SN

# 中华人民共和国出入境检验检疫行业标准

SN/T 2224—2008

# 进出口动物源性食品中利福西明残留量检测方法 液相色谱-质谱/质谱法

**Determination of rifaximin residue in foodstuffs of animal origin for export and import—LC-MS/MS method**

2008-11-18 发布　　2009-06-01 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均为资料性附录。

本标准由国家认证认可监督管理委员会提出并归口。

本标准由中华人民共和国辽宁出入境检验检疫局、中华人民共和国黑龙江出入境检验检疫局负责起草。

本标准主要起草人：李一尘、林维宣、康庆贺。

本标准系首次发布的出入境检验检疫行业标准。

# 进出口动物源性食品中利福西明残留量检测方法 液相色谱-质谱/质谱法

## 1 范围

本标准规定了动物源性食品中利福西明残留量的液相色谱-质谱/质谱检测方法。

本标准适用于猪肉、猪肝、猪肾、牛奶中利福西明残留量的检测和确证。

## 2 规范性引用

下列文件中的条款通过本标准的引用而成为本标准的条款。凡是注日期的引用文件，其随后所有的修改单(不包括勘误的内容)或修订版均不适用于本标准，然而，鼓励根据本标准达成协议的各方研究是否可使用这些文件的最新版本。凡是不注明日期的引用文件，其最新版本适用于本标准。

GB/T 6682 分析实验室用水规格和试验方法。(GB/T 6682—2008，ISO 3696:1987，MOD)

## 3 方法提要

样品中利福西明残留物用乙腈提取，正己烷除去提取液中脂肪，固相萃取柱净化，洗脱液氮气吹干后，用液相色谱串联质谱测定，外标法定量。

## 4 试剂和材料

除另有说明外，所用试剂均为分析纯，水为 GB/T 6682 规定的一级水。

4.1 乙腈：高效液相色谱级。

4.2 正己烷：高效液相色谱级。

4.3 异丙醇。

4.4 甲酸：高效液相色谱级。

4.5 无水硫酸钠：650 ℃灼烧 4 h，置于干燥器中备用。

4.6 乙腈饱和正己烷：100 mL 乙腈中加入 100 mL 正己烷，充分振荡后，静置分层，取上层液体。

4.7 0.1%甲酸溶液：准确量取 1 mL 甲酸(4.4)于 1 L 容量瓶中，用水定容至 1 L。

4.8 乙腈-水溶液(40+60，体积比)：量取 40 mL 乙腈加入 60 mL 水中混匀。

4.9 利福西明标准品(Rifaximin，CAS No. 80621-81-4)：纯度大于等于 95%。

4.10 利福西明标准储备溶液：称取约 0.01 g 利福西明标准品(精确至 0.000 1 g)，用乙腈溶解定容至 100 mL，此标准溶液浓度为 100 μg/mL，置于－18 ℃冰箱避光保存。保存期半年。

4.11 利福西明标准工作溶液(1.0 μg/mL)：吸取适量的利福西明标准储备溶液(4.10)，用乙腈配成 1.0 μg/mL的标准工作溶液。标准工作溶液置于 4 ℃避光贮存。

4.12 固相萃取小柱：Waters oasisHLB 柱，500 mg，6 mL，或相当者。

4.13 微孔滤膜：0.45 μm，有机相。

## 5 仪器和设备

5.1 高效液相色谱-质谱/质谱仪：配有电喷雾离子源。

5.2 组织捣碎机。

5.3 分析天平:感量分别为0.1 mg和0.01 g。

5.4 旋转蒸发器。

5.5 固相萃取装置。

5.6 离心机:4 000 r/min。

5.7 旋涡混合器。

5.8 均质器。10 000 r/min。

## 6 样品制备与保存

### 6.1 猪肉、猪肝和猪肾

取约500 g代表性样品,用组织捣碎机充分捣碎,均分成两份,分别装入洁净容器中,密封,并标明标记。

### 6.2 牛奶

所取约500 g样品,充分均匀,均分成两份,分别装入洁净容器中,密封,并标明标记。

### 6.3 样品保存

猪肉、猪肝和猪肾试样于−18 ℃以下冷冻避光存放。牛奶试样于0 ℃～4 ℃冷藏避光存放。

在制样的操作过程中,应防止样品受到污染或发生残留物含量的变化。

## 7 测定步骤

### 7.1 提取

称取5 g试样(精确至0.01 g)于50 mL具塞离心管中,加入5 g无水硫酸钠(4.5)和20 mL乙腈(4.1),均质30 s,4 000 r/min离心5 min,将上清液转移至另一50 mL离心管中,用玻棒捣碎离心管中的沉淀。加入10 mL乙腈提取,旋涡混合1 min,4 000 r/min离心5 min,合并上清液。加入10 mL乙腈饱和正己烷(4.6),振荡5 min,静置10 min。收集下层液体于100 mL浓缩瓶中,加入10 mL异丙醇,40 ℃旋转蒸发至干,用3 mL乙腈溶解,待净化。

### 7.2 净化

用5 mL乙腈淋洗固相萃取柱(4.12),将提取液(7.1)上柱,控制洗脱流速为1 mL/min,收集流出液于10 mL试管中。再用5 mL乙腈洗柱,控制洗脱流速为1 mL/min,合并洗脱液,于45 ℃下氮气吹干,用乙腈水溶液(4.8)溶解定容至1.0 mL。过0.45 μm滤膜,供液相色谱-质谱/质谱测定。

### 7.3 利福西明基质校准标准溶液的配制

称取七个基质空白样品(称样量为5 g)于50 mL具塞离心管中,其中六个空白样品中分别加入适量利福西明标准工作溶液(4.11),按照样品操作步骤7.1、7.2同步操作,使最终样品溶液中利福西明的浓度分别为10 ng/mL、50 ng/mL、100 ng/mL、200 ng/mL、250 ng/mL、500 ng/mL。所得系列利福西明基质标准校准溶液即配即用,用以制作标准曲线。

### 7.4 测定

#### 7.4.1 液相色谱条件

7.4.1.1 色谱柱:Agilent $C_{18}$柱,4.6mm(内径)×150 mm,5 μm,或相当者。

7.4.1.2 流动相:A组分是0.1%甲酸溶液;B组分是乙腈。梯度洗脱程序见表1。

**表1 梯度洗脱程序**

| 时间/min | 流速/(mL/min) | 组分A/% | 组分B/% |
|---|---|---|---|
| 0.00 | 0.6 | 65.0 | 35.0 |
| 3.00 | 0.6 | 5.0 | 95.0 |
| 5.00 | 0.6 | 5.0 | 95.0 |

表 1（续）

| 时间/min | 流速/(mL/min) | 组分 A/% | 组分 B/% |
|---|---|---|---|
| 5.01 | 0.6 | 65.0 | 35.0 |
| 10.00 | 0.6 | 65.0 | 35.0 |

7.4.1.3 流速：0.6 mL/min。

7.4.1.4 进样量：20 μL。

7.4.2 质谱条件

7.4.2.1 离子源：电喷雾离子源。

7.4.2.2 扫描方式：正离子扫描。

7.4.2.3 检测方式：多反应监测。

7.4.2.4 雾化气、气帘气、辅助气、碰撞气均为高纯氮气；使用前应调节个参数使质谱灵敏度达到检测要求，质谱参考条件参见附录 A。

7.4.3 定性测定

在相同试验条件下，样品中待测物质与同时检测的浓度相近的基质校准标准溶液具有相同的保留时间，并且所选择的离子相对丰度比相一致或在表 2 规定的允许偏差范围内，则可判断样品中存在对应的被测物。在上述实验条件下，利福西明的保留时间约为 5.44 min，定性离子对(m/z)为 786.5/754.4；786.5/151.0，定量离子对 786.5/754.4，参考离子相对丰度比(%)分别为 100.00；23.8，相应的谱图参见附录 B。

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 |

7.4.4 定量测定

在仪器最佳工作条件下，对利福西明的基质校准标准溶液分别进样，以峰面积为纵坐标，系列基质校准标准溶液浓度为横坐标绘制标准工作曲线，用标准工作曲线对样品进行定量，样品溶液中待测物的响应值均应在仪器测定的线性范围内。

7.4.5 空白试验

除不加试样外，均按上述操作步骤进行。

## 8 结果计算

按式(1)计算试样中利福西明残留量：

$$X = \frac{(c - c_0) \cdot V}{m} \qquad \cdots\cdots(1)$$

式中：

$X$——试样中利福西明残留量，单位为微克每千克(μg/kg)；

$c$——从标准曲线中得到的被测组分溶液浓度，单位为纳克每毫升(ng/mL)；

$c_0$——从标准曲线中得到的空白试验中被测组分的溶液浓度，单位为纳克每毫升(ng/mL)；

$V$——样品溶液最终定容体积，单位为毫升(mL)；

$m$——最终样液所代表试样质量，单位为克(g)。

## 9 测定低限和回收率

### 9.1 测定低限

本方法的测定低限为 10 μg/kg。

## 9.2 回收率

不同样品基质添加浓度及回收率范围见表3。

**表3 利福西明的回收率**

| 化合物 | 添加浓度/(μg/kg) | 回收率/% | | | |
|---|---|---|---|---|---|
| | | 猪肉 | 猪肝 | 猪肾 | 牛奶 |
| 利福西明 | 10 | 72.3～86.4 | 73.5～89.3 | 73.9～87.5 | 71.5～88.7 |
| | 20 | 71.0～93.5 | 72.5～94.0 | 72.5～87.5 | 71.6～91.6 |
| | 50 | 73.8～85.2 | 74.2～94.8 | 78.0～92.4 | 77.2～93.2 |

# 附 录 A
（资料性附录）
质谱参考条件参数[1)]

质谱参考条件参数：

a） 电喷雾电压(IS)：5 500 V；

b） 雾化气压力(GS1)：310.275 kPa(45 psi)；

c） 气帘气压力(CUR)：137.9 kPs(20 psi)；

d） 辅助气压力(GS2)：379.225 kPa(55 psi)；

e） 离子源温度(TEM)：650 ℃；

f） 利福西明的定性离子对、定量离子对、去簇电压(DP)、碰撞气能量(CE)及碰撞室出口电压(CXP)见表 A.1。

**表 A.1 定性离子对、定量离子对、去簇电压、碰撞气能量和碰撞室出口电压**

| 组分名称 | 定性离子对 m/z | 定量离子对 m/z | 去簇电压 (DP)/V | 碰撞能量 (CE)/V | 碰撞室出口电压 (CXP)/V |
|---|---|---|---|---|---|
| 利福西明 | 786.5/754.4 | 786.5/754.4 | 90 | 33 | 10 |
| | 786.5/151.0 | | 90 | 50 | 10 |

1） 非商业性声明：附录 A 所列参数是在 API4000 质谱仪完成的，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不同厂家和型号的仪器。

# 附　录　B
（资料性附录）
## 利福西明标准品的多反应监测色谱图

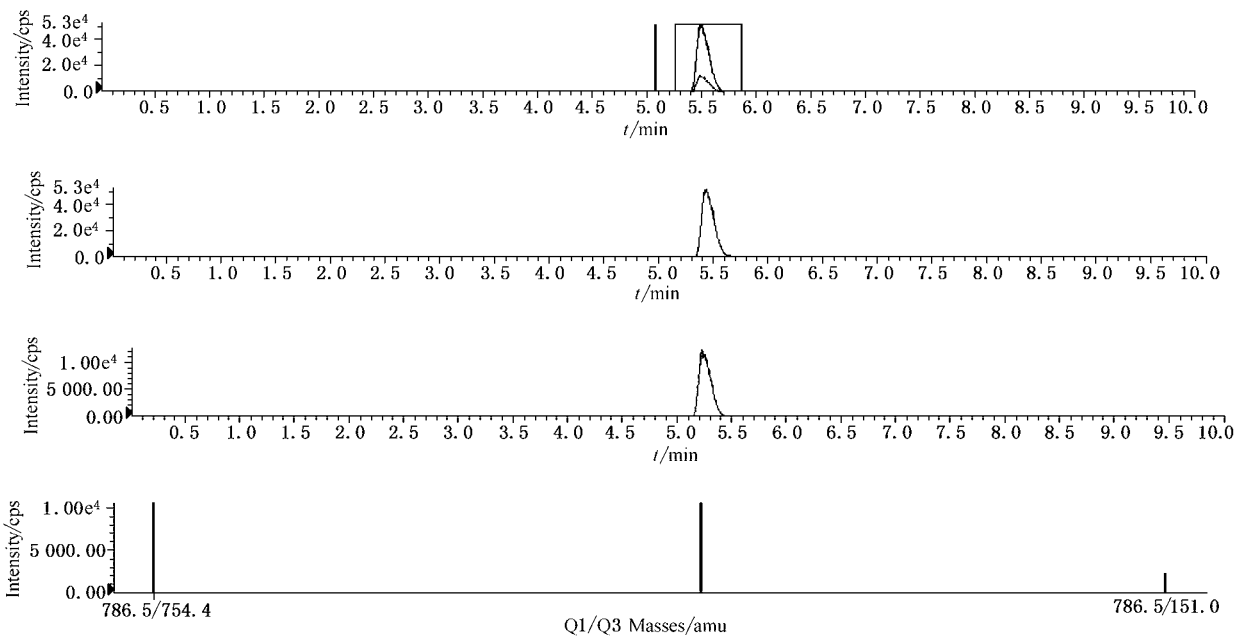


**图 B.1　利福西明标准品的多反应监测色谱图**

# 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 administation of the People's Republic of China.

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

The standard was mainly drafted by Yichen Li，Weixuan Lin，Qinghe Kang.

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

# Determination of rifaximin residue in foodstuffs of animal origin for export and import—LC-MS/MS method

## 1 Scope

The standard specifies the methods of determination by LC-MS/MS of Rifaximin residue in foodstaffs of animal origin.

This standard is applicable to the determination of Rifaximin residue in swine, liver, kidney and milk.

## 2 Reference

For the files with an indication of date, the sequencely modified versions (disincluding the contents of errata) or revised versions of them would not be applicable to this standard. However, it is encouraged for those have reached an agreement according to this standard to research whether to use the latest version of these documents. For the files without an indication of date, their lastest versions would be applicable to this standard.

GB/T 6682 Specification and test method for water used in analytical lab (GB/T 6682—2008, ISO 3696:1987, MOD)

## 3 Method of determination

Rifaximin residues are extracted from the sample with acetonitrile. It is defatted with n-hexane and cleaned up with $C_{18}$ column. Finally it is determined by LC-MS/MS. External standard method is used.

## 4 Reagents and materials

Unless otherwise specified, all the reagent is analystic grade and "water" is deionized water.

4.1 Acetonitrile: HPLC grade.

4.2 *n*-Hexane: HPLC grade.

4.3 Isopropanol.

4.4 Formic acid: HPLC grade.

4.5 Anhydrous sodium sulphate: Ignite for 4 h at 650 ℃, cool to room temperature in desiccator and keep in a tightly closed container.

4.6 *n*-hexane saturated with acetonitrile: Add 100 mL *n*-hexane into 100 mL acetonitrile, mix adequately, then wait for delamination, use the substrate layer.

4.7 0.1% formic acid solution: Add 1 mL formic acid(4.4) into 1 L volumetric flask, then dilute to 1 L with water.

4.8 Acetonitrile-water: (40+60, *V*/*V*).

4.9 Rifaximin(CAS No. 80621-81-4): Purity≥95%.

4.10 Stock standard solution: accurately weigh 0.01 g standard, dissolve with Acetonitrile and quantitatively on 100 mL volumetric flask individually, the concentration of solutions are 100 μg/mL. It should be stored at −18 ℃ in refrigerator.

4.11 Working standard solution: 1.0 μg/mL. Certain amount of rifaximin stock solution of rifaximin(4.10) was diluted with Acetonitrile into a kind of rifaximin working solution which concentration is 1.0 μg/mL. The solution could be available if it is stored at 4 ℃ in refrigerator avoiding light.

4.12 Oasis HLB SPE cartridge: 6 mL, 500 mg, or equivalent.

4.13 Film: 0.45 μm, Organic phase.

## 5 Apparatus and equipment

5.1 liquid chromatograhy with electrospray ionization mass spectrometry.

5.2 Meat Mixer.

5.3 Balance whose sensitivity should be 0.1 mg or 0.01 g.

5.4 Rotary vacuum evaporator.

5.5 SPE.

5.6 Centrifuge. 4 000 r/min.

5.7 Vortex mixer.

5.8 Homogenizer 10 000 r/min.

## 6 Preparation and storage of test sample

### 6.1 Swine,liver,kidney

Take the representative portions from the whole primary sample. It is about 500 g and ground in a blender. Keep the prepared sample into two sample bottles,seal and label. The test sample is stored in −18 ℃ refrigerator.

### 6.2 Milk

Take the representative portions from the whole primary sample. It is about 500 g and ground in a blender. Keep the prepared sample into two sample bottles,seal and label. The test sample is stored in 4 ℃ refrigerator.

### 6.3 Storage of test sample

Swine,liver and kidney are stored in −18 ℃ refrigerator. Milk is stored in 4 ℃ refrigerator. It is important for the detecting analyte to avoiding pollution and keep its concentration without varied during the whole storing period.

## 7 Procedure

### 7.1 Extraction

Weigh ca 5.00 g of the test sample into a 50 mL centrifugal tube,add 5 g anhydrous sodium sulphate (4.5),add 20 mL acetonitrile. Homogenize for 30 s. Then Centrifugal 5 min at 4 000 r/min. Then displace the upper clear solution into a 50 mL centrifugal tube. Add 10 mL acetonitrile into another centrifugal tube and then wash the reamer of homogenizer. Triturate the deposition in centrifugal tube with glass stick. After adding solution which was used for washing the reamer of homogenizer, surge in vortex vibrating implement for 1 min,centrifugal separate in 4 000 r/min for 5 min and displace the upper clear solution into the 50 mL. centrifugal tube too. Add 10 mL n-hexane saturated with acetonitrile(4.6),shake for 5 min and then wait for 10 min. Collect the substrate layer. Add 10 mL isopropanol and evaporate to nearly dryness in 40 ℃ water bath under vacuum. Dissolve the residue with 3 mL aceonitrile,preparing cleaning up.

### 7.2 Clean up

Through the solid extract pillar which has been pretreated with the rate of 1 mL/min,collect the

fraction. Then elute with 5 mL of acetonitrile,collect the elution. Concetrate the collection at 45 ℃ under vacuum,dissolve the residue with 1 mL of solution(4.8). Filter the sample solution through a syringe filter for determination and confirmation of HPLC-MS/MS.

7.3 Preparation of Tiamulin standard solution calibrated by Matrix

Weigh seven negative matrix samples whose weight is 5 g respectively into 50 mL centrifuge tube, and spike a certain amount of rifaximin working solution(4.12) into six of them. Then process these samples according to the analytic steps 7.1 and 7.2 as the procedure mensioned above to prepare the standard solutions which concentration are 10 ng/mL,50 ng/mL,100 ng/mL,200 ng/mL,250 ng/mL, 500 ng/mL respectively. The rifaximin standard solutions calibrated by Matrix are made for obtaining a standard curve and should be used after prepared timely.

7.4 Determination

7.4.1 HPLC operating conditions

7.4.1.1 Column:Agilent $C_{18}$,5 μm,250 mm×4.6 mm(i.d.),or the equivalent.

7.4.1.2 Mobile phase:A:0.1% formic acid solution;B:Acetonitrile. See table 1.

Table 1—Gradient of mobile phase

| Time/min | Rate/(mL/min) | A/% | B/% |
|---|---|---|---|
| 0.00 | 0.6 | 65.0 | 35.0 |
| 3.00 | 0.6 | 5.0 | 95.0 |
| 5.00 | 0.6 | 5.0 | 95.0 |
| 5.01 | 0.6 | 65.0 | 35.0 |
| 10.00 | 0.6 | 65.0 | 35.0 |

7.4.1.3 Flow rate:0.6 mL/min.

7.4.1.4 Injection volume:20 μL.

7.4.2 Mass spectral acquisition

7.4.2.1 Source:ESI.

7.4.2.2 Polarity:Positive.

7.4.2.3 Mode:Multiple reaction monitoring.

7.4.2.4 Gas1、Gas2、Curtain gas are nitrogen. See annex A.

7.4.3 Confirmation of LC-MS/MS

Under the same LC-MS/MS conditions, the standard working solutions and the test sample solutions are injected. If the retention time and the relative abundance ratios for the precursor and the product ion pairs of the test sample solution match that of the standard working solution which has calibrated by matrix within a proper range respectively(see table 2), the corresponding analyte must be presented in the test samples. At the test conditions montioned above, the retention time of rifaximin is about 5.44 min; the precursor and the product ion pairs(m/z) for qualification are 786.5/754.4 and 786.5/151.0 respectively, the precursor ion pairs(m/z) for quantitation is 786.5/754.4, and the relative abundance ratio(%) of them are about 100.00, and 23.8 respectively. Those ralative graphically information was presented in Annex B.

Table 2—Maxium allowable deviation of relative abundance ratio while confirmation

| Relative abundance ratio/% | >50 | >20～50 | >10～20 | ≤10 |
|---|---|---|---|---|
| Maximum allowable deviation/% | ±20 | ±25 | ±30 | ±50 |

7.4.4 Quantitation of LC-MS/MS

Under the optimal condition of LC-MS/MS, a serial of standard solutions of rifaximin which have been calibrated by matrix is injected to obtain a standard working curve whose y-axis represents peak area and x-axis the concentration. The analyte concentration in the test samples solution should be quantified depending on the curve and its response value should be within the linear range of the instrument.

7.4.5 Blank test

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

## 8 Calculation and expression of result

Calculation the content of rifaximin residue in the test sample according to the formula(1).

$$X = \frac{(c - c_0) \cdot V}{m} \qquad \cdots\cdots(1)$$

Where:

$X$—the residue content of tiamulin in the test sample, μg/kg;

$c$—the rifaximin concentration of test sample obtained from the standard working curve, ng/mL;

$c_0$—the rifaximin concentration of test sample in the blank test obtained from the standard; working curve, ng/mL;

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

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

## 9 Limit of quantification(LOQ) and recovery

### 9.1 Limit of quantification

The limit of rifaximin is 10.0 μg/kg.

### 9.2 Recovery

According to the experimental data,the corresponding recoveries of fortifying concentrations are in Table 3.

Table 3—Recovery

| | Spike/(μg/kg) | Recovery/% | | | |
|---|---|---|---|---|---|
| | | Swine | Liver | Kidney | Milk |
| Rifaximin | 10 | 72.3～86.4 | 73.5～89.3 | 73.9～87.5 | 71.5～88.7 |
| | 20 | 71.0～93.5 | 72.5～94.0 | 72.5～87.5 | 71.6～91.6 |
| | 50 | 73.8～85.2 | 74.2～94.8 | 78.0～92.4 | 77.2～93.2 |

# Annex A
(informative annex)
Mass spectral acquisition[1)]

Mass spectral acquisition:

a) IS:5 500 V;

b) GS1:310.275 kPa(45 psi);

c) CUR:137.9 kPa(20 psi);

d) GS2:379.225 kPa(55 psi);

e) TEM:650 ℃;

f) Transitions for confirmation and quantification of rifaximin DP,CE,CXP sees table A.1.

Table A.1—Transitions for confirmation and quantification of rifaximin DP,CE,CXP

| Compound | Transitions for confirmation m/z | Transitions for quantification m/z | DP/V | CE/V | CXP/V |
|---|---|---|---|---|---|
| rifaximin | 786.5/754.4 | 786.5/754.4 | 90 | 33 | 10 |
| | 786.5/151.0 | | 90 | 50 | 10 |

1) The parameter listed in annex A is obtained from the instrument of API 4000 mass spectrometry. The instrument type amentioned above is aimed to proving some reference for user and do not involve in the commercial activity.

# Annex B
（informative annex）
MRM Chromatograms

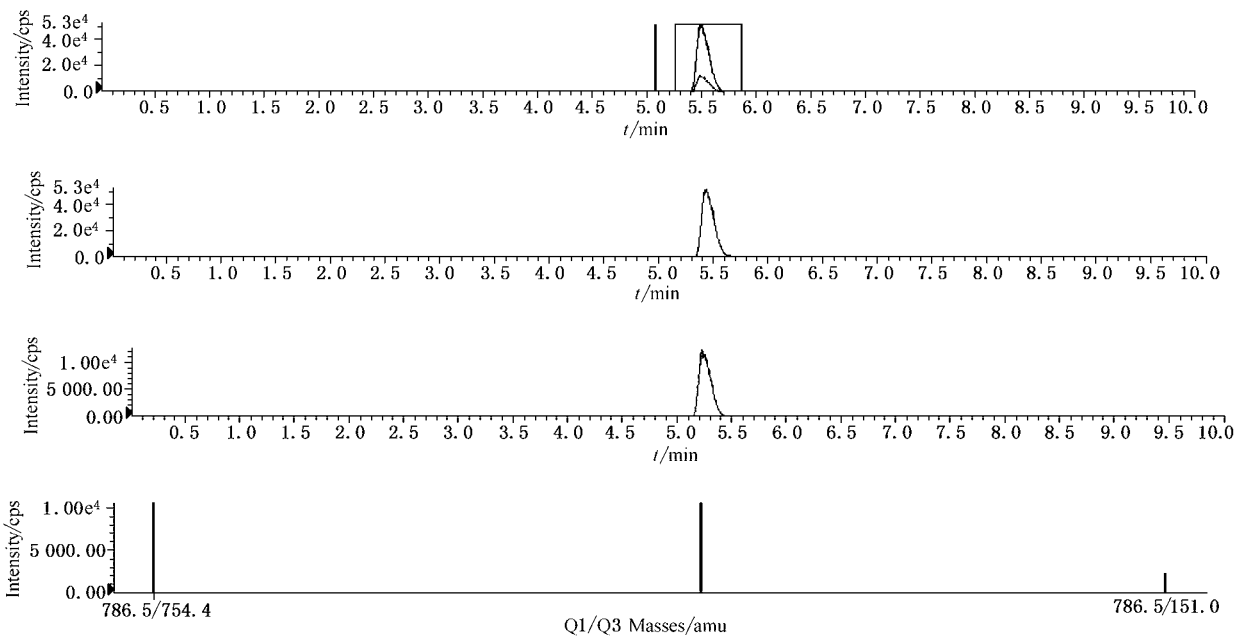


Figure B.1—Selected ion chromatograms of rifaximin standards(MRM)

中华人民共和国出入境检验检疫
行 业 标 准
**进出口动物源性食品中利福西明残留量检测方法 液相色谱-质谱/质谱法**
SN/T 2224—2008
*
中 国 标 准 出 版 社 出 版
北京复兴门外三里河北街 16 号
邮政编码:100045
网址 www.spc.net.cn
电话:68523946 68517548
中国标准出版社秦皇岛印刷厂印刷
*
开本 880×1230 1/16 印张 1.25 字数 28 千字
2009 年 2 月第一版 2009 年 2 月第一次印刷
印数 1—2 000
*
书号:155066·2-19513 定价 12.00 元