SN

中华人民共和国出入境检验检疫行业标准

SN/T 2578—2010

进出口蜂王浆中15种喹诺酮类药物残留量的检测方法 液相色谱-质谱/质谱法

Determination of fifteen quinolones residues in royal jelly for import and export—LC-MS/MS

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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。

本标准由中华人民共和国浙江出入境检验检疫局负责起草。

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进出口蜂王浆中15种喹诺酮类药物残留量的检测方法 液相色谱-质谱/质谱法

1 范围

本标准规定了进出口蜂王浆中喹诺酮残留量测定的制样和液相色谱-质谱/质谱测定方法。

本标准适用于蜂王浆中麻保沙星、依诺沙星、氧氟沙星、诺氟沙星、环丙沙星、达氟沙星、恩诺沙星、洛美沙星、奥比沙星、双氟沙星、沙拉沙星、司帕沙星、恶喹酸、萘啶酸、氟甲喹残留量的检测。

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

用甲醇沉淀样品中蛋白质，上清液用Oasis(HLB)固相萃取小柱净化，液相色谱-质谱/质谱测定，外标法或内标法定量。

4 试剂和材料

除另有规定外，所有试剂均为分析纯，水为GB/T 6682规定的一级水。

4.1 乙腈：高效液相色谱级。

4.2 甲醇：高效液相色谱级。

4.3 甲酸：高效液相色谱级。

4.4 氨水：25%～28%。

4.5 甲醇水溶液：甲醇-水(1+9，体积比)。

4.6 5%氨水甲醇溶液：氨水-甲醇(5+95，体积比)。

4.7 麻保沙星、依诺沙星、氧氟沙星、诺氟沙星、环丙沙星、达氟沙星、恩诺沙星、洛美沙星、奥比沙星、双氟沙星、沙拉沙星、司帕沙星、恶喹酸、萘啶酸、氟甲喹、诺氟沙星-D5、环丙沙星-D8标准品：纯度大于等于96%，见附录A表A.1。

4.8 标准储备溶液：分别称取经折算适量标准品(4.7)(精确至0.1 mg)，分别用甲醇溶解定容，溶液浓度为100 μg/mL，1 ℃～4 ℃冰箱保存。有效期3个月。

4.9 诺氟沙星-D5、环丙沙星-D8同位素内标标准储备溶液：分别称取适量标准品(4.7)，分别用甲醇溶解定容，溶液浓度为100 μg/mL，1 ℃～4 ℃冰箱保存。

4.10 空白样品提取液：用不含15种喹诺酮的蜂王浆样品，按照第7章制备空白样品溶液。

4.11 标准工作溶液：根据需要用空白样品提取液(4.10)将标准储备液稀释成 2 ng/mL、2.5 ng/mL、5 ng/mL、10 ng/mL、20 ng/mL 的混合标准工作溶液，相当于样品中含有 4 μg/kg、5 μg/kg、10 μg/kg、20 μg/kg、40 μg/kg 喹诺酮类药物。内标溶液浓度为 7.5 ng/mL。

4.12 Oasis(HLB)固相萃取小柱，500 mg，5 mL 或相当者。使用前依次用 5 mL 甲醇，5 mL 水预洗。

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 天平：感量为 0.000 1 g 和 0.01 g。

5.3 固相萃取装置。

5.4 离心机：大于等于 6 000 r/min。

5.5 旋涡混合器。

5.6 旋转蒸发器。

5.7 具塞离心管：聚四氟乙烯，50 mL。

6 试样制备与保存

取 500 g 代表性蜂王浆样品，在室温下解冻，等样品全部融化后搅匀，将试样均分成两份，分别装入样品瓶中，密封，并标明标记。一份作为试验样，另一份在－18 ℃保存。在制样的操作过程中，应防止样品污染或发生残留物含量的变化。

7 测定步骤

7.1 提取

称取 2 g 试样(精确到 0.01 g)置于 50 mL 具塞离心管中，加 0.2 mL 同位素内标混合标准溶液(150 ng/mL)和 10 mL 水，混匀，静置 2 min，再加甲醇至 20 mL，于旋涡混合器上以 2 000 r/min 混匀 1 min，以 6 000 r/min 离心 5 min，过滤，移取 5.0 mL 上清液，加 25 mL 水，混匀，待净化。

7.2 净化

将溶液转移至 Oasis HLB 固相萃取小柱中(4.12)，弃之流出液，加 5 mL 水和 5 mL 甲醇水溶液(4.5)淋洗，抽干，用 4 mL 5%氨水甲醇洗脱(4.6)，控制流速 1 mL/min～2 mL/min，收集全部洗脱液于 10 mL 离心管中，将洗脱液用乙腈转移至浓缩瓶中，在 45 ℃以下水浴下减压浓缩至近干，用 1.0 mL 乙腈-甲醇－0.15%甲酸(2+20+78，体积比)溶解残渣，混匀，过 0.45 μm，滤膜，供液相色谱-质谱/质谱仪测定。

7.3 测定

7.3.1 液相色谱-串联质谱条件

a) 色谱柱：C_8 柱，150 mm×4.6 mm(内径)，5 μm 或相当者；

b) 流动相梯度洗脱程序见表 1；

表 1 梯度洗脱程序

时间/min	乙腈/%	甲醇/%	0.15%甲酸水溶液/%
0	2	20	78
4	5	20	75
8	10	20	70
10	40	20	40
15	40	20	40
15.5	2	20	78
22	2	20	78

c) 流速:0.4 mL/min;
d) 进样量:50 μL;
e) 离子源:电喷雾离子源;
f) 扫描方式:正离子扫描;
g) 检测方式:多反应监测;
h) 雾化气压力(GS1)、气帘气压力(CUR)、辅助气流速(GS2)均为高纯氮气或其他合适气体;使用前应调节各气体流量以及离子源温度(TEM)使质谱灵敏度达到检测要求,参考条件及监测离子对(m/z)参见附录 B。

7.3.2 高效液相色谱-串联质谱测定

根据试样中被测样液的含量,选定浓度相近的混合基质标准溶液,待测物的响应值应在仪器检测的线性范围内。对混合基质标准溶液及样液等体积参插进样测定。在上述色谱条件下麻保沙星、依诺沙星、氧氟沙星、诺氟沙星、环丙沙星、达氟沙星、恩诺沙星、洛美沙星、奥比沙星、双氟沙星、沙拉沙星、司帕沙星、噁喹酸、萘啶酸、氟甲喹的参考保留时间分别约为 12.4 min、13.3 min、13.8 min、14.5 min、14.8 min、14.8 min、15.1 min、15.1 min、15.2 min、15.5 min、15.5 min、15.5 min、17.0 min、19.1 min、19.4 min,标准溶液的选择性离子流图参见附录 C 中图 C.1。

7.3.3 定性测定

按照上述条件测定样品和混合基质标准工作液,如果检测的质量色谱峰保留时间与混合基质标准工作液一致,允许偏差小于±2.5%。定性离子对的相对丰度与浓度相当混合基质标准工作液的相对丰度一致,相对丰度偏差不超过表 2 的规定,则可判断样品中存在相应的被测物。

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

7.3.4 空白试验

除不加试样外,均按上述操作步骤进行。

8 结果计算和表述

用色谱数据处理机或按式(1)计算试样中喹诺酮类药物的残留含量，计算结果需扣除空白值：

$$X_i = \frac{c_i \times V}{m} \quad \cdots\cdots(1)$$

式中：

X_i ——试样中喹诺酮类药物残留量，单位为微克每千克(μg/kg)；

c_i ——基质标准溶液中喹诺酮类药物残留量的浓度，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)；

m ——最终样液代表的试样质量，单位为克(g)。

9 方法的测定低限(LOQ)和回收率

9.1 测定低限

喹诺酮类药物残留量测定低限为5 μg/kg。

9.2 回收率

回收率的实验数据(在不同添加浓度范围内)见表3。

表3 15种喹诺酮类药物添加回收率范围(n=6)

化合物	添加浓度/(μg/kg)	回收率/%
麻保沙星	5	87.8～96.6
	10	78.5～94.3
	20	80.0～96.5
依诺沙星	5	72.8～103.2
	10	79.6～105.0
	20	85.0～106.0
氧氟沙星	5	71.0～91.2
	10	75.4～96.9
	20	72.0～94.0
诺氟沙星	5	72.0～99.6
	10	75.0～104.0
	20	73.0～102.5
环丙沙星	5	80.6～108.4
	10	71.0～108.0
	20	78.0～107.0
达氟沙星	5	71.4～98.8
	10	75.8～98.7
	20	74.5～91.5

表 3（续）

化合物	添加浓度/(μg/kg)	回收率/%
恩诺沙星	5	72.2～101.8
	10	71.3～107.0
	20	71.0～104.0
洛美沙星	5	60.0～76.8
	10	61.2～74.7
	20	61.5～80.5
奥比沙星	5	72.0～98.6
	10	72.5～98.1
	20	73.0～99.0
双氟沙星	5	62.6～71.2
	10	60.3～81.9
	20	62.0～74.5
沙拉沙星	5	61.6～78.0
	10	61.2～73.4
	20	61.5～83.0
司帕沙星	5	74.2～108.6
	10	73.4～104.0
	20	71.5～99.0
噁喹酸	5	77.8～97.6
	10	74.4～98.3
	20	70.1～97.0
萘啶酸	5	71.4～97.4
	10	75.7～96.8
	20	72.5～98.0
氟甲喹	5	51.6～81.6
	10	51.8～70.0
	20	50.5～71.5

附 录 A
（规范性附录）
喹诺酮类药物标准品信息

表 A.1 喹诺酮类药物标准品信息

中文名称	英文名称	CAS 编号	分子式	相对分子质量
麻保沙星	marbofloxacin	115550-35-1	$C_{17}H_{19}N_4O_4F$	362.36
依诺沙星	enoxacin	74011-58-8	$C_{15}H_{17}FN_4O_3$	320.32
氧氟沙星	ofloxacin	82419-36-1	$C_{18}H_{20}FN_3O_4$	361.37
诺氟沙星	norfloxacin	70458-96-7	$C_{16}H_{18}FN_3O_3$	319.33
环丙沙星	ciprofloxacin hydrochloride	86393-32-0	$C_{17}H_{18}FN_3O_3HCl$	367.81
达氟沙星	danofloxacin mesylate	119478-55-6	$C_{20}H_{24}FN_3O_6S$	453.6
恩诺沙星	enrofloxacine	93106-60-6	$C_{19}H_{22}FN_3O_3$	359.4
洛美沙星	lomefloxacin hydrochloride	98079-52-8	$C_{17}H_{19}F_2N_3O_3 \cdot HCl$	387.8
奥比沙星	orbifloxacin	113617-63-3	$C_{19}H_{20}F_3N_3O_3$	395.38
双氟沙星	difloxacin	98106-17-3	$C_{21}H_{19}F_2N_3O_3$	400.16
沙拉沙星	sarafloxacin hydrochloride	98105-99-8	$C_{20}H_{17}F_2N_3O_3HCl$	421.83
司帕沙星	sparfloxacin	111542-93-9	$C_{19}H_{22}F_2N_4O_3$	392.40
噁喹酸	oxolinic acid	14698-29-4	$C_{13}H_{11}NO_5$	261.23
萘啶酸	nalidixic acid	389-08-2	$C_{12}H_{12}N_2O_3$	232.23
氟甲喹	flumequine	42835-25-6	$C_{14}H_{12}FNO_3$	261.25
诺氟沙星-D5	norfloxacin-D5	—	—	324.33
环丙沙星-D8	ciprofloxacin-D8	—	—	339.81

附 录 B
（资料性附录）
API 4000 LC-MS/MS 系统电喷雾离子源参考条件[1)]

API 4000 LC-MS/MS 系统电喷雾离子源参考条件：

a) 电喷雾电压(IS)：5 500 V；

b) 雾化气压力(GS1)：289.59 kPa(42 psi)；

c) 气帘气压力(CUR)：172.375 kPa(25 psi)；

d) 辅助气流速(GS2)：310.275 kPa(45 psi)；

e) 离子源温度(TEM)：550 ℃；

f) 碰撞气(CAD)：6；

g) 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 B.1。

表 B.1 定性离子对、定量离子对、去簇电压(DP)、碰撞气能量和碰撞室出口电压

名称	离子对 m/z	去簇电压(DP)/V	碰撞气能量(CE)/V	碰撞室出口电压(CXP)/V	内标化合物名称
麻保沙星	363.2/320.2[a]	63	23	10	
	363.2/345.3	63	25	10	
伊诺沙星	321.2/303.1[a]	67	29	10	环丙沙星-D8
	321.2/234.4	67	32	10	
氧氟沙星	362.2/318.3[a]	75	27	8	诺氟沙星-D5
	362.2/261.2	75	39	6	
诺氟沙星	320.0/233.3[a]	74	35	11	诺氟沙星-D5
	320.0/276.3	74	25	14	
环丙沙星	332.1/288.3[a]	85	27	7	环丙沙星 D8
	332.1/230.9	85	50	12	
达氟沙星	358.2/340.3[a]	65	35	9	环丙沙星-D8
	358.2/283.1	65	33	9	
恩诺沙星	360.2/316.2[a]	76	29	7	环丙沙星-D8
	360.2/245.4	76	35	9	
洛美沙星	352.3/265.1[a]	80	35	10	—
	352.3/308.2	80	24	10	
奥比沙星	396.0/352.0[a]	76	27	9	—
	396.0/295.0	76	35	9	
双氟沙星	400.0/356.0[a]	76	29	9	环丙沙星-D8
	400.0/299.0	76	42	9	
沙拉氟沙星	386.1/342.4[a]	87	27	8	环丙沙星-D8
	386.1/299.2	87	39	7	

1) 非商业性声明：附录 B 所列参数是在 API 4000 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。

表 B.1（续）

名称	离子对 m/z	去簇电压(DP)/V	碰撞气能量(CE)/V	碰撞室出口电压(CXP)/V	内标化合物名称
司帕沙星	393.2/292.2[a] 393.2/349.2	94 94	36 28	10 10	环丙沙星-D8
噁喹酸	262.0/244.4[a] 262.0/216.3	75 75	25 40	18 18	环丙沙星-D8
萘啶酸	233.2/215.0[a] 233.2/187.0	53 53	21 35	10 10	环丙沙星-D8
氟甲喹	262.1/244.0[a] 262.1/202.1	73 73	27 49	10 10	环丙沙星-D8
诺氟沙星-D5	325.4/281.2	65	25	9	—
环丙沙星-D8	340.1/296.5	90	27	10	—
[a] 该离子对为定量离子对。					

附 录 C
（资料性附录）
喹诺酮类药物标准溶液选择性离子流图

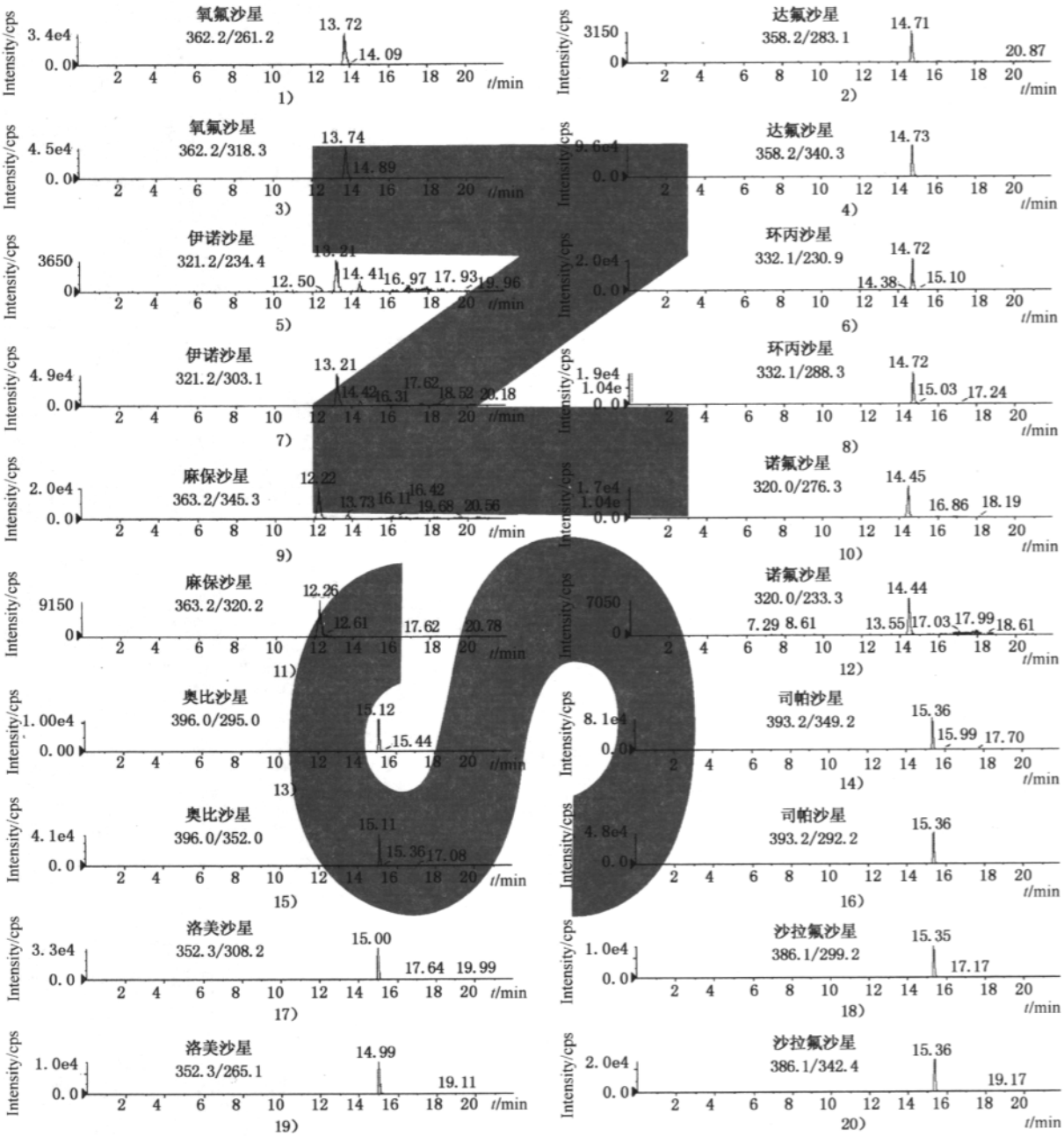

图 C.1 喹诺酮类药物(2.5 ng/mL)的标准品的选择性离子流图

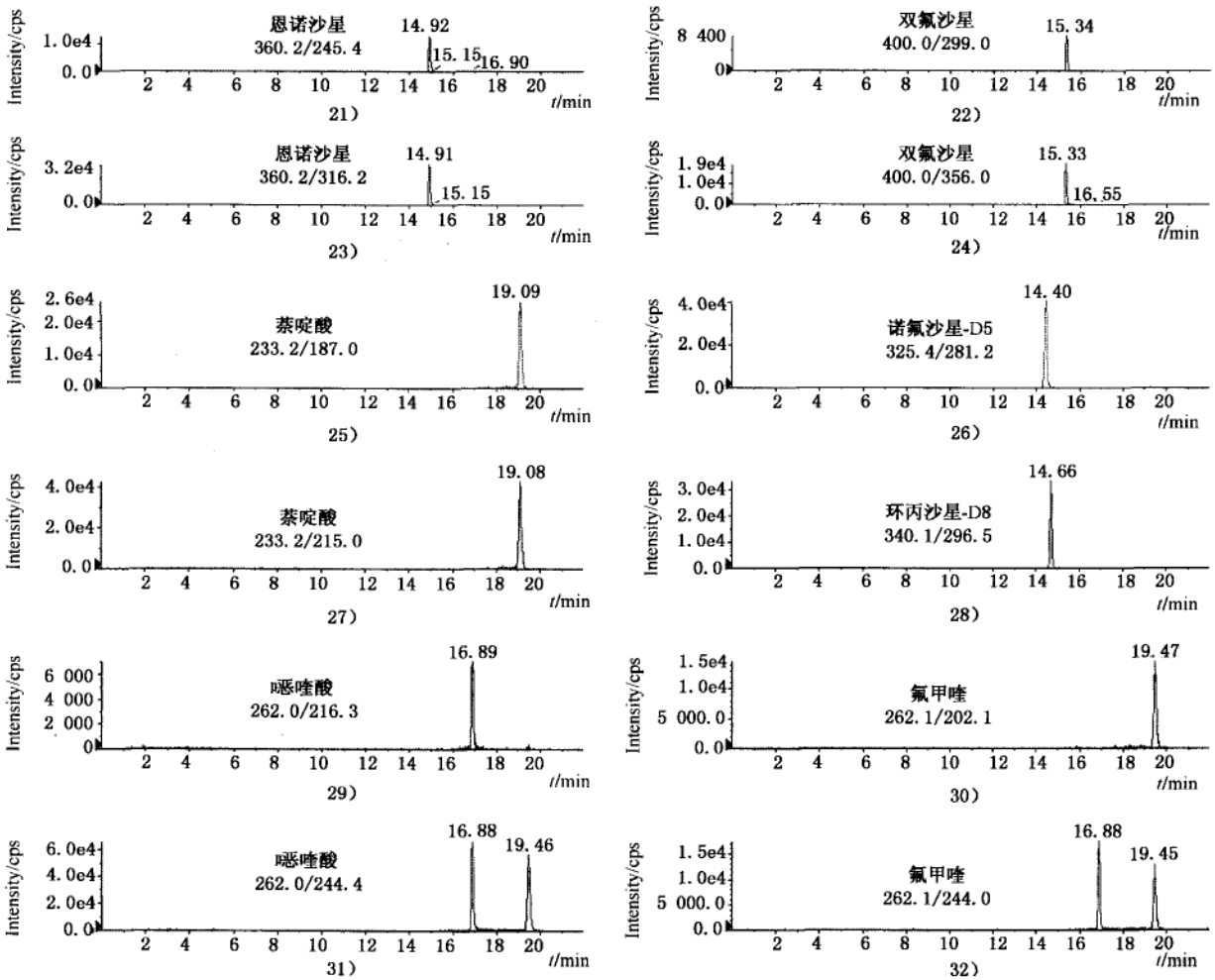
恩诺沙星
360.2/245.4
14.92
15.15
16.90
21)
恩诺沙星
360.2/316.2
14.91
15.15
23)
萘啶酸
233.2/187.0
19.09
25)
萘啶酸
233.2/215.0
19.08
27)
噁喹酸
262.0/216.3
16.89
29)
噁喹酸
262.0/244.4
16.88
19.46
31)
双氟沙星
400.0/299.0
15.34
22)
双氟沙星
400.0/356.0
15.33
16.55
24)
诺氟沙星-D5
325.4/281.2
14.40
26)
环丙沙星-D8
340.1/296.5
14.66
28)
氟甲喹
262.1/202.1
19.47
30)
氟甲喹
262.1/244.0
16.88
19.45
32)
Intensity/cps
t/min

图 C.1（续）

Foreword

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 Zhejiang Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The standard was mainly drafted by Xie Wen, Chen Xiaomei, Ding Huiying, Qian Yan, Liu Haishan, Zhang Huimin.

Determination of fifteen quinolones residues in royal jelly for import and export—LC-MS/MS

1 Scope

The standard specifies the method of sample preparation and determination of fifteen quinolones residues in royal jelly by LC-MS/MS.

This standard is applicable to the determination of residues of marbofloxacin, enoxacin, ofloxacin, norfloxacin, ciprofloxacin, danofloxacin mesylate, enrofloxacine, lomefloxacin, orbifloxacin, difloxacin, sarafloxacin, sparfloxacin, oxolinic acid, nalidixic acid, flumequine in royal jelly.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this professional standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based in this professional 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

Methanol is used to precipitate protein. Then the supernatant is cleaned up with HLB cartridge. The residues are determined by LC-MS/MS and quantified by external standard method or 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 Acetonitrile: HPLC grade.

4.2 Methanol: HPLC grade.

4.3 Formic acid: HPLC grade.

4.4 Ammonia water: 25%～28%.

4.5 Methanol aqueous solution: Methanol-water (1+9, *V*/*V*).

4.6 5% Ammonia in methanol solution: Ammonia water-methanol (5+95, *V*/*V*).

4.7 Marbofloxacin, enoxacin, ofloxacin, norfloxacin, ciprofloxacin hydrochloride, danofloxacin mesylate, enrofloxacine, lomefloxacin hydrochloride, orbifloxacin, difloxacin, sarafloxacin hydrochloride, sparfloxacin, oxolinic acid, nalidixic acid, flumequine, norfloxacin-D5 and ciprofloxacin-D8 standards: Purity ≥96%. Other information see annex A table A.1.

4.8 Standard stock solutions: Accurately weigh appropriate amount of each standard (4.7) (accurate to 0.1 mg), dissolve with methanol respectively, the concentration of solution is 100 μg/mL. Standard stock solutions should be stored at 1 ℃～4 ℃ in refrigerator. Standard stock solutions are stable for three months.

4.9 Isotope standard stock solutions of norfloxacin-D5 and ciprofloxacin-D8: Accurately weigh appropriate amount of each standard (4.7), dissolve with methanol respectively, the concentration of solution is 100 μg/mL. Standard stock solutions should be stored at 1 ℃～4 ℃ in refrigerator.

4.10 Blank sample solution: According to section 7, blank sample solution is prepared with royal jelly sample without fifteen quinolones.

4.11 Calibration curve standard working solutions: According to the requirement, dilute the standard stock solution to 2 ng/mL, 2.5 ng/mL, 5 ng/mL, 10 ng/mL, and 20 ng/mL, mix standard working solution with blank solution of sample (4.10) before using. It is same as 4 μg/kg, 5 μg/kg, 10 μg/kg, 20 μg/kg, and 40 μg/kg quinolones in sample. The concentrations of norfloxacin-D5 and ciprofloxacin-D8 are 7.5 ng/mL.

4.12 Oasis (HLB) solid-phase extraction (SPE) cartridge: 500 mg, 5 mL or equivalent. It should be conditioned with 5 mL methanol followed by 5 mL water before use.

4.13 Membrane filter: 0.45 μm, organic type.

5 Apparatus and equipment

5.1 Liquid chromatography-tandem mass spectrometry, equipped with electrospray ion source.

5.2 Analytical balance, accuracy: 0.000 1 g, 0.01 g.

5.3 Solid phase extraction vacuum container.

5.4 Centrifuge: ≥6 000 r/min.

5.5 Vortex mixer.

5.6 Rotary vacuum evaporator.

5.7 Centrifuge tube: Polytetrafluoroethylene, 50 mL.

6 Sample preparation and storage

Royal jelly is about 500 g. The sample is melted under room temperature. Keep the prepared sample into two sample bottles, seal and label. The test sample is stored at room temperature. The rest sample is stored in −18 ℃ refrigerator. In the course of sample preparation, precautions shall be taken to avoid contamination or any factors, which may cause the change of residue content.

7 Analytical Procedure

7.1 Extraction

Weigh ca 2 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube. Add 0.2 mL isotope standard solution of norfloxacin-D5 and ciprofloxacin-D8 (150 ng/mL) and 10 mL water, mix it. Standing for 2 min. Add to 20 mL with methanol. Vortex for 1 min under 2 000 r/min. Centrifuge for 5 min under 6 000 r/min, then filtrate and transfer 5.0 mL supernatant layer into a container. Add 25 mL water, mix it.

7.2 Clean up

Transfer the above solution into the Oasis HLB cartridge (4.12), discard the eluate. Rinse the cartridge with 5 mL water and 5 mL methanol aqueous solution (4.5), discard the eluate. The cartridge is dried to "dryness". Elute the cartridge with 4 mL 5% ammonia methanol (4.6). The elute is transferred to flask with acetonitrile. The solution is evaporated to nearly dryness in a water bath below 45 ℃. The residues are reconstituted in 1.0 mL acetonitrile-methanol-0.15% formic acid (2+20+78, *V*/*V*/*V*), mix it. The solution is passed through 0.45 μm filter. The filtrate is ready for LC-MS/MS determination.

7.3 Determination

7.3.1 LC-MS/MS operating conditions

a) Column: C_8, 150 mm × 4.6 mm(i.d.), 5 μm or the equivalent.

b) Mobile phase: See table 1.

Table 1—Gradient of mobile phase

Time/(min)	Acetonitrile/%	Methanol/%	0.15% formic acid/%
0	2	20	78
4	5	20	75
8	10	20	70
10	40	20	40
15	40	20	40
15.5	2	20	78
22	2	20	78

c) Flow rate: 0.4 mL/min.

d) Injection volume: 50 μL.

e) Ion source: Electrospray ionization.

f) Polarity: Positive.

g) Monitoring model: Multiple reaction monitor (MRM).

h) Nebulizer gas (GS1), curtain gas (CUR), auxiliary heater gas (GS2) are all high purity nitrogen (N2) or equivalent. Optimize the flow rate of each gas and ion source temperature to reach the requirement of the sensitivity of mass spectrometry. Detailed parameters, qualifier and quantifier MRM are listed in annex B.

7.3.2 LC-MS/MS determination

According to the concentrations of quinolones in sample solution, select the standard working solution of similar concentration to that of sample solution. The responses of quinolones in the sample solution should be within the linear range of the calibration curve. The standard working solution should be injected randomly in between the injections of the sample solution of equal volume. Under the above LC-MS/MS operating condition, the retention time of marbofloxacin, enoxacin, ofloxacin, norfloxacin, ciprofloxacin, danofloxacin, enrofloxacine, lomefloxacin,

orbifloxacin, difloxacin, sarafloxacin, sparfloxacin, oxolinic acid, nalidixic acid and flumequine is 12.4 min, 13.3 min, 13.8 min, 14.5 min, 14.8 min, 14.8 min, 15.1 min, 15.1 min, 15.2 min, 15.5 min, 15.5 min, 15.5 min, 17.0 min, 19.1 min, 19.4 min. Selected ion chromatograms of the standards are shown in figure C.1 of annex C.

7.3.3 LC-MS/MS confirmation

Under above determination condition, the variation range of the retention time for the peak of analyte in unknown sample and in the standard working solution can not be out of range of ±0.25%. 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 can not be out of range of table 2, and then the corresponding analyte must be present in the sample.

Table 2—Maximum permitted tolerances for relative ion intensities while confirmation

Relative intensity/%	>50	>20~50	>10~20	≤10
Permitted tolerances/%	±20	±25	±30	±50

7.3.4 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

Calculate the content of quinolones residues in the test sample by LC-MS/MS data processor or according to the following formula (1), the blank value should be subtracted from the above result of calculation.

$$X_i = \frac{c_i \times V}{m} \qquad \cdots\cdots(1)$$

Where:

X_i —the residue content of quinolones residues in the test sample, μg/kg;

c_i —the concentration of quinolones residues obtained from calibration curve, ng/mL;

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

m —mass of test sample of final sample solution, g.

9 Limit of quantification (LOQ) and recovery

9.1 Limit of quantification

The limit of quantifications of quinolones are 5 μg/kg.

9.2 Recovery

According to the experimental data, the corresponding recoveries of fortifying concentrations see table 3.

Table 3—Recoveries of quinolones residues ($n=6$)

Compound	Spiked level/(μg/kg)	Recovery/%
marbofloxacin	5	87.8～96.6
	10	78.5～94.3
	20	80.0～96.5
enoxacin	5	72.8～103.2
	10	79.6～105.0
	20	85.0～106.0
ofloxacin	5	71.0～91.2
	10	75.4～96.9
	20	72.0～94.0
norfloxacin	5	72.0～99.6
	10	75.0～104.0
	20	73.0～102.5
ciprofloxacin	5	80.6～108.4
	10	71.0～108.0
	20	78.0～107.0
danofloxacin	5	71.4～98.8
	10	75.8～98.7
	20	74.5～91.5
enrofloxacine	5	72.2～101.8
	10	71.3～107.0
	20	71.0～104.0
lomefloxacin	5	60.0～76.8
	10	61.2～74.7
	20	61.5～80.5

Table 3 (continued)

Compound	Spiked level/(μg/kg)	Recovery/%
orbifloxacin	5	72.0～98.6
	10	72.5～98.1
	20	73.0～99.0
difloxacin	5	62.6～71.2
	10	60.3～81.9
	20	62.0～74.5
sarafloxacin	5	61.6～78.0
	10	61.2～73.4
	20	61.5～83.0
sparfloxacin	5	74.2～108.6
	10	73.4～104.0
	20	71.5～99.0
oxolinic acid	5	77.8～97.6
	10	74.4～98.3
	20	70.1～97.0
nalidixic acid	5	71.4～97.4
	10	75.7～96.8
	20	72.5～98.0
flumequine	5	51.6～81.6
	10	51.8～70.0
	20	50.5～71.5

Annex A
(Normative annex)
Standard information

Table A. 1—Standard information of quinolones

Name	CAS No.	Molecular formula	Molecular weight
marbofloxacin	115550-35-1	$C_{17}H_{19}N_4O_4F$	362. 36
enoxacin	74011-58-8	$C_{15}H_{17}FN_4O_3$	320. 32
ofloxacin	82419-36-1	$C_{18}H_{20}FN_3O_4$	361. 37
norfloxacin	70458-96-7	$C_{16}H_{18}FN_3O_3$	319. 33
ciprofloxacin hydrochloride	86393-32-0	$C_{17}H_{18}FN_3O_3HCl$	367. 81
danofloxacin mesylate	119478-55-6	$C_{20}H_{24}FN_3O_6S$	453. 6
enrofloxacine	93106-60-6	$C_{19}H_{22}FN_3O_3$	359. 4
lomefloxacin hydrochloride	98079-52-8	$C_{17}H_{19}F_2N_3O_3$ HCl	387. 8
orbifloxacin	113617-63-3	$C_{19}H_{20}F_3N_3O_3$	395. 38
difloxacin	98106-17-3	$C_{21}H_{19}F_2N_3O_3$	400. 16
sarafloxacin hydrochloride	98105-99-8	$C_{20}H_{17}F_2N_3O_3 \cdot HCl$	421. 83
sparfloxacin	111542-93-9	$C_{19}H_{22}F_2N_4O_3$	392. 40
oxolinic acid	14698-29-4	$C_{13}H_{11}NO_5$	261. 23
nalidixic acid	389-08-2	$C_{12}H_{12}N_2O_3$	232. 23
flumequine	42835-25-6	$C_{14}H_{12}FNO_3$	261. 25
norfloxacin-D5	—	—	324. 33
ciprofloxacin-D8	—	—	339. 81

Annex B
(Informative annex)
API 4000 LC-MS/MS conditions[1)]

API 4000 LC-MS/MS conditions:

a) Electrospray capillary voltage:5 500 V;

b) GS1:289.59 kPa(42 psi);

c) CUR:172.375 kPa(25 psi);

d) GS2:310.275 kPa(45 psi);

e) Ion source temperature:550 ℃;

f) Collision gas (CAD):6;

g) Qualifier and quantifier MRM, Declustering potential (DP), Collision energy (CE), Collision cell exit potential (CXP) are shown in table B.1.

Table B.1—Transitions, DP, CE and CXP

Compound	Transitions m/z	DP/V	CE/V	CXP/V	Internal compound
marbofloxacin	363.2/320.2[a]	63	23	10	—
	363.2/345.3	63	25	10	
enoxacin	321.2/303.1[a]	67	29	10	ciprofloxacin-D8
	321.2/234.4	67	32	10	
ofloxacin	362.2/318.3[a]	75	27	8	norfloxacin-D5
	362.2/261.2	75	39	6	
norfloxacin	320.0/233.3[a]	74	35	11	norfloxacin-D5
	320.0/276.3	74	25	14	
ciprofloxacin	332.1/288.3[a]	85	27	7	ciprofloxacin-D8
	332.1/230.9	85	50	12	
danofloxacin	358.2/340.3[a]	65	35	9	ciprofloxacin-D8
	358.2/283.1	65	33	9	

1) Non-commercial statement: Parameters listed in annex B are accomplished by API 4000 LC-MS/MS. The equipment and its type involved in the standard method is only for reference and not related to commercial aims, and the analysts are encouraged to use equipments of different corporation or different type.

Table B. 1 (continued)

Compound	Transitions m/z	DP/V	CE/V	CXP/V	Internal compound
enrofloxacine	360. 2/316. 2[a] 360. 2/245. 4	76 76	29 35	7 9	ciprofloxacin-D8
lomefloxacin	352. 3/265. 1[a] 352. 3/308. 2	80 80	35 24	10 10	—
orbifloxacin	396. 0/352. 0[a] 396. 0/295. 0	76 76	27 35	9 9	—
difloxacin	400. 0/356. 0[a] 400. 0/299. 0	76 76	29 42	9 9	ciprofloxacin-D8
sarafloxacin	386. 1/342. 4[a] 386. 1/299. 2	87 87	27 39	8 7	ciprofloxacin-D8
sparfloxacin	393. 2/292. 2[a] 393. 2/349. 2	94 94	36 28	10 10	ciprofloxacin-D8
oxolinic acid	262. 0/244. 4[a] 262. 0/216. 3	75 75	25 40	18 18	ciprofloxacin-D8
nalidixic acid	233. 2/215. 0[a] 233. 2/187. 0	53 53	21 35	10 10	ciprofloxacin-D8
flumequine	262. 1/244. 0[a] 262. 1/202. 1	73 73	27 49	10 10	ciprofloxacin-D8
norfloxacin-D5	325. 4/281. 2	65	25	9	—
ciprofloxacin-D8	340. 1/296. 3	90	27	10	—
[a] product ion is used for quantification.					

Annex C
(Informative annex)
Selected ion chromatograms of quinolones standards

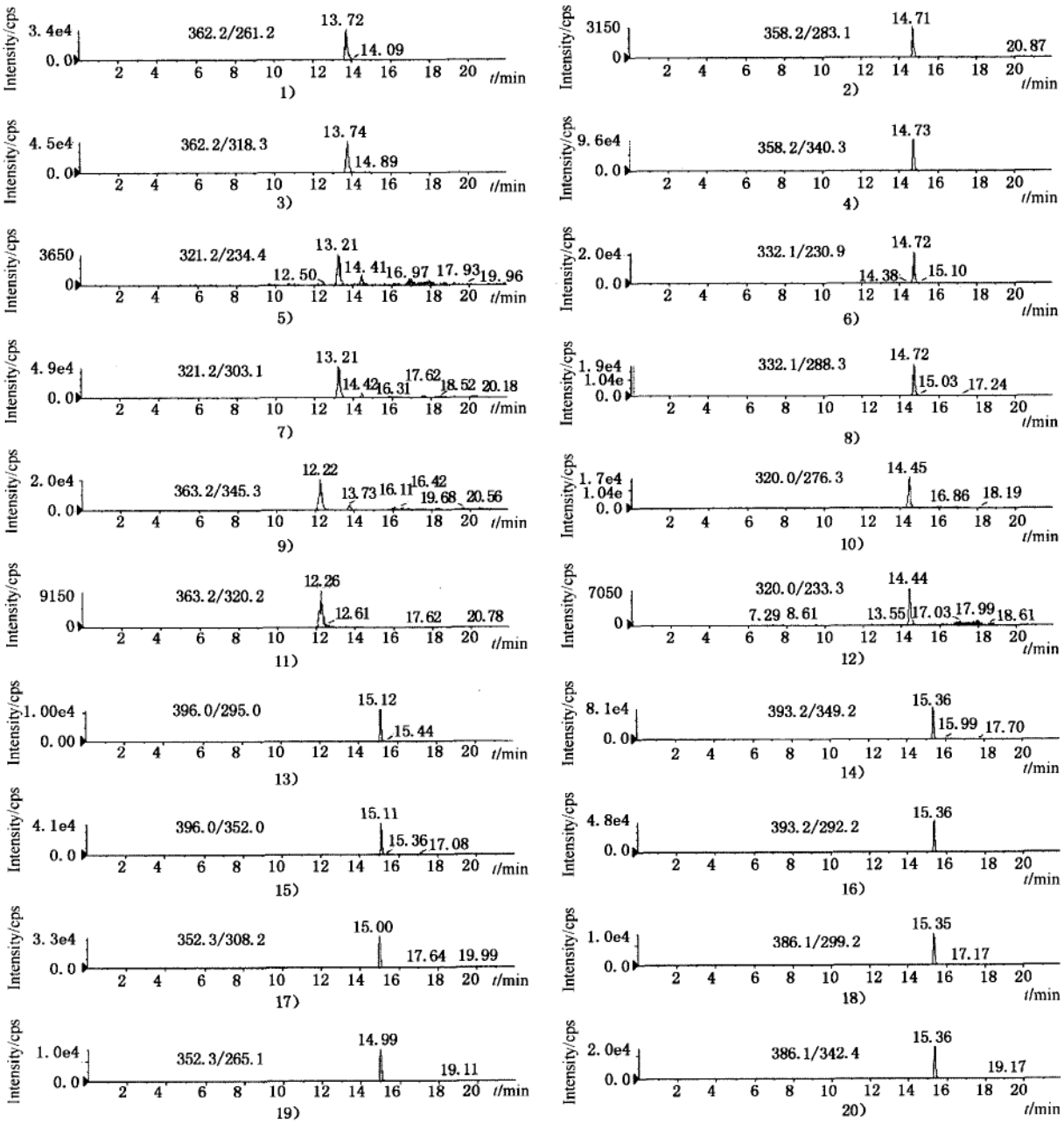

Figure C. 1—Selected ion chromatograms of quinolones standards (2.5 ng/mL)

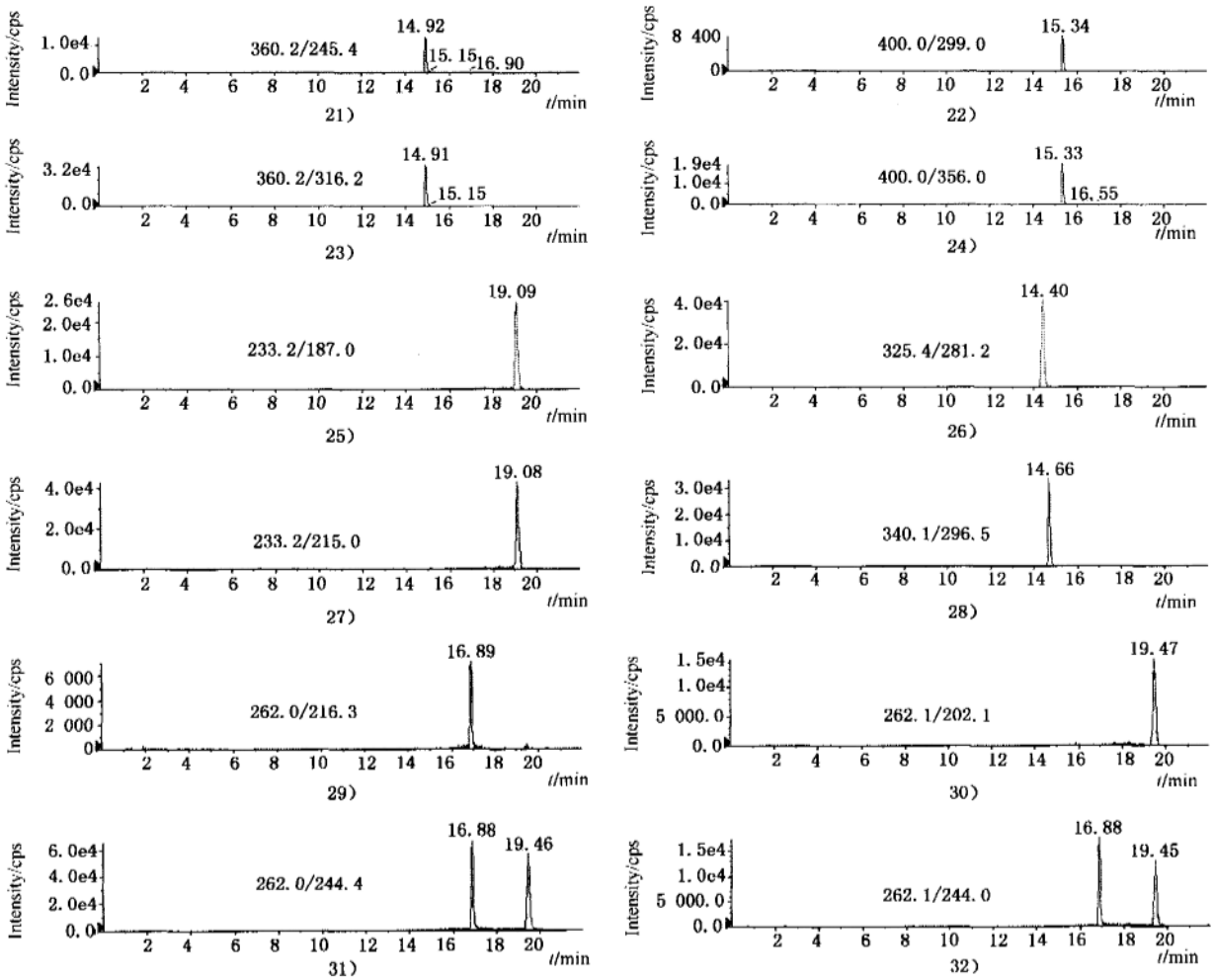

Figure C.1 (continued)