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中华人民共和国出入境检验检疫行业标准

SN/T 4519—2016

出口动物源食品中利巴韦林残留量的 测定 液相色谱-质谱/质谱法

Determination of ribavirin residue 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。

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、中华人民共和国东山出入境检验检疫局、中华人民共和国厦门出入境检验检疫局、中华人民共和国绍兴出入境检验检疫局、福建圣农发展股份有限公司。

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出口动物源食品中利巴韦林残留量的 测定 液相色谱-质谱/质谱法

1 范围

本标准规定了动物源食品中利巴韦林残留量的液相色谱-质谱/质谱的测定方法。

本标准适用于鸡肉、肝脏、肾、蛋、猪肉、鳗鱼、虾等动物源食品中利巴韦林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

试样中残留的利巴韦林代谢物经酸性磷酸酯酶水解成利巴韦林原药,与样品中残留的利巴韦林原药一起,经三氯乙酸-乙腈混合溶液提取,经苯硼酸固相萃取小柱净化,液相色谱-质谱/质谱进行测定,同位素内标法定量。

4 试剂和材料

除非另有说明,所用试剂均为分析纯,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:色谱纯。

4.4 三氯乙酸。

4.5 氨水:含量为 25%~28%。

4.6 乙酸铵:色谱级纯。

4.7 酸性磷酸酯酶(phosphatase acid, from wheat germ, 活力: ≥ 0.4 unit/mg, CAS 号: 9001-77-8)。

4.8 酸性磷酸酯酶溶液(100 $\mu\text{g/mL}$):准确称取酸性磷酸酯酶(4.7)5 mg,置 50 mL 容量瓶中,用水溶解并稀释至刻度,摇匀,配制成 100 $\mu\text{g/mL}$ 的酶解液,4 $^{\circ}\text{C}$ ~8 $^{\circ}\text{C}$ 冰箱中保存。

4.9 三氯乙酸溶液(20 g/L, pH 4.8):称取 20 g 三氯乙酸,加水约 950 mL 使其溶解,用氨水调 pH 至 4.8(± 0.1),再加水定容至 1 000 mL。

4.10 PBA 苯硼酸固相萃取小柱洗脱液,甲酸-水-甲醇溶液(体积比 2:8:90):准确量取 2 mL 甲酸和 8 mL 水加入 90 mL 的甲醇中,混匀后备用。

4.11 乙酸铵缓冲溶液(2.0 mol/L, pH 4.8):称取乙酸铵 77.0 g,加水约 450 mL 使其溶解,用乙酸调 pH 至 4.8(± 0.1),再加水定溶至 500 mL。

4.12 乙酸铵缓冲溶液(0.25 mol/L, pH 8.5):称取乙酸铵 9.06 g,加水约 450 mL 使其溶解,用氨水调 pH 至 8.5(± 0.1),再加水定容至 500 mL。

4.13 标准物质:利巴韦林(ribavirin;CAS号:36791-04-5),纯度大于98.0%。

4.14 同位素内标标准物质:利巴韦林-¹³C₅ 纯度大于99.0%。

4.15 标准储备液(100 μg/mL):准确称取利巴韦林标准品(4.13)10 mg(精确至0.1 mg),置100 mL容量瓶中,用甲醇溶解并稀释至刻度,摇匀,配制成100 μg/mL的标准储备液,-18℃冰箱中保存,有效期为1年。

4.16 标准中间液(1.0 μg/mL):用移液管吸取标准储备液(4.15)1 mL置100 mL容量瓶中,用甲醇稀释至刻度,摇匀得到1.0 μg/mL标准中间液,置4℃~8℃冰箱中保存,有效期为3个月。

4.17 内标储备液:准确称取利巴韦林-¹³C₅ 标准品(4.14)10 mg(精确至0.1 mg),置100 mL容量瓶中,用甲醇溶解并稀释至刻度,摇匀,配制成100 μg/mL的内标标准储备液,-18℃冰箱中保存,有效期为1年。

4.18 内标中间溶液(1.0 μg/mL):移取1.00 mL的内标标准储备液(4.17)至100 mL容量瓶中,用甲醇稀释至刻度,置4℃~8℃冰箱中保存,有效期为3个月。

4.19 标准工作液:临用时根据需要,移取适量的标准中间溶液(4.16)和内标中间溶液(4.18),用乙腈稀释至合适浓度,使用前配制。

4.20 PBA 苯硼酸固相萃取小柱:100 mg/3 mL,或相当者;使用前依次用3 mL 乙腈,3 mL 乙腈-1%甲酸(体积比,3:1),3 mL 乙酸铵缓冲溶液(4.12)活化,并保持柱体湿润。

注:可采用商品化的 Bond Elut PBA 固相萃取小柱[Bond Elut PBA, part No:12102127, USA],或等同性能的其他小柱。

4.21 滤膜:0.22 μm,有机系。

5 仪器和设备

5.1 液相色谱-质谱/质谱仪:带电喷雾电离(ESI)源。

5.2 天平:感量0.1 mg和0.01 g。

5.3 组织捣碎机。

5.4 高速冷冻离心机:转速≥8 000 r/min。

5.5 离心机:5 000 r/min。

5.6 漩涡振荡器。

5.7 pH计。

5.8 氮吹仪。

5.9 固相萃取装置。

6 试样制备与保存

6.1 试样制备

肌肉、肝脏、肾放入组织捣碎机均质,充分混匀,均分成两份,分别装入清洁容器内,并标明标记;蛋类,虾去壳后取可食部分后放入组织捣碎机均质,充分混匀,均分成两份,分别装入清洁容器内,并标明标记。

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。

6.2 试样保存

试样于-18℃以下保存,新鲜或冷冻的组织样品可在2℃~6℃贮存72 h。

7 测定步骤

7.1 提取

称取约 5 g(精确至 0.01 g)试样,置于 50 mL 具螺旋盖的聚丙烯离心管中,添加 100 μ L 内标标准工作溶液(0.1 μ g/mL),加入 12 mL 三氯乙酸溶液(4.9)和 2.5 mL 乙腈,振荡混匀 3.0 min 后在超声波发生器中超声 10 min,于 15 000 r/min 离心 5 min,取出上清液,再加入 10 mL 三氯乙酸溶液(4.9)重复提取一次,离心后合并上清液定容至 25 mL 的容量瓶中。

7.2 酶解

准确移取 5 mL 以上提取液,加入 1.0 mL 乙酸铵溶液(4.11),混匀,再加入 100 μ L 酸性磷酸酯酶(4.8),加盖后涡旋 1 min,于恒温烘箱中 37 $^{\circ}$ C 培养 2 h。取出后冷却至室温,用氨水调 pH 至 8.5 (\pm 0.1),混匀,4 000 r/min 离心 5 min,取上清液备用。

7.3 净化

取上清液(7.2),上样到活化过的 PBA 固相萃取小柱,控制上样流速小于 3 mL/min;依次用 5 mL 含有 10%乙腈-乙酸铵缓冲溶液(4.12)和 2.0 mL 5%氨水甲醇淋洗,然后真空抽干 5 min,以 4.0 mL 洗脱溶液(4.10)洗脱至 10 mL 玻璃管中,在 45 $^{\circ}$ C 用氮气浓缩仪吹干。准确加入 1.0 mL 乙腈-水(体积比 90 : 10)溶液溶解残渣,过 0.22 μ m 滤膜,供液相色谱-质谱/质谱仪测定。

7.4 测定

7.4.1 液相色谱条件

- 7.4.1.1 色谱柱:两性离子型亲水相互作用色谱柱,100 mm \times 3.0 mm(内径),粒度 2.7 μ m,或相当者。
7.4.1.2 流动相:A 为 5 mmol/L 乙酸铵溶液(含 0.2%甲酸),B 为乙腈,梯度洗脱,洗脱程序见表 1。

表 1 液相色谱的梯度洗脱程序

时间/min	流动相 A/%	流动相 B/%
0	5	95
2	5	95
4	30	70
5	60	40
6	5	95
10	5	95

- 7.4.1.3 流速:0.4 mL/min。
7.4.1.4 柱温:30 $^{\circ}$ C。
7.4.1.5 进样量:2 μ L。

7.4.2 质谱条件

- 7.4.2.1 离子化模式:电喷雾电离(ESI)正离子模式;
7.4.2.2 质谱扫描方式:多反应监测(MRM);
7.4.2.3 其他参考质谱条件参见附录 A。

7.4.3 定性测定

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标准校准溶液中对浓度标准校准溶液的保留时间偏差在±2.5%之内; 且样品谱图中各组分定性离子的相对丰度与浓度接近的标准校准溶液谱图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
允许的最大偏差/%	±20	±25	±30	±50

7.4.4 定量测定

内标法定量: 用标准工作溶液分别进样, 以分析化合物和内标化合物的峰面积比为纵坐标, 以分析化合物和内标化合物的浓度比为横坐标作标准工作曲线, 用标准工作曲线对样品进行定量, 标准工作液和待测液中利巴韦林的响应值均应在仪器线性响应范围内。利巴韦林的保留时间、母离子和子离子参见附录 A 表 A.1。利巴韦林的标准品选择离子流色谱图参见附录 B 图 B.1。

7.5 空白试验

除不加试样外, 均按 7.1~7.4 操作步骤进行。

8 结果计算和表述

试样中利巴韦林的残留总量利用数据处理系统计算或按式(1)计算, 计算结果需扣除空白值。

$$X = \frac{c \times c_i \times A \times A_{s_i} \times V}{c_{s_i} \times A_i \times A_s \times m} \times \frac{1\,000}{1\,000} \dots\dots\dots(1)$$

式中:

- X ——样品中利巴韦林残留量, 单位为微克每千克(μg/kg);
- c ——标准工作溶液中利巴韦林的浓度, 单位为纳克每毫升(ng/mL);
- c_i ——样液中内标物的浓度, 单位为纳克每毫升(ng/mL);
- A ——样液中利巴韦林的峰面积;
- A_{s_i} ——标准工作溶液中内标物的峰面积;
- V ——样品溶液最终定容体积, 单位为毫升(mL);
- c_{s_i} ——标准工作溶液中内标物的浓度, 单位为纳克每毫升(ng/mL);
- A_i ——样液中内标物的峰面积;
- A_s ——标准工作溶液中利巴韦林的峰面积;
- m ——最终样液代表的试样质量, 单位为克(g)。

9 测定低限和回收率

9.1 测定低限

本方法中利巴韦林的测定低限为 1.0 μg/kg。

9.2 正确度(回收率)

不同基质中利巴韦林残留量在不同添加水平下的回收率试验数据参见附录 C 表 C.1。

附 录 A
(资料性附录)
质谱参考参数¹⁾

- 质谱参考参数如下：
- a) ESI 正离子模式；
 - b) 多反应监测(MRM)；
 - c) 电喷雾电压(IS):5 500.0 V；
 - d) 离子源温度(TEM):500 ℃；
 - e) 雾化气压力(GS1):345 kPa(50.00 psi)；
 - f) 辅助气压力(GS2):345 kPa(50.00 psi)；
 - g) 气帘气压力(CUR):241 kPa(35.00 psi)；
 - h) 监测离子对、去簇电压(DP)、碰撞电压(CE)见表 A.1。

表 A.1 利巴韦林保留时间、定性定量离子对及去簇电压、碰撞能量

药物名称	保留时间 min	定性离子对 m/z	定量离子对 m/z	去簇电压 V	碰撞电压 eV
利巴韦林	2.1	245.1>113.1	245.1>113.1	100	15
		245.1>96.0			35
利巴韦林- ¹³ C ₅	2.1	250.1>113.0	250.1>113.0	100	15

1) 非商业性声明:附录 A 所列参考质谱条件是在 Waters Ultra UPLC-API5500 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。

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附录 B
(资料性附录)

利巴韦林标准物质的多反应监测(MRM)色谱图

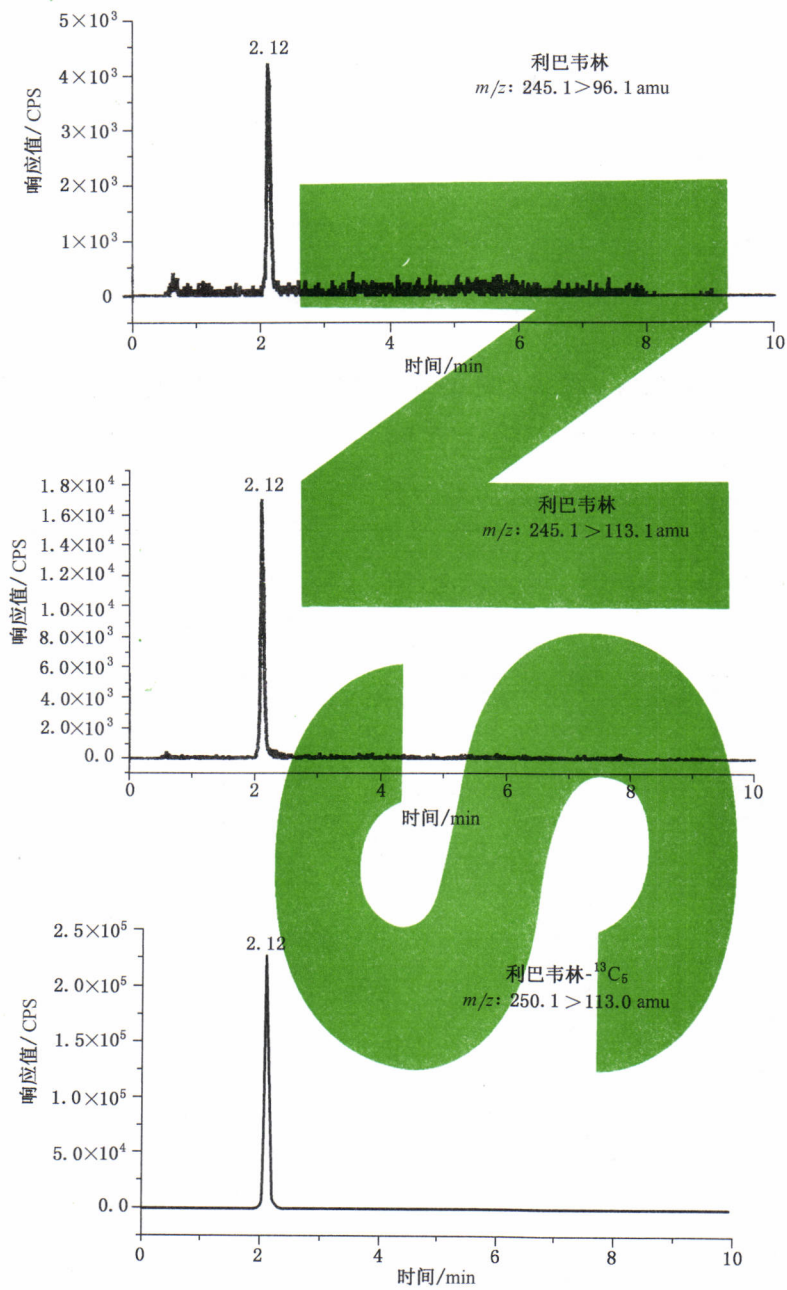


图 B.1 利巴韦林以及内标标准品(2 ng/mL)的反应监测(MRM)色谱图

附 录 C
(资料性附录)
回收率

利巴韦林在鸡肉、鸡蛋、鸡肝、鸡肾、猪肉、鳗鱼、虾、蒸煮鸡尾串、皮蛋中回收率范围的试验数据,见表 C.1。

表 C.1 利巴韦林在不同基质中不同添加水平下的回收率范围

样品名称	添加水平/($\mu\text{g/kg}$)	回收率范围/%
鸡肉	1.0	77.8~107.0
	2.0	81.0~104.0
	10.0	82.7~99.1
鸡蛋	1.0	81.9~102.0
	2.0	82.5~101.5
	10.0	80.3~100.0
鸡肝	1.0	78.3~102.0
	2.0	82.0~100.5
	10.0	80.4~93.4
鸡肾	1.0	78.6~102.0
	2.0	78.5~95.0
	10.0	76.6~89.9
猪肉	1.0	81.8~101.0
	2.0	82.0~100.5
	10.0	80.7~93.8
鳗鱼	1.0	81.7~103.0
	2.0	80.0~103.5
	10.0	83.5~97.0
虾	1.0	80.7~106.0
	2.0	80.5~104.0
	10.0	82.0~98.3
蒸煮鸡尾串	1.0	81.4~107.0
	2.0	80.0~101.5
	10.0	77.6~91.8
皮蛋	1.0	79.7~103.0
	2.0	82.0~98.0
	10.0	80.9~95.9

Foreword

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

Please pay attention that some contents in this standard may refer to patents, The institution doesn't take on the responsibility to indentify 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, Dongshan Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Xiamen Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Shaoxing Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Fujian sunner development CO.,LTD

This standard was mainly drafted by Liu Zhengcai, Yang Fang, Pan Yinfen, Lin Yonghui, Chen Xudong, Xun Dunming, Ren Mingxing, Lv Xiaoling.

Note: This English version, a translation from the Chine text, is solely for guidance.

Determination of ribavirin residue in foodstuffs of animal origin for export— LC-MS/MS method

1 Scope

The standard specifies the sample preparation and determination of the ribavirin by LC-MS/MS in animal tissues for export.

This standard is applicable to the determination and confirmation of ribavirin in residues in chicken, liver, kidney, eggs, pork, fish, and shrimps.

2 Normative references

The following documents are necessary for this standard. For dated references, only dated editions shall apply to this standard. For undated references, the latest edition of the normative document (including subsequent amendments) referred to applies.

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

3 Principle

Trichloroacetic acid-acetonitrile solution is used to extract the total residues of ribavirin in tested-samples and then was digested by acid phosphatase. The extract was purified using phenyl boronic acid(PBA) SPE cartridge. The ribavirin is determined by LC-MS/MS, quantified by internal standard method.

4 Reagents and materials

Unless otherwise specified, all the reagent used should be analytical grade, water is the first grade water prescribed by GB/T 6682.

4.1 Methanol: HPLC grade.

4.2 Acetonitrile:HPLC grade.

4.3 Formic acid:HPLC grade.

4.4 Trichloroacetic acid.

4.5 Ammonia (25%~28%) .

4.6 Ammonium acetate:HPLC grade.

4.7 Acid phosphatase (Phosphatase Acid, From Wheat Germ, Vitality: ≥ 0.4 unit/mg, CAS No:9001-77-8).

4.8 Stock solutions of acid phosphatase (100 $\mu\text{g/mL}$): Weigh about 5.0 mg acid phosphatase materials (4.7) dissolve with water to a volume of 50 mL. The stock solutions store at approximately 4 $^{\circ}\text{C}$ ~8 $^{\circ}\text{C}$.

4.9 Trichloroacetic acid solution (20 g/L, pH 4.8): Weigh 20 g trichloroacetic acid, dissolve with 950 mL water and adjusted pH 4.8 (± 0.1) with ammonia and dilute to 1 000 mL, then mix well.

4.10 The eluent of PBA SPE (formic acid/water/methanol 2 : 8 : 90, V/V/V): Pipet 2.0 mL formic acid and 8.0 mL water into 90 mL methanol and mix well.

4.11 2.0 mol/L ammonium acetate buffer solution: Weigh ammonium acetate 77.0 g, dissolve with 450 mL water and adjusted pH 4.8 (± 0.1) with ammonia and dilute to 500 mL.

4.12 0.25 mol/L ammonium acetate buffer solution: Weigh ammonium acetate 9.06 g, dissolve with 450 mL water and adjusted pH 8.5 (± 0.1) with ammonia and dilute to 500 mL.

4.13 Standard: ribavirin, CAS No. 36791-04-5, purity $\geq 98.0\%$.

4.14 Isotope internal standard: ribavirin- $^{13}\text{C}_5$, purity $\geq 99.0\%$.

4.15 Stock solutions of ribavirin (100 $\mu\text{g/mL}$): Weigh about 10.0 mg ribavirin (4.13), dissolve with methanol and then dilute with methanol to a volume of 100 mL. The stock solutions store at approximately -18 $^{\circ}\text{C}$, can use for 12 months.

4.16 Middle standard solution (1 $\mu\text{g/mL}$): Dilute 1.00 mL the stock standard solution (4.15) to 100 mL with methanol. The stock solutions store at approximately 4 $^{\circ}\text{C}$ ~8 $^{\circ}\text{C}$, can use for three months.

4.17 Stock solutions of ribavirin- $^{13}\text{C}_5$ (100 $\mu\text{g/mL}$): Weigh about 10.0 mg ribavirin- $^{13}\text{C}_5$ (4.14), dissolve with methanol and then dilute with methanol to a volume of 100 mL. The stock solutions store

at approximately $-18\text{ }^{\circ}\text{C}$, can use for 12 months.

4.18 Middle Isotope internal standard solution($1\text{ }\mu\text{g/mL}$): Dilute 1.00 mL the stock standard solution (4.17) to 100 mL with methanol. The stock solutions store at approximately $4\text{ }^{\circ}\text{C}\sim 8\text{ }^{\circ}\text{C}$, can use for three months.

4.19 Working standard solution: Dilute the middle standard solution (4.16) and internal standard solution (4.18) to acquired concentration with acetonitrile. The stock solutions should be prepared before using.

4.20 Phenyl boronic acid (PBA) SPE cartridge: 100 mg/ 3 mL or equivalent. , the extraction cartridge is conditioned using 3 mL acetonitrile, 3 mL acetonitrile-1% formic acid(v/v 3 : 1) and 3 mL ammonium acetate solution(4.12) before use, prevent the columns from tuning dry.

Note: Can use commercial SPE cartridge [Bond Elut PBA, part No: 12102127, USA], or equivalent.

4.21 Membrane filter: Organic, $0.22\text{ }\mu\text{m}$.

5 Apparatus

5.1 High Performance Liquid Chromatography-Mass Spectrometer equipment: equipped with electrospray (ESI) LC interface.

5.2 Analytical balance: sensibility reciprocal is 0.01 g and 0.1 mg respectively.

5.3 Tissue blender.

5.4 Refrigerated centrifuge: Cooling to $4\text{ }^{\circ}\text{C}$, $\geq 8\text{ }000\text{ r/min}$ or equivalent.

5.5 Centrifuge: $\geq 5\text{ }000\text{ r/min}$.

5.6 Vortex mixer.

5.7 pH meter: $\pm 0.02\text{ pH}$.

5.8 Nitrogen evaporators.

5.9 Solid phase extraction equipment.

6 Sample preparation and storage

6.1 The requirements of sample preparation

Take approximately representative sample of the chicken, liver, fish blended in a high speed blender, mix thoroughly, divide into two portions and placed into a clean container, sealed and labeled. Take the chicken for the chicken product and the edible part for the egg and shrimp blended in a high speed blender, mix thoroughly, divide into two portions and placed into a clean container, sealed and labeled.

Precaution measures should be taken to avoid contamination or other factors may cause the change of residues concentration in samples.

6.2 Sample preparation

The sample should be stored at $-18\text{ }^{\circ}\text{C}$, the fresh and cryogenic can be stored at $2\text{ }^{\circ}\text{C}\sim 6\text{ }^{\circ}\text{C}$ in 72 h.

7 Method of determination

7.1 Extraction

Weigh 5 g tested sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 0.1 mL internal standard working standard solution ($0.1\text{ }\mu\text{g/mL}$), 12 mL trichloroacetic acid solution (4.9) and 2.5 mL acetonitrile. After mix-vortexing for 3 min and ultrasonic 10 min in ultrasonic generator, then centrifuge at 15 000 r/min for 5 min under the condition of $4\text{ }^{\circ}\text{C}$. Transfer the supernatant into 25 mL flask. Add 10 mL extracting solution (4.9) and extract again. Combine the supernatant and dilute to scale with acetonitrile.

7.2 Enzymatic

A portion of the combined extract (5 mL) was transferred to a clean glass tube; add 1 mL ammonium acetate solution (4.11) and 100 μL acid phosphatase (4.8), mix-vortexing for 1 min. Enzyme digestion to convert all phosphorylated metabolites to ribavirin was accomplished to the sample and incubating at $37\text{ }^{\circ}\text{C}$ for 2 h. The pH of this solution was adjusted to approximately 8.5 (± 0.1) by adding a solution of ammonia after digestion, then centrifuge at 4 000 r/min for 5 min.

7.3 Clean-up

All of the supernatant (7.2) is loaded onto phenyl boronic acid (PBA) SPE cartridge. After penetration, the column is washed successively with 5 mL acetonitrile-ammonium acetate solution (4.12) ($v/v\ 1:9$) and 2.0 mL methanol-ammonia solution ($v/v\ 5:95$), and vacuum dried for 5 min by suck-

ing air. The cartridge is eluted with 4 mL methanol-water-formic acid solution (4:10) after dried under vacuum 5 min. The elute is evaporated to dryness at 45 °C under a stream of nitrogen and redissolved in 1 mL of the dissolving solvent (acetonitrile /water v/v 90 : 10). This solution is filtered on a 0.22 µm filter prior to LC-MS/MS analysis.

7.4 Determination

7.4.1 LC operation conditions

7.4.1.1 Column: Hydrophilic interaction liquid chromatography (HILIC) bonded amphoteric functional groups (3.0 mm × 100 mm, 2.7 µm), or equivalent.

7.4.1.2 Mobile phase: A: 5 mmol/L ammonium acetate buffer solution (0.2% formic acid), B: acetonitrile. The gradient elution program see table 1.

Table.1 The gradient elution program

Time/min	A/%	B/%
0	5	95
2	5	95
4	30	70
5	60	40
6	5	95
10	5	95

7.4.1.3 Flow rate: 0.4 mL/min.

7.4.1.4 Column temperature: 30 °C.

7.4.1.5 Injection volume: 2 µL.

7.4.2 MS operation conditions

7.4.2.1 Ion source: ESI, positive ionization mode.

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

7.4.2.3 Referenced conditions see annex A.

7.4.3 Qualification determination

Under the same determination conditions, the variation range of the retention time for the peak of analyte in unknown sample and in the standard working solution cannot be out of range of ±2.5%. 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. Then the corresponding analyte must be present in the sample.

Table 2 Maximum permitted tolerances for relative ion intensities

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

7.4.4 Quantitation determination

According to the approximate concentration of the ribavirin in sample solution, select the standard working solution with similar responses to that of sample solution. The responses of the analyte in the standard working solution and the sample solution should be within the linear range of the instrument detection. The working standard solution and the sample solution should be injected with equal volume alternatively. Under the above LC-MS/MS operating condition, the MRM transition, quantitative ion pair and retention time of the ribavirin see table A.1 in annex A, reconstituted (MRM) chromatogram of standard working solution see Figure B.1 in annex B.

7.5 Blank test

The operation of the recovery test is according to the steps 7.1—7.4 described in the method.

8 Calculation and expression of the result

The calculation of ribavirin in the sample is according to formula (1); the blank value should be subtracted from the result of calculation:

$$X = \frac{c \times c_i \times A \times A_{s_i} \times V}{c_{s_i} \times A_i \times A_s \times m} \times \frac{1\ 000}{1\ 000} \dots\dots\dots (1)$$

Where:

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

c —the concentration of ribavirin in standard working solution, ng/mL ;

c_i —the concentration of internal standard in sample solution, ng/mL ;

A —the peak area of ribavirin in sample solution;

A_{s_i} —the internal standard peak area of ribavirin in standard working solution;

V —the final volume of sample solution, mL ;

c_{s_i} —the concentration of internal standard in standard working solution, ng/mL;

A_i —the internal standard peak area of ribavirin in sample solution;

A_s —the peak area of ribavirin in standard working solution;

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

9 Limit of quantification and accuracy

9.1 Limit of quantification

The limit of quantification of the method is 1.0 $\mu\text{g/kg}$.

9.2 Accuracy (recovery)

The averages recoveries of ribavirin total residual in different samples see table C. 1.

Annex A
(Informative)
LC-MS/MS referenced conditions¹⁾

Main mass parameters:

- a) ESI positive ion mode (ESI⁺);
- b) Multiple reaction monitoring (MRM);
- c) Electrospray voltage (IS): 5 500.0 V;
- d) Ion source temperature (TEM): 500 °C;
- e) GAS1 (GS1): 345 kPa(50.00 psi);
- f) GAS 2 (GS2): 345 kPa(50.00 psi);
- g) Curtain GAS(CUR): 241 kPa(35.00 psi).

Precursor ion, product ion, collision energy (CE) and declustering potential (DP) see table A. 1

Table A. 1 Other important parameters of MS for ribavirin

Compounds	RT min	MRM Transition <i>m/z</i>	Quantitative ion pair <i>m/z</i>	DP (V)	CE (eV)
ribavirin	2.1	245.1>113.1	245.1>113.1	100	15
		245.1>96.0			35
ribavirin- ¹³ C ₅	2.1	250.1>113.0	250.1>113.0	100	15

1) Non-commercial statement: The equipments and their type Waters Ultra UPLC-API5500 involved in the standard method are not related to commercial aims, and it is encouraged to use equipment of different corporation or different type.

Annex B
(Informative)

Multiple reaction monitoring(MRM)chromatograms of ribavirin

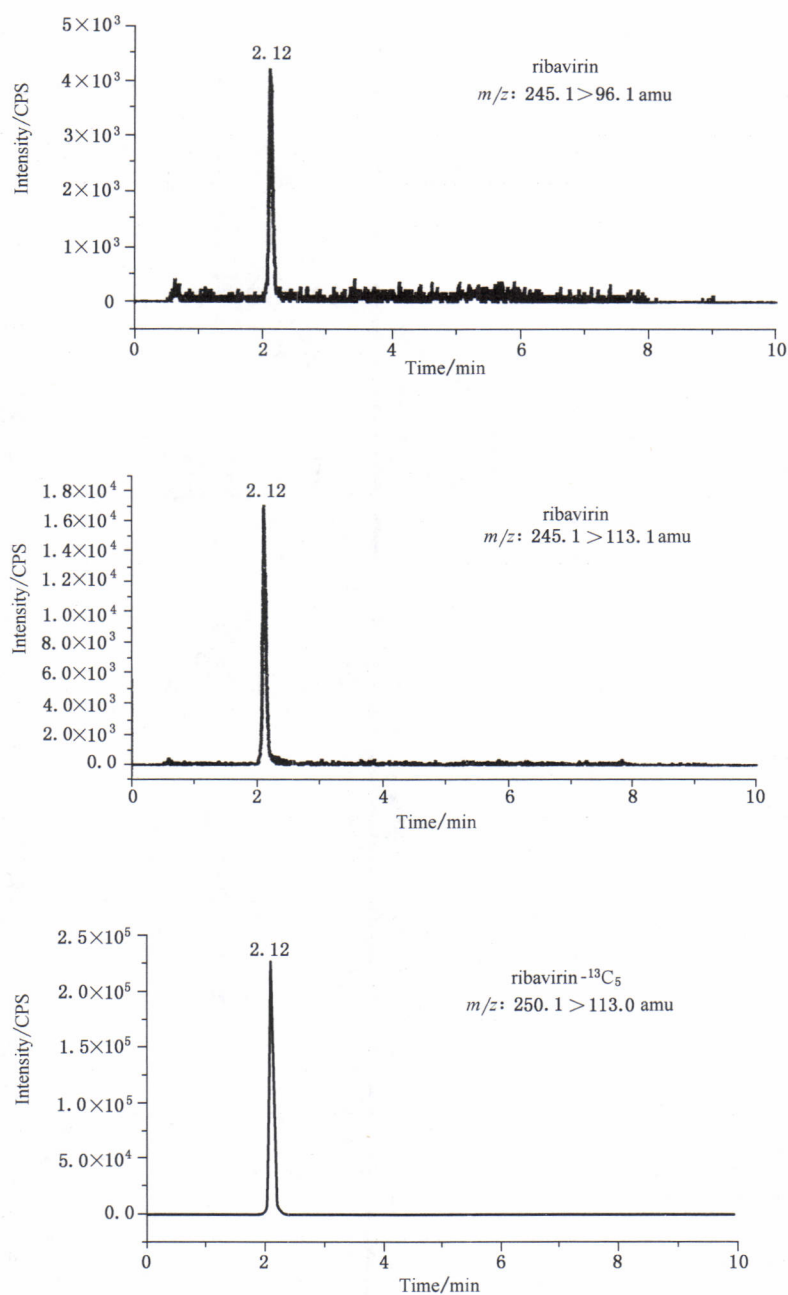


Figure B.1 Multiple reaction monitoring(MRM)chromatograms of ribavirin(2 ng/mL)

Annex C

(Informative)

Recovery

Date of the average recoveries of ribavirin in chicken, egg, liver, kidney, pork, eel, shrimp, preserved egg, and chicken product see table C.1.

Table C.1 The average recoveries of ribavirin in different samples (n=6)

Sample Name	Levels/($\mu\text{g/kg}$)	The average recoveries/%
chicken	1.0	77.8—107.0
	2.0	81.0—104.0
	10.0	82.7—99.1
egg	1.0	81.9—102.0
	2.0	82.5—101.5
	10.0	80.3—100.0
liver	1.0	78.3—102.0
	2.0	82.0—100.5
	10.0	80.4—93.4
kidney	1.0	78.6—102.0
	2.0	78.5—95.0
	10.0	76.6—89.9
pork	1.0	81.8—101.0
	2.0	82.0—100.5
	10.0	80.7—93.8
eel	1.0	81.7—103.0
	2.0	80.0—103.5
	10.0	83.5—97.0
shrimp	1.0	80.7—106.0
	2.0	80.5—104.0
	10.0	82.0—98.3
Cooking chicken tail string	1.0	81.4—107.0
	2.0	80.0—101.5
	10.0	77.6—91.8
Preserved egg	1.0	79.7—103.0
	2.0	82.0—98.0
	10.0	80.9—95.9

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