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## 中华人民共和国出入境检验检疫行业标准

SN/T 4584—2016

### 出口动物源性食品中沃尼妙林和泰妙菌素 残留量的测定 液相色谱-质谱/质谱法

Determination of valnemulin and tiamulin residues in foodstuffs of  
animal origin for export—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。

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：中华人民共和国秦皇岛出入境检验检疫局。

本标准主要起草人：刘晓茂、李学民、常巧英、王飞、葛娜、杨志伟、张守军、曹彦忠、张进杰。

## 出口动物源性食品中沃尼妙林和泰妙菌素 残留量的测定 液相色谱-质谱/质谱法

### 1 范围

本标准规定了出口动物源性食品中沃尼妙林和泰妙菌素残留量的液相色谱-质谱/质谱测定方法。

本标准适用于动物肌肉组织、肝脏、鱼、蛋和奶中沃尼妙林和泰妙菌素残留量的定量测定和定性确证。

### 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

用乙腈提取试样中的沃尼妙林和泰妙菌素残留,经 MCX SPE 小柱净化,  $C_{18}$  色谱柱分离,以电喷雾离子源(ESI)正离子扫描方式,在多反应监测(MRM)模式下进行测定,外标法定量。

### 4 试剂和材料

除另有说明外,所用试剂均为优级纯,水为 GB/T 6682 规定的一级水。

4.1 甲醇:色谱纯。

4.2 乙腈:色谱纯。

4.3 甲酸。

4.4 正己烷。

4.5 氨水:25%。

4.6 氯化钠。

4.7 无水硫酸钠:650 °C 灼烧 4 h,在干燥器内冷却至室温,贮于密封瓶中备用。

4.8 甲酸-水(1+19,体积比):取 5 mL 甲酸(4.3)用水定容至 100 mL。

4.9 氨水-甲醇(1+19,体积比):取 5 mL 氨水(4.5)用甲醇(4.1)定容至 100 mL。

4.10 乙腈-水(1+3,体积比):取 25 mL 乙腈(4.2)用水定容至 100 mL。

4.11 标准物质:沃尼妙林(CAS 号:101312-92-9);纯度大于或等于 98%;泰妙菌素(CAS 号:55297-95-5);纯度大于或等于 98%。

4.12 沃尼妙林标准贮备溶液:1.0 mg/mL。称取适量的沃尼妙林标准物质,用甲醇溶解并配制成 1.0 mg/mL 的标准贮备液。避光保存于 -18 °C 冰柜中。

4.13 泰妙菌素标准贮备溶液:1.0 mg/mL。称取适量的泰妙菌素标准物质,用甲醇溶解并配制成 1.0 mg/mL 的标准贮备液。避光保存于 -18 °C 冰柜中。

4.14 沃尼妙林和泰妙菌素混合标准工作溶液:1.0 µg/mL。吸取适量沃尼妙林标准贮备溶液(4.12)和

泰妙菌素标准贮备溶液(4.13),用乙腈-水(4.10)稀释成 $1.0\text{ }\mu\text{g/mL}$ 的标准工作溶液,4℃冷藏避光保存。

4.15 MCX 固相萃取小柱:60 mg,3 mL,或相当者。

4.16 微孔滤膜: $0.22\text{ }\mu\text{m}$ ,水相。

## 5 仪器

5.1 高效液相色谱-串联质谱仪,配有电喷雾离子源(ESI)。

5.2 分析天平:感量分别为 $0.01\text{ mg}$ 和 $0.01\text{ g}$ 。

5.3 均质器: $15\text{ }000\text{ r/min}$ 。

5.4 振荡器。

5.5 离心机: $10\text{ }000\text{ r/min}$ 。

5.6 旋转蒸发器。

5.7 固相萃取装置。

5.8 真空泵:真空度应达到 $80\text{ kPa}$ 。

## 6 试样的制备与保存

### 6.1 动物肌肉组织、肝脏、鱼

从原始样品取出有代表性样品约 $500\text{ g}$ ,用组织捣碎机充分捣碎混匀,装入洁净容器内,密封作为试样,注明标记。将试样于 $-18\text{ }^{\circ}\text{C}$ 冷冻保存。试样在制备和保存过程中避免受到污染或待测物含量发生变化。

### 6.2 蛋

从原始样品取出有代表性样品约 $500\text{ g}$ ,去壳后用组织捣碎机充分混匀,装入洁净容器内。密封作为试样,注明标记。将试样置于 $4\text{ }^{\circ}\text{C}$ 冷藏避光保存。试样在制备和保存过程中避免受到污染或待测物含量发生变化。

### 6.3 奶

从原始样品取出有代表性样品约 $500\text{ g}$ ,充分混匀,装入洁净容器内。密封作为试样,注明标记。将试样置于 $4\text{ }^{\circ}\text{C}$ 冷藏避光保存。试样在制备和保存过程中避免受到污染或待测物含量发生变化。

## 7 测定步骤

### 7.1 提取

#### 7.1.1 动物肌肉组织、肝脏和鱼

称取 $2\text{ g}$ (精确至 $0.01\text{ g}$ )样品至离心管中,加入 $5\text{ g}$ 无水硫酸钠(4.7)、 $10\text{ mL}$ 乙腈,用均质器于 $10\text{ }000\text{ r/min}$ 速率下均质 $2\text{ min}$ ,振荡 $10\text{ min}$ ,以 $4\text{ }000\text{ r/min}$ 离心 $10\text{ min}$ 。上层清液转移至鸡心瓶中,用 $10\text{ mL}$ 乙腈重复上述提取、离心操作,合并两次提取的上清液,在 $40\text{ }^{\circ}\text{C}$ 下浓缩至近干,用 $5\text{ mL}$ 甲酸-水(4.8)溶解,混匀,待净化。



7.1.2 蛋和奶

称取 5 g(精确至 0.01 g)样品至离心管中,加入 2 g 氯化钠、10 mL 乙腈,振荡 20 min,以 4 000 r/min离心 5 min。取上层清液 5 mL,于鸡心瓶中,在 40 ℃下浓缩至近干,用 5 mL 甲酸-水(4.8)溶解,混匀,待净化。

7.2 净化

依次用 3 mL 氨水-甲醇(4.9)、3 mL 甲醇和 10 mL 水淋洗 MCX 固相萃取小柱(4.15),弃去淋洗液。将上述提取液过柱,再依次用 3 mL 甲酸-水(4.8)、3 mL 甲醇,3 mL 正己烷淋洗小柱,弃去全部流出液,减压抽干,最后用 5 mL 氨水-甲醇(4.9)洗脱,收集洗脱液,40 ℃氮气吹干,1 mL 乙腈-水(4.10)溶液定容,过膜待测。

7.3 基质标准溶液的制备

称取 5 份 2 g(精确至 0.01 g)阴性试样,按照 7.1 和 7.2 操作,制成基质空白溶液。在基质空白溶液中添加适量沃尼妙林和泰妙菌素标准工作溶液(4.14),制成系列基质标准溶液。

7.4 测定

7.4.1 液相色谱参考条件

液相色谱参考条件如下:

- a) 色谱柱: C<sub>18</sub>, 1.7 μm, 100 mm×2.1 mm(内径)或相当者;
- b) 柱温: 35 ℃;
- c) 进样量: 10 μL;
- d) 流动相: A: 甲酸-水(1+999)溶液; B: 乙腈。梯度洗脱条件见表 1。

表 1 液相色谱梯度洗脱条件

时间/min	流速/(μL/min)	A/%	B/%
0	300	95	5
0.8	300	60	40
2.3	300	40	60
2.4	300	5	95
4	300	5	95

7.4.2 质谱参考条件

质谱参考条件如下:

- a) 离子源: 电喷雾离子源;
- b) 扫描方式: 正离子扫描;
- c) 检测方式: 多反应监测(MRM);
- d) 雾化气、气帘气、辅助加热气、碰撞气均为高纯氮气;使用前应调节各气体流量以使质谱灵敏度达到检测要求,参考条件参见附录 A;
- e) 喷雾电压、去集簇电压、碰撞能量等参数应优化至最优灵敏度,参考条件参见附录 A。

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7.4.3 液相色谱-串联质谱测定

7.4.3.1 定性确证

被测组分选择 1 个母离子,2 个以上子离子,在相同实验条件下,样品中待测物质的保留时间与混合基质标准校准溶液中对应组分的保留时间偏差在±2.5%之内;且样品谱图中被测组分的相对离子丰度与浓度接近的基质标准校准溶液谱图中对应的相对离子丰度进行比较,偏差不超过表 2 规定的范围,则可判定样品中存在对应的待测物。

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 K	K>50%	20%<K≤50%	10%<K≤20%	K≤10%
允许的最大偏差	±20%	±25%	±30%	±50%

7.4.3.2 定量测定

外标法定量:在仪器最佳工作条件下,对沃尼妙林和泰妙菌素的基质校准标准溶液进样测定,以基质标准校准溶液浓度为横坐标,以峰面积为纵坐标,绘制标准工作曲线,用标准工作曲线对待测样品进行定量,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。沃尼妙林和泰妙菌素的标准物质多反应监测(MRM)色谱图参见图 B.1。

7.5 空白实验

除不称取试样外,均按上述步骤进行。

7.6 结果计算与表述

按式(1)计算试样中沃尼妙林和泰妙菌素的含量:

$$X = \frac{(A - A_0) \times c \times V \times 1\,000}{A_s \times m \times 1\,000}$$

.....( 1 )

式中:

- X —— 试样中被测化合物的含量:单位为微克每千克(μg/kg);
- A —— 样液中被测化合物的峰面积;
- A<sub>0</sub> —— 空白实验中被测化合物的峰面积;
- c —— 标准工作溶液中被测化合物的浓度,单位为纳克每毫升(ng/mL);
- V —— 样液最终定容体积,单位为毫升(mL);
- A<sub>s</sub> —— 标准工作溶液中被测化合物的峰面积;
- m —— 最终样液所代表的试样量,单位为克(g)。

8 测定低限和回收率

8.1 测定低限

本方法测定低限:沃尼妙林和泰妙菌素的测定低限均为 5.0 μg/kg。

8.2 回收率

鸡肉、鱼肉、猪肝、牛奶和鸡蛋中沃尼妙林和泰妙菌素添加浓度及其回收率实验数据见表 3。

表3 沃尼妙林和泰妙菌素的添加浓度及平均回收率( $n=10$ )

样品	添加浓度/( $\mu\text{g}/\text{kg}$ )	平均回收率范围/%	
		沃尼妙林	泰妙菌素
鸡肉	5.0	72.40~98.60	74.60~101.20
	10.0	78.50~95.40	76.90~96.80
	50.0	82.38~95.40	76.90~95.26
	100.0	82.56~96.71	76.69~93.44
鱼肉	5.0	73.20~99.60	73.80~98.20
	10.0	76.70~95.60	75.80~96.90
	50.0	77.48~95.64	75.64~94.64
	100.0	82.76~96.87	77.41~98.97
猪肝	5.0	75.20~99.20	73.40~102.20
	10.0	74.70~96.30	74.10~99.30
	50.0	79.14~95.26	75.14~93.78
	100.0	82.33~95.75	78.60~96.25
	6 000.0	84.20~101.6	85.30~100.4
鸡蛋	5.0	75.60~96.40	73.40~99.80
	10.0	80.60~97.80	75.80~94.50
	50.0	77.12~94.52	72.98~97.20
	100.0	82.33~98.58	76.30~95.33
牛奶	5.0	74.20~98.60	73.40~97.20
	10.0	76.50~94.30	76.40~106.20
	50.0	73.12~93.52	73.58~93.06
	100.0	77.88~95.47	74.56~92.63



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附 录 A  
(资料性附录)  
参考质谱条件<sup>1)</sup>

参考质谱条件:

- a) 离子源:电喷雾离子源;
- b) 扫描方式:正离子扫描;
- c) 检测方式:多反应监测(MRM);
- d) 毛细管电压:5 400 V;
- e) 气帘气压力流速:3.5 L/min;
- f) 雾化气压力流速:6 L/min;
- g) 辅助气压力流速:8 L/min;
- h) 辅助气温度:550 ℃;
- i) 监测离子对、采集时间、保留时间、去簇电压和碰撞能量见表 A.1。

表 A.1 沃尼妙林和泰妙菌素的质谱参数

化合物	定性离子对 $m/z$	定量离子对 $m/z$	采集时间/ ms	保留时间/ min	去簇电压 (DP)/V	碰撞能量 (CE)/V
泰妙菌素	494.30/192.10	494.30/192.10	80	1.97	234	25
	494.30/119.00					38
沃尼妙林	565.30/263.10	565.30/263.10	80	2.07	100	25
	565.30/285.20					29

1) 非商业性声明:附录 A 所列参数是在 API5500 质谱仪上完成的,此处列出试验用仪器型号仅是为了提供参考,并不涉及商业目的,鼓励标准使用者尝试采用不同厂家或型号的仪器。



附录 B  
(资料性附录)

沃尼妙林和泰妙菌素的多反应监测(MRM)色谱图

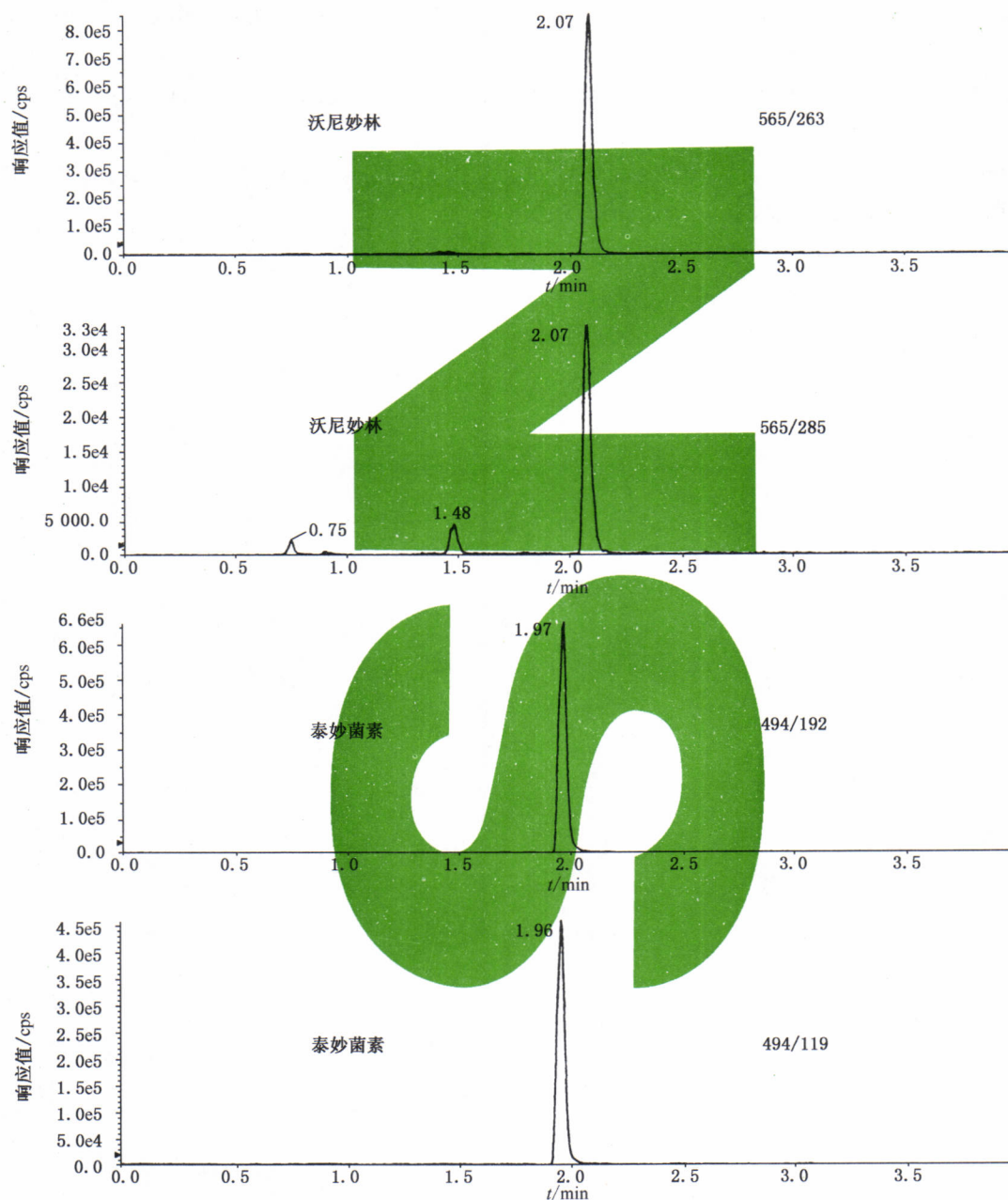


图 B.1 沃尼妙林和泰妙菌素的多反应监测(MRM)色谱图

## Foreword

This standard is drafted accordance with the rules given by the GB/T 1.1—2009.

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

The standard was drafted by Qinhuangdao Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Liu Xiaomao, Li Xuemin, Chang Qiaoying, Wang Fei, Ge Na, Yang Zhiwei, Zhang Shoujun, Cao Yanzhong, Zhang Jinjie.

# Determination of valnemulin and tiamulin residues in foodstuffs of animal origin for export—LC-MS/MS method

## 1 Scope

This standard specifies the method of determination of valnemulin and tiamulin residues in foodstuffs of animal origin for export.

This standard is applicable to the determination of valnemulin and tiamulin residues in animal muscle, animal liver, fish, egg and milk.

## 2 Normative reference

The following normative documents contain provisions which, through reference in this text, constitute provisions of this standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, protocol to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies.

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

## 3 Principle

Valnemulin and tiamulin residues in the samples are extracted with acetonitrile, and cleaned by MCX solid phase extraction, determined by LC-MS/MS (ESI<sup>+</sup>), quantified by external standard method.

## 4 Regents and materials

Unless otherwise specified, all reagents used are A.R. and “water” is deionized water.

4.1 Methanol; HPLC grade.

4.2 Acetonitrile; HPLC grade.

4.3 Formic acid.

4.4 n-Hexane.

4.5 Ammonia; 25%.

4.6 Sodium chloride.

4.7 anhydrous sodium sulfate; Ignite for 4 h at 650 °C, cool to room temperature in desiccator and keep in a tightly closed container.

4.8 Formic acid-water ( 1 + 19, V : V ): 5 mL Formic acid(4.3) mix with 95 mL water.

4.9 Ammonia-Methanol ( 1 + 19, V : V ): 5 mL Ammonia(4.5) mix with 95 mL Methanol(4.1).

4.10 Acetonitrile-water ( 1 + 3, V : V ): 25 mL Acetonitrile(4.2) mix with 75 mL water.

4.11 Standards: valnemulin (CASNO.: 101312-92-9), purity  $\geq 98\%$ ; tiamulin (CASNO.: 55297-95-5), purity  $\geq 98\%$ .

4.12 Valnemulin stock standard solutions (1.0 mg/mL): Separately accurately weigh an adequate amount of valnemulin standards, dissolve in methanol and prepare a solution of 1.0 mg/mL as the stock standard solutions. Solutions are stable at  $-18\text{ }^{\circ}\text{C}$ .

4.13 Tiamulin stock standard solutions (1.0 mg/mL): Separately accurately weigh an adequate amount of tiamulin standards, dissolve in methanol and prepare a solution of 1.0 mg/mL as the stock standard solutions. Solutions are stable at  $-18\text{ }^{\circ}\text{C}$ .

4.14 Working standard solutions (1.0  $\mu\text{g/mL}$ ): According to the requirement, pipette adequate amount of valnemulin stock standard solutions(4.12) and tiamulin stock standard solutions(4.13), and dissolve in Acetonitrile-water (4.10) and prepare a solution of 1.0  $\mu\text{g/mL}$ . Stored below  $4\text{ }^{\circ}\text{C}$  avoiding sunlight.

4.15 MCX solid phase extraction cartridges; 60 mg, 3 mL, or equivalent.

4.16 Membrane filter; 0.20  $\mu\text{m}$ .

## 5 Apparatus and equipment

5.1 LC-MS/MS; equipped with ESI source.

5.2 Balance; 0.1 mg and 0.01 g sensitivity.

5.3 Homogenizer; 15 000 r/min.



5.4 Shaker.

5.5 Centrifuge; 10 000 r/min.

5.6 Rotary vacuum evaporator.

5.7 Solid phase extraction.

5.8 Vacuum pump; vacuum to 80 kPa.

## 6 Sample preparation and storage

### 6.1 Animal muscle, animal liver, fish

All primary sample is reduced to 500 g as the representative sample, then grinded and blended to produce homogenous samples, and then divided into two equal portions. Each portion is placed in clean containers as the test sample, which is sealed and labeled. The test sample should be stored below in  $-18\text{ }^{\circ}\text{C}$ . In the course of sample preparation, precautions should be taken to avoid contamination or any factors which may cause the change of residue content.

### 6.2 Egg

All primary sample is reduced to 500 g as the representative sample. Deseeded and blended to produce homogenous samples, and then divided into two equal portions. Each portion is placed in clean containers as the test sample, which is sealed and labeled. The test sample should be stored below in  $4\text{ }^{\circ}\text{C}$ . In the course of sample preparation, precautions should be taken to avoid contamination or any factors which may cause the change of residue content.

### 6.3 Milk

All primary sample is reduced to 500 g as the representative sample, and blended to produce homogenous samples, and then divided into two equal portions. Each portion is placed in clean containers as the test sample, which is sealed and labeled. The test sample should be stored below in  $4\text{ }^{\circ}\text{C}$ . In the course of sample preparation, precautions should be taken to avoid contamination or any factors which may cause the change of residue content.

## 7 Procedure

### 7.1 Extraction

### 7.1.1 Animal tissue, animal liver, fish

Weigh 2 g of test sample (accurate to 0.01 g) into a plastic centrifuge tube, then add 5 g anhydrous sodium sulfate (4.7) and 10 mL Acetonitrile into the centrifuge tube. Homogenize at 10 000 r/min for 2 min, then centrifuge at 4 000 r/min for 10 min. Transfer the supernatant to a evaporated flask, extract again with 10 mL Acetonitrile. Combine the supernatants, evaporate to dryness at 40 °C. The residue was dissolved with 5 mL formic acid-water (4.8), then it is ready for cleaned-up.

### 7.1.2 Egg and milk

Weigh 5 g of test sample (accurate to 0.01 g) into a plastic centrifuge tube, then add 2 g Sodium chloride (4.6) and 10 mL Acetonitrile into the centrifuge tube. shake for 20 min, then centrifuge at 4 000 r/min for 10 min. Transfer 5 mL supernatant to a evaporated flask, evaporate to dryness at 40 °C. The residue was dissolved with 5 mL formic acid-water (4.8), then it is ready for cleaned-up.

## 7.2 Clean up

The MCX SPE cartridge (4.15) is conditioned with 3 mL Ammonia-Methanol (4.9), 3 mL methanol and 10 mL water in sequence. The above-mentioned extract solution is loaded, wash the cartridge with 3 mL Formic acid-water (4.8), 3 mL methanol and 3 mL *n*-Hexane, discard the effluent. Dry the column with vacuum pump. Finally, elute with 5 mL Ammonia-Methanol (4.9) into test tube. Evaporate the elute solution to dryness under nitrogen at 40 °C. Add accurately 1 mL acetonitrile-water (4.10) to dissolve the residue. Filter with 0.21 µm syringe filter and determined by LC-MS/MS.

## 7.3 Preparation of matrix standard solutions

Weigh five negative test samples whose weight is 2 g (accurate to 0.01 g) Then process these samples according to the analyt steps 7.1 and 7.2 to prpare the matrix blank solutions. add adequate amount of valnemulin and tiamulin Working standard solutions (4.14) into matrix blank solutions and prepare them into a series of matrix standard solutions.

## 7.4 Determination

### 7.4.1 HPLC operation conditions

HPLC operation conditions:

- a) Column: C<sub>18</sub>, 50 mm × 2.1 mm (i.d), 1.7 µm, or equivalent;
- b) Column temperature: 35 °C;
- c) Injection volume: 10 µL;

- d) Mobile phase: A: Formic acid-H<sub>2</sub>O (1 + 999); B: Acetonitrile. Mobile phase and flow rate rate see Table 1.

Table 1 Mobile phase and flow rate

Time/(min)	Flow rate/( $\mu$ L/min)	A/%	B/%
0.00	300	95	5
0.80	300	60	40
2.30	300	40	60
2.40	300	5	95
4.00	300	5	95

#### 7.4.2 MS operation conditions

MS operation conditions:

- a) Ion source: ESI;
- b) Scan mode: positive mode;
- c) Monitor mode: Multiple reaction monitoring (MRM);
- d) Other parameters are listed Annex A.

#### 7.4.3 LC-MS/MS determination

##### 7.4.3.1 Qualitative determination

The qualitative ions for analyst include one precursor ion and two product ions at least. Under the same determination conditions, The ratio of the chromatographic retention time of the analyte to that of the external standard, i.e. the relative retention time of the analyte, shall correspond to that of the calibration solution at a tolerance of  $\pm 2.5\%$ . The relative intensities of the detected ions of analyst, shall correspond to those of the calibration standard at comparable concentrations, within the tolerances shown in Table 2, then the corresponding analyte must be present in the sample.

Table 2 Maximum permitted tolerances for relative ion intensities while confirmation

Relative ion intensities $K$	$K > 50\%$	$20\% \leq K \leq 50\%$	$10\% < K \leq 20\%$	$K \leq 10\%$
Maximum permitted tolerances	$\pm 20\%$	$\pm 25\%$	$\pm 30\%$	$\pm 50\%$



### 7.4.3.2 Quantitative determination

Under the best working conditions of LC-MS/MS, inject standard working solutions and a linear regression curve is calculated using the chromatographic peak area and the concentration of the analyte. Quantify the sample solution by standard working curve. The responses of the analyte in the standard working solution and the sample solution should be within the linear range of the instrument detection. Quantified by external standard method. The MRM chromatogram of valnemulin and tiamulin are shown in Figure B1 of Annex B.

### 7.5 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.

### 7.6 Calculation and expression of result

Calculate the results according to formula (1):

$$X = \frac{(A - A_0) \times c \times V}{A_s \times m} \dots\dots\dots (1)$$

where

$X$  — the content of compound in the test sample,  $\mu\text{g}/\text{kg}$ ;

$A$  — the peak area of compound derivative in the sample solution,

$A_0$  — the peak area of compound derivative in the blank test;

$c$  — the concentration of compound in the mixed standard working solution,  $\text{ng}/\text{mL}$ ;

$V$  — the final volume of sample solution,  $\text{mL}$ ;

$A_s$  — the peak area of compound derivative in the mixed standard working solution;

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

## 8 Limit of determination and recovery

### 8.1 Limit of quantification

The limits of quantification of this method are  $5.0 \mu\text{g}/\text{kg}$  respectively for valnemulin and tiamulin.



## 8.2 Recovery

The results of recoveries were showed on Table 3.

Table 3 Recovery of four levels in different samples ( $n = 10$ )

Sample	Spiking level/( $\mu\text{g}/\text{kg}$ )	Average recovery rang/%	
		Valnemulin	Tiamulin
Chicken	5.0	72.40~98.60	74.60~101.20
	10.0	78.50~95.40	76.90~96.80
	50.0	82.38~95.40	76.90~95.26
	100.0	82.56~96.71	76.69~93.44
Fish	5.0	73.20~99.60	73.80~98.20
	10.0	76.70~95.60	75.80~96.90
	50.0	77.48~95.64	75.64~94.64
	100.0	82.76~96.87	77.41~98.97
Liver	5.0	75.20~99.20	73.40~102.20
	10.0	74.70~96.30	74.10~99.30
	50.0	79.14~95.26	75.14~93.78
	100.0	82.33~95.75	78.60~96.25
	6 000.0	84.20~101.6	85.30~100.4
Egg	5.0	75.60~96.40	73.40~99.80
	10.0	80.60~97.80	75.80~94.50
	50.0	77.12~94.52	72.98~97.20
	100.0	82.33~98.58	76.30~95.33
Milk	5.0	74.20~98.60	73.40~97.20
	10.0	76.50~94.30	76.40~106.20
	50.0	73.12~93.52	73.58~93.06
	100.0	77.88~95.47	74.56~92.63

**Annex A**  
(Informative annex)  
**LC-MS/MS conditions<sup>1)</sup>**

LC-MS/MS conditions:

- a) Ion source: ESI;
- b) Scan mode: positive mode;
- c) Monitor mode: Multiple reaction monitoring (MRM);
- d) Ion spray voltage: 5 400 V;
- e) Curtain gas: 3.5 L/min;
- f) Nebulizer gas: 6 L/min;
- g) Auxiliary gas: 8 L/min;
- h) Auxiliary gas temp: 550 °C ;
- i) Quality ions, quantity ions, dwell time, retention time, declustering potential and collision energy see Table A.1.

**Table A.1 MRM condition**

Compound	Quality ions <i>m/z</i>	Quantity ions <i>m/z</i>	Dwell time/ms	Retention time/min	Declustering potential/V	Collision energy/V
Tiamulin	494.30/192.10 494.30/119.00	494.30/192.10	80	1.97	234	25 38
Valnemulin	565.30/263.10 565.30/285.20	565.30/263.10	80	2.07	100	25 29

1) Non-commercial statement; the equipments and their type AB SCIEX QTRAP 5500 involved in the standard method are not related to commercial aims, and the analysts are encouraged to equipments of different corporation or different type.

**Annex B**  
(Informative annex)  
**MRM chromatogram of the standard**

The MRM chromatogram of valnemulin and tiamulin standard see Figure B.1.

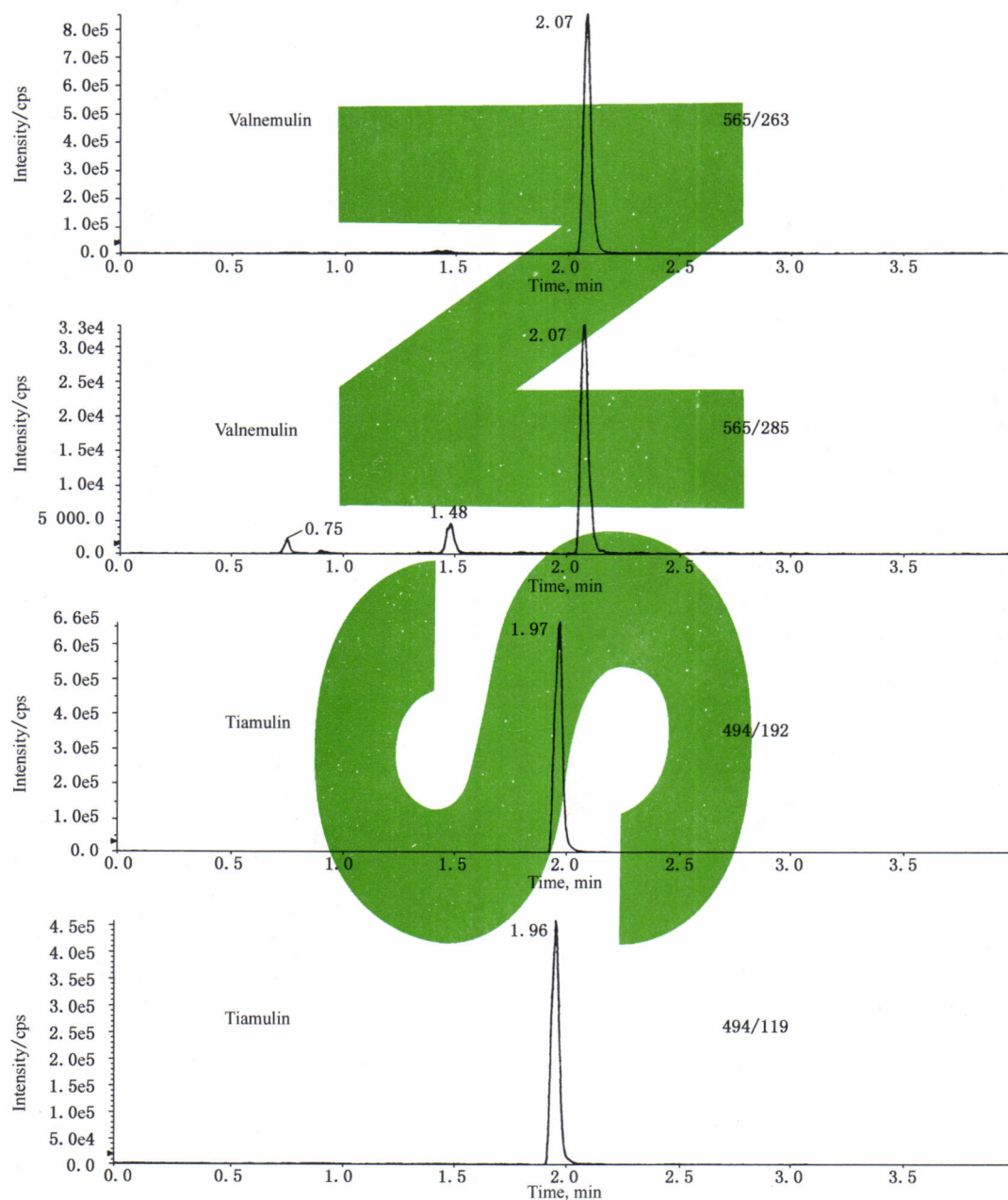


Figure B.1 MRM chromatogram of valnemulin and tiamulin

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