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# 中华人民共和国出入境检验检疫行业标准

SN/T 3264—2012

# 出口食品中鱼藤酮和印楝素残留量的检测方法　液相色谱-质谱/质谱法

**Determination of rotenone and azadirachtin residues in food for export—LC-MS/MS method**

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中华人民共和国
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# 前　言

本标准按照 GB/T 1.1—2009 给出的规则起草。

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

本标准由国家认证认可监督管理委员会提出并归口。

本标准起草单位：中华人民共和国福建出入境检验检疫局、中国检验检疫科学研究院。

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# 出口食品中鱼藤酮和印楝素残留量的检测方法　液相色谱-质谱/质谱法

## 1　范围

本标准规定了出口食品中鱼藤酮和印楝素残留量的液相色谱-质谱/质谱检测方法。

本标准适用于大米、花椰菜、苹果、木耳、茶叶、蜂蜜、猪肝、鱼肉、虾肉、鸡肉、牛奶中鱼藤酮和印楝素残留量的测定和确证。

## 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

试样中残留的鱼藤酮和印楝素采用乙腈提取，提取液经氯化钠盐析后经正己烷除脂，以聚苯乙烯-二乙烯基苯-吡咯烷酮聚合物填料的固相萃取小柱净化，液相色谱-质谱/质谱仪检测及确证，外标法定量。

## 4　试剂和材料

除非另有规定，所用试剂均为分析纯，水为 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　甲醇：色谱纯。

4.4　甲酸：色谱纯。

4.5　氯化钠。

4.6　乙酸铵。

4.7　碳酸氢钠。

4.8　饱和碳酸氢钠溶液：称取一定量碳酸氢钠溶于水中至饱和。

4.9　5 mmol/L 乙酸铵缓冲液：称取 0.38 g 乙酸铵溶于 800 mL 水中，加入 2 mL 甲酸，以水定容至 1 000 mL。

4.10　标准物质(鱼藤酮：英文名 Rotenone，分子式 $C_{23}H_{22}O_6$，CAS No. 83-79-4，相对分子质量 394.42；印楝素：英文名 Azadirachtin，分子式 $C_{35}H_{44}O_{16}$，CAS No. 11141-17-6，分子量 720.71)：纯度≥98%。

4.11　鱼藤酮和印楝素标准贮备液(100 mg/L)：准确称取 0.010 0 g 鱼藤酮和印楝素标准物质，用甲醇溶解并定容至 100 mL，该标准储备液于 4 ℃避光保存不超过 1 个月。

4.12　鱼藤酮和印楝素标准工作液：根据需要取适量标准贮备液，以 20%乙腈水溶液稀释成适当浓度的标准工作液，临用现配。

4.13　聚苯乙烯-二乙烯基苯-吡咯烷酮聚合物填料的固相萃取柱:60 mg,3 mL。使用前依次以 3 mL 甲醇、3 mL 水预处理。

4.14　滤膜:0.22 μm,有机系。

## 5　仪器和设备

5.1　液相色谱-质谱/质谱联用仪,配电喷雾(ESI)源。

5.2　分析天平,感量分别为 0.1 mg 与 0.01 g。

5.3　离心机:4 500 r/min,配有 50 mL 的具塞塑料离心管。

5.4　粉碎机。

5.5　组织捣碎机。

5.6　涡旋混合器。

5.7　超声波清洗器。

5.8　固相萃取装置。

5.9　氮吹仪。

## 6　试样制备和保存

### 6.1　试样制备

#### 6.1.1　水果蔬菜类

取有代表性样品 500 g,将可食部分切碎后(不可水洗),用组织捣碎机将样品加工成浆状,混匀,分成 2 份,装入洁净容器内,密封并标识。

#### 6.1.2　茶叶、粮谷与坚果类

取有代表性样品 500 g,用粉碎机粉碎并通过直径 2.0 mm 的筛,混匀,分成 2 份,装入洁净容器内,密封并标识。

#### 6.1.3　肉及肉制品

取有代表性样品 500 g,切碎后用组织捣碎机将样品加工成浆状,混匀,装入洁净容器内,分成 2 份,密封并标识。

#### 6.1.4　蜂蜜

取有代表性样品 500 g,对于无结晶的蜂蜜样品,将其搅拌均匀。对有结晶的样品,在密闭情况下,置于不超过 60 ℃的水浴中温热,振荡,待样品全部融化后搅匀,迅速冷却至室温,分成 2 份,装入洁净样品瓶中,密封并标识。

#### 6.1.5　牛奶

取有代表性的样品 500 g,充分混匀,分为 2 份,装入洁净样品瓶中,密封并标识。

### 6.2　试样保存

茶叶、果酒、蜂蜜、牛奶、粮谷于 0 ℃～4 ℃保存,蔬菜、水果、肉及肉制品于－18 ℃保存。在制样过程中,应防止样品受到污染或发生残留物含量的变化。

## 7 测定步骤

### 7.1 提取

7.1.1 茶叶、谷物及坚果等样品：称取 1 g(精确至 0.01 g)试样，置于 50 mL 具塞塑料离心管中，加入 5 mL 饱和碳酸氢钠溶液(4.8)振摇后避光浸泡 10 min，加入 15 mL 乙腈(4.1)，涡动 30 s 后超声提取 10 min，4 500 r/min 离心 3 min，移出有机相，残渣再加入 10 mL 乙腈重复提取 1 次，合并提取液，加入约 3 g 氯化钠(4.5)盐析，4 500 r/min 离心 3 min 离心，取上清液，加入 2 mL 经乙腈饱和后的正己烷(4.2)，振摇 1 min，4 500 r/min 离心 3 min 离心，弃去正己烷层，乙腈层于 45 ℃减压旋转蒸发至近干，以 5 mL 20%甲醇水溶解残渣，按 7.2 步骤净化。

7.1.2 蔬菜与水果样品：称取 2 g(精确至 0.01 g)试样，置于 50 mL 具塞塑料离心管中，加入约 2 g 碳酸氢钠和 15 mL 乙腈，振摇均匀后超声提取 10 min，4 500 r/min 离心 3 min，移出有机相，残渣再加入 10 mL 乙腈重复提取 1 次，合并提取液，加入约 3 g 氯化钠盐析，4 500 r/min 离心 3 min，取上清液，于 45 ℃减压旋转蒸发至近干，以 5 mL 20%甲醇水溶解残渣，按 7.2 步骤净化。

7.1.3 蜂蜜：称取 2 g(精确至 0.01 g)试样置于 50 mL 具塞塑料离心管中，加入 5 mL 饱和碳酸氢钠溶液振摇，加入 15 mL 乙腈，涡动 30 s 后超声提取 10 min，4 500 r/min 离心 3 min，移出有机相，残渣再加入 10 mL 乙腈重复提取 1 次，合并提取液，加入约 3 g 氯化钠盐析，4 500 r/min 离心 3 min，取上清液，于 45 ℃减压旋转蒸发至近干，以 5 mL 20%甲醇水溶解残渣，按 7.2 步骤净化。

7.1.4 牛奶、果汁及葡萄酒等：称取 2 g(精确至 0.01 g)试样置于 50 mL 具塞塑料离心管中，加入约 2 g 碳酸氢钠和 15 mL 乙腈，涡动 30 s 后超声提取 10 min，4 500 r/min 离心 3 min，移出有机相，残渣再加入 10 mL 乙腈重复提取 1 次，合并提取液，加入约 3 g 氯化钠盐析，4 500 r/min 离心 3min，取上清液，于 45 ℃减压旋转蒸发至近干，以 5 mL 20%甲醇水溶解残渣，按 7.2 步骤净化。

7.1.5 鱼、肉及肉制品：称取 2 g(精确至 0.01 g)试样置于 50 mL 具塞塑料离心管中，加入 5 mL 饱和碳酸氢钠溶液振摇，加入 15 mL 乙腈，涡动 30 s 后超声提取 10 min，4 500 r/min 离心 3 min，移出有机相，残渣再加入 10 mL 乙腈重复提取 1 次，合并提取液，加入约 3 g 氯化钠盐析，4 500 r/min 离心 3 min，取上清液，加入 5 mL 正己烷，振摇 1 min，4 500 r/min 离心 3 min，弃去正己烷层，乙腈层于 45 ℃ 减压旋转蒸发至近干，以 5 mL 20%甲醇水溶解残渣，按 7.2 步骤净化。

### 7.2 净化

将样品提取液上柱，用 5 mL 水淋洗，弃去全部淋洗液，抽干，以 5 mL 乙腈洗脱，保持流速约为 1 mL/min，收集洗脱液，于 45 ℃氮吹至近干，以 20%乙腈水溶液定容 1 mL，过 0.22 μm 滤膜(4.14)，供液相色谱-质谱/质谱测定。

### 7.3 液相色谱-质谱/质谱法测定

#### 7.3.1 色谱条件

7.3.1.1 色谱柱：Phenomenex Luna $C_{18}$柱，150 mm×2.0 mm(内径)，3 μm，或相当者。

7.3.1.2 柱温：35 ℃。

7.3.1.3 流动相：乙腈-5 mmol/L 乙酸铵缓冲液(4.9) (35+65，体积分数)。

7.3.1.4 流速：400 μL/min。

7.3.1.5 进样量：10 μL。

#### 7.3.2 质谱条件

7.3.2.1 离子源：电喷雾源(ESI)，正离子模式。

7.3.2.2　扫描方式：多反应监测(MRM)。

7.3.2.3　其他参考质谱条件参见表 A.1。

### 7.3.3　液相色谱-质谱/质谱测定

根据试样中被测物的含量情况，选取响应值适宜的标准工作液进行色谱分析。标准工作液和待测样液中鱼藤酮和印楝素的响应值均应在仪器线性响应范围内。按式(1)进行结果计算。在本方法条件下，鱼藤酮和印楝素的保留时间约为 5.6 min 和 4.2 min，鱼藤酮和印楝素标准品的多反应监测(MRM)色谱图参见附录 B。

### 7.3.4　定性标准

进行样品测定时，如果检出的色谱峰保留时间与标准样品一致，并且在扣除背景后的样品谱图中，各定性离子的相对丰度与浓度接近的同样条件下得到的标准溶液谱图相比，最大允许相对偏差不超过表 1 中规定的范围，则可判断样品中存在对应的被测物。

**表 1　定性确证时相对离子丰度的最大允许偏差**

| 相对离子丰度/% | >50 | >20～50 | >10～20 | ≤10 |
|---|---|---|---|---|
| 允许的相对偏差/% | ±20 | ±25 | ±30 | ±50 |

## 7.4　空白实验

除不加试样外，均按上述步骤进行。

## 8　结果计算和表述

用数据处理软件或按式(1)计算试样中鱼藤酮和印楝素药物的残留量，计算结果需扣除空白值：

$$X = c \times \frac{V}{m} \qquad \cdots\cdots(1)$$

式中：

$X$ ——试样中鱼藤酮或印楝素残留量，单位为微克每千克(μg/kg)；

$c$ ——从标准工作曲线得到的鱼藤酮和印楝素溶液浓度，单位为微克每升(μg/L)；

$V$ ——样品溶液最终定容体积，单位为毫升(mL)；

$m$ ——最终样液所代表的试样质量，单位为克(g)。

## 9　测定低限、回收率

### 9.1　测定低限

鱼藤酮和印楝素测定低限分别为 0.5 μg/kg 和 2 μg/kg。

### 9.2　回收率

食品中鱼藤酮和印楝素残留的添加回收数据见表 2。

表 2　鱼藤酮和印楝素药物残留的添加回收率数据

| 样品名称 | 鱼藤酮 | | 印楝素 | |
|---|---|---|---|---|
| | 添加浓度<br>μg/kg | 回收率范围<br>% | 添加浓度<br>μg/kg | 回收率范围<br>% |
| 大米 | 0.5 | 83.5～102.0 | 2 | 75.2～104.5 |
| | 1 | 84.6～101.8 | 4 | 79.6～105.2 |
| | 5 | 79.3～97.8 | 20 | 80.5～101.3 |
| 花椰菜 | 0.5 | 81.0～103.0 | 2 | 84.8～106.5 |
| | 1 | 79.4～96.8 | 4 | 80.1～102.4 |
| | 5 | 80.8～108.7 | 20 | 78.9～100.3 |
| 苹果 | 0.5 | 81.0～96.0 | 2 | 81.4～102.8 |
| | 1 | 79.6～98.4 | 4 | 86.6～106.5 |
| | 5 | 78.4～100.3 | 20 | 90.1～102.4 |
| 木耳 | 0.5 | 81.0～99.0 | 2 | 82.1～108.2 |
| | 1 | 79.0～99.8 | 4 | 85.6～104.4 |
| | 5 | 81.4～104.5 | 20 | 81.4～104.8 |
| 茶叶 | 0.5 | 78.0～103.0 | 2 | 74.6～107.6 |
| | 1 | 78.8～102.2 | 4 | 76.2～100.3 |
| | 5 | 77.4～99.7 | 20 | 78.9～104.8 |
| 蜂蜜 | 0.5 | 84.0～101.0 | 2 | 79.4～102.8 |
| | 1 | 80.2～97.6 | 4 | 80.5～103.2 |
| | 5 | 80.5～99.7 | 20 | 80.5～100.5 |
| 牛奶 | 0.5 | 76.2～104.3 | 2 | 78.6～109.6 |
| | 1 | 76.4～106.6 | 4 | 76.2～95.5 |
| | 5 | 76.2～102.8 | 20 | 81.9～106.8 |
| 猪肝 | 0.5 | 75.0～98.0 | 2 | 77.2～106.5 |
| | 1 | 78.4～103.2 | 4 | 76.3～101.5 |
| | 5 | 76.8～100.1 | 20 | 81.8～100.7 |
| 鱼肉 | 0.5 | 79.0～105.0 | 2 | 77.5～100.3 |
| | 1 | 79.6～101.2 | 4 | 76.2～101.7 |
| | 5 | 80.7～103.0 | 20 | 80.9～98.4 |
| 虾肉 | 0.5 | 81.0～97.0 | 2 | 77.2～102.8 |
| | 1 | 80.6～101.8 | 4 | 79.2～105.3 |
| | 5 | 80.3～96.3 | 20 | 82.7～101.4 |
| 鸡肉 | 0.5 | 83.0～97.0 | 2 | 78.6～104.5 |
| | 1 | 80.4～104.2 | 4 | 79.3～93.8 |
| | 5 | 81.2～96.9 | 20 | 81.9～106.8 |

# 附　录　A[1)]
（资料性附录）
参考质谱条件

A.1　毛细管电压:4 kV。

A.2　屏蔽气温度:320 ℃。

A.3　屏蔽气流量:10 L/min。

A.4　干燥气流量:3 L/min。

A.5　碰撞气压:50 psi。

A.6　其他质谱参数见表 A.1。

表 A.1　鱼藤酮和印楝素的主要参考质谱参数

| 化合物 | 监测离子 | 驻留时间<br>ms | 电压<br>V | 碰撞能量<br>eV |
|---|---|---|---|---|
| 鱼藤酮 | 395>213 | 50 | 160 | 24 |
| | 395>192 | | | 25 |
| 印楝素 | 743>725 | 50 | 140 | 28 |
| | 743>625 | | | 36 |
| 注：表中黑体字显示的离子为定量离子；对于不同质谱仪器，仪器参数可能存在差异，测定前应将质谱参数优化到最佳。 | | | | |

1)　非商业性表明:附录 A 所列参考质谱条件是在 Agilent 6460 型液质联用仪上完成的，此处列出试验用仪器型号仅为提供参考，并不涉及商业目的，鼓励标准使用者尝试不同厂家或型号的仪器。

# 附 录 B
（资料性附录）
## 鱼藤酮和印楝素标准溶液的多反应监测色谱图

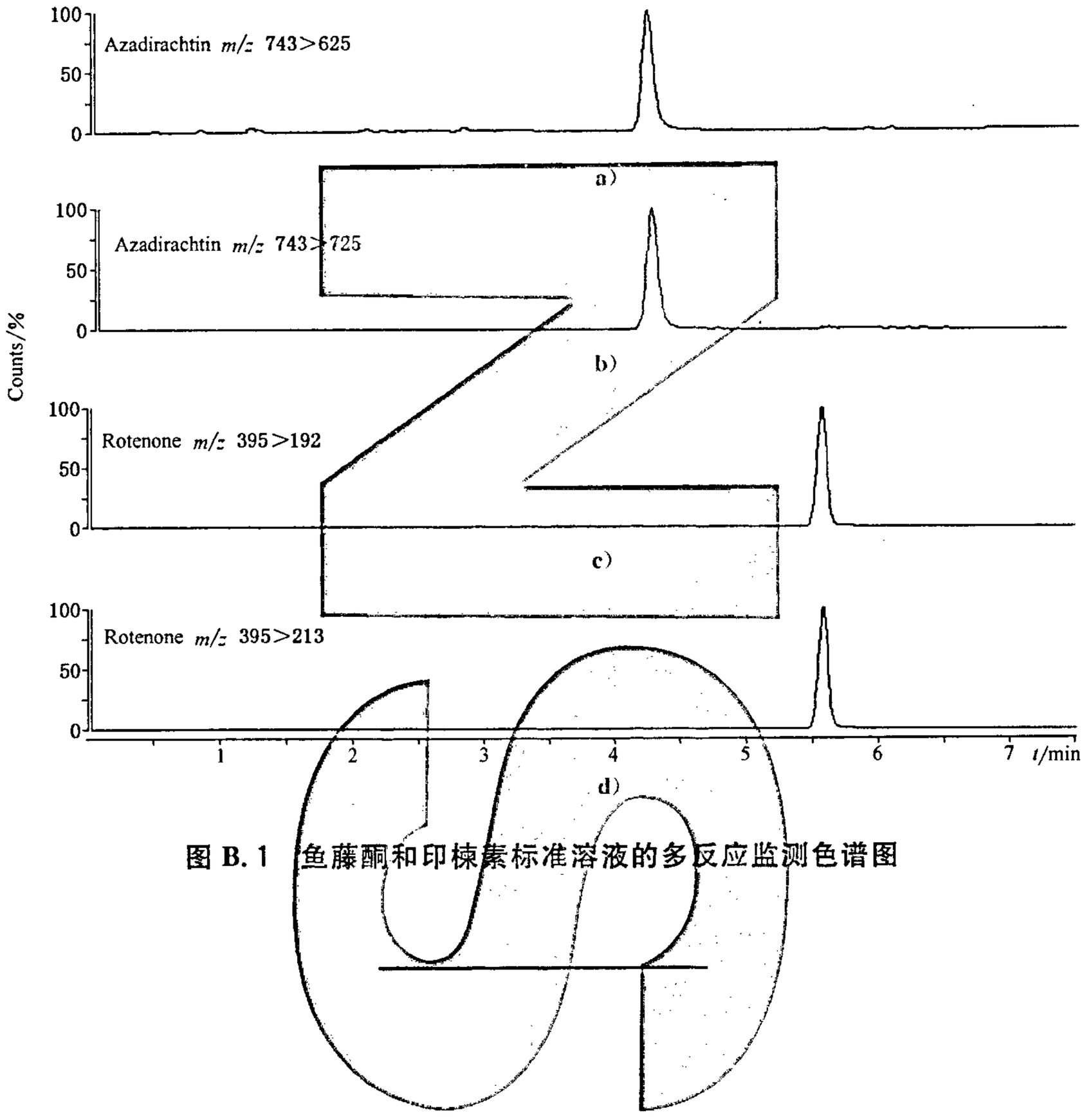


图 B.1 鱼藤酮和印楝素标准溶液的多反应监测色谱图

# Foreword

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

Please note that some of the content of this document may involve patent, The publisher of this document does not assume responsibility for identifying these patents.

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

This standard is drafted by Fujian Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Chinese Academy Inspection and Quarantine.

The main drafters of this standard are Yang Fang, Zhang Feng, Tong Yugui, Liu Zhengcai, Zhao Jianhui and Zhiming Xue.

# Determination of rotenone and azadirachtin residues in food for export—LC-MS/MS method

## 1 Scope

The standard specifies the method of determination of rotenone and azadirachtin residue in foodstuffs for export by liquid chromatography-tandem mass spectrometry method.

The method is applicable to determine rotenone and azadirachtin residue in rice, cauliflower, apple, mushroom, tea, honey, liver, fish, shrimp and chicken.

## 2 References

This standard refer to the following standards and the latest versions are recommended:

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

## 3 Principle

Extraction of the rotenone and azadirachtin residue with acetonitrile. After salting-out of acetonitrile from aqueous solution with sodium chloride, the extracts were defatted using *n*-hexane and then purification with a polymer solid phase extraction(SPE) cartridge. The rotenone and azadirachtin residues were determined by liquid chromatography-tandem mass spectrometry.

## 4 Reagents and Materials

Unless otherwise specified, all regents used are A. R. grade, and water is the first grade according to GB/T 6682.

**4.1** Acetonitrile: HPLC grade.

**4.2** *n*-Hexane: HPLC grade.

**4.3** Methanol: HPLC grade

**4.4** Formic acid: HPLC grade.

**4.5** Sodium Chloride.

**4.6** Ammonium acetate.

**4.7** Sodium bicarbonate.

**4.8** Saturated sodium bicarbonate solution: Weigh appropriate amount of sodium bicarbonate, dissolve in water till saturation.

**4.9** 5 mmol/L ammonium acetate: Weigh 0.38 g ammonium acetate in 800 mL water, piping 2 mL formic acid, and then adding water to a volume of 1 000 mL.

**4.10** Standard of rotenone and azadirachtin: Rotenone molecular formula $C_{15}H_8C_{12}FNO$, CAS No. 124495-18-7, molecular weight 308.14; Azadirachtin molecular formula $C_{35}H_{44}O_{16}$, CAS No. 11141-17-6, molecular weight 720.71, purity≥98%.

**4.11** Stock solutions of rotenone and azadirachtin (100 mg/L): Accurately weigh rotenone and azadirachtin standard material, dissolve with methanol to a volume of 100 mL, and store at approximately 4 ℃ in darkness for a maximum period of 1 months.

**4.12** Calibration solutions of rotenone and azadirachtin: Dilute appropriate volume of stock solutions to a intended concentration with acetonitrile-water (2+8, *V*/*V*), and prepare freshly.

**4.13** Polymer solid phase extraction catridge: 60 mg, 3 mL.

**4.14** Filter membrane: 0.22 μm, nylon syringe filter.

## 5 Apparatus

**5.1** Liquid chromatography-tandem mass spectrometry: Equipped with electrospray (ESI) LC interface.

**5.2** Electronic balance: Accuracy 0.1 mg and 0.01 g, respectively.

**5.3** Centrifuge: 4 000 r/min.

**5.4** Grinder.

**5.5** Tissues homogenizer.

**5.6** Vortex mixer.

**5.7** Ultrasonic machine.

5.8 Solid phase extraction equipment.

5.9 Pressured gas blowing concentrator.

## 6 Sample preparation and storage

### 6.1 Sample preparation

#### 6.1.1 Fruits and vegetables

Collect *ca* 500 g the representative samples the edible portions are cut up(without washing by water) and mixed well with a tissure homogenizer,divide the prepared samples,seal in two clean containers and label.

#### 6.1.2 Tea,grains and nuts

Collect *ca* 500 g the representative samples and crush with a grinder,let them pass through a 20 mesh sieve,divide the prepared samples,eal in two clean containers and label.

#### 6.1.3 Meats and Meat products

Collect *ca* 500 g the representative samples and the edible portions are mixed well with a tissure homogenizer,divide the prepared samples,seal in two clean containers and label.

#### 6.1.4 Honey

Collect *ca* 500 g the representative samples,the crystalline sample should be melted with water bath under 60 ℃. Divide the prepared sample into two sample bottles,seal and label.

### 6.2 Sample storage

Samples such tea,wine,honey and grains store at 0 ℃～4 ℃,samples such as vegetables,fruits,meats and meat products store at －18 ℃. In the course of sample preparation,precautions must be taken to avoid contamination or any factors,which may cause the change of residue content.

## 7 Method of Determination

### 7.1 Extraction

**7.1.1** Tea,grains and nuts:Weigh 1 g of the prepared test samples into a 50 mL stoppered plastic centrifuge tube,add 5 mL saturated sodium bicarbonate solution(4.9)and let stand in darkness for

10 min,add 15 mL acetonitrile(4. 1),extract with ultersonic machine for 10 min,and then,transfer the supernatant to another clean tube and repeat the extraction procedure again using 10 mL acetonitrile. Collect the entire supernatants,adding ca 3 g sodium chlorium(4. 6) for salting-out and after centrifuging at 4 500 r/min for 3 min,collecting the supernatants and defatting with 5 mL *n*-hexane. Collect the acetonitrile layer and concentrate to dryness at 45 ℃,Dissolve the residues in 5 mL of methanol-water(2+8, *V*/*V*),then clean-up the extracts according to clean-up step 7. 2.

**7. 1. 2** Vegetables and fruits:Weigh 2 g of the prepared test samples into a 50 mL stoppered plastic centrifuge tube,add 15 mL acetonitrile(4. 1),extract with ultersonic machine for 10 min,and then, transfer the supernatant to another clean tube and repeat the extraction procedure again using 10 mL acetonitrile. Collect the entire supernatants,adding ca 3 g sodium chlorium(4. 6) for salting-out and after centrifuging at 4 500 r/min for 3 min,collecting the supernatants and concentrate to dryness at 45 ℃,Dissolve the residues in 5 mL of methanol-water(2+8, *V*/*V*),then clean-up the extracts according to clean-up step 7. 2.

**7. 1. 3** Honey:Weigh 2 g of the prepared test samples into a 50 mL stoppered plastic centrifuge tube,adding 5 mL saturated sodium bicarbonate solution and mixed well,then adding 15 mL acetonitrile,extract with ultersonic machine for 10 min,and then,transfer the supernatant to another clean tube and repeat the extraction procedure again using 10 mL acetonitrile. Collect the entire supernatants,adding ca 3 g sodium chlorium for salting-out and after centrifuging at 4 500 r/min for 3 min, collecting the supernatants and concentrate to dryness at 45 ℃,Dissolve the residues in 5 mL of methanol-water(2+8, *V*/*V*),then clean-up the extracts according to clean-up step 7. 2.

**7. 1. 4** Milk,Juice,and wine:weigh 2 g

**7. 1. 5** Meat and meat products:Weigh 2 g of the prepared test samples into a 50 mL stoppered plastic centrifuge tube,adding 5 mL saturated sodium bicarbonate solution(4. 9) and mixed well, Then adding 15 mL acetonitrile(4. 1),extract with ultersonic machine for 10 min,and then,transfer the supernatant to another clean tube and repeat the extraction procedure again using 10 mL acetonitrile. Collect the entire supernatants,adding ca 3 g sodium chlorium(4. 6) for salting-out and after centrifuging at 4 500 r/min for 3 min,collecting the supernatants and defatting with 5 mL *n*-hexane. Collect the acetonitrile layer and concentrate to dryness at 45 ℃,Dissolve the residues in 5 mL of methanol-water(2+8, *V*/*V*),then clean-up the extracts according to clean-up step 7. 2.

## 7. 2 Clean-up

Condition a polymer SPE column with 3 mL methanol and 3 mL water,apply the solution to the column and elute the column with 5 mL water,discard the effluent. Elute the column with 5 mL acetonitrile. Collect the entire volumn eo effluent and concentrate to dryness at 45 ℃ under a strean of nitrogen and redissolve in l mL of 20% acetonitrile-water,filtered on a 0. 22 μm nylon springe filter for HPLC-MS/MS analysis.

### 7.3 Determination by liquid chromatography-tandem mass spectrometry

#### 7.3.1 LC operation conditions

7.3.1.1 Column:Phenomenex Luna $C_{18}$ column,150 mm×2.0 mm(i.d.),3 μm,or equivalent.

7.3.1.2 Column temperature:35 ℃.

7.3.1.3 Mobile phase:acetonitrile-5 mmol ammonium acetate buffer (4.9)(35+65,*V*/*V*).

7.3.1.4 Flow rate:400 μL/min.

7.3.1.5 Injection volume:10 μL.

#### 7.3.2 MS operation conditions

7.3.2.1 Ion source:ESI,positive ionisation mode.

7.3.2.2 Scan mode:multiple reaction monitoring(MRM) mode.

7.3.2.3 Other reference mass operating conditions are listed in table A. 1 in Annex A.

#### 7.3.3 LC-MS detection

Prepare standard solutions containing rotenone and azadirachtin at appropriate concentrations according to the analyte in sample extracts. The referenced retention times for rotenone and azadirachtin are 5.6 min and 4.2 min. Annex B are the reconstituted ion chromatograms of rotenone and azadirachtin standard solution.

#### 7.3.4 Confirmation test

The qualification ions of the analyze must be found,and at least include one precursor ion and two daughter ions. For the same analysis batch and the same analyze,the variation range of the ion ratio between the two daughter ions for the unknown samples and the standard working solutions at the similar concentration can not be out of range of table 1 under the same determination conditions.

**Table 1—Maximum permitted tolerances for relative ion intensities**

| Relative intensity(of base peak)/% | >50 | >20~50 | >10~20 | ≤10 |
|---|---|---|---|---|
| Maximum permitted tolerances for relative ion intensities/% | ±20 | ±25 | ±30 | ±50 |

### 7.4 Blank test

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

## 8 Calculation and expression of the result

Calculate the concentration of the rotenone and azadirachtin residue in sample according to the following equation:

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

Where:

$X$ —the residues content of rotenone or azadirachtin in the test sample, μg/kg;

$c$ —the concentration of rotenone or azadirachtin gotten from the calibration curve, μg/L;

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

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

## 9 Limit of determination and recovery

### 9.1 Limit of determination

The limit of determination of rotenone is 0.5 μg/kg. The limit of determination of azadirachtin is 2 μg/kg.

### 9.2 Recovery

The recovery data of rotenone and azadirachtin residues determined by HPLC -MS/MS is listed in table 2.

Table 2—Recovery for rotenone and azadirachtin residue

| Matrix | Rotenone | | Azadirachtin | |
|---|---|---|---|---|
| | Spiked level μg/kg | Recovery % | Spiked level μg/kg | Recovery % |
| rice | 0.5 | 83.2～104.5 | 2 | 93.3～103.2 |
| | 1 | 86.2～92.9 | 4 | 87.8～98.6 |
| | 5 | 81.8～93.0 | 20 | 82.3～112.3 |
| caulifolwer | 0.5 | 86.6～106.5 | 2 | 82.9～95.0 |
| | 1 | 90.1～102.4 | 4 | 80.2～112.6 |
| | 5 | 94.9～100.3 | 20 | 94.9～100.3 |
| apple | 0.5 | 81.4～102.8 | 2 | 81.4～102.8 |
| | 1 | 83.2～98.0 | 4 | 86.6～106.5 |
| | 5 | 86.6～98.4 | 20 | 90.1～102.4 |
| mushroom | 0.5 | 89.6～108.2 | 2 | 94.9～100.3 |
| | 1 | 84.2～104.4 | 4 | 81.4～102.8 |
| | 5 | 88.9～104.8 | 20 | 83.5～98.0 |
| tea | 0.5 | 77.2～102.8 | 2 | 78.6～108.4 |
| | 1 | 79.2～98.2 | 4 | 89.6～108.2 |
| | 5 | 82.8～97.2 | 20 | 96.3～108.8 |
| honey | 0.5 | 84.6～107.6 | 2 | 79.2～107.5 |
| | 1 | 86.2～100.3 | 4 | 80.4～84.4 |
| | 5 | 81.9～94.8 | 20 | 81.1～93.3 |
| liver | 0.5 | 79.4～102.8 | 2 | 81.6～86.8 |
| | 1 | 91.9～99.7 | 4 | 79.9～88.3 |
| | 5 | 84.8～95.6 | 20 | 82.4～100.9 |
| fish | 0.5 | 84.6～107.6 | 2 | 74.6～106.5 |
| | 1 | 92.6～105.4 | 4 | 76.3～101.5 |
| | 5 | 78.6～83.6 | 20 | 95.7～114.1 |
| shrimp | 0.5 | 89.5～95.9 | 2 | 88.9～97.9 |
| | 1 | 83.6～95.7 | 4 | 93.0～108.5 |
| | 5 | 85.7～104.1 | 20 | 76.4～91.9 |
| chicken | 0.5 | 88.9～97.9 | 2 | 86.4～95.3 |
| | 1 | 83.0～108.5 | 4 | 81.0～94.8 |
| | 5 | 83.4～91.9 | 20 | 95.7～114.1 |

# Annex A[1)]
# (Informative)
# Reference mass conditions

A.1 Capillary voltage:4 kV.

A.2 Sheath gas Temperature:320 ℃.

A.3 Sheath gas flow:10 L/min.

A.4 Drying gas flow:3 L/min.

A.5 Nebulizer pressure:50 psi.

A.6 Other mass operating conditions are listed in table A.1.

**Table A.1—Main Mass parameters of rotenone and azadirachtin**

| Compounds | MRM Transition | Dwell time ms | Voltage V | Collision energy eV |
|---|---|---|---|---|
| Rotenone | **395>213** | 50 | 160 | 24 |
| | 395>192 | | | 25 |
| Azadirachtin | **743>725** | 50 | 140 | 28 |
| | 743>625 | | | 36 |
| **Note:** The major MRM transition for quantification is indicated in bold font. | | | | |

1) Declaration for non-commercial: The reference mass conditions listed in annex A are performed on Agilent 6 460 mass spectrum. The type of the equipment mentioned here is only for reference and not for commercial purpose. Encourage users to try different manufactures or models of equipments.

# Annex B
(Informative)
MRM chromatogram of rotenone and azadirachtin standard solution

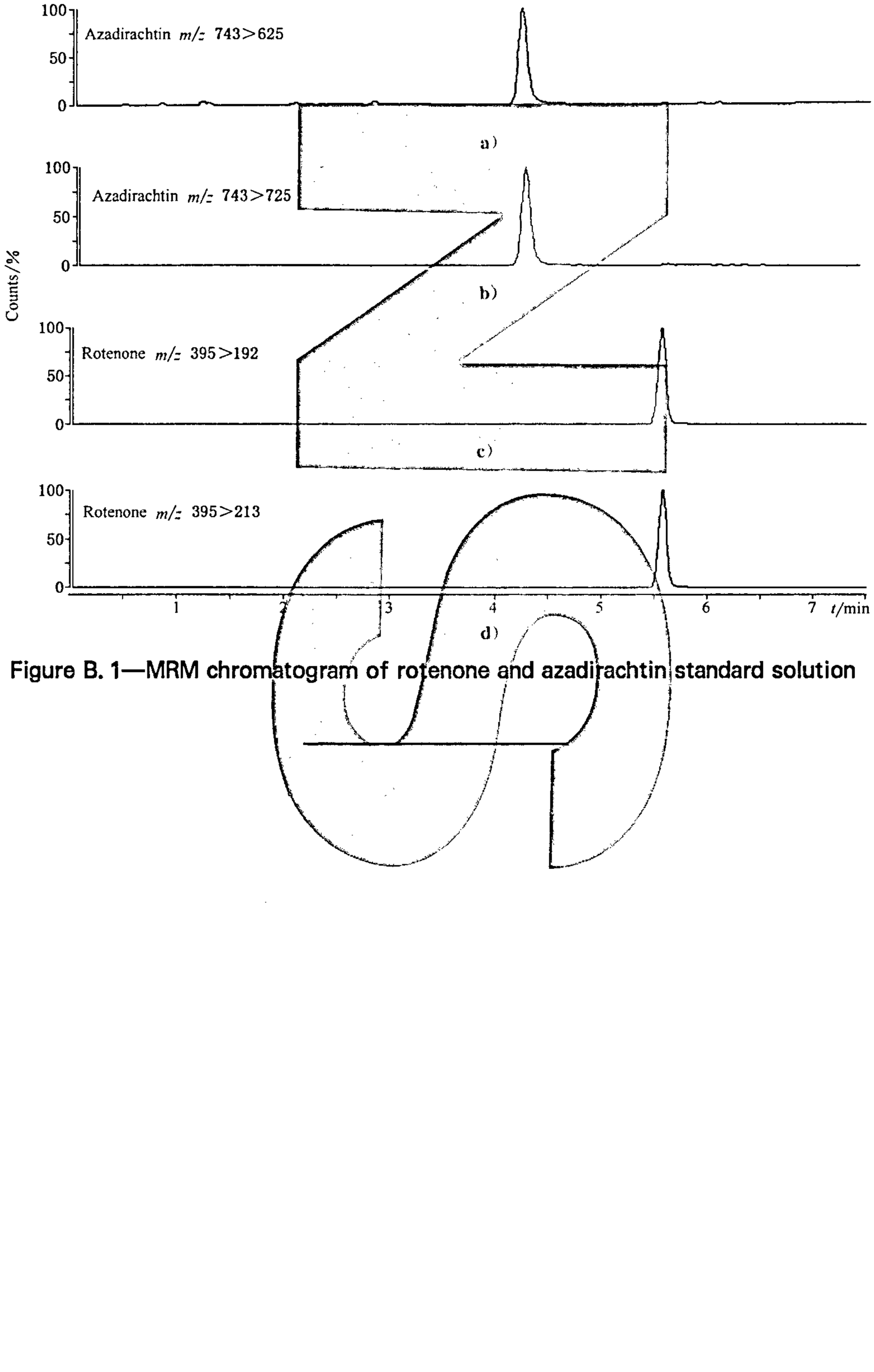


**Figure B. 1—MRM chromatogram of rotenone and azadirachtin standard solution**