.0 μg/mL、10 μg/mL、20 μg/mL、50 μg/mL 和 100 μg/mL 标准工作液。

13.6 碱性氧化铝固相萃取柱:1 g,3 mL,临用前用 10 mL 乙酸乙酯活化。

## 14 仪器和设备

除特殊注明外,所有设备同 5.2~5.8。

14.1 高效液相色谱仪:配有紫外检测器或二极管阵列检测器。

14.2 旋转蒸发仪。

## 15 试样的制备与保存

荔枝、白菜、葡萄干、大豆和小麦样品的制备与保存同第 6 章中所述。

## 16 样品处理

### 16.1 样品提取

#### 16.1.1 荔枝、白菜、葡萄干

准确称取 10 g(精确至 0.01 g,葡萄干样品相当于 5 g 样品)样品,置于 50 mL 离心管中,准确加入 20 mL 乙腈,20 000 r/min 均质提取 3 min,加入 6.0 g 无水硫酸镁,1.0 g 无水乙酸钠,混匀,静置 2 min,7 000 r/min 离心 3 min。准确吸取 10 mL 提取液于试管中,于 40 ℃水浴氮气吹干,加入 2 mL 乙酸乙酯(13.1)超声溶解残渣,4 000 r/min 离心 3 min,上清液待净化。

#### 16.1.2 大豆、小麦

准确称取 5 g(精确至 0.01 g)样品,置于 50 mL 离心管中,加入 8 mL 水,混匀,静置 30 min,接下来按照 16.1.1 中“准确加入 20 mL 乙腈……”的步骤进行。

### 16.2 样品净化

将样品待净化液转移碱性氧化铝柱(13.6),收集流出液于 100 mL 旋蒸瓶中,残渣再用 2 mL 乙酸乙酯洗涤两次,离心,上清液转移上柱,再用 25 mL 乙酸乙酯淋洗固相萃取柱并收集洗脱液,洗脱液于 40 ℃水浴减压浓缩至约 2 mL,小心转移至 10 mL 试管中,用 5 mL 乙酸乙酯少量多次洗涤旋蒸瓶并转移洗涤液至试管中,于 40 ℃水浴氮气吹干,用 50%乙腈(荔枝、白菜样品加入 1.00 mL,葡萄干、大豆和小麦样品加入 0.50 mL)超声溶解残渣,加入 0.5 mL 正己烷,涡旋混合 30 s,4 000 r/min 离心 3 min,取下层溶液过微孔滤膜,用液相色谱测定。

### 16.3 测定

#### 16.3.1 液相色谱参考条件

a) 色谱柱: $C_{18}$ 柱,250 mm(柱长)×4.6 mm(内径),粒度 5 μm,或相当者;

- b) 流动相:乙腈-水(60+40,体积比)(13.2);
- c) 流速:1.0 mL/min;
- d) 柱温:30 ℃;
- e) 进样量:20 μL;
- f) 波长:225 nm。

16.3.2 液相色谱测定

按 16.3.1 液相色谱条件测定标准工作液和样液,以外标法测定样液中螺虫乙酯的含量。如果样品中螺虫乙酯的含量超出标准曲线范围,应用流动相稀释后再进行分析。在上述色谱条件下,螺虫乙酯色谱峰保留时间约为:5.79 min。螺虫乙酯的标准色谱图见附录 C 中图 C.2。

17 空白试验

除不加试样外,均按上述操作步骤进行。

18 结果计算

用色谱数据处理机或按照式(3)计算样品中螺虫乙酯的残留量。

$$X = \frac{(c - c_0) \times V}{m \times \frac{10}{20}} \times \frac{1\,000}{1\,000} \dots\dots\dots (3)$$

- 式中:
- X ——试样中螺虫乙酯的含量,单位为毫克每千克(mg/kg);
  - c ——试样溶液中螺虫乙酯的浓度,单位为微克每毫升(μg/mL);
  - c<sub>0</sub> ——空白试验溶液中螺虫乙酯的浓度,单位为微克每毫升(μg/mL);
  - V ——样液定容体积,单位为毫升(mL);
  - m ——试样质量,单位为克(g)。

19 测定低限

大豆、小麦、白菜、荔枝和葡萄干样品中螺虫乙酯的测定低限均为 0.1 mg/kg。

20 回收率

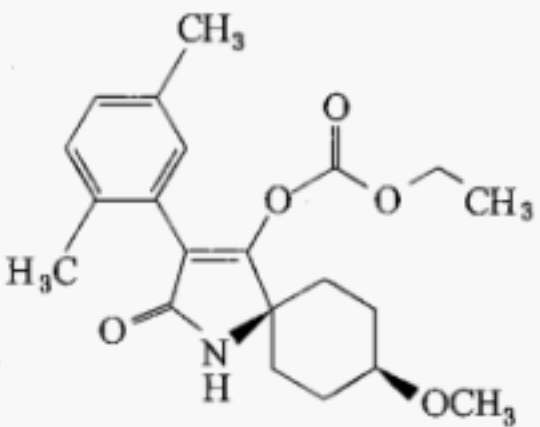
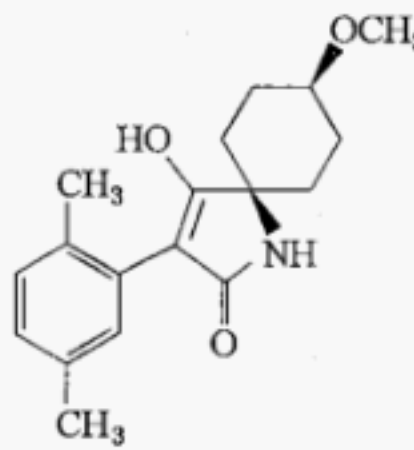
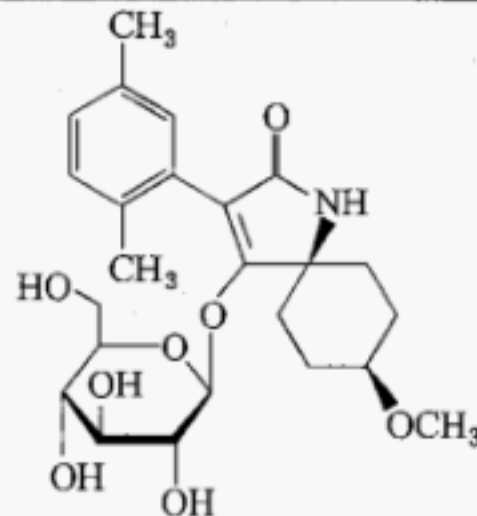
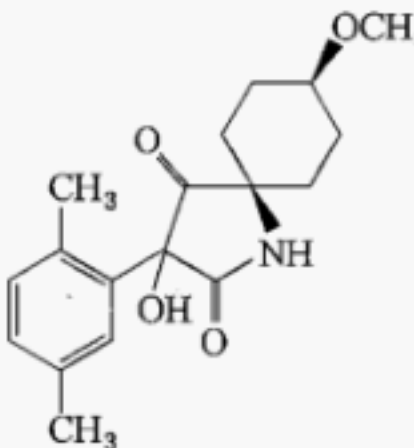
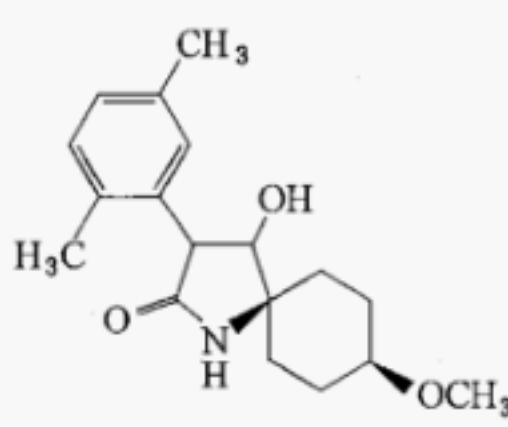
不同样品基质中螺虫乙酯的回收率试验数据见参见附录 D 中表 D.2。

## 附录 A

(资料性附录)

## 螺虫乙酯及其代谢物标准品信息

表 A.1 螺虫乙酯及其代谢物详细信息

| 英文名  | 中文名   | CAS 号        | 分子式                | 相对分子质量 | 结构式   |
|--|---|--------------|--------------------|--------|---|
| Spirotetramat<br>(BYI08330)  | 螺虫乙酯, 顺式-3-(2,5-二甲基苯基)-8-甲氧基-2-氧代-1-氮杂螺[4.5]癸-3-烯-4-基碳酸乙酯     | 203313-25-1  | $C_{21}H_{27}NO_5$ | 373.44 |   |
| cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one<br>(BYI08330-cis-enol)                      | 顺式-3-(2,5-二甲基苯基)-4-羟基-8-甲氧基-1-氮杂螺[4.5]癸-3-烯-2-酮               | 203312-38-3  | $C_{18}H_{23}NO_3$ | 301.38 |  |
| cis-3-(2,5-Dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl β-D-glucopyranoside<br>(BYI08330-enol-glucoside) | 顺式-3-(2,5-二甲基苯基)-8-甲氧基-2-氧代-1-氮杂螺[4.5]癸-3-烯-4-基碳酸 yl β-D-葡萄糖苷 | 1172614-86-6 | $C_{24}H_{33}NO_8$ | 463.52 |  |
| cis-3-(2,5-Dimethylphenyl)-3-hydroxy-8-methoxy-1-azaspiro[4.5]decane-2,4-dione<br>(BYI08330-cis-keto-hydroxy)            | 顺式-3-(2,5-二甲基苯基)-3-羟基-8-甲氧基-1-氮杂螺[4.5]癸-2,4-二酮                | 1172134-11-0 | $C_{18}H_{23}NO_4$ | 317.38 |  |
| cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]decan-2-one<br>(BYI08330-mono-hydroxy)                     | 顺式-3-(2,5-二甲基苯基)-4-羟基-8-甲氧基-1-氮杂螺[4.5]癸-2-酮                   | 1172134-12-1 | $C_{18}H_{25}NO_3$ | 303.40 |  |

附 录 B  
(资料性附录)  
参考质谱条件<sup>1)</sup>

质谱参考条件:

- a) 电喷雾电压(IS): 5 500 V;
- b) 离子源温度(TEM): 550 °C;
- c) 气帘气压力(CUR): 0.3 MPa(45 psi);
- d) 雾化气压力(GS1): 0.5 MPa(70 psi);
- e) 辅助气压力(GS2): 0.45 MPa(65 psi);
- f) 碰撞室入口电压(EP): 10 V;
- g) 去簇电压(DP)、碰撞电压(CE)和碰撞室出口电压(CXP)见表 B.1;
- h) 驻留时间: 30 ms。

表 B.1 待测物母离子、子离子、去簇电压(DP)、碰撞电压(CE)和碰撞室出口电压(CXP)

| 化合物                        | 母离子( $m/z$ ) | 子离子<br>( $m/z$ ) | 去簇电压(DP)<br>锥孔电压/V | 碰撞电压(CE)<br>/V | 碰撞室出口电压<br>(CXP)/V |
|----------------------------|--------------|------------------|--------------------|----------------|--------------------|
| 螺虫乙酯                       | 374.1        | 302.3*           | 74                 | 23             | 15                 |
|                            |              | 330.4            | 74                 | 21             | 18                 |
| BYI08330 enol-glucoside    | 464.1        | 216.4            | 50                 | 62             | 9                  |
|                            |              | 302.4*           | 50                 | 16             | 13                 |
| BYI08330-mono-hydroxy      | 304.3        | 131.2            | 82                 | 25             | 17                 |
|                            |              | 254.3*           | 82                 | 36             | 22                 |
| BYI08330-cis-enol          | 302.2        | 216.3*           | 105                | 38             | 10                 |
|                            |              | 270.1            | 105                | 30             | 13                 |
| BYI0-8330-cis-keto-hydroxy | 318.3        | 268.3            | 60                 | 28             | 13                 |
|                            |              | 300.3*           | 60                 | 18             | 15                 |
| 注：“*”为定量离子。                |              |                  |                    |                |                    |

1) 非商业性声明:附录 B 所列参考质谱条件是在 AB API4000 QTRAP 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)  
色谱图

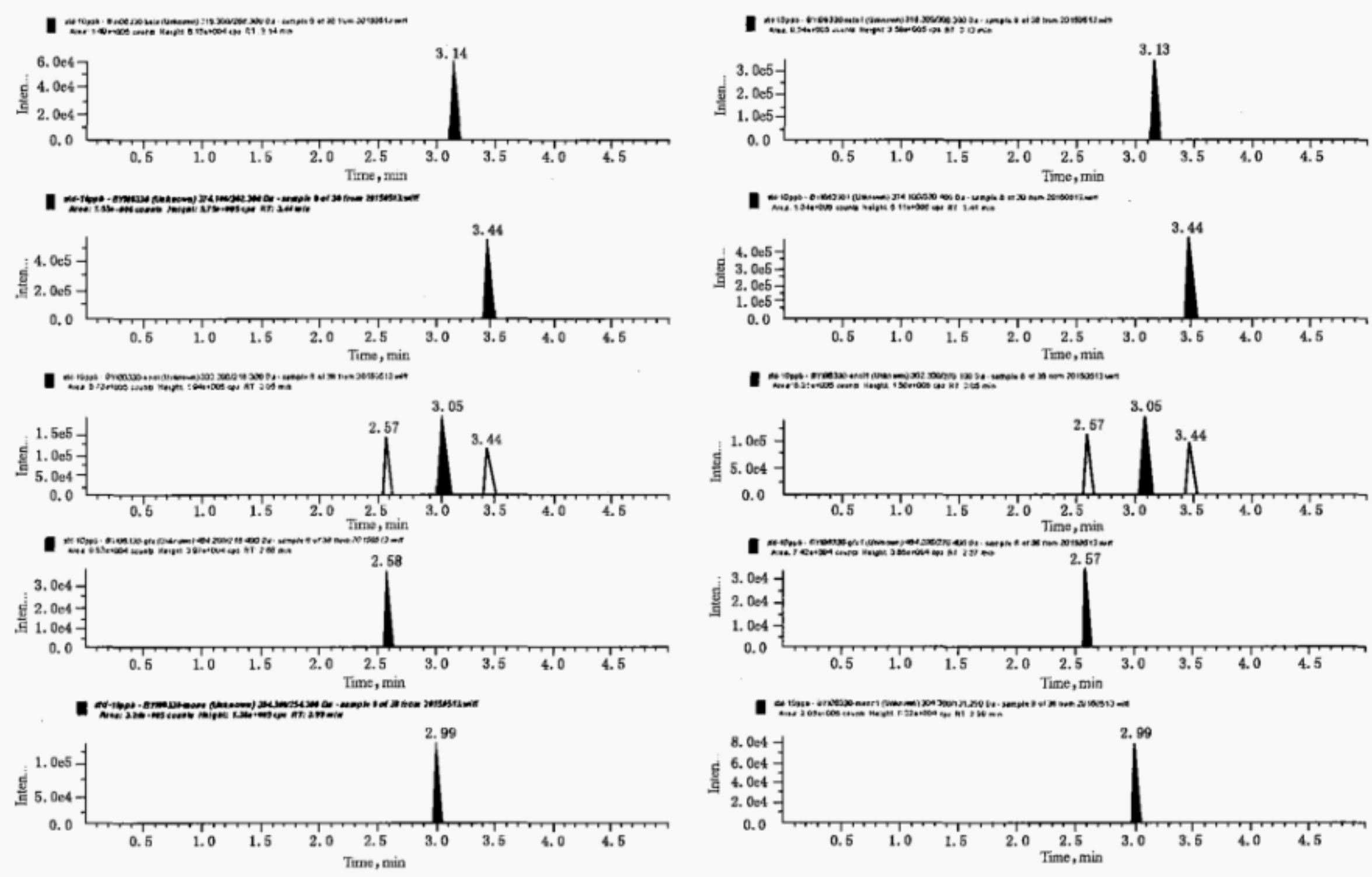


图 C.1 螺虫乙酯及其代谢物标准品多反应监测(MRM)色谱图(10 ng/mL)

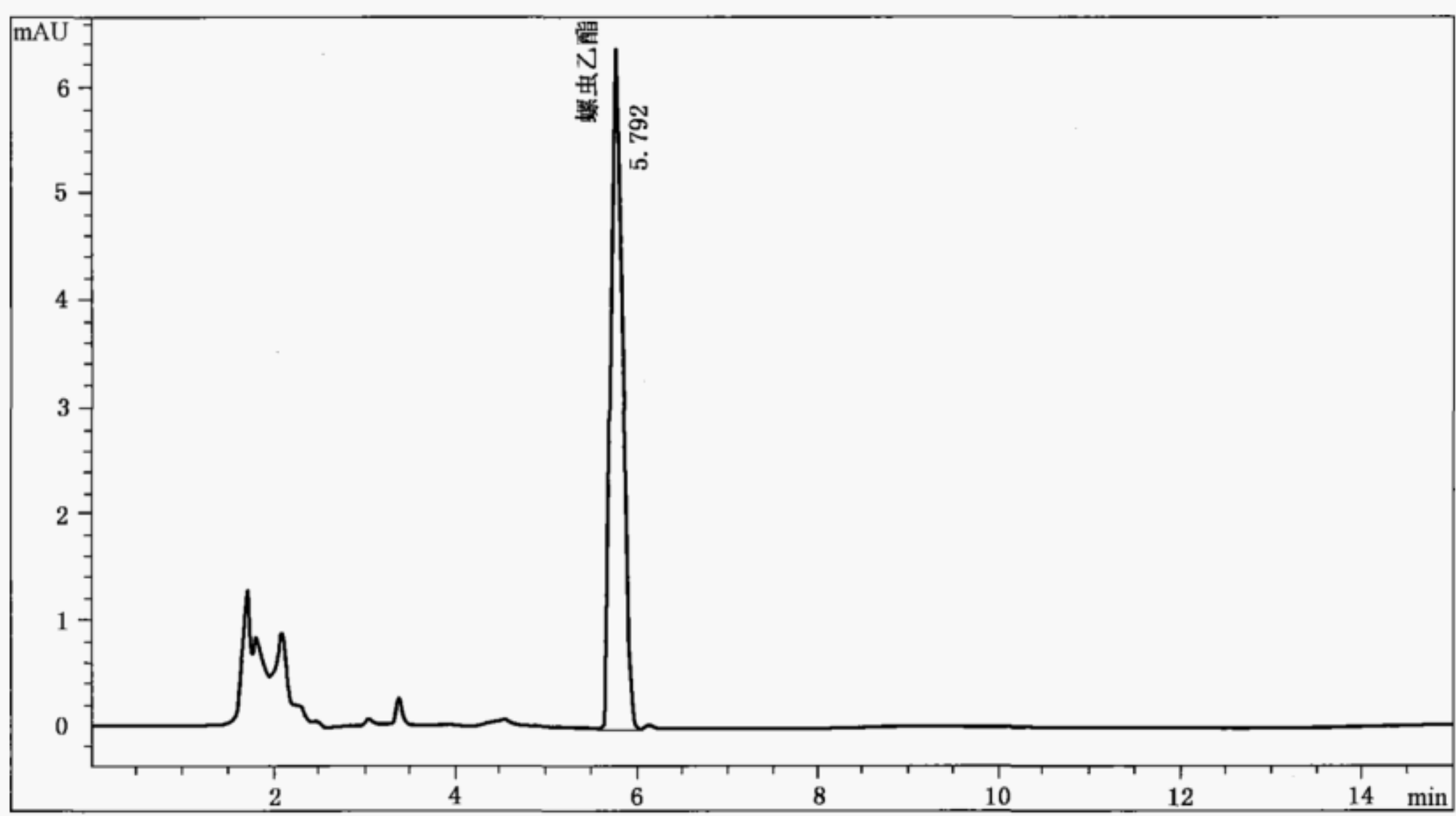


图 C.2 螺虫乙酯标准溶液液相色谱图(1.0 µg/mL)

附 录 D  
(资料性附录)  
回收率试验数据

表 D.1 液相色谱-质谱/质谱法测定螺虫乙酯残留的添加回收率试验数据

| 基质  | 添加水平<br>( $\mu\text{g/kg}$ ) | 回收率范围/%   |                             |                                |                       |                           |
|-----|------------------------------|-----------|-----------------------------|--------------------------------|-----------------------|---------------------------|
|     |                              | 螺虫乙酯      | BYI08330-<br>enol-glucoside | BYI0-8330-cis-<br>keto-hydroxy | BYI08330-cis-<br>enol | BYI08330-mono-<br>hydroxy |
| 猪肉  | 1.0                          | 86.9~109  | 66.6~80.4                   | 75.1~113                       | 88.6~106              | 86.6~108                  |
|     | 2.0                          | 69.0~88.0 | 62.5~79.0                   | 76.0~95.0                      | 87.0~92.5             | 86.5~91.0                 |
|     | 10                           | 74.4~93.8 | 63.9~73.7                   | 77.7~93.0                      | 86.7~100              | 80.2~92.9                 |
|     | 50                           | 81.6~87.8 | 61.2~70.2                   | 83.4~92.2                      | 84.8~93.6             | 78.0~84.0                 |
| 猪肝  | 1.0                          | 64.4~82.1 | 63.8~82.7                   | 78.0~100                       | 74.1~98.2             | 71.7~95.3                 |
|     | 2.0                          | 68.5~87.5 | 75.5~84.5                   | 79.5~98.0                      | 72.5~87.0             | 78.0~102                  |
|     | 10                           | 64.7~84.9 | 64.8~79.3                   | 75.9~87.3                      | 60.3~83.0             | 65.3~91.0                 |
|     | $7.0 \times 10^2$            | 72.1~90.7 | 75.7~91.4                   | 76.4~91.4                      | 76.4~92.1             | 77.1~90.7                 |
| 猪脂肪 | 1.0                          | 97.7~116  | 94.7~113                    | 98.4~117                       | 95.0~120              | 96.0~118                  |
|     | 2.0                          | 106~116   | 96.0~117                    | 98.5~116                       | 98.0~115              | 96.5~113                  |
|     | 20                           | 93.0~111  | 96.5~102                    | 96.0~109                       | 93.5~110              | 94.5~109                  |
| 牛奶  | 1.0                          | 72.5~111  | 86.7~119                    | 86.9~108                       | 86.5~112              | 88.9~114                  |
|     | 2.0                          | 69.5~97.0 | 94.5~118                    | 79.5~110                       | 84.0~113              | 81.5~107                  |
|     | 10                           | 87.2~103  | 98.0~119                    | 97.7~111                       | 93.1~116              | 101~118                   |
| 鸡蛋  | 1.0                          | 61.4~76.3 | 68.2~95.5                   | 84.3~97.9                      | 86.9~114              | 87.2~101                  |
|     | 2.0                          | 60.5~72.5 | 65.5~83.0                   | 81.0~98.5                      | 83.0~112              | 81.5~109                  |
|     | 20                           | 60.5~72.0 | 65.5~86.0                   | 87.5~99.5                      | 89.0~104              | 80.5~93.5                 |
| 蜂蜜  | 1.0                          | 83.0~110  | 67.9~99.1                   | 97.1~112                       | 101~113               | 98.7~109                  |
|     | 2.0                          | 102~112   | 63.5~84.5                   | 91.0~106                       | 92.0~115              | 94.5~108                  |
|     | 10                           | 96.7~106  | 63.1~75.4                   | 93.1~97.3                      | 94.3~100              | 90.1~101                  |
|     | 50                           | 85.2~101  | 68.4~80.4                   | 94.8~104                       | 93.4~102              | 92.0~96.8                 |
| 大豆  | 1.0                          | 78.8~95   | 82.3~95                     | 78.0~108                       | 102~119               | 96.5~118                  |
|     | 2.0                          | 65.0~79   | 70.5~88.5                   | 78.5~105                       | 90.0~114              | 87.5~106                  |
|     | 10                           | 70.4~77.5 | 85.0~101                    | 87.8~98                        | 109~119               | 105~112                   |
|     | $1.6 \times 10^4$            | 85.9~96.3 | 84.4~98.1                   | 91.6~101                       | 87.2~101              | 90.0~102                  |
| 小麦  | 1.0                          | 93.2~116  | 94.6~120                    | 82.2~117                       | 96.0~112              | 99.2~119                  |
|     | 2.0                          | 92.5~103  | 71.5~98.0                   | 89.0~109                       | 87.5~102              | 87.0~110                  |
|     | 10                           | 89.8~99.8 | 72.6~108                    | 86.2~106                       | 86.4~113              | 89.6~108                  |
|     | $1.0 \times 10^2$            | 80.2~88.0 | 78.8~89.6                   | 78.8~95.4                      | 81.6~94.0             | 84.8~89.2                 |

表 D.1 (续)

| 基质  | 添加水平<br>( $\mu\text{g}/\text{kg}$ ) | 回收率范围/%   |                             |                                |                       |                           |
|-----|-------------------------------------|-----------|-----------------------------|--------------------------------|-----------------------|---------------------------|
|     |                                     | 螺虫乙酯      | BYI08330-<br>enol-glucoside | BYI0-8330-cis-<br>keto-hydroxy | BYI08330-cis-<br>enol | BYI08330-mono-<br>hydroxy |
| 白菜  | 1.0                                 | 70.2~95.2 | 70.2~96.0                   | 79.6~96.8                      | 73.4~97.0             | 74.1~93.4                 |
|     | 2.0                                 | 78.5~94.5 | 73.5~91.0                   | 84.0~106                       | 80.5~92.5             | 81.0~104                  |
|     | 10                                  | 80.1~94.3 | 78.3~92.1                   | 87.2~105                       | 86.5~100              | 87.3~108                  |
|     | $1.0\times 10^4$                    | 81.0~92.5 | 80.5~93.0                   | 90.5~105                       | 91.0~105              | 91.5~102                  |
| 荔枝  | 1.0                                 | 76.2~93.9 | 69.8~90.1                   | 80.3~99.4                      | 77.4~99.4             | 82.0~97.6                 |
|     | 2.0                                 | 74.5~92.5 | 69.0~83.0                   | 82.5~97.0                      | 82.5~104              | 80.0~102                  |
|     | 10                                  | 77.3~93.9 | 75.6~85.6                   | 84.2~102                       | 87.4~107              | 91.2~106                  |
|     | $1.5\times 10^4$                    | 84.0~92.0 | 81.3~93.7                   | 95.0~108                       | 93.7~101              | 88.3~101                  |
| 葡萄干 | 1.0                                 | 70.9~92.1 | 65.1~90.5                   | 82.1~114                       | 74.9~96.3             | 79.4~112                  |
|     | 2.0                                 | 74.0~97.0 | 77.5~89.5                   | 80.0~103                       | 75.5~94.0             | 82.0~102                  |
|     | 10                                  | 78.6~95.8 | 77.4~87.7                   | 86.3~109                       | 80.3~102              | 82.7~102                  |
|     | $4.0\times 10^3$                    | 85.3~100  | 82.3~93.5                   | 95.5~109                       | 93.5~106              | 91.5~108                  |

表 D.2 液相色谱法测定螺虫乙酯残留的添加回收率试验数据

| 基质  | 添加水平/( $\text{mg}/\text{kg}$ ) | 回收率范围/%   |
|-----|--------------------------------|-----------|
| 大豆  | 0.10                           | 81.6~102  |
|     | 0.20                           | 84.5~101  |
|     | 1.0                            | 84.4~103  |
|     | 16                             | 86.9~99.4 |
| 小麦  | 0.10                           | 80.4~103  |
|     | 0.20                           | 83.0~99.5 |
|     | 1.0                            | 83.8~102  |
| 白菜  | 0.10                           | 86.6~103  |
|     | 0.20                           | 87.0~103  |
|     | 1.0                            | 89.4~104  |
|     | 10                             | 89.4~100  |
| 荔枝  | 0.10                           | 88.8~106  |
|     | 0.20                           | 87.0~101  |
|     | 1.0                            | 88.8~101  |
|     | 15                             | 88.0~103  |
| 葡萄干 | 0.10                           | 85.2~102  |
|     | 0.20                           | 84.0~104  |
|     | 4.0                            | 87.8~102  |

## Foreword

This standard was drafted in accordance with the GB/T 1.1—2009.

It is noted that some contents in this file may involve in patent authority. Agency regarding distribution and publication of this file does not be responsible for the identification of these patents.

This standard was proposed by and was under the charged of National Regulatory Commission for Certification and Accreditation.

This standard was drafted by Chongqing Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Wanzhou Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

This standard was mainly drafted by Tang Bobin, Xia Mingxing, Li Guangman, Xi Cunxian, Cao Shurui, Zheng Xiaoling, Zhang Lei, Li Xianliang, Wang Guomin.

# Determination of Spirotetramat residue in foodstuffs for export—HPLC and LC-MS/MS method

## 1 Scope

The first method in this standard specifies the method of determination of Spirotetramat and its four metabolites residue in foodstuffs for export by LC-MS/MS.

The second method in this standard specifies the method of determination of Spirotetramat residue in foodstuffs for export by HPLC.

The first method in this standard is applicable to the determination and confirmation of Spirotetramat and its four metabolites residue in pork, liver, fat, milk, egg, honey, bean, wheat, cabbage, litchi and raisins.

The second method in this standard is applicable to the determination of Spirotetramat residue in bean, wheat, Chinese cabbage, litchi and raisins.

## 2 Normative reference

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB/T 6682—2008 Water for analytic laboratory use - Specification and test methods

### The First Method LC-MS/MS Method

## 3 Principle

The residues of Spirotetramat and its four metabolites in the sample are extracted with acetonitrile. The extracted is concentrated and clean up with *n*-Hexane. The extraction solution is cleaned by passing SPE cartridges. The purified solution is determined by LC-MS/MS, quantified by external standard method.

## 4 Reagents and materials

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

4.1 Acetonitrile: HPLC grade.

4.2 *n*-Hexane: HPLC grade.

4.3 Formic acid: HPLC grade.

4.4 Ammonium acetate: HPLC grade.

4.5 Magnesium sulfate anhydrous.

4.6 Sodium acetate anhydrous.

4.7 50% Acetonitrile: volume 50 mL acetonitrile, then add 50 mL water, mix them.

4.8 2 mmol/L Ammonium acetate (Contain 0.1% Formic acid): Weigh 0.154 g Ammonium acetate (4.4), Dissolve with 950 mL water, add 1.0 mL Formic acid (4.3) and dilute to 1 000 mL.

4.9 Standard chemical: Spirotetramat and its four metabolites, purity  $\geq 99\%$ , Other information see annex A Table A. 1.

4.10 Stock standard solution (100 mg/L): Accurately weigh an adequate amount of Spirotetramat and its four metabolites(4.9) (accurate to 0.1 mg), dissolved in acetonitrile and prepare a solution of 100 mg/L as the standard stock solution respectively, stored at  $-18\text{ }^{\circ}\text{C}$ .

4.11 Intermediate standard solution (1  $\mu\text{g/mL}$ ): Accurately pipet 1 mL stock standard solution (100 mg/L) (4.10) into a 100 mL volumetric flask, dissolve and dilute to volume with 50% acetonitrile (4.7).

4.12 Standard working solution: Accurately pipet an adequate amount of intermediate standard solution(4.11) prepare the solution of 0.5 ng/mL, 1.0 ng/mL, 2.0 ng/mL, 5.0 ng/mL, 10 ng/mL, 20 ng/mL and 50 ng/mL with 50% acetonitrile just before use.

4.13 Membrane filter: Nylon 66, 0.22  $\mu\text{m}$ , organic.

## 5 Apparatus and equipment

5.1 Liquid chromatography tandem mass spectrometer equipped with electrospray ionization source (ESI).

5.2 Analytical balance, sensitivity: 0.01 g, 0.1 mg.

5.3 High speed homogenizer, 20 000 r/min.

5.4 Vortex mixer.

5.5 Centrifuge: 10 000 r/min.

5.6 Kinematica.

5.7 Evaporator with nitrogen flow.

5.8 Ultrasonic extractor.

## 6 Sample preparation and storage

### 6.1 Pork, liver, egg, cabbage and litchi

Pork, liver should be removed the fat. Egg should be removed the shell. Litchi should be removed both the pee l and stone. About 500 g representative samples should be taken from all samples, the grinded and blended by a tissue blender to produce homogenous samples, put in suitable clean container. After being sealed and labeled the samples should be stored at below  $-18^{\circ}\text{C}$ .

### 6.2 Bean, wheat

About 500 g representative samples should be taken from all samples, the grinded and blended by a tissue blender to produce homogenous samples, put in suitable clean container. After being sealed and labeled the samples should be stored at below room temperature.

### 6.3 Raisins

About 500 g representative samples should be taken from all samples, add 500 g water and stand for one night, the grinded and blended by a tissue blender to produce homogenous samples, put in suitable clean container. After being sealed and labeled the samples should be stored at below  $-18^{\circ}\text{C}$ .

#### 6.4 Milk, honey

About 500 g representative samples should be taken from all samples, then mixed to produce homogenous samples, put in suitable clean container. After being sealed and labeled the samples should be stored at below 4 °C.

#### 6.5 Fat

About 500 g representative samples should be taken from all samples, heat, discard the residues and keep the oil, the grinded and blended by a tissue blender to produce homogenous samples, put in suitable clean container. After being sealed and labeled the samples should be stored at below 4 °C.

In the course of sample preparation and sample storage, precaution must be taken to avoid contamination.

### 7 Analytical procedure

#### 7.1 Extract procedure

##### 7.1.1 Cabbage, litchi and raisins

Accurately weigh 10 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 20 mL acetonitrile (4.1), homogen for 3 min at 20 000 r/min, then add anhydrous  $\text{MgSO}_4$  (6 g) and sodium acetate anhydrous (1 g) to the tube, mix and stand for 2 min. After being centrifuged for 3 min at 7 000 r/min, transfer the supernatant to 50 mL volumetric flask. 20 mL acetonitrile were used to wash high speed homogenizer, and then transferred to extract the residue by ultrasonic extraction for 30 min (Shake every 10 minutes), then centrifuge at 7 000 r/min for 3 min. Combine the supernatant to 50 mL volumetric flask, add acetonitrile to scale, mix homogeneity to cleanup.

##### 7.1.2 Bean, wheat

Accurately weigh 5 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 8 mL water, mix and stand for 30 min, add 20 mL acetonitrile. Next, according to the procedures" add anhydrous  $\text{MgSO}_4$  (6 g) ..... mix homogeneity to cleanup ." in 7.1.1.

##### 7.1.3 Egg, milk

Accurately weigh 10 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 20 mL acetonitrile, and ultrasonic extraction for 30 min. Next, according to the procedures" add anhydrous  $\text{MgSO}_4$  (6 g) ..... mix homogeneity to cleanup ." in 7.1.1.

#### 7.1.4 Honey

Accurately weigh 10 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 10 mL water, mix, then add 20 mL acetonitrile, vortex extract 2 min, centrifuge at 7 000 r/min for 3 min, transfer the supernatant to 50 mL volumetric flask. Repeat the extraction above with another addition of acetonitrile. Combine the supernatant to 50 mL volumetric flask, added acetonitrile to scale, mix homogeneity to cleanup.

#### 7.1.5 Fat

Accurately weigh 10 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 10 mL *n*-Hexane (4.2), mix, then add 20 mL acetonitrile, vortex extract 2 min, centrifuge at 7 000 r/min for 3 min, transfer the acetonitrile solution to 50 mL volumetric flask. Repeat the extraction above with another addition of acetonitrile. Combine the supernatant to 50 mL volumetric flask, added acetonitrile to scale, mix homogeneity to cleanup.

### 7.2 Cleanup procedure

Transfer 5.00 mL extracts of pork, liver, fat, milk, egg, honey, cabbage and litchi, 10.00 mL extracts of bean, wheat and raisins to 10 mL tube. Evaporate the extracts to dry at 40 °C under nitrogen flow, then dissolved the residue with 1.0 mL 50% acetonitrile (4.7), added 1 mL *n*-Hexane, vortex 30 s, centrifuge at 4 000 r/min for 3 min. After being filtrated with 0.22 μm filter (4.13), and final solution is ready for analysis by LC-MS/MS.

### 7.3 Matrix curve

Weigh seven blank sample, according to the procedures in 7.1 and 7.2, dissolved the residue with 1.0 mL standard working solution, added 1 mL *n*-Hexane, vortex 30 s, centrifuge at 4 000 r/min for 3 min. After being filtrated with 0.22 μm filter, and final solution is ready for analysis by LC-MS/MS.

### 7.4 Determination

#### 7.4.1 LC-MS/MS confirmation condition

LC-MS/MS confirmation condition is as follows:

- a) LC column: C18, 100 mm × 2.1 mm (i.d.), 1.7 μm (or other conformable column);
- b) Mobile phase: Acetonitrile (A)-2 mmol/L Ammonium acetate solution (contain 0.1% formic acid) (4.8) (B), the elution gradient is listed in Table 1;
- c) Flow rate: 0.3 mL/min;

- d) Column temperature: 40 °C ;
- e) Injector volume: 5  $\mu$ L;
- f) Ion source: electrospray ionization (ESI);
- g) Ionization mode: ESI+ ;
- h) Scan mode: MRM;
- i) Other reference mass operating conditions are list in Annex B.

Table 1—Elution gradient of LC-MS/MS

| Time/min                            | A/% | B/% |
|-------------------------------------|-----|-----|
| 0.0                                 | 10  | 90  |
| 0.5                                 | 10  | 90  |
| 2.0                                 | 80  | 20  |
| 4.0                                 | 80  | 20  |
| 4.5                                 | 10  | 90  |
| 5                                   | 10  | 90  |
| NOTE 2 min for column regeneration. |     |     |

#### 7.4.2 Qualitative determination

Under the same conditions of experiment, the retention time of the unknown sample is the same as the standard working solution; the qualification ions for every compound must be found. For the same analysis batch and the same compound, the variation range of the ion ratio between the two daughter ions for the unknown sample and the standard working solution at the similar concentration cannot be out of range of Table 2.

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% |

#### 7.4.3 Quantitative determination

According to the method of 7.4.1, detect the residues of Spirotetramat and its four metabolites in the samples solution and matrix curve. The response should be in the linear of the instrumental de-

tection. If the response is out of the linear range, the sample should be diluted with the blank matrix extract solution to suitable concentration. Under the above chromatograph conditions, the reference retention time of BYI08330 enol-glucoside: 2.50 min, BYI08330-mono-hydroxy: 2.93 min, BYI08330-cis-enol: 3.05 min, BYI0-8330-cis-keto-hydroxy: 3.12 min, Spirotetramat: 3.43 min. chromatogram of standard solution, see annex C, Figure C. 1.

## 8 Blank test

The operation of the blank test is the same as that is described while there is no sample involved.

## 9 Calculation and expression of result

9.1 Calculating the content of Spirotetramat and its four metabolites concentration according to the formula(1):

$$X = \frac{(c - c_0) \times V_2}{m \times \frac{V_1}{50}} \times \frac{1\,000}{1\,000} \dots\dots\dots (1)$$

where

$X$  is the residue content of analyte in the test sample,  $\mu\text{g}/\text{kg}$ ;

$c$  is the concentration of analyte in the test sample which is quantified by standard calibration curve,  $\text{ng}/\text{mL}$ ;

$c_0$  is the concentration of analyte in the blank sample which is quantified by standard calibration curve,  $\text{ng}/\text{mL}$ ;

$V_1$  is the volume of cleanup,  $\text{mL}$ ;

$V_2$  is the final volume of sample solution,  $\text{mL}$ ;

$m$  is the corresponding mass of test sample in the final sample solution,  $\text{g}$ .

9.2 Calculating the total of Spirotetramat and its four metabolites (calculated as Spirotetramat) according to the formula (2):

$$X_T = X_{\text{BYI}} + X_{\text{glu}} \times 0.8057 + X_{\text{mono}} \times 1.231 + X_{\text{enol}} \times 1.239 + X_{\text{keto}} \times 1.177 \dots\dots\dots (2)$$

where

$X_T$  is the total of Spirotetramat and its four metabolites (calculated as Spirotetramat) in the test sample,  $\mu\text{g}/\text{kg}$ ;

$X_{\text{BYI}}$  is the content of Spirotetramat in the test sample,  $\mu\text{g/kg}$ ;

$X_{\text{glu}}$  is the residue content of BYI08330-enol-glucoside in the test sample,  $\mu\text{g/kg}$ ;

$X_{\text{mono}}$  is the residue content of BYI08330-mono-hydroxy in the test sample,  $\mu\text{g/kg}$ ;

$X_{\text{enol}}$  is the residue content of BYI08330-cis-enol in the test sample,  $\mu\text{g/kg}$ ;

$X_{\text{keto}}$  is the residue content of BYI0-8330-cis-keto-hydroxy in the test sample,  $\mu\text{g/kg}$ .

## 10 Limit of quantification (LOQ)

The limit of quantification for Spirotetramat and its four metabolites are all  $1.0 \mu\text{g/kg}$ .

## 11 Recovery

The recovery and the fortifying concentration of Spirotetramat and its four metabolites in different matrix are listed in annex D, table D.1.

### The second method HPLC method

## 12 Principle

The residues of Spirotetramat and its four metabolites in the sample are extracted with acetonitrile. After being purification with  $\text{B-Al}_2\text{O}_3$  solid phase extraction column, the analyte are determined by high performance liquid chromatography, quantified by external standard method.

## 13 Reagents and materials

Except for special note, the other reagents and materials are the same as section 4.1, 4.2, 4.5~4.7, 4.13.

13.1 Ethyl acetate: HPLC grade

13.2 60% Acetonitrile: volume 60 mL acetonitrile, then add 40 mL water, mix them.

13.3 Standard chemical: Spirotetramat, purity  $\geq 99\%$ , Other information see annex A Table A. 1.

**13.4** Stock standard solution (500 mg/L): Accurately weigh an adequate amount of Spirotetramat (accurate to 0.1 mg), dissolved in acetonitrile and prepare a solution of 500 mg/L as the standard stock solution respectively, stored at  $-18^{\circ}\text{C}$ .

**13.5** Standard working solution: Accurately pipet an adequate amount of stock standard solution (13.4) prepare the solution of 0.2  $\mu\text{g/mL}$ , 0.5  $\mu\text{g/mL}$ , 1.0  $\mu\text{g/mL}$ , 2.0  $\mu\text{g/mL}$ , 5.0  $\mu\text{g/mL}$ , 10  $\mu\text{g/mL}$ , 20  $\mu\text{g/mL}$ , 50  $\mu\text{g/mL}$  and 100  $\mu\text{g/mL}$  with 50% acetonitrile just before use.

**13.6** Basic alumina column: (1 g, 3 mL) or equivalent, activated by 10 mL ethyl acetate (13.1) before use.

## 14 Apparatus and equipment

Except for special note, the other apparatus are the same as section 5.2~5.8.

**14.1** High performance chromatography with photodiode array detector.

**14.2** Rotary evaporator.

## 15 Sample preparation and storage

The section is the same as 6.

## 16 Sample preparation

### 16.1 Extract procedure

#### 16.1.1 Cabbage, litchi and raisins

Accurately weigh 10 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 20 mL acetonitrile, homogen for 3 min at 20 000 r/min, add anhydrous  $\text{MgSO}_4$  (6 g) and sodium acetate anhydrous (1 g) to the tube, mix and stand for 2 min. After being centrifuged for 3 min at 7 000 r/min, transfer 10 mL extracts to 10 mL tube. Evaporate the extracts to dry at  $40^{\circ}\text{C}$  under nitrogen flow, then dissolved the residue with 2 mL ethyl acetate (13.1), centrifuged for 3 min at 4 000 r/min, the supernatant use to cleanup.

#### 16.1.2 Bean, wheat

Accurately weigh 5 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 8 mL water, mix and stand for 30 min, add 20 mL acetonitrile. Next, according to the procedures" add an-

hydrous  $\text{MgSO}_4$  (6 g) ..... mix homogeneity to cleanup ." in 16.1.1.

## 16.2 Cleanup procedure

Load the solution to the basic alumina column (13.6), dissolved the residue with 2 mL ethyl acetate twice and centrifuged for 3 min at 4 000 r/min, the supernatant added into the column, then eluted with 25 mL ethyl acetate, collect the total eluate. Evaporate the eluate to dryness at 40 °C, then dissolved the residue of cabbage and litchi with 1 mL 50% acetonitrile, dissolved the residue of bean, wheat and raisins with 0.5 mL 50% acetonitrile, added 0.5 mL *n*-Hexane, vortex 30 second, centrifuge at 4 000 r/min for 3 min. After being filtrated with 0.22  $\mu\text{m}$  filter, and final solution is ready for analysis by HPLC.

## 16.3 Determination

### 16.3.1 HPLC operation condition

HPLC operation condition is as follows:

- a) LC column: C18, 250 mm  $\times$  4.6 mm (i.d.), 5  $\mu\text{m}$ , (or other conformable column);
- b) Mobile phase: 60% Acetonitrile ( 13.2 );
- c) Flow rate: 1.0 mL/min;
- d) Column temperature: 30 °C ;
- e) Injector volume: 20  $\mu\text{L}$ ;
- f) Detection wavelength: 225 nm.

### 16.3.2 HPLC determination

According to 16.3.1 HPLC conditions, standard working solution and samples of liquid sample measured with equal volume. The sample analyte concentration should be within the scope of the standard curve, if the content beyond the scope of the standard curve should be determined after appropriate dilution. Under these conditions, the reference retention times of Spirotetramat is 5.79 min, chromatogram of standard solution, see annex C, Figure C.2.

## 17 Blank test

The operation of the blank test is the same as that is described while there is no sample involved.

## 18 Calculation and expression of result

Calculating the content of Spirotetramat concentration according to the formula (3):

$$X = \frac{(c - c_0) \times V}{m \times \frac{10}{20}} \times \frac{1\ 000}{1\ 000} \dots\dots\dots (3)$$

where

$X$  is the residue content of Spirotetramat in the test sample, mg/kg;

$c$  is the concentration of analyte which is quantified by standard calibration curve,  $\mu\text{g/mL}$ ;

$c_0$  is the concentration of blank test which is quantified by standard calibration curve,  $\mu\text{g/mL}$ ;

$V$  is the final volume of sample solution, mL;

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

## 19 Limit of quantification and recovery

The limit of quantification for Spirotetramat is 0.1 mg/kg.

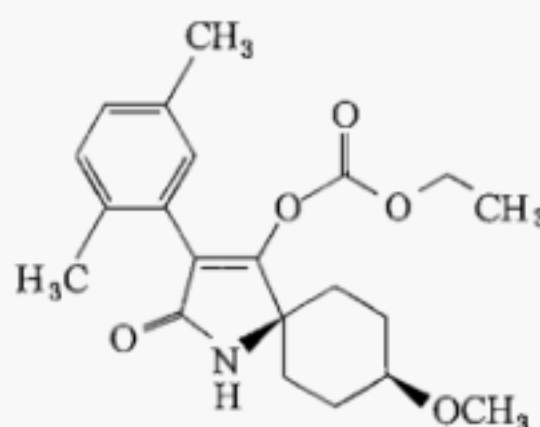
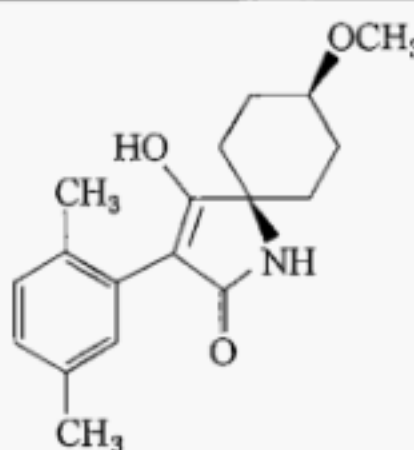
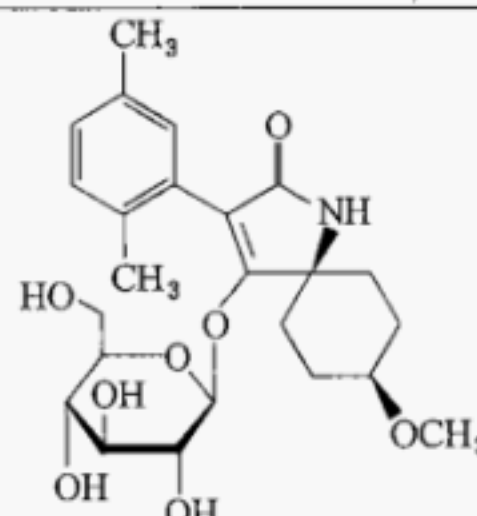
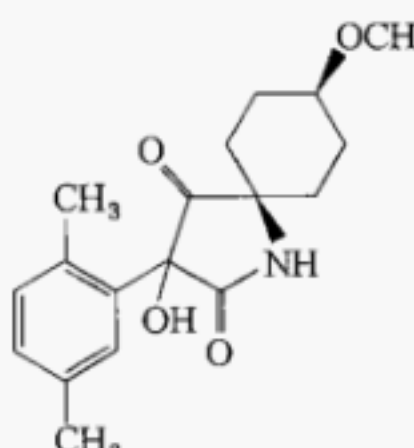
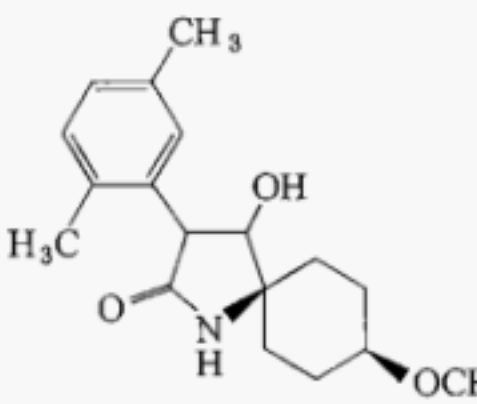
## 20 Recovery

The recovery and the fortifying concentration of Spirotetramat in different matrix are listed in annex D, Table D.2.

**Annex A**  
(informative)

**Information of Spirotetramat and its four metabolites**

**Table A.1**—The detailed information of Spirotetramat and its four metabolites

| Compound   | CAS NO.      | Molecular formula  | Molecular weight | Structural formula  |
|--|--------------|--------------------|------------------|---|
| Spirotetramat<br>(BYI08330)  | 203313-25-1  | $C_{21}H_{27}NO_5$ | 373.44           |   |
| cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]dec-3-en-2-one<br>(BYI08330-cis-enol)                      | 203312-38-3  | $C_{18}H_{23}NO_3$ | 301.38           |  |
| cis-3-(2,5-Dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl β-D-glucopyranoside<br>(BYI08330-enol-glucoside) | 1172614-86-6 | $C_{24}H_{33}NO_8$ | 463.52           |  |
| cis-3-(2,5-Dimethylphenyl)-3-hydroxy-8-methoxy-1-azaspiro[4.5]decane-2,4-dione<br>(BYI08330-cis-keto-hydroxy)            | 1172134-11-0 | $C_{18}H_{23}NO_4$ | 317.38           |  |
| cis-3-(2,5-Dimethylphenyl)-4-hydroxy-8-methoxy-1-azaspiro[4.5]decan-2-one<br>(BYI08330-mono-hydroxy)                     | 1172134-12-1 | $C_{18}H_{25}NO_3$ | 303.40           |  |

**Annex B**  
(informative)  
Reference mass conditions<sup>1)</sup>

## Reference mass conditions

- a) Electrospray voltage(IS): 5 500 V;
- b) Temperature (TEM): 550 °C;
- c) Curtain gas pressure(CUR): 0.3 MPa(45 psi);
- d) Ion source gas 1(GSI): 0.5 MPa(70 psi);
- e) Ion source gas 2(GS2): 0.45 MPa(65 psi);
- f) Entrance potential (EP): 10 V;
- g) Declustering potential (DP)、Collision energy (CE) and Collision cell exit potential (CXP) see Table B.1;
- h) Dwell time:30 ms.

Table B.1—Qualitative ions, quantitative ion pairs, DP, CE and CXP

| Compound  | Q1( <i>m/z</i> ) | Q3( <i>m/z</i> ) | DP/V | CE/V | CXP/V |
|---|------------------|------------------|------|------|-------|
| Spirotetramat                                     | 374.1            | 302.3 *          | 74   | 23   | 15    |
|   |                  | 330.4            | 74   | 21   | 18    |
| BYI08330-enol-glucoside                           | 464.1            | 216.4            | 50   | 62   | 9     |
|   |                  | 302.4 *          | 50   | 16   | 13    |
| BYI08330-mono-hydroxy                             | 304.3            | 131.2            | 82   | 25   | 17    |
|   |                  | 254.3 *          | 82   | 36   | 22    |
| BYI08330-cis-enol                                 | 302.2            | 216.3 *          | 105  | 38   | 10    |
|   |                  | 270.1            | 105  | 30   | 13    |
| BYI0-8330-cis-keto-hydroxy                        | 318.3            | 268.3            | 60   | 28   | 13    |
|   |                  | 300.3 *          | 60   | 18   | 15    |
| NOTE the quantification of ion with“ * ”in table. |                  |                  |      |      |       |

1) Non-commercial Statement: Reference mass spectrometry conditions in Annex B was Completed by liquid chromatography-mass spectrometry of AB API4000 QTRAP. Test instruments are listed here only to provide a reference model. Involve commercial purposes, and encourage users to try a different standard or model of instrument manufacturers.

# Annex C

## (informative)

### Chromatograms

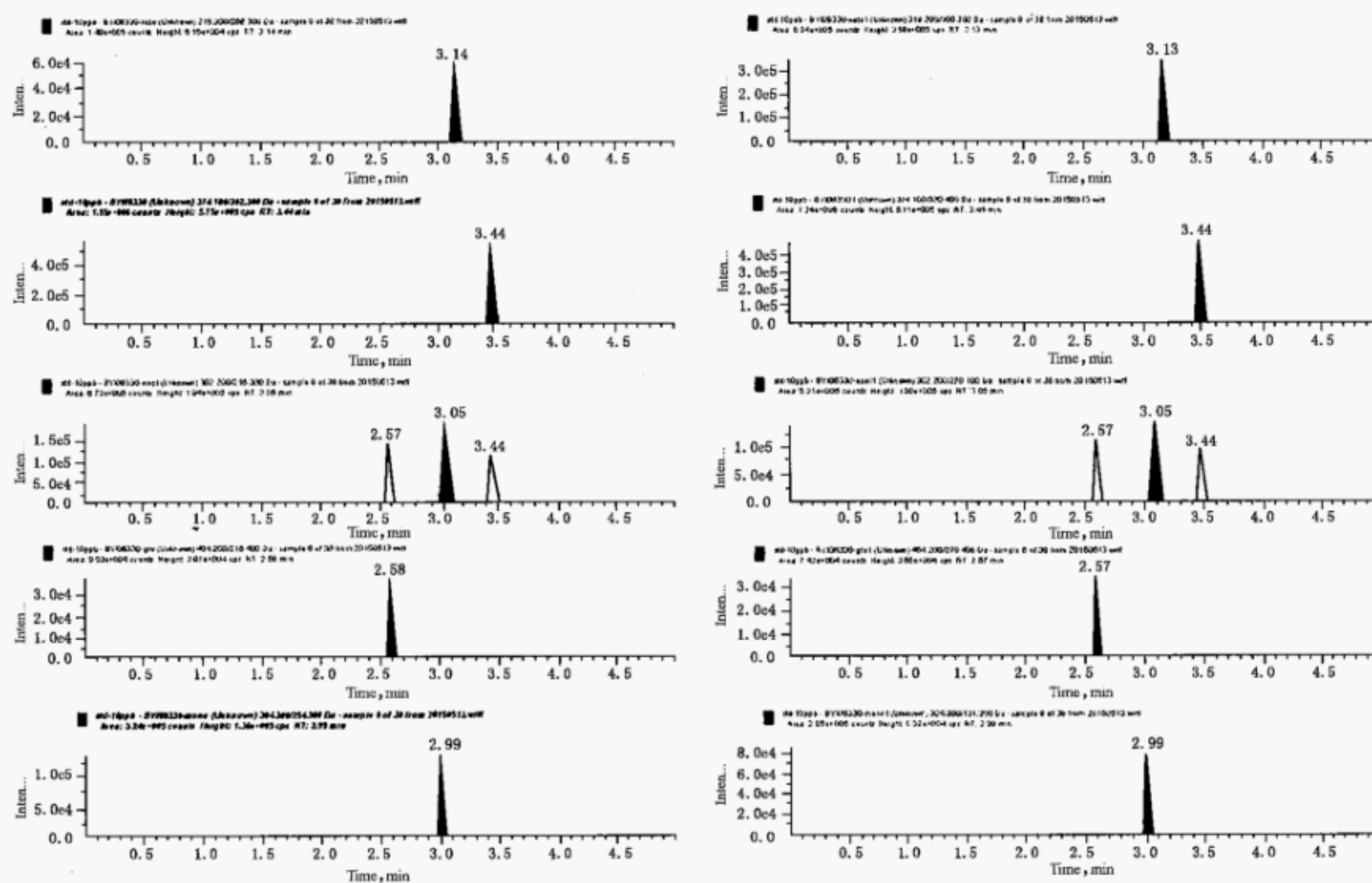


Figure C.1—Multiple reaction monitoring (MRM) chromatogram of standard solution (10 ng/L)

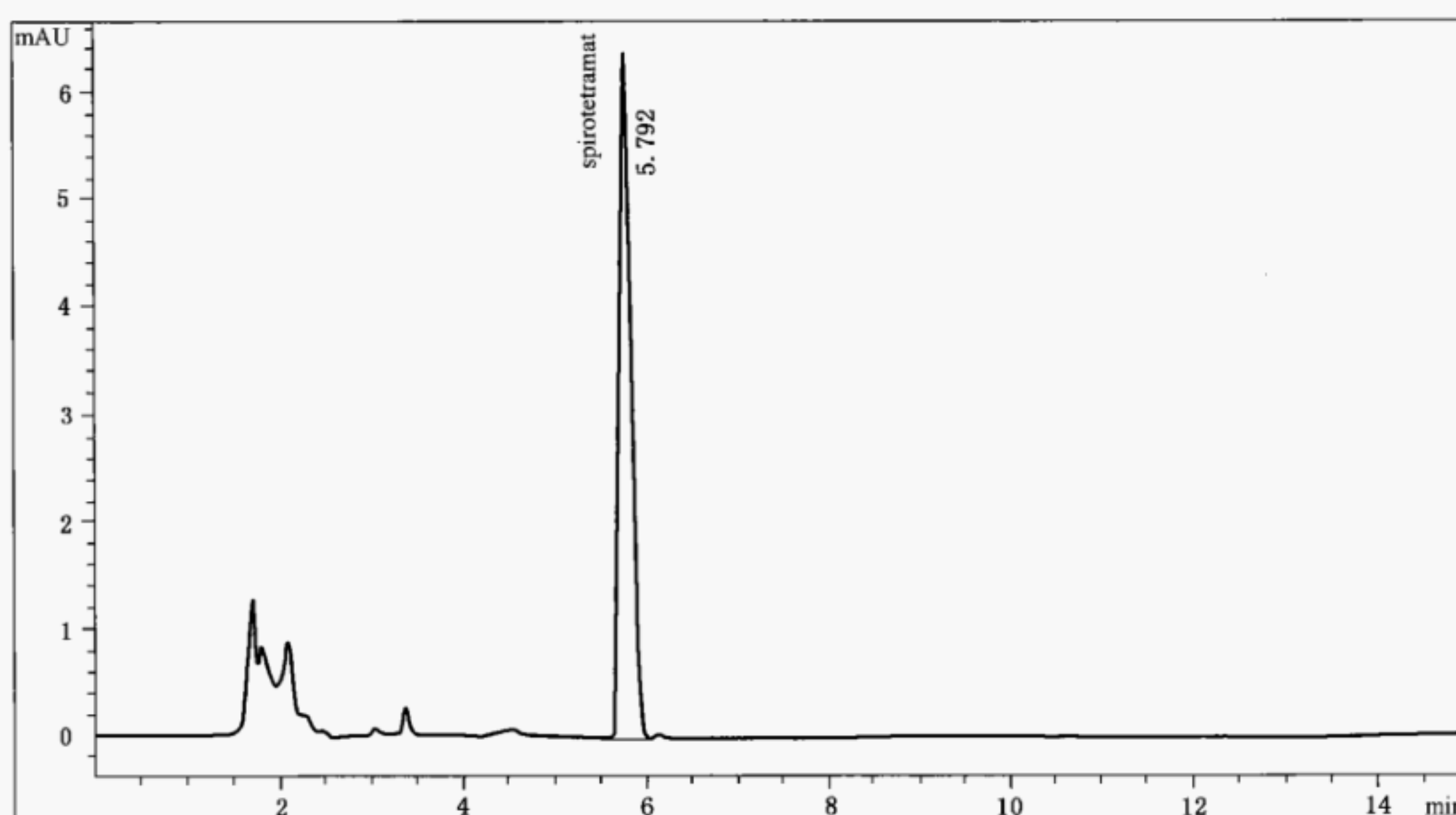


Figure C.2—Liquid chromatogram of standard solution (1.0 µg/mL)

**Annex D**  
**(informative)**  
**Recoveries**

Table D.1—Recoveries of Spirotetramat and its four metabolites(LC-MS/MS method)

| Sample | Spiked level<br>( $\mu\text{g}/\text{kg}$ ) | Recovery range/% |                         |                            |                   |                       |
|--------|---|------------------|-------------------------|----------------------------|-------------------|-----------------------|
|        |   | Spirotetramat    | BYI08330-enol-glucoside | BYI0-8330-cis-keto-hydroxy | BYI08330-cis-enol | BYI08330-mono-hydroxy |
| Pork   | 1.0   | 86.9 to 109      | 66.6 to 80.4            | 75.1 to 113                | 88.6 to 106       | 86.6 to 108           |
|        | 2.0   | 69.0 to 88.0     | 62.5 to 79.0            | 76.0 to 95.0               | 87.0 to 92.5      | 86.5 to 91.0          |
|        | 10  | 74.4 to 93.8     | 63.9 to 73.7            | 77.7 to 93.0               | 86.7 to 100       | 80.2 to 92.9          |
|        | 50  | 81.6 to 87.8     | 61.2 to 70.2            | 83.4 to 92.2               | 84.8 to 93.6      | 78.0 to 84.0          |
| Liver  | 1.0   | 64.4 to 82.1     | 63.8 to 82.7            | 78.0 to 100                | 74.1 to 98.2      | 71.7 to 95.3          |
|        | 2.0   | 68.5 to 87.5     | 75.5 to 84.5            | 79.5 to 98.0               | 72.5 to 87.0      | 78.0 to 102           |
|        | 10  | 64.7 to 84.9     | 64.8 to 79.3            | 75.9 to 87.3               | 60.3 to 83.0      | 65.3 to 91.0          |
|        | $7.0 \times 10^2$                           | 72.1 to 90.7     | 75.7 to 91.4            | 76.4 to 91.4               | 76.4 to 92.1      | 77.1 to 90.7          |
| Fat    | 1.0   | 97.7 to 116      | 94.7 to 113             | 98.4 to 117                | 95.0 to 120       | 96.0 to 118           |
|        | 2.0   | 106 to 116       | 96.0 to 117             | 98.5 to 116                | 98.0 to 115       | 96.5 to 113           |
|        | 20  | 93.0 to 111      | 96.5 to 102             | 96.0 to 109                | 93.5 to 110       | 94.5 to 109           |
| Milk   | 1.0   | 72.5 to 111      | 86.7 to 119             | 86.9 to 108                | 86.5 to 112       | 88.9 to 114           |
|        | 2.0   | 69.5 to 97.0     | 94.5 to 118             | 79.5 to 110                | 84.0 to 113       | 81.5 to 107           |
|        | 10  | 87.2 to 103      | 98.0 to 119             | 97.7 to 111                | 93.1 to 116       | 101 to 118            |
| Egg    | 1.0   | 61.4 to 76.3     | 68.2 to 95.5            | 84.3 to 97.9               | 86.9 to 114       | 87.2 to 101           |
|        | 2.0   | 60.5 to 72.5     | 65.5 to 83.0            | 81.0 to 98.5               | 83.0 to 112       | 81.5 to 109           |
|        | 20  | 60.5 to 72.0     | 65.5 to 86.0            | 87.5 to 99.5               | 89.0 to 104       | 80.5 to 93.5          |
| Honey  | 1.0   | 83.0 to 110      | 67.9 to 99.1            | 97.1 to 112                | 101 to 113        | 98.7 to 109           |
|        | 2.0   | 102 to 112       | 63.5 to 84.5            | 91.0 to 106                | 92.0 to 115       | 94.5 to 108           |
|        | 10  | 96.7 to 106      | 63.1 to 75.4            | 93.1 to 97.3               | 94.3 to 100       | 90.1 to 101           |
|        | 50  | 85.2 to 101      | 68.4 to 80.4            | 94.8 to 104                | 93.4 to 102       | 92.0 to 96.8          |
| Bean   | 1.0   | 78.8 to 95       | 82.3 to 95              | 78.0 to 108                | 102 to 119        | 96.5 to 118           |
|        | 2.0   | 65.0 to 79       | 70.5 to 88.5            | 78.5 to 105                | 90.0 to 114       | 87.5 to 106           |
|        | 10  | 70.4 to 77.5     | 85.0 to 101             | 87.8 to 98                 | 109 to 119        | 105 to 112            |
|        | $1.6 \times 10^4$                           | 85.9 to 96.3     | 84.4 to 98.1            | 91.6 to 101                | 87.2 to 101       | 90.0 to 102           |
| Wheat  | 1.0   | 93.2 to 116      | 94.6 to 120             | 82.2 to 117                | 96.0 to 112       | 99.2 to 119           |
|        | 2.0   | 92.5 to 103      | 71.5 to 98.0            | 89.0 to 109                | 87.5 to 102       | 87.0 to 110           |
|        | 10  | 89.8 to 99.8     | 72.6 to 108             | 86.2 to 106                | 86.4 to 113       | 89.6 to 108           |
|        | $1.0 \times 10^2$                           | 80.2 to 88.0     | 78.8 to 89.6            | 78.8 to 95.4               | 81.6 to 94.0      | 84.8 to 89.2          |

表 D.1(continued)

| Sample  | Spiked level<br>( $\mu\text{g}/\text{kg}$ ) | Recovery range/% |                             |                                |                       |                           |
|---------|---|------------------|-----------------------------|--------------------------------|-----------------------|---------------------------|
|         |   | Spirotetramat    | BYI08330-<br>enol-glucoside | BYI0-8330-cis-<br>keto-hydroxy | BYI08330-cis-<br>enol | BYI08330-mono-<br>hydroxy |
| Cabbage | 1.0   | 70.2 to 95.2     | 70.2 to 96.0                | 79.6 to 96.8                   | 73.4 to 97.0          | 74.1 to 93.4              |
|         | 2.0   | 78.5 to 94.5     | 73.5 to 91.0                | 84.0 to 106                    | 80.5 to 92.5          | 81.0 to 104               |
|         | 10  | 80.1 to 94.3     | 78.3 to 92.1                | 87.2 to 105                    | 86.5 to 100           | 87.3 to 108               |
|         | $1.0 \times 10^4$                           | 81.0 to 92.5     | 80.5 to 93.0                | 90.5 to 105                    | 91.0 to 105           | 91.5 to 102               |
| Litchi  | 1.0   | 76.2 to 93.9     | 69.8 to 90.1                | 80.3 to 99.4                   | 77.4 to 99.4          | 82.0 to 97.6              |
|         | 2.0   | 74.5 to 92.5     | 69.0 to 83.0                | 82.5 to 97.0                   | 82.5 to 104           | 80.0 to 102               |
|         | 10  | 77.3 to 93.9     | 75.6 to 85.6                | 84.2 to 102                    | 87.4 to 107           | 91.2 to 106               |
|         | $1.5 \times 10^4$                           | 84.0 to 92.0     | 81.3 to 93.7                | 95.0 to 108                    | 93.7 to 101           | 88.3 to 101               |
| Raisins | 1.0   | 70.9 to 92.1     | 65.1 to 90.5                | 82.1 to 114                    | 74.9 to 96.3          | 79.4 to 112               |
|         | 2.0   | 74.0 to 97.0     | 77.5 to 89.5                | 80.0 to 103                    | 75.5 to 94.0          | 82.0 to 102               |
|         | 10  | 78.6 to 95.8     | 77.4 to 87.7                | 86.3 to 109                    | 80.3 to 102           | 82.7 to 102               |
|         | $4.0 \times 10^3$                           | 85.3 to 100      | 82.3 to 93.5                | 95.5 to 109                    | 93.5 to 106           | 91.5 to 108               |

Table D.2—Recoveries of Spirotetramat(HPLC method)

| Sample  | Spiked level/( $\text{mg}/\text{kg}$ ) | Recovery range/% |
|---------|--|------------------|
| Bean    | 0.10                                   | 81.6 to 102      |
|         | 0.20                                   | 84.5 to 101      |
|         | 1.0                                    | 84.4 to 103      |
|         | 16                                     | 86.9 to 99.4     |
| Wheat   | 0.10                                   | 80.4 to 103      |
|         | 0.20                                   | 83.0 to 99.5     |
|         | 1.0                                    | 83.8 to 102      |
| Cabbage | 0.10                                   | 86.6 to 103      |
|         | 0.20                                   | 87.0 to 103      |
|         | 1.0                                    | 89.4 to 104      |
|         | 10                                     | 89.4 to 100      |
| Litchi  | 0.10                                   | 88.8 to 106      |
|         | 0.20                                   | 87.0 to 101      |
|         | 1.0                                    | 88.8 to 101      |
|         | 15                                     | 88.0 to 103      |
| Raisins | 0.10                                   | 85.2 to 102      |
|         | 0.20                                   | 84.0 to 104      |
|         | 4.0                                    | 87.8 to 102      |

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