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中华人民共和国出入境检验检疫行业标准

SN/T 3155—2012

出口猪肉、虾、蜂蜜中多类药物残留量的测定　液相色谱-质谱/质谱法

Determination of multi-veterinary drugs residues in pork, shrimp and honey 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：中华人民共和国浙江出入境检验检疫局。

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1　范围

本标准规定了猪肉、虾和蜂蜜中多类药物残留测定的制样和液相色谱-质谱/质谱测定方法。

本标准适用于猪肉、虾和蜂蜜中磺胺嘧啶、磺胺噻唑、磺胺吡啶、磺胺甲基嘧啶、磺胺二甲基嘧啶、磺胺-5-(对)甲氧嘧啶、磺胺甲噻二唑、磺胺甲氧哒嗪、磺胺氯哒嗪、磺胺-6-(间)甲氧嘧啶、磺胺邻二甲氧嘧啶、磺胺甲基异恶唑、磺胺二甲异恶唑、苯酰磺胺、磺胺二甲氧嘧啶、磺胺喹噁啉、羟基甲硝唑、2-甲基-5-硝基咪唑、羟基二甲硝眯唑、甲硝唑、二甲硝咪唑、洛硝哒唑、氯甲硝咪唑、硝基苯并咪唑、羟基异丙硝唑、异丙硝唑、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、螺旋霉素、替米考星、竹桃霉素、泰乐菌素、红霉素、罗红霉素、交沙霉素、吉他霉素、泰妙菌素、林可霉素、氯林可霉素、吡利霉素和吡喹酮残留量的检测。

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　方法原理

猪肉、虾样品经乙腈提取，正己烷脱脂，再用 C_{18} 固相萃取小柱净化；蜂蜜经磷酸盐缓冲溶液稀释后直接用 HLB 固相萃取小柱净化，液相色谱-质谱/质谱法测定，内标法或外标法进行定量。

4　试剂和材料

除另有说明外，所用试剂均为色谱级，水为符合 GB/T 6682 规定的一级水。

4.1　乙腈。

4.2　甲醇。

4.3　甲酸。

4.4　正己烷。

4.5　无水硫酸钠：分析纯。650 ℃灼烧 4 h，在干燥器内冷却至室温，贮于密封瓶中备用。

4.6　磷酸二氢钠：分析纯。

4.7　氢氧化钠：分析纯。

4.8　0.1 mol/L 氢氧化钠溶液：称取 4 g 氢氧化钠，并用水稀释至 1 L。

4.9　磷酸盐缓冲溶液：溶解 13.8 g 磷酸二氢钠于 950 mL 水中，用 0.1 mol/L 氢氧化钠溶液调节溶液 pH 值到 8.0，最后用水稀释至 1 L。

4.10　兽药标准品：磺胺类药物纯度大于等于 98%，硝基咪唑类药物纯度大于等于 98%，喹诺酮类药物

纯度大于等于96%，大环内酯类药物纯度大于等于95%，林可酰胺类药物纯度大于等于86%，吡喹酮纯度大于等于98%，详细标准物质信息见表A.1。

4.11 同位素内标标准品：磺胺嘧啶-D4、磺胺噻唑-D6、磺胺吡啶-D6、磺胺二甲基嘧啶-D4、磺胺甲基异恶唑-D6、磺胺二甲氧嘧啶-D4、磺胺喹噁啉-D6、羟基二甲硝咪唑-D3、二甲硝咪唑-D3、洛硝哒唑-D3、羟基异丙硝唑-D3、异丙硝唑-D3、依诺沙星-D8、氧氟沙星-D3、诺氟沙星-D5、环丙沙星-D8、恩诺沙星-D5、双氟沙星-D4、沙拉沙星-D8同位素标准品：纯度大于等于98%，详细信息见表A.1。

4.12 标准储备溶液的配制：分别称取经折算的适量标准品(4.10)(精确至0.1 mg)，用甲醇溶解定容，各类化合物溶液浓度均为1 mg/mL，1 ℃～4 ℃冰箱避光保存。

4.13 同位素内标标准储备溶液的配制：分别称取适量同位素内标标准品(4.11)，用甲醇溶解定容，各类化合物溶液浓度均为1 mg/mL，1 ℃～4 ℃冰箱避光保存。

4.14 中间标准混合溶液的配制：用甲醇稀释标准储备溶液(4.12)至混合标准溶液中最终含有磺胺类药物浓度为500 ng/mL，硝基咪唑类药物浓度为500 ng/mL，喹诺酮类药物浓度为1 000 ng/mL，大环内酯类药物浓度为1 500 ng/mL，林可酰胺类药物浓度为1 000 ng/mL，吡喹酮药物浓度为150 ng/mL，1 ℃～4 ℃冰箱避光保存。

4.15 空白样品提取液：用不含待测药物的猪肉、虾或蜂蜜样品，按照7.1制备空白样品溶液。

4.16 基质混合标准工作溶液：根据需要用空白基质溶液(4.15)将中间标准混合溶液(4.14)稀释成合适浓度的混合标准工作溶液，现用现配。

4.17 无水硫酸钠柱：80 mm×40 mm(内径)筒形漏斗，底部垫5 mm脱脂棉，再装40 mm无水硫酸钠。

4.18 C_{18}固相萃取小柱：500 mg，3 mL或相当者，使用前用5 mL甲醇预洗。

4.19 Oasis(HLB)固相萃取小柱，500 mg，6 mL或相当者。使用前依次用5 mL甲醇，5 mL磷酸盐缓冲溶液(4.9)预洗。

4.20 微孔滤膜：0.22 μm，有机相。

5 仪器和设备

5.1 液相色谱-质谱/质谱仪：配有电喷雾离子源(ESI)。

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 减压浓缩仪。

5.7 具塞离心管：聚丙烯，50 mL。

6 试样制备与保存

6.1 制备与保存要求

在制样的操作过程中，应防止样品受到污染或发生药物残留含量的变化。

6.2 猪肉、虾

猪肉、虾样品：取500 g代表性猪肉和虾样品，在室温下解冻，待样品全部融化后破碎，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 ℃以下保存。

6.3 蜂蜜

取500 g代表性蜂蜜样品，未结晶的样品将其用力搅拌均匀，有结晶析出的样品可将样品瓶盖塞紧

后，置于不超过 60 ℃的水浴中温热，等样品全部熔化后搅匀，迅速冷却至室温。在熔化时应注意防止水分挥发。制备好的试样均分成两份，分别装入样品瓶中，密封，并标明标记，于常温下存放。

7 分析步骤

7.1 提取与净化

7.1.1 猪肉、虾

称取 5 g 试样(精确到 0.01 g)置于 50 mL 具塞离心管中(5.7)，加入同位素内标溶液(磺胺类同位素内标 8 ng，硝基咪唑类同位素内标 40 ng，喹诺酮类同位素内标 30 ng)，加入 20 mL 乙腈，混匀，以 3 000 r/min离心 3 min，上清液转移至另一 50 mL 具塞离心管中；残渣再加入 15 mL 乙腈重复上述操作，合并乙腈提取液，以 7 000 r/min 离心 5 min，取乙腈上清液，加入 15 mL 正己烷，混匀，4 000 r/min 离心 3 min，弃去正己烷层，再加入 15 mL 正己烷，重复上述操作，乙腈提取液过无水硫酸钠柱(4.17)，在40 ℃以下水浴中减压浓缩至近干，加入 4 mL 甲醇溶解残渣，定量转移至 C_{18} 固相萃取小柱中(4.18)，控制流速 1 mL/min～2 mL/min，同时接收甲醇溶液，再分批加入 6 mL 甲醇进行洗脱，收集洗脱液，在 40 ℃以下水浴中减压浓缩至近干，用 2.0 mL 甲醇-水(3+7，体积比)定容，混匀，溶液过 0.22 μm 滤膜(4.20)，供液相色谱-质谱/质谱仪测定。

7.1.2 蜂蜜

称取 5 g 试样(精确到 0.01 g)置于 50 mL 具塞离心管中(5.7)，加入同位素内标溶液(磺胺类同位素内标 8 ng，硝基咪唑类同位素内标 40 ng，喹诺酮类同位素内标 30 ng)，加 20 mL 磷酸盐缓冲溶液(4.9)稀释，混匀，定量转移至 HLB 固相萃取小柱中(4.19)，控制流速 1 mL/min～2 mL/min，弃去流出液，加 20 mL 水淋洗，抽干，6 mL 甲醇洗脱，收集洗脱液，在 40 ℃以下水浴中减压浓缩至近干，用2.0 mL甲醇-水(3+7，体积比)定容，混匀，溶液过 0.22 μm 滤膜(4.20)，供液相色谱-质谱/质谱仪测定。

7.2 测定

7.2.1 液相色谱条件

液相色谱条件如下：

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

b) 流动相梯度洗脱程序见表 1；

c) 流速：0.8 mL/min；

d) 进样量：50 μL。

表 1 流动相洗脱梯度表

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	38	20	42
16	38	20	42
16.5	2	20	78
23	2	20	78

7.2.2 质谱条件

质谱条件如下：

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；

d) 雾化气、气帘气、辅助气、碰撞气均为高纯氮气；使用前应调节各参数使质谱灵敏度达到检测要求，参考条件及监测离子对(m/z)参见表 B.1。

7.3 液相色谱-质谱/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。在上述色谱条件下的待测药物的总离子流色谱图参见图 C.1，参考保留时间参见表 B.1，标准溶液中待测药物的多反应监测色谱图参见图 C.2～图 C.6(用本方法检测确证存在的待测药物，建议采用其他定量方法再次进行测定)。

7.4 液相色谱-质谱/质谱确证

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 定性测定时相对离子丰度最大容许偏差 %

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

7.5 空白试验

除不加试样外，均按上述操作步骤进行。

8 结果计算和表述

用色谱数据处理机或按式(1)计算试样中分析物的含量，计算结果需扣除空白值。

$$X_i = \frac{c_i \times V}{m} \times \frac{1\,000}{1\,000} \qquad \cdots\cdots(1)$$

式中：

X_i ——试样中分析物的含量，单位为微克每千克(μg/kg)；

c_i ——从基质混合标准曲线上得到的样液中分析物的含量，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 测定低限、回收率

9.1 测定低限

本标准方法测定低限分别为磺胺类药物 1.0 μg/kg，硝基咪唑类药物 1.0 μg/kg，喹诺酮类药物

2.0 μg/kg，大环内酯类药物 3.0 μg/kg，林可酰胺类药物 2.0 μg/kg，吡喹酮残留量 0.3 μg/kg。

9.2 添加回收率

试验方法的回收率和精密度数据(在不同添加浓度范围内)参见表 D.1。

附 录 A
（规范性附录）
药物标准品信息

表 A.1 药物标准品信息

编号	中文名称	英文名称	CAS号	分子式	相对分子质量
磺胺类药物(16种)					
1	磺胺嘧啶	Sulfadiazine	68-35-9	$C_{10}H_{10}N_4O_2S$	250.27
2	磺胺噻唑	Sulfathiazloe	72-14-0	$C_9H_9N_3O_2S_2$	255.31
3	磺胺吡啶	Sulfapyridine	144-83-2	$C_{11}H_{11}N_3O_2S$	249.28
4	磺胺甲基嘧啶	Sulfamerazine	127-79-7	$C_{11}H_{12}N_4O_2S$	264.30
5	磺胺二甲基嘧啶	Sulfamethazine	57-68-1	$C_{12}H_{14}N_4O_2S$	278.32
6	磺胺-5-(对)甲氧嘧啶	Sulfameter	651-06-9	$C_{11}H_{12}N_4O_3S$	280.30
7	磺胺甲噻二唑	Sulfamethizole	144-82-1	$C_9H_{10}N_4O_2S_2$	270.32
8	磺胺甲氧哒嗪	Sulfamethoxypyridazine	80-35-3	$C_{11}H_{12}N_4O_3S$	280.30
9	磺胺氯哒嗪	Sulfachloropyridazine	80-32-0	$C_{10}H_9ClN_4O_2S$	284.72
10	磺胺-6-(间)甲氧嘧啶	Sulfamonomethoxine	1220-83-3	$C_{11}H_{12}N_4O_3S$	280.30
11	磺胺邻二甲氧嘧啶	Sulfadoxine	2447-57-6	$C_{12}H_{14}N_4O_4S$	310.32
12	磺胺甲基异恶唑	Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253.27
13	磺胺二甲异恶唑	Sulfafurazole	127-69-5	$C_{11}H_{13}N_3O_3S$	267.30
14	苯酰磺胺	Sulfabenzamide	127-71-9	$C_{13}H_{12}N_2O_3S$	276.31
15	磺胺二甲氧嘧啶	Sulfadimethoxine	122-11-2	$C_{12}H_{14}N_4O_4S$	310.32
16	磺胺喹噁啉	Sulfaquinoxaline	59-40-5	$C_{14}H_{12}N_4O_2S$	300.33
	磺胺嘧啶-D4	Sulfadiazine-D4	1020719-78-1	$C_{10}H_6D_4N_4O_2S$	254.30
	磺胺噻唑-D6	Sulfathiazole-D6		$C_9H_3D_6N_3O_2S_2$	261.05
	磺胺吡啶-D6	Sulfapyridine-D6		$C_{11}H_5D_6N_3O_2S$	255.09
	磺胺二甲基嘧啶-D4	Sulfamethazine-D4		$C_{12}H_{10}D_4N_4O_2S$	282.36
	磺胺甲基异恶唑-D6	Sulfamethoxazole-D6		$C_{10}H_5D_6N_3O_3S$	259.09
	磺胺二甲氧嘧啶-D4	Sulfadimethoxine-D4		$C_{12}H_{10}D_4N_4O_4S$	314.35
	磺胺喹噁啉-D6	Sulfaquinoxaline-D6		$C_{14}H_6D_6N_4O_2S$	306.11
硝基咪唑类药物(10种)					
17	羟基甲硝唑	1-(2-Hydroxyethyl)-2-hydroxy-methyl-5-nitroimidazol(MNZOH)	4812-40-2	$C_6H_9N_3O_4$	187.15
18	2-甲基-5-硝基咪唑	2-Methyl-5-nitroimidazole	88054-22-2	$C_4H_5N_3O_2$	127.10

表 A.1（续）

编号	中文名称	英文名称	CAS号	分子式	相对分子质量
19	羟基二甲硝咪唑	2-Hydroxymethyl-1-methyl-5-nitroimidazole(DMZOH、HMMNI)	936-05-0	$C_5H_7N_3O_3$	157.13
20	甲硝唑	Metronidazole(MNZ)	443-48-1	$C_6H_9N_3O_3$	171.15
21	二甲硝咪唑	Dimetridazole(DMZ)	551-92-8	$C_5H_7N_3O_2$	141.13
22	洛硝哒唑	Ronidazole(RNZ)	7681-76-7	$C_6H_8N_4O_4$	200.15
23	氯甲硝咪唑	5-Chloro-1-methyl-4-nitroimidazole	4897-25-0	$C_4H_4ClN_3O_2$	161.55
24	苯硝咪唑	5-Nitrobenzimidazole	94-52-0	$C_7H_5N_3O_2$	163.14
25	羟基异丙硝唑	Ipronidazole-OH(IPZOH)	35175-14-5	$C_7H_{11}N_3O_3$	185.18
26	异丙硝唑	Ipronidazole(IPZ)	14885-29-1	$C_7H_{11}N_3O_2$	169.18
	羟基二甲硝咪唑-D3	DMZOH-D3		$C_5H_4D_3N_3O_3$	160.2
	二甲硝咪唑-D3	DMZ-D3		$C_5H_4D_3N_3O_2$	144.2
	洛硝哒唑-D3	RNZ-D3		$C_6H_5D_3N_4O_4$	203.2
	羟基异丙硝唑-D3	IPZOH-D3		$C_7H_8D_3N_3O_3$	188.2
	异丙硝唑-D3	IPZ-D3		$C_7H_8D_3N_3O_2$	172.2
喹诺酮类药物(15种)					
27	麻保沙星	Marbofloxacin	115550-35-1	$C_{17}H_{19}FN_4O_4$	362.36
28	依诺沙星	Enoxacin	74011-58-8	$C_{15}H_{17}FN_4O_3$	320.32
29	氧氟沙星	Ofloxacin	82419-36-1	$C_{18}H_{20}FN_3O_4$	361.37
30	诺氟沙星	Norfloxacin	70458-96-7	$C_{16}H_{18}FN_3O_3$	319.33
31	环丙沙星	Ciprofloxacin hydrochloride	86393-32-0	$C_{17}H_{18}FN_3O_3HCl$	367.81
32	达氟沙星	Danofloxacin mesylate	119478-55-6	$C_{20}H_{24}FN_3O_6S$	453.6
33	恩诺沙星	Enrofloxacine	93106-60-6	$C_{19}H_{22}FN_3O_3$	359.4
34	洛美沙星	Lomefloxacin hydrochloride	98079-52-8	$C_{17}H_{19}F_2N_3O_3HCl$	387.8
35	奥比沙星	Orbifloxacin	113617-63-3	$C_{19}H_{20}F_3N_3O_3$	395.38
36	双氟沙星	Difloxacin	98106-17-3	$C_{21}H_{19}F_2N_3O_3$	400.16
37	沙拉沙星	Sarafloxacin hydrochloride	98105-99-8	$C_{20}H_{17}F_2N_3O_3HCl$	421.83
38	司帕沙星	Sparfloxacin	111542-93-9	$C_{19}H_{22}F_2N_4O_3$	392.40
39	噁喹酸	Oxolinic acid	14698-29-4	$C_{13}H_{11}NO_5$	261.23
40	萘啶酸	Nalidixic acid	389-08-2	$C_{12}H_{12}N_2O_3$	232.23
41	氟甲喹	Flumequine	42835-25-6	$C_{14}H_{12}FNO_3$	261.25
	依诺沙星-D8	Enoxacin-D8		$C_{15}H_9D_8FN_4O_3$	328.18

表 A.1（续）

编号	中文名称	英文名称	CAS号	分子式	相对分子质量
	氧氟沙星-D3	Ofloxacin-D3		$C_{18}H_{17}D_3FN_3O_4$	364.16
	诺氟沙星-D5	Norfloxacin-D5		$C_{16}H_{13}D_5FN_3O_3$	324.33
	环丙沙星-D8	Ciprofloxacin-D8		$C_{17}H_{10}D_8FN_3O_3$	339.81
	恩诺沙星-D5	Enrofloxacine-D5		$C_{19}H_{17}D_5FN_3O_3$	364.20
	双氟沙星-D4	Difloxacin-D4		$C_{21}H_{15}D_4F_2N_3O_3$	403.16
	沙拉沙星-D8	Sarafloxacin-D8		$C_{20}H_9D_8F_2N_3O_3$	393.17
大环内酯类药物（9种）					
42	螺旋霉素	Spiramycin	8025-81-8	$C_{43}H_{74}N_2O_{14}$	843.06
43	替米考星	Tilmicosin	108050-54-0	$C_{46}H_{80}N_2O_{13}$	869.13
44	竹桃霉素磷酸盐	Oleandomycin phosphate	7060-74-4	$C_{35}H_{61}NO_{12}H_3PO_4 \cdot 2H_2O$	687.4
45	泰乐菌素酒石酸盐	Tylosin tartrate	74610-55-2	$C_{50}H_{83}NO_{23}$	915.5
46	红霉素A二水合物	Erythromycin	59319-72-1	$C_{37}H_{67}NO_{13}$	733.9
47	罗红霉素	Roxithromycin	80214-83-1	$C_{41}H_{76}N_2O_{15}$	837.0
48	交沙霉素	Josamycin	16846-24-5	$C_{42}H_{69}NO_{15}$	827.9
49	吉他霉素	Kitasamycin	1392-21-8	$C_{39}H_{65}NO_{14}$	771.93
50	泰妙菌素	Tiamulin	55297-95-5	$C_{28}H_{47}NO_4S$	493.74
林可酰胺类药物（3种）					
51	林可霉素盐酸盐	Lincomycin hydrochloride monhydrate	859-18-7	$C_{18}H_{34}N_2O_6S \cdot HCl$	443.0
52	氯林可霉素盐酸盐	Clindamycin hydrochloride	21462-39-5	$C_{18}H_{33}ClN_2O_5S \cdot HCl$	461.4
53	吡利霉素盐酸盐	Pirlimycin hydrochloride	79548-73-5	$C_{17}H_{31}ClN_2O_5S \cdot HCl$	446.0
54	吡哇酮	Praziquantel	55268-74-1	$C_{19}H_{24}N_2O_2$	312.41

附 录 B
（资料性附录）
API 4 000 LC-MS/MS 系统电喷雾离子源参考条件[1)]

API 4 000 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)：358.28 kPa(52 psi)；

c) 气帘气压力(CUR)：172.25 kPa(25 psi)；

d) 辅助气流速(GS2)：378.95 kPa(55 psi)；

e) 离子源温度(TEM)：550 ℃；

f) 碰撞气(CAD)：6；

g) 定量离子对、定性离子对、去簇电压(DP)、碰撞气能量(CE)，碰撞室出口电压(CXP)及相对保留时间见表 B.1。

表 B.1 定量离子对、定性离子对、去簇电压、碰撞气能量、碰撞室出口电压和保留时间

编号	化合物	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	保留时间 min
1	磺胺嘧啶	251.1/156.3* 251.1/108.2	65	22 33	10	磺胺嘧啶-D4	4.35
2	磺胺噻唑	255.8/156.3* 255.8/108.2	70	22 30	10	磺胺噻唑-D6	4.90
3	磺胺吡啶	249.9/156.2* 249.9/184.4	70	24	10	磺胺吡啶-D6	5.29
4	磺胺甲基嘧啶	265.1/156.3* 265.1/172.2	65	25 23	10	磺胺嘧啶-D4	6.06
5	磺胺二甲基嘧啶	279.2/156.3* 279.2/186.1	70	26	10	磺胺二甲基嘧啶-D4	7.78
6	磺胺-5-(对)甲氧嘧啶	281.2/156.3* 281.2/215.4	70	25 26	10	磺胺二甲基嘧啶-D4	8.35
7	磺胺甲噻二唑	271.0/156.2* 271.0/108.2	70	24 32	10	磺胺二甲基嘧啶-D4	8.75
8	磺胺甲氧哒嗪	281.2/156.3* 281.2/215.4	70	25 26	10	磺胺二甲基嘧啶-D4	9.29
9	磺胺氯哒嗪	285.0/156.3* 285.0/108.2	70	22 35	10	磺胺二甲氧嘧啶-D4	11.5
10	磺胺-6-(间)甲氧嘧啶	281.2/156.3* 281.2/215.4	70	25 26	10	磺胺二甲基嘧啶-D4	11.9

1) 非商业性声明：表 B.1 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）

编号	化合物	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	保留时间 min
11	磺胺邻二甲氧嘧啶	311.2/156.3* 311.2/108.2	70	30 37	10	磺胺二甲氧嘧啶-D4	12.1
12	磺胺甲基异恶唑	254.1/156.3* 254.1/108.2	65	22 36	10	磺胺甲基异恶唑-D6	12.1
13	磺胺二甲异恶唑	268.0/156.3* 268.0/113.2	70	22	10	磺胺二甲氧嘧啶-D4	12.5
14	苯酰磺胺	277.0/156.2* 277.0/92.0	68	19 41	7 8	磺胺二甲氧嘧啶-D4	12.8
15	磺胺二甲氧嘧啶	311.2/156.3* 311.2/108.2	70	30 37	10	磺胺二甲氧嘧啶-D4	13.1
16	磺胺喹噁啉	301.2/156.3* 301.2/108.2	55	24 37	13 9	磺胺喹噁啉-D6	13.1
	磺胺嘧啶-D4	255.2/160.3	56	22	12		
	磺胺噻唑-D6	262.0/97.9	72	39	8		
	磺胺吡啶-D6	256.2/97.9	70	38	7		
	磺胺二甲基嘧啶-D4	283.1/186.3	63	29	10		
	磺胺甲基异恶唑-D6	260.2/162.3	75	22	20		
	磺胺二甲氧嘧啶-D4	315.3/156.3	65	29	10		
	磺胺喹噁啉-D6	307.3/162.3	80	24	12		
17	羟基甲硝唑	188.1/123.0* 188.1/126.0	53	19 26	10	羟基二甲硝咪唑-D3	3.34
18	2-甲基-5-硝基咪唑	128.0/82.0* 128.0/42.0	60	26 51	10		3.59
19	羟基二甲硝咪唑	158.0/140.1* 158.0/55.0	48	16 31	10	羟基二甲硝咪唑-D3	4.14
20	甲硝唑	172.0/128.2* 172.0/82.1	62	21 35	11 14	羟基二甲硝咪唑-D3	4.07
21	二甲硝咪唑	142.0/96.0* 142.0/81.0	62	23 36	8 14	二甲硝咪唑-D3	4.83
22	洛硝哒唑	201.1/140.2* 201.1/110.2	37	17 25	8	洛硝哒唑-D3	4.65
23	氯甲硝咪唑	162.0/116.0* 162.0/145.0	68	26 24	10		7.09
24	苯硝咪唑	164.0/118.0* 164.0/91.0	64	32 52	10		7.51
25	羟基异丙硝唑	186.3/168.1* 186.3/122.3	45	19 29	9 5	羟基异丙硝唑-D3	10.5

表 B.1（续）

编号	化合物	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	保留时间 min
26	异丙硝唑	170.2/109.0* 170.2/124.1	63	37 26	10	异丙硝唑-D3	12.5
	羟基二甲硝咪唑-D3	161.2/143.1	51	18	6		
	二甲硝咪唑-D3	145.2/99.0	85	25	10		
	洛硝哒唑-D3	204.2/143.3	50	17	10		
	羟基异丙硝唑-D3	189.2/171.2	60	20	9		
	异丙硝唑-D3	173.1/127.2	67	28	6		
27	麻保沙星	363.2/320.2* 363.2/345.3	63	23 25	10		5.58
28	伊诺沙星	321.2/303.1* 321.2/234.4	67	29 32	10	伊诺沙星-D8	6.38
29	氧氟沙星	362.2/318.3* 362.2/261.2	75	27 39	8 6	氧氟沙星-D3	6.76
30	诺氟沙星	320.0/233.3* 320.0/276.3	74	35 25	11 14	诺氟沙星-D5	7.51
31	环丙沙星	332.1/288.3* 332.1/230.9	85	27 50	7 12	环丙沙星-D8	8.00
32	达氟沙星	358.2/340.3* 358.2/283.1	65	35 33	9 9		8.17
33	恩诺沙星	360.2/316.2* 360.2/245.4	76	29 35	7 9	恩诺沙星-D5	8.38
34	洛美沙星	352.3/265.1* 352.3/308.2	80	35 24	10 10		8.70
35	奥比沙星	396.0/352.0* 396.0/295.0	76	27 35	9 9		8.92
36	双氟沙星	400.0/356.0* 400.0/299.0	76	29 42	9	双氟沙星-D4	10.6
37	沙拉沙星	386.1/342.4* 386.1/299.2	87	27 39	8 7	沙拉沙星-D8	11.2
38	司帕沙星	393.2/292.2* 393.2/349.2	94	36 28	10 10		11.7
39	噁喹酸	262.0/244.4* 262.0/216.3	75	25 40	18 18		12.9
40	萘啶酸	233.2/215.0* 233.2/187.0	53	21 35	10 10		14.1
41	氟甲喹	262.1/244.0* 262.1/202.1	73	27 49	10 10		14.3

表 B.1（续）

编号	化合物	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	保留时间 min
	依诺沙星-D8	329.4/311.2	66	28	7		
	氧氟沙星-D3	365.2/321.4	65	26	8		
	诺氟沙星-D5	325.4/281.2	65	25	9		
	环丙沙星-D8	340.1/296.5	90	27	10		
	恩诺沙星-D5	365.1/321.5	70	28	8		
	双氟沙星-D4	404.3/360.2	76	28	9		
	沙拉沙星-D8	394.1/376.5	70	30	8		
42	螺旋霉素	843.6/174.3* 843.6/318.5	110	50 40	14 9		11.8
43	替米考星	869.7/174.4* 869.7/156.6	120	58 68	15 12		12.1
44	竹桃霉素	688.6/158.4* 688.6/544.6	65	40 23	13 19		12.6
45	泰乐菌素	916.5/174.2* 916.5/772.7	96	53 41	15		12.7
46	红霉素	734.6/158.5* 734.6/576.5	80	40 27	13 17		12.7
47	罗红霉素	837.6/679.6* 837.6/158.4	90	48 30	14 22		13.3
48	交沙霉素	828.8/174.3* 828.8/229.3	105	45 42	14		13.6
49	吉他霉素	772.7/174.3* 772.7/109.0	115	47 63	8 10		13.0
50	泰妙菌素	494.3/192.5* 494.3/119.2	60	26 54	10		12.9
51	林可霉素	407.2/126.3* 407.2/359.4	90	26 28	10 9		5.15
52	氯林可霉素	425.9/126.1* 425.9/378.2	90	48 28	10		12.4
53	吡利霉素	411.2/112.1* 411.2/363.1	90	44 24	10		12.3
54	吡喹酮	313.5/203.2* 313.5/83.2	74	25 39	4 6		17.1

注：带“*”为定量离子对。

附 录 C
（资料性附录）
各类药物标准品离子流图

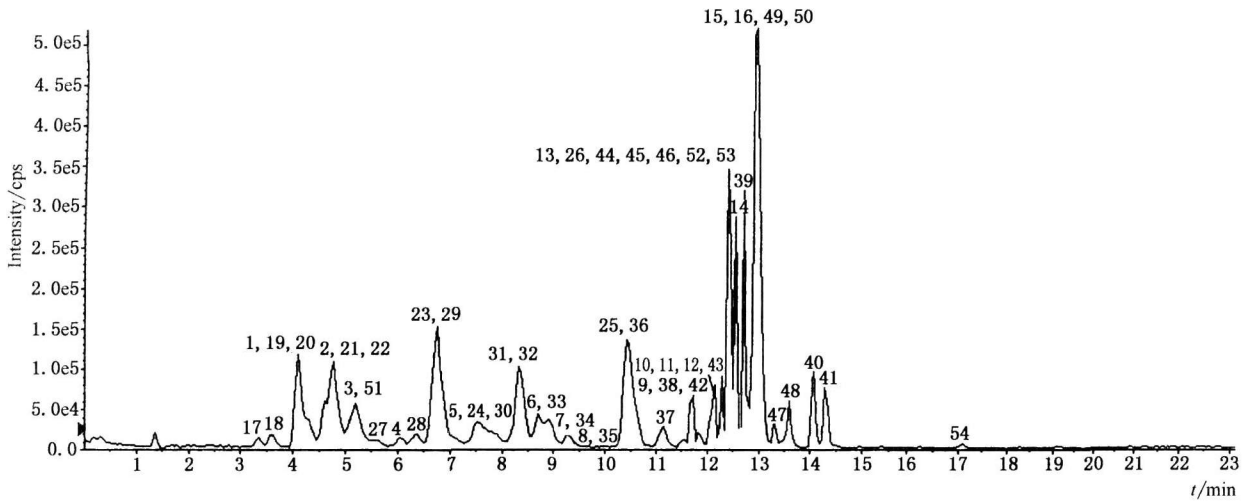

图 C.1 各类药物混合标准品的总离子流图（药物浓度：磺胺类药物 1.0 μg/kg，硝基咪唑类药物 1.0 μg/kg，喹诺酮类药物 2.0 μg/kg，大环内酯类药物 3.0 μg/kg，林可酰胺类药物 2.0 μg/kg，吡喹酮 0.3 μg/kg）

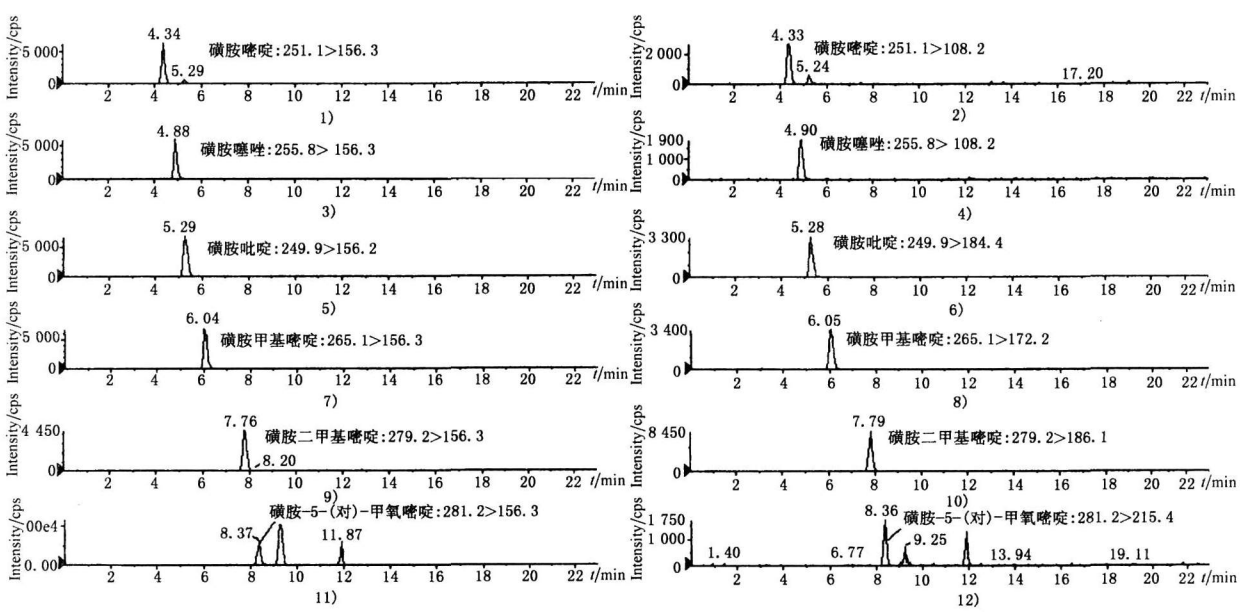

图 C.2 磺胺类药物混合标准品（1.0 μg/kg）的选择性离子流图

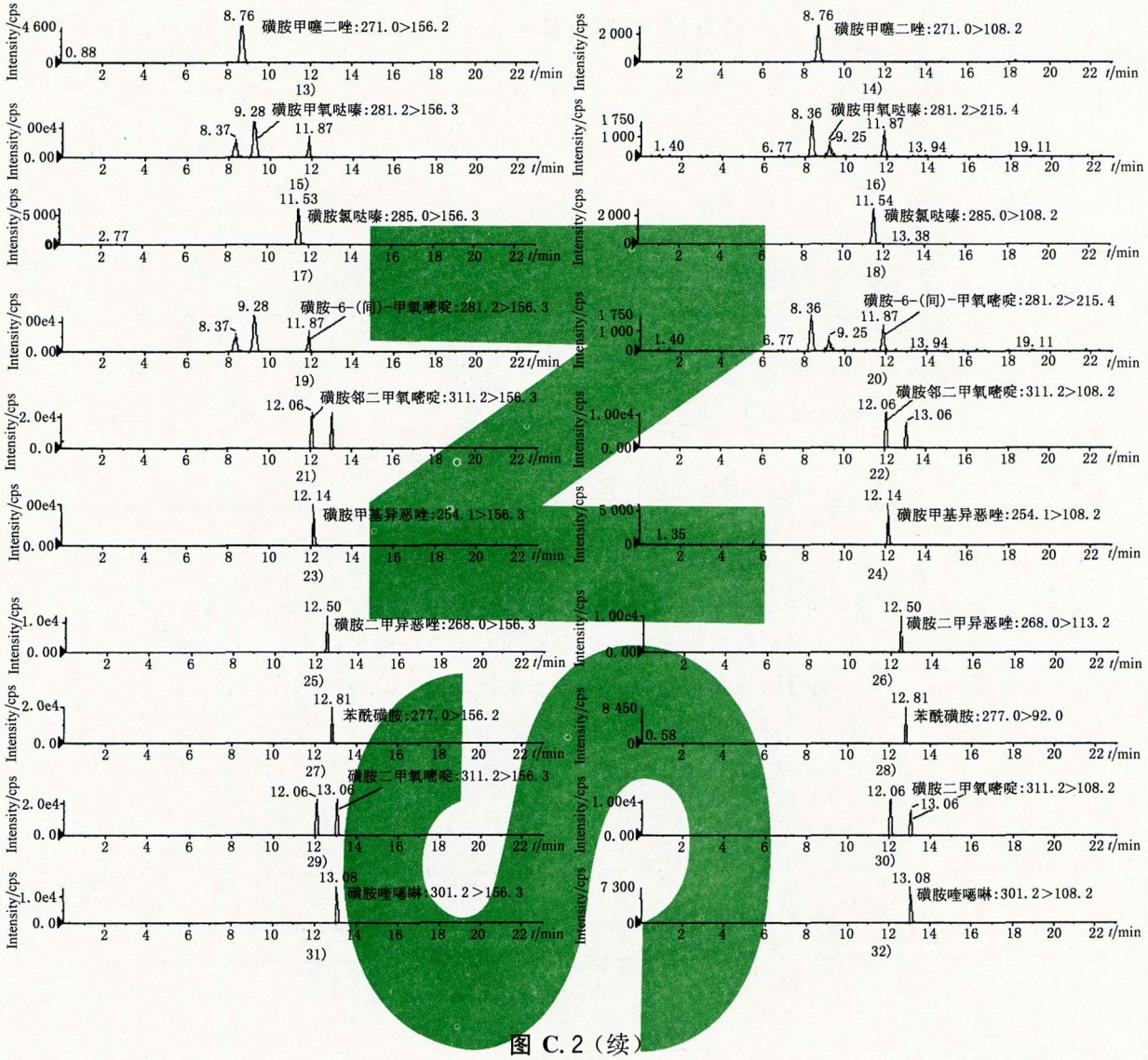

图 C.2（续）

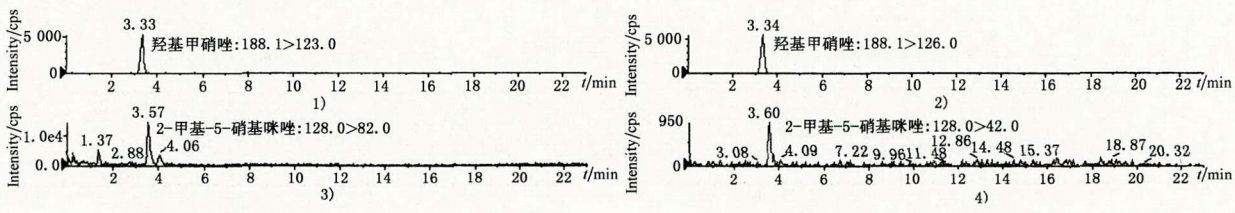

图 C.3　硝基咪唑类药物混合标准品(1.0 μg/kg)的选择性离子流图

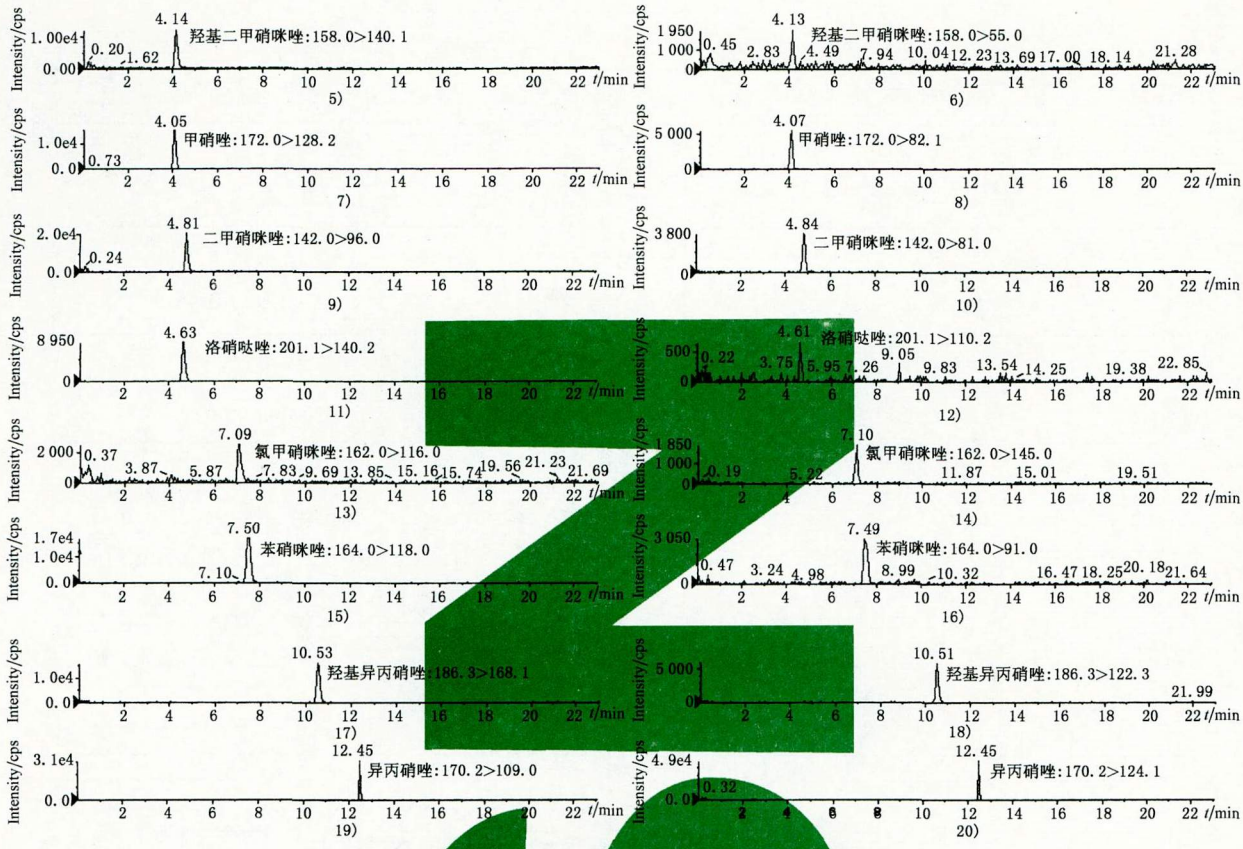

图 C.3（续）

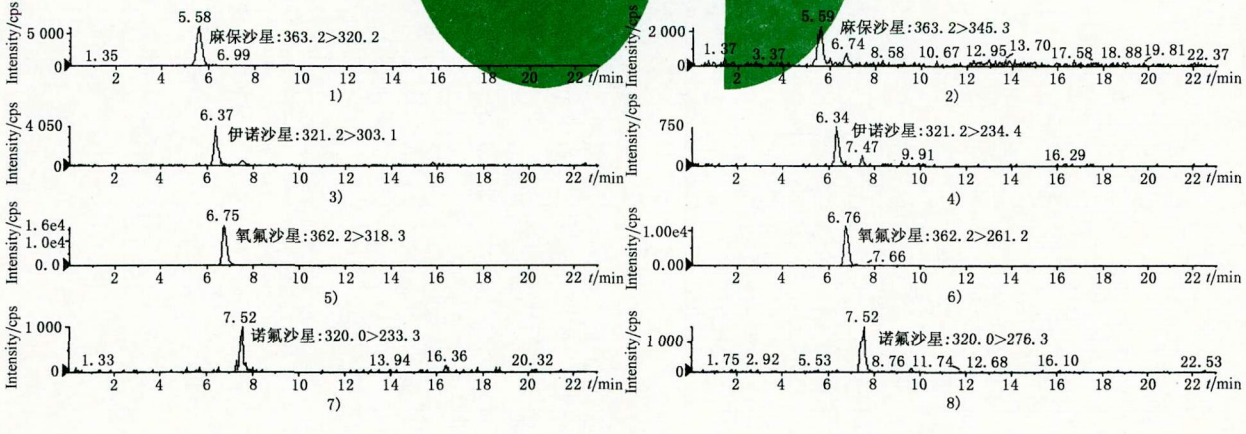

图 C.4　喹诺酮类药物混合标准品(2.0 μg/kg)的选择性离子流图

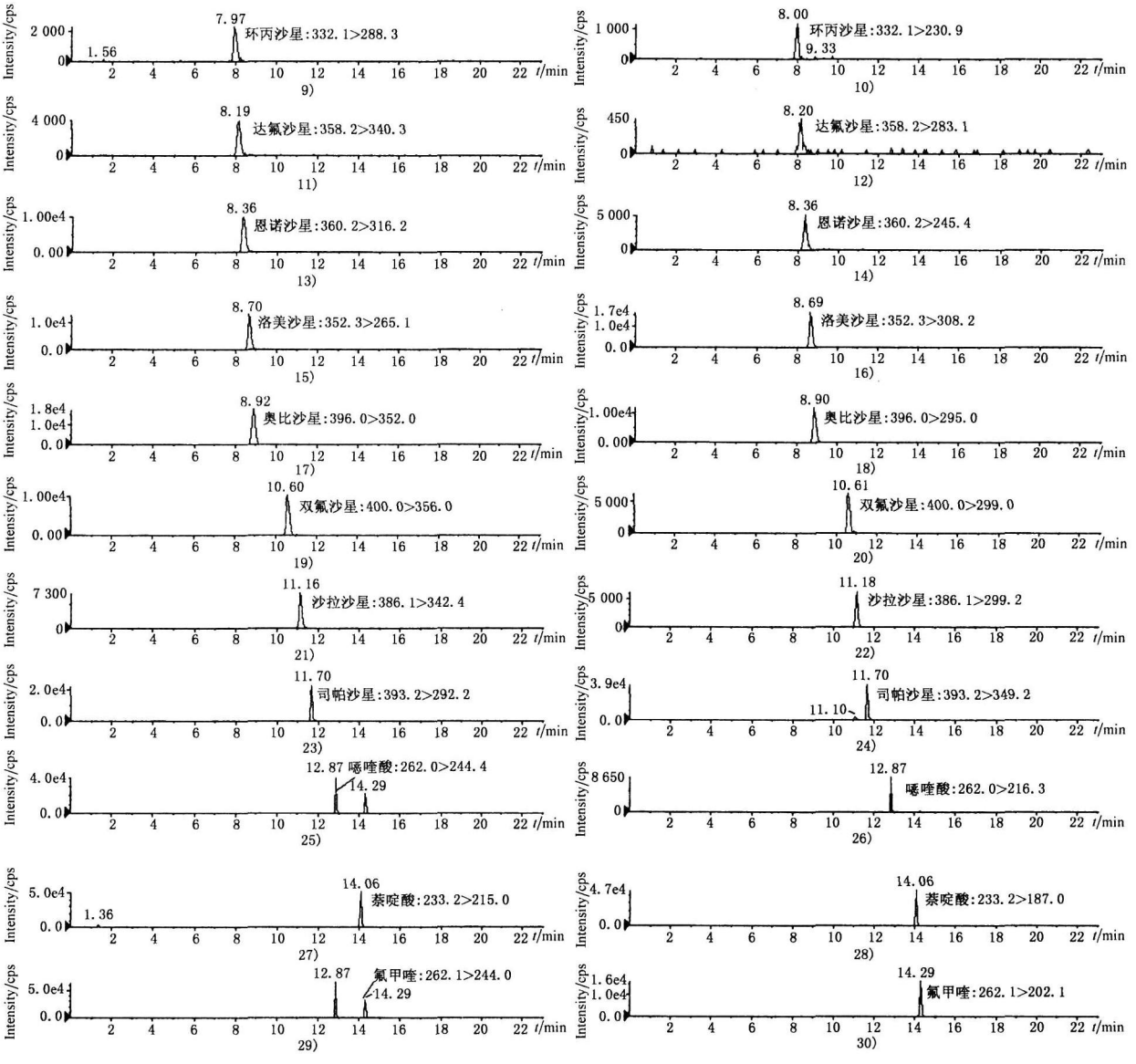
Intensity/cps
t/min
9)
7.97
环丙沙星:332.1>288.3
1.56
10)
8.00
环丙沙星:332.1>230.9
9.33
11)
8.19
达氟沙星:358.2>340.3
12)
8.20
达氟沙星:358.2>283.1
13)
8.36
恩诺沙星:360.2>316.2
14)
8.36
恩诺沙星:360.2>245.4
15)
8.70
洛美沙星:352.3>265.1
16)
8.69
洛美沙星:352.3>308.2
17)
8.92
奥比沙星:396.0>352.0
18)
8.90
奥比沙星:396.0>295.0
19)
10.60
双氟沙星:400.0>356.0
20)
10.61
双氟沙星:400.0>299.0
21)
11.16
沙拉沙星:386.1>342.4
22)
11.18
沙拉沙星:386.1>299.2
23)
11.70
司帕沙星:393.2>292.2
24)
11.70
司帕沙星:393.2>349.2
11.10
25)
12.87 噁喹酸:262.0>244.4
14.29
26)
12.87
噁喹酸:262.0>216.3
27)
14.06
萘啶酸:233.2>215.0
1.36
28)
14.06
萘啶酸:233.2>187.0
29)
12.87
氟甲喹:262.1>244.0
14.29
30)
14.29
氟甲喹:262.1>202.1

图 C.4（续）

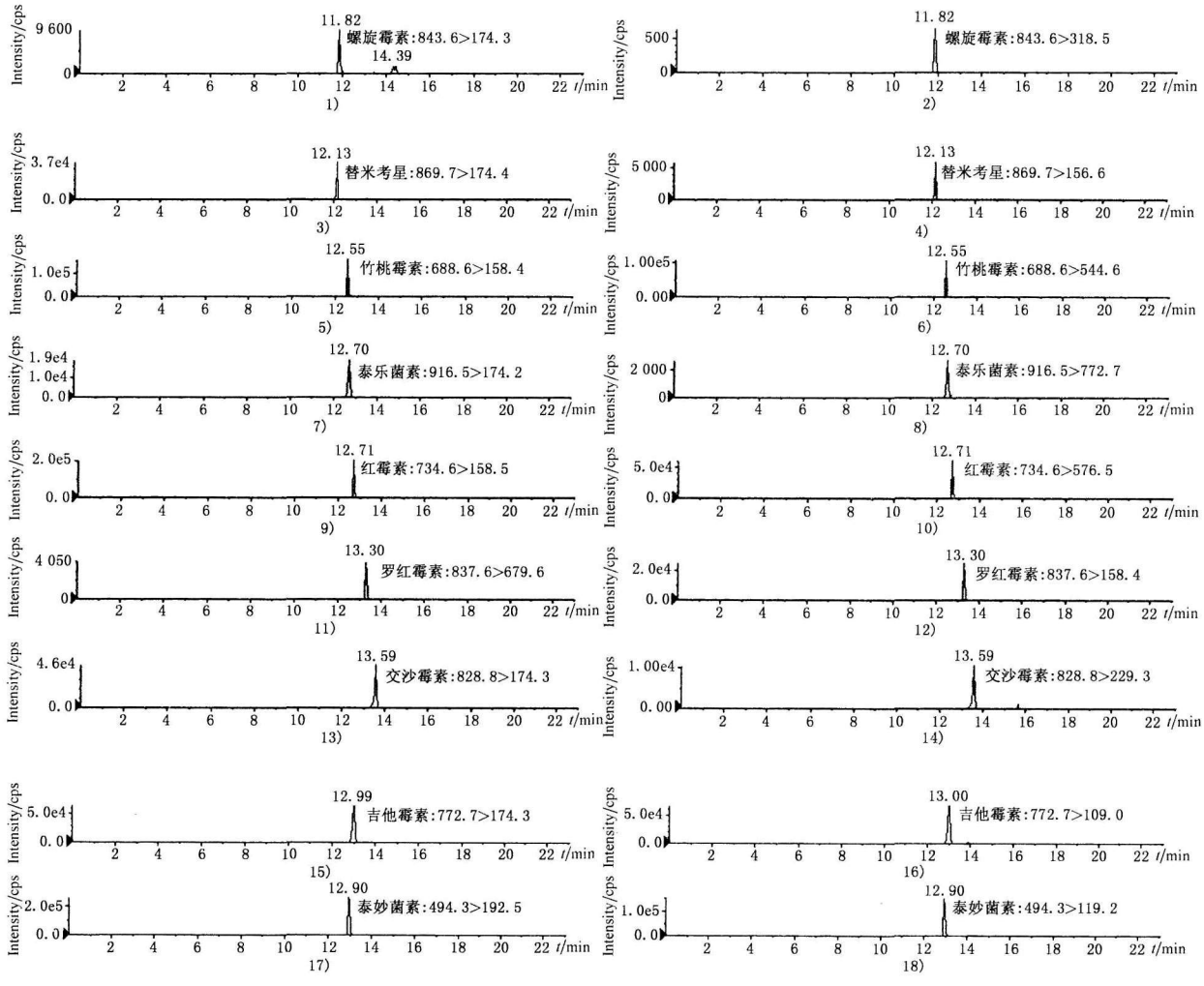

图 C.5　大环内酯类药物混合标准品(3.0 μg/kg)的选择性离子流图

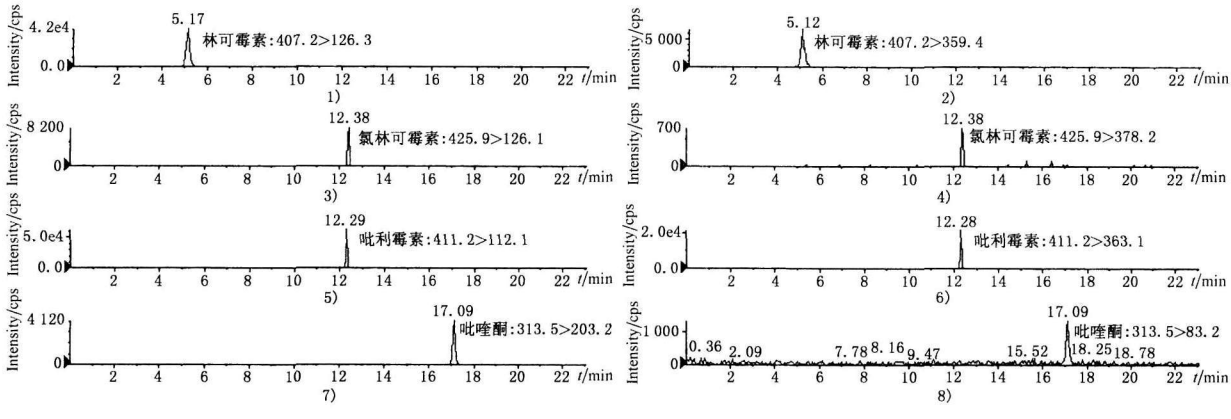

图 C.6　林可酰胺类药物混合标准品(2.0 μg/kg)和吡喹酮标准品(0.3 μg/kg)的选择性离子流图

附 录 D
（资料性附录）
回 收 率

表 D.1 猪肉、虾和蜂蜜中多类药物添加浓度及回收率（$n=6$）

化合物	添加水平 μg/kg	回收率（猪肉） %	回收率（虾） %	回收率（蜂蜜） %
磺胺嘧啶	1.0	88.9～106	76.3～102	96.5～109
	2.0	93.0～109	81.1～107	95.0～98.6
	4.0	97.9～111	76.5～107	96.1～106
磺胺噻唑	1.0	86.7～107	77.7～113	98.9～110
	2.0	93.3～110	90.5～106	89.7～104
	4.0	88.5～106	90.7～109	84.4～110
磺胺吡啶	1.0	84.5～112	86.5～106	86.5～105
	2.0	87.7～107	78.9～106	80.0～95.8
	4.0	79.4～109	81.1～109	85.0～108
磺胺甲基嘧啶	1.0	86.8～105	98.6～110	90.8～106
	2.0	85.5～109	77.7～108	86.8～94.2
	4.0	85.6～108	90.4～100	93.0～105
磺胺二甲基嘧啶	1.0	79.2～103	95.0～114	86.5～108
	2.0	90.0～109	102～114	91.4～101
	4.0	95.1～108	103～111	92.2～110
磺胺-5-（对）甲氧嘧啶	1.0	89.4～105	92.4～112	94.5～107
	2.0	89.7～110	98.6～109	91.1～106
	4.0	90.5～107	87.9～111	92.6～110
磺胺甲噻二唑	1.0	73.2～97.7	81.9～103	85.0～94.3
	2.0	85.4～106	71.4～99.1	86.1～99.1
	4.0	84.6～100	77.5～95.8	84.7～96.1
磺胺甲氧哒嗪	1.0	71.9～101	79.8～118	80.9～94.8
	2.0	75.1～106	95.7～114	78.8～94.0
	4.0	78.0～93.2	97.4～118	84.5～94.7
磺胺氯哒嗪	1.0	84.7～109	66.2～97.3	64.7～76.4
	2.0	79.0～103	74.3～99.2	82.5～97.0
	4.0	83.0～107	72.6～98.0	81.1～94.5

表 D.1（续）

化合物	添加水平 μg/kg	回收率(猪肉) %	回收率(虾) %	回收率(蜂蜜) %
磺胺-6-(间)甲氧嘧啶	1.0	98.1～109	73.5～104	86.4～99.8
	2.0	87.8～110	91.6～109	88.0～110
	4.0	83.4～107	93.3～116	90.4～114
磺胺邻二甲氧嘧啶	1.0	86.7～107	81.7～109	77.2～104
	2.0	88.1～104	92.0～103	69.0～80.4
	4.0	88.2～108	86.7～105	78.9～98.1
磺胺甲基异恶唑	1.0	85.9～108	88.4～105	79.2～95.2
	2.0	86.8～102	72.3～99.2	81.8～108
	4.0	91.7～109	78.3～94.2	83.4～99.4
磺胺二甲异恶唑	1.0	88.7～106	79.3～107	54.5～78.0
	2.0	78.8～107	78.6～92.9	55.8～75.1
	4.0	80.0～108	77.8～97.2	60.9～88.2
苯酰磺胺	1.0	82.8～111	73.8～106	52.5～73.7
	2.0	77.8～97.7	82.7～105	54.2～72.2
	4.0	78.4～107	82.9～105	53.6～65.4
磺胺二甲氧嘧啶	1.0	95.7～106	91.0～107	81.5～94.8
	2.0	77.4～111	106～112	81.4～104
	4.0	81.3～107	101～111	81.5～109
磺胺喹噁啉	1.0	91.5～107	75.6～109	78.5～100
	2.0	90.9～113	65.0～79.5	71.1～90.3
	4.0	92.3～111	73.5～106	83.8～104
羟基甲硝唑	1.0	88.8～109	92.2～114	99.5～108
	2.0	88.1～114	91.0～109	95.5～108
	4.0	78.8～105	98.4～115	97.7～107
2-甲基-5-硝基咪唑	1.0	75.3～99.8	74.9～103	64.4～82.3
	2.0	78.2～99.9	78.2～109	74.8～99.6
	4.0	74.7～89.0	75.3～90.5	74.2～94.2
羟基二甲硝咪唑	1.0	85.2～111	75.4～103	83.2～104
	2.0	91.1～101	83.7～106	92.4～110
	4.0	85.8～101	86.1～112	85.7～107
甲硝唑	1.0	97.3～111	94.8～109	99.7～107
	2.0	98.5～104	85.6～107	95.8～110
	4.0	99.1～107	89.2～104	94.6～106

表 D.1（续）

化合物	添加水平 μg/kg	回收率(猪肉) %	回收率(虾) %	回收率(蜂蜜) %
二甲硝咪唑	1.0	77.7～104	94.3～109	90.2～103
	2.0	96.5～102	88.3～107	85.8～96.9
	4.0	91.8～106	90.5～106	91.1～102
洛硝哒唑	1.0	86.4～101	93.0～113	85.6～98.2
	2.0	87.4～96.3	95.9～113	90.5～102
	4.0	88.7～102	89.8～104	87.3～105
氯甲硝咪唑	1.0	72.5～94.1	72.5～100	72.4～99.7
	2.0	71.2～97.4	70.7～88.3	80.9～94.1
	4.0	72.5～87.0	78.8～97.8	84.2～97.5
硝基苯并咪唑	1.0	65.0～74.2	60.9～80.1	62.8～85.4
	2.0	60.5～69.1	53.7～74.4	72.0～84.3
	4.0	60.9～78.7	53.0～70.7	83.2～89.2
羟基异丙硝唑	1.0	89.1～105	98.1～113	85.6～105
	2.0	91.8～103	104～113	82.4～102
	4.0	86.6～110	90.6～109	87.8～101
异丙硝唑	1.0	90.6～106	96.3～105	94.9～106
	2.0	97.6～105	99.0～111	82.9～104
	4.0	90.3～109	90.1～113	92.9～109
麻保沙星	2.0	73.8～92.7	65.2～90.5	84.8～102
	4.0	63.4～78.4	59.2～83.4	74.9～96.2
	8.0	60.2～74.9	57.9～72.5	74.3～86.0
依诺沙星	2.0	79.8～95.9	90.1～105	73.2～79.0
	4.0	85.3～104	88.6～115	74.8～90.6
	8.0	92.7～101	88.2～118	85.5～98.3
氧氟沙星	2.0	84.7～105	93.1～117	95.7～104
	4.0	92.7～112	93.8～107	94.8～100
	8.0	92.8～109	97.4～116	93.5～110
诺氟沙星	2.0	60.4～82.7	90.3～110	70.3～95.2
	4.0	72.7～99.4	72.8～109	74.3～89.0
	8.0	76.7～86.4	82.2～116	77.5～89.0
环丙沙星	2.0	65.4～88.2	69.5～103	81.3～98.1
	4.0	64.3～85.8	84.0～115	80.7～102
	8.0	69.3～100	82.6～112	92.8～101

表 D.1（续）

化合物	添加水平 μg/kg	回收率(猪肉) %	回收率(虾) %	回收率(蜂蜜) %
达氟沙星	2.0	52.9～79.1	75.0～102	80.1～110
	4.0	49.0～65.9	67.0～95.6	76.7～100
	8.0	51.8～70.3	61.8～88.6	71.6～96.7
恩诺沙星	2.0	86.6～102	97.8～105	92.0～108
	4.0	95.6～103	92.2～118	93.7～105
	8.0	95.6～110	102～113	96.6～105
洛美沙星	2.0	36.1～51.1	50.1～76.0	67.5～80.6
	4.0	45.8～62.2	49.0～68.8	63.1～75.4
	8.0	53.6～72.7	53.8～63.1	71.5～76.4
奥比沙星	2.0	52.5～79.8	57.0～83.9	71.7～87.5
	4.0	56.8～81.2	50.5～70.9	80.1～89.6
	8.0	64.0～81.7	61.9～79.6	81.6～99.6
双氟沙星	2.0	85.3～118	77.2～110	97.9～113
	4.0	89.0～121	79.7～98.2	93.2～110
	8.0	85.5～109	79.4～107	95.7～108
沙拉沙星	2.0	87.0～111	72.7～113	76.6～98.4
	4.0	88.5～107	69.6～95.5	72.0～102
	8.0	87.3～105	77.2～101	75.9～109
司帕沙星	2.0	48.8～72.0	62.2～83.5	80.7～107
	4.0	51.0～73.3	53.6～71.8	86.4～107
	8.0	56.4～71.8	57.3～76.0	82.0～103
噁喹酸	2.0	60.6～88.5	62.8～92.2	68.0～101
	4.0	74.2～93.9	70.0～95.0	75.6～109
	8.0	75.1～93.1	74.7～85.1	84.7～98.7
萘啶酸	2.0	68.2～79.4	51.5～70.6	83.1～98.5
	4.0	63.0～85.3	60.0～87.4	87.2～100
	8.0	66.4～82.6	64.2～76.8	93.6～109
氟甲喹	2.0	68.7～76.0	63.6～88.7	71.1～99.4
	4.0	62.2～85.1	65.5～82.1	73.1～101
	8.0	57.5～79.3	62.2～70.7	80.9～102
螺旋霉素	3.0	21.1～29.8	41.0～57.0	62.6～83.5
	6.0	24.5～28.9	33.3～45.6	48.9～66.4
	12	31.1～43.2	44.3～53.8	67.7～85.5

表 D.1（续）

化合物	添加水平 μg/kg	回收率(猪肉) %	回收率(虾) %	回收率(蜂蜜) %
替米考星	3.0	30.2～47.3	44.1～59.7	73.2～105
	6.0	42.5～51.5	40.2～50.4	82.0～97.6
	12	57.6～78.6	60.4～87.5	91.4～111
竹桃霉素	3.0	37.8～59.6	35.7～53.0	82.0～106
	6.0	47.9～59.1	37.2～55.8	82.3～107
	12	69.8～87.6	58.7～84.6	81.3～105
泰乐菌素	3.0	34.1～42.7	40.0～50.0	74.2～93.3
	6.0	35.3～43.1	32.4～40.7	83.1～108
	12	41.2～52.9	44.5～62.7	85.3～109
红霉素	3.0	33.9～53.6	33.7～49.8	32.6～70.9
	6.0	41.9～63.5	45.4～63.7	37.1～69.1
	12	47.5～66.4	45.2～61.2	42.7～72.4
罗红霉素	3.0	51.0～78.0	48.9～71.1	85.8～108
	6.0	53.4～75.7	58.3～71.7	93.3～114
	12	66.1～88.3	54.7～76.2	96.4～110
交沙霉素	3.0	41.4～59.1	34.0～44.0	88.8～110
	6.0	41.2～59.7	30.8～40.0	88.0～111
	12	44.2～60.3	36.1～50.1	91.6～109
吉他霉素	3.0	28.3～38.9	35.1～51.1	42.0～58.6
	6.0	26.1～35.4	31.3～45.4	47.3～63.8
	12	31.1～42.9	33.0～47.9	50.8～61.4
泰妙菌素	3.0	46.1～59.8	41.4～54.0	53.3～68.7
	6.0	50.3～62.5	32.2～41.5	70.7～86.9
	12	55.4～69.1	38.6～52.8	58.8～75.4
林可霉素	2.0	32.7～50.3	46.2～63.8	78.3～89.4
	4.0	41.4～62.6	50.5～69.5	81.2～86.5
	8.0	46.2～57.8	45.3～60.4	78.3～89.6
氯林可霉素	2.0	34.1～47.8	37.8～52.6	90.2～107
	4.0	38.7～53.3	35.4～48.7	76.0～93.9
	8.0	44.2～58.4	32.3～48.1	76.6～107
吡利霉素	2.0	20.9～37.8	36.2～42.6	64.1～94.1
	4.0	26.6～43.7	39.5～56.1	61.3～75.9
	8.0	27.3～38.4	38.5～47.9	60.0～84.7

表 D.1（续）

化合物	添加水平 μg/kg	回收率(猪肉) %	回收率(虾) %	回收率(蜂蜜) %
吡喹酮	0.3	41.8～55.9	35.9～52.9	62.3～78.5
	0.6	37.5～47.5	37.1～49.9	69.3～79.5
	1.2	39.7～47.3	39.5～50.8	67.2～85.6

Foreword

This standard was drafted in accordance with GB/T 1. 1—2009"Directives for standardization—Part 1:The structure and drafting of standards".

Please note that some of the elements of this standard may involve patents,but the standards organization does not assume responsibility for identifying these patents.

This standard was proposed by and is under the charged of Certification and Accreditation Administration 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 main drafters of this standard are Chen Xiaomei,Xie Wen,Hou Jianbo,Xi Junyang,Qian Yan, Wang Feng. and Han Chao.

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

Determination of multi-veterinary drugs residues in pork, shrimp and honey for export—LC-MS/MS method

1 Scope

This standard specifies the method of determination multi-veterinary drugs residues in pork, shrimp and honey for import and export by LC-MS/MS.

This standard is applicable to the determination and comfirmation of sulfadiazine, sulfathiazloe, sulfapyridine, sulfamerazine, sulfamethazine, sulfameter, sulfamethizole, sulfamethoxypyridazine, sulfachloropyridazine, sulfamonomethoxine, sulfadoxine, sulfamethoxazole, sulfafurazole, sulfabenzamide, sulfadimethoxine, sulfaquinoxaline, 1-(2-hydroxyethyl)-2-hydroxy-methyl-5-nitroimidazol, 2-methyl-5-nitroimidazole, 2-hydroxymethyl-1-methyl-5-nitroimidazole, metronidazole, dimetridazole, ronidazole, 5-chloro-1-methyl-4-nitroimidazole, 5-nitrobenzimidazole, hydroxyipronidazole, ipronidazole, marbofloxacin, enoxacin, ofloxacin, norfloxacin, ciprofloxacin, danofloxacin, enrofloxacine, lomefloxacin, orbifloxacin, difloxacin, sarafloxacin, sparfloxacin, oxolinic acid, nalidixic acid, flumequine, spiramycin, tilmicosin, oleandomycin, tylosin, erythromycin, roxithromycin, josamycin, kitasamycin, tiamulin, lincomycin, clindamycin, pirlimycin and praziquantel residues in pork, shrimp and honey.

2 Normative references

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 referred to applies.

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

3 Principle

The residues are extracted with acetonitrile from pork or shrimp sample, and is defatted with *n*-hexane. Then the extract is cleaned up by C_{18} solid phase extraction column. The residues are diluted with phosphate buffer solution from honey sample. It is cleaned up by HLB solid phase extraction column. The residues are determined by LC-MS/MS and quantified by internal standard method or external standard method.

4 Reagents and materials

Unless otherwise specified, all reagents used shall be HPLC grade. "Water" is first-grade water prescribed by GB/T 6682.

4.1 Acetonitrile.

4.2 Methanol.

4.3 Formic acid.

4.4 *n*-Hexane.

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

4.6 Sodium dihydrgen phosphate: Analytical grade.

4.7 Sodium hydroxide: Analytical grade.

4.8 Sodium hydroxide solution (0.1 mol/L): Dissolve 4 g of sodium hydroxide in water and dilute to 1 L.

4.9 *Phosphate buffer solution*: Weigh that 13.8 g of sodium dihydrgen phosphate dissolved in 950 mL water, adjust pH to 8.0 with 0.1 mol/L sodium hydroxide solution, dilute to 1 L.

4.10 Standards: Purity of sulfanilamide and nitroimidazole are≥98%, qluinolones are≥96%, macrolide antibiotics are≥95%, lincosamides are≥86% and praziquantel is≥98%. Other information see annex A table A.1.

4.11 The isotope internal standards: Sulfadiazine-D4, sulfathiazole-D6, sulfapyridine-D6, sulfamethazine-D4, sulfamethoxazole-D6, sulfadimethoxine-D4, sulfaquinoxaline-D6, 2-hydroxymethyl-1-methyl-5-nitroimidazole-D3, dimetridazole-D3, ronidazole-D3, ipronidazole-OH-D3, ipronidazole-D3, enoxacin-D8, ofloxacin-D8, norfloxacin-D5, ciprofloxacin-D8, enrofloxacine-D5, difloxacin-D4, sarafloxacin-D8. Purity are≥98%. Other information see annex A table A.1.

4.12 *Standard stock solution*: Accurately weigh an adequate amount of each standard (4.10) (accurate to 0.1 mg), dissolve in methanol individually, and prepare a solution of 1 mg/mL as the standard stock solution respectively. It shall be stored in brown volumtric flask at 1 ℃～4 ℃.

4.13 Isotope internal standards stock solution: Weigh an adequate amount of each isotope internal standard(4.11), dissolve in methanol individually. The concentrations of isotope internal standard is 1 mg/mL. It shall be stored in brown volumtric flask at 1 ℃～4 ℃.

4.14 Intermediate mixed-standard working solution: Diluted the standard stock solution (4.12) by methanol. The concentrations of sulfanilamides and nitroimidazoles are 500 ng/mL, qluinolones are 1 000 ng/mL, macrolide antibiotics are 1 500 ng/mL, lincosamides are 1 000 ng/mL and praziquantel is 150 ng/mL. It shall be stored in brown volumtric flask at 1 ℃～4 ℃.

4.15 Matrix blank solution: According to section 7.1, the blank solutions are prepared with pork, shrimp and honey without sulfanilamide, nitroimidazole, qluinolones, macrolide antibiotics, lincosamides and praziquantel.

4.16 Matrix standard solution: According to the requirement, dilute intermediate mixed-standard working solution(4.14) to appropriate concentration with matrix blank solution (4.15). It is prepared before using.

4.17 Anhydrous sodium sulfate column: 80 mm × 40 mm(id) cylinder funnel, pack with ca 5 mm absorbent cotton at the bottom of the column and fill in 40 mm anhydrous sodium sulfate.

4.18 C_{18} solid phase extraction (SPE) column: 500 mg, 3 mL or equivalent. It shall be conditioned with 3 mL methanol before use.

4.19 Oasis (HLB) solid phase extraction (SPE) column: 500 mg, 6 mL or equivalent. It shall be conditioned with 5 mL methanol followed by 5 mL phosphate buffer solution (4.9).

4.20 Membrane filter: 0.22 μm, organic type.

5 Apparatus and equipment

5.1 Liquid chromatography-tandem mass spectrometry: Equipped with electrospray ionization source (ESI).

5.2 Analytical balances: Accuracy 0.000 1 g and 0.01 g.

5.3 Solid phase extraction vacuum container.

5.4 Centrifuge: ≥6 000 r/min.

5.5 Vortex mixter.

5.6 Rotary vacuum evaporator.

5.7 Stoppered centrifuge tube: Polypropylene, 50 mL.

6 Sample preparation and storage

6.1 Requinement

In the course of sample preparation, precautions shall be taken to avoid contamination or any factors

which may cause the change of residue content.

6.2 Pork and Shrimp

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

6.3 Honey

Honey sample is about 500 g. The sample, which is not crystallized, shall be stirred well to make homogeneous. If the sample is crystallized, it must be warmed in a water-bath below 60 ℃ with the sample bottle covered tightly, mix thoroughly when all sample has melted, then cool immediately to room temperature. In the course of melting the sample, precautions must be taken to avoid evaporation of water from the sample. Keep the prepared sample into two sample bottles, seal and label. The honey test sample is stored at room temperature.

7 Analysis procedure

7.1 *Extraction and clean up*

7.1.1 Pork and Shrimp

Weigh ca 5 g test sample (accurate to 0.01 g) into a 50 mL centrifuge tube(5.7), add isotope internal standards solution(isotope internal standards of sulfanilamides are 8 ng, nitroimidazoles are 40 ng, qluinolones are 30 ng). Add 20 mL acetonitrile, vortex for 0.5 min, mix it, centrifuge at 3 000 r/min for 3 min. Transfer upper layer solution into another centrifuge tube. Repeat the extraction in the same way with 15 mL acetonitrile and combined the solution, centrifuge at 7 000 r/min for 5 min. Transfer the upper layer into a centrifuge tube, add and 15 mL *n*-hexane, mix it. Centrifuge at 4 000 r/min for 3 min, discard *n*-hexane layer. Repeat the extraction in the same way with 15 mL *n*-hexane. The acetonitrile layer was passed through anhydrous sodium sulphate column (4.17) into flask. It is evaporated to nearly dryness in a water bath below 40 ℃. The residues was dissolved in 4 mL methanol and transfer the solution into C_{18} column(4.18), flow rate is 1 mL/min～2 mL/min, collect the eluate. Elute the column with 6 mL methanol. Combine the solution, evaporated to nearly dryness in a water bath below 40 ℃, The residues was reconstituted in 2.0 mL methanol-water (3+7, *V*/*V*), mix it. The solution is passed through a 0.22 μm filter (4.20). The filtrate is ready for LC-MS/MS determination.

7.1.2 Honey

Weight ca 5 g test sample(accurate to 0.01 g) into a 50 mL centrifuge tube(5.7), add isotope internal standards solution(isotope internal standards of sulfanilamides are 8 ng, nitroimidazoles are 40 ng, qluinolones are 30 ng). Add 20 mL phosphate buffer solution (4.9), mix it. Transfer the solution into

HLB column, flow rate is 1 mL/min～2 mL/min, discard the eluate. The column is rinsed with 20 mL water. The column is dried to "dryness". Elute the column with 6 mL methanol. The solution is evaporated to nearly dryness in a water bath below 40 ℃ to dryness. The residues was reconstituted in 2.0 mL methanol-water (3+7, *V*/*V*), mix it. The solution is passed through a 0.22 μm filter (4.20). The filtrate is ready for LC-MS/MS determination.

7.2 Determination

7.2.1 HPLC operating condition

HPLC operating condition as following:

a) LC column: C_8, 150 mm × 4.6 mm(id), 5 μm, or equivalent column.

b) Mobile phase: See table 1.

c) Flow rate: 0.8 mL/min.

d) Injector volumn: 50 μL.

Table 1—Gradient program of mobile phase

Time min	Acetonitrile %	Methanol %	0.15% formic acid water solution %
0	2	20	78
4	5	20	75
8	10	20	70
10	38	20	42
16	38	20	42
16.5	2	20	78
23	2	20	78

7.2.2 MS/MS operating condition

MS/MS operating condition as following:

a) Ion source: Electrospray ionization source (ESI).

b) Polarity: Positive-ion mode.

c) Monitor model: Multiple reaction monitoring (MRM).

d) Nebulizer gas(GS 1), curtain gas(CUR), auxiliary heater gas(GS2)and collision gas are all high purity nitrogen (N_2)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 and monitoring ion pairs (m/z)are listed in annex B.

7.3 LC-MS/MS determination

According to the concentrations of compounds in sample solution, select the standard working solution of similar concentration to that of sample solution. The responses of compounds in the sample solution shall be within the linear range of the calibration curve. The standard working solution shall be injected randomly in between the injections of the sample solution of equal volume. Under the above LC-MS/MS operating condition, the TLC figure and retention time of these compounds are shown in C. 1 of annex C and table B. I of annex B. Selected ion chromatograms of the standards are shown in Figure C. 2-C. 6 of annex C. The positive samples confirmed by this method are suggested to be determined by another method for further quantitation.

7.4 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 ±2.5%. 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

7.5 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.

8 Calculation and expression of result

Calculate the content of multi-veterinary drugs residue in the test sample by LC-MS/MS data processor or using the followed formula (1), the blank value shall be subtracted from the about result of calculation.

$$X_i = \frac{c_i \times V}{m} \times \frac{1\,000}{1\,000} \quad \cdots\cdots(1)$$

X_i ——the residue content of analyte in the test samples (μg/kg);

c_i ——the concentration of analyte in the matrix standard working solution (ng/mL);

V ——the final volume of sample solution (mL);

m ——the corresponding 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 quantification sulfanilamides and nitroimidazoles are 1.0 μg/kg, qluinolones are 2.0 μg/kg, macrolide antibiotics are 3.0 μg/kg, lincosamides are 2.0 μg/kg, and praziquantel is 0.3 μg/kg.

9.2 Recovery

The recoveries of spiked concentrations are shown in table D.1 of annex D.

Annex A
(Normative annex)
Standard information

Table A. 1—Information of the standards

NO.	Compound	CAS No.	Formula	Molecular weight
Sulfanilamides(16)				
1	Sulfadiazine	68-35-9	$C_{10}H_{10}N_4O_2S$	250. 27
2	Sulfathiazloe	72-14-0	$C_9H_9N_3O_2S_2$	255. 31
3	Sulfapyridine	144-83-2	$C_{11}H_{11}N_3O_2S$	249. 28
4	Sulfamerazine	127-79-7	$C_{11}H_{12}N_4O_2S$	264. 30
5	Sulfamethazine	57-68-1	$C_{12}H_{14}N_4O_2S$	278. 32
6	Sulfameter	651-06-9	$C_{11}H_{12}N_4O_3S$	280. 30
7	Sulfamethizole	144-82-1	$C_9H_{10}N_4O_2S_2$	270. 32
8	Sulfamethoxypyridazine	80-35-3	$C_{11}H_{12}N_4O_3S$	280. 30
9	Sulfachloropyridazine	80-32-0	$C_{10}H_9ClN_4O_2S$	284. 72
10	Sulfamonomethoxine	1220-83-3	$C_{11}H_{12}N_4O_3S$	280. 30
11	Sulfadoxine	2447-57-6	$C_{12}H_{14}N_4O_4S$	310. 32
12	Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253. 27
13	Sulfafurazole	127-69-5	$C_{11}H_{13}N_3O_3S$	267. 30
14	Sulfabenzamide	127-71-9	$C_{13}H_{12}N_2O_3S$	276. 31
15	Sulfadimethoxine	122-11-2	$C_{12}H_{14}N_4O_4S$	310. 32
16	Sulfaquinoxaline	59-40-5	$C_{14}H_{12}N_4O_2S$	300. 33
	Sulfadiazine-D4	1020719-78-1	$C_{10}H_6D_4N_4O_2S$	254. 30
	Sulfathiazole-D6		$C_9H_3D_6N_3O_2S_2$	261. 05
	Sulfapyridine-D6		$C_{11}H_5D_6N_3O_2S$	255. 09
	Sulfamethazine-D4		$C_{12}H_{10}D_4N_4O_2S$	282. 36
	Sulfamethoxazole-D6		$C_{10}H_5D_6N_3O_3S$	259. 09
	Sulfadimethoxine-D4		$C_{12}H_{10}D_4N_4O_4S$	314. 35
	Sulfaquinoxaline-D6		$C_{14}H_6D_6N_4O_2S$	306. 11
Nitroimidazoles(10)				
17	1-(2-Hydroxyethyl)-2-hydroxy-methyl-5-nitroimi dazol(MNZOH)	4812-40-2	$C_6H_9N_3O_4$	187. 15
18	2-Methyl-5-nitroimidazole	88054-22-2	$C_4H_5N_3O_2$	127. 10

Table A. 1 (continued)

NO.	Compound	CAS No.	Formula	Molecular weight
19	2-Hydroxymethyl-1-methyl-5-nitroimidazole (DMZOH、HMMNI)	936-05-0	$C_5H_7N_3O_3$	157. 13
20	Metronidazole(MNZ)	443-48-1	$C_6H_9N_3O_3$	171. 15
21	Dimetridazole(DMZ)	551-92-8	$C_5H_7N_3O_2$	141. 13
22	Ronidazole(RNZ)	7681-76-7	$C_6H_8N_4O_4$	200. 15
23	5-Chloro-1-methyl-4-nitroimidazole	4897-25-0	$C_4H_4ClN_3O_2$	161. 55
24	5-Nitrobenzimidazo1e	94-52-0	$C_7H_5N_3O_2$	163. 14
25	Ipronidazole-OH(IPZOH)	35175-14-5	$C_7H_{11}N_3O_3$	185. 18
26	Ipronidazole(IPZ)	14885-29-1	$C_7H_{11}N_3O_2$	169. 18
	DMZOH-D3		$C_5H_4D_3N_3O_3$	160. 2
	DMZ-D3		$C_5H_4D_3N_3O_2$	144. 2
	RNZ-D3		$C_6H_5D_3N_4O_4$	203. 2
	IPZOH-D3		$C_7H_8D_3N_3O_3$	188. 2
	IPZ-D3		$C_7H_8D_3N_3O_2$	172. 2
Qluinolones(15)				
27	Marbofloxacin	115550-35-1	$C_{17}H_{19}FN_4O_4$	362. 36
28	Enoxacin	74011-58-8	$C_{15}H_{17}FN_4O_3$	320. 32
29	Ofloxacin	82419-36-1	$C_{18}H_{20}FN_3O_4$	361. 37
30	Norfloxacin	70458-96-7	$C_{16}H_{18}FN_3O_3$	319. 33
31	Ciprofloxacin hydrochloride	86393-32-0	$C_{17}H_{18}FN_3O_3HCl$	367. 81
32	Danofloxacin mesylate	119478-55-6	$C_{20}H_{24}FN_3O_6S$	453. 6
33	Enrofloxacine	93106-60-6	$C_{19}H_{22}FN_3O_3$	359. 4
34	Lomefloxacin hydrochloride	98079-52-8	$C_{17}H_{19}F_2N_3O_3HCl$	387. 8
35	Orbifloxacin	113617-63-3	$C_{19}H_{20}F_3N_3O_3$	395. 38
36	Difloxacin	98106-17-3	$C_{21}H_{19}F_2N_3O_3$	400. 16
37	Sarafloxacin hydrochloride	98105-99-8	$C_{20}H_{17}F_2N_3O_3HCl$	421. 83
38	Sparfloxacin	111542-93-9	$C_{19}H_{22}F_2N_4O_3$	392. 40
39	Oxolinic acid	14698-29-4	$C_{13}H_{11}NO_5$	261. 23
40	Nalidixic acid	389-08-2	$C_{12}H_{12}N_2O_3$	232. 23
41	Flumequine	42835-25-6	$C_{14}H_{12}FNO_3$	261. 25
	Enoxacin-D8		$C_{15}H_9D_8FN_4O_3$	328. 18
	Ofloxacin-D3		$C_{18}H_{17}D_3FN_3O_4$	364. 16
	Norfloxacin-D5		$C_{16}H_{13}D_5FN_3O_3$	324. 33
	Ciprofloxacin-D8		$C_{17}H_{10}D_8FN_3O_3$	339. 81

Table A. 1 (continued)

NO.	Compound	CAS No.	Formula	Molecular weight
	Enrofloxacine-D5		$C_{19}H_{17}D_5FN_3O_3$	364. 20
	Difloxacin-D4		$C_{21}H_{15}D_4F_2N_3O_3$	403. 16
	Sarafloxacin-D8		$C_{20}H_9D_8F_2N_3O_3$	393. 17
Macrolide antibiotics(9)				
42	Spiramycin	8025-81-8	$C_{43}H_{74}N_2O_{14}$	843. 06
43	*Tilmicosin*	108050-54-0	$C_{46}H_{80}N_2O_{13}$	869. 13
44	Oleandomycin phosphate	7060-74-4	$C_{35}H_{61}NO_{12}$ $H_3PO_4 \cdot 2H_2O$	687. 4
45	Tylosin tartrate	74610-55-2	$C_{50}H_{83}NO_{23}$	915. 5
46	Erythromycin	59319-72-1	$C_{37}H_{67}NO_{13}$	733. 9
47	Roxithromycin	80214-83-1	$C_{41}H_{76}N_2O_{15}$	837. 0
48	Josamycin	16846-24-5	$C_{42}H_{69}NO_{15}$	827. 9
49	*Kitasamycin*	1392-21-8	$C_{39}H_{65}NO_{14}$	771. 93
50	Tiamulin	55297-95-5	$C_{28}H_{47}NO_4S$	493. 74
Lincosamides(3)				
51	Lincomycin hydrochloride monhydrate	859-18-7	$C_{18}H_{34}N_2O_6S \cdot HCl$	443. 0
52	Clindamycin hydrochloride	21462-39-5	$C_{18}H_{33}ClN_2O_5S \cdot HCl$	461. 4
53	Pirlimycin hydrochloride	79548-73-5	$C_{17}H_{31}ClN_2O_5S \cdot HCl$	446. 0
54	Praziquantel	55268-74-1	$C_{19}H_{24}N_2O_2$	312. 41

Annex B
(Informative annex)
API 4 000 LC-MS/MS conditions[1)]

API 4 000 LC-MS/MS conditions:

a) Electrospray capillary voltage(IS):5 500 V;

b) Nebulizer gas(GS1):358. 28 kPa (52 psi);

c) Curtain gas(CUR):172. 25 kPa (25 psi);

d) Auxiliary heater gas(GS2):378. 95 kPa (55 psi);

e) Ion Source Temperature(TEM):550 ℃;

f) Collision-activated dissociation (CAD):6;

g) Monitoring ion pairs, declustering potential(DP), collision energy(CE), collision cell exit potential (CXP)and retention time of the compounds are shown in table B. 1.

Table B. 1—Detailed parameters (DP, CE, CXP), monitoring ion pairs(*m/z*)and retention time of the compounds

NO.	Compound	Ion pairs *m/z*	Declustering Potential (DP) V	Collision Energy (CE) V	Collision Cell Exit Potential (CXP) V	Isotope internal compound	Retention time min
1	Sulfadiazine	251. 1/156. 3* 251. 1/108. 2	65	22 33	10	Sulfadiazine-D4	4. 35
2	Sulfathiazloe	255. 8/156. 3* 255. 8/108. 2	70	22 30	10	Sulfathiazole-D6	4. 90
3	Sulfapyridine	249. 9/156. 2* 249. 9/184. 4	70	24	10	Sulfapyridine- D6	5. 29
4	Sulfamerazine	265. 1/156. 3* 265. 1/172. 2	65	25 23	10	Sulfadiazine-D4	6. 06
5	Sulfamethazine	279. 2/156. 3* 279. 2/186. 1	70	26	10	Sulfamethazine-D4	7. 78

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)

NO.	Compound	Ion pairs *m/z*	Declustering Potential (DP) V	Collision Energy (CE) V	Collision Cell Exit Potential (CXP) V	Isotope internal compound	Retention time min
6	Sulfameter	281. 2/156. 3* 281. 2/215. 4	70	25 26	10	Sulfamethazine-D4	8. 35
7	Sulfamethizole	271. 0/156. 2* 271. 0/108. 2	70	24 32	10	Sulfamethazine-D4	8. 75
8	Sulfamethoxypyridazine	281. 2/156. 3* 281. 2/215. 4	70	25 26	10	Sulfamethazine-D4	9. 29
9	Sulfachloropyridazine	285. 0/156. 3* 285. 0/108. 2	70	22 35	10	Sulfadimethoxine-D4	11. 5
10	Sulfamonomethoxine	281. 2/156. 3* 281. 2/215. 4	70	25 26	10	Sulfamethazine-D4	11. 9
11	Sulfadoxine	311. 2/156. 3* 311. 2/108. 2	70	30 37	10	Sulfadimethoxine-D4	12. 1
12	Sulfamethoxazole	254. 1/156. 3* 254. 1/108. 2	65	22 36	10	Sulfamethoxazole-D6	12. 1
13	Sulfafurazole	268. 0/156. 3* 268. 0/113. 2	70	22	10	Sulfadimethoxine-D4	12. 5
14	Sulfabenzamide	277. 0/156. 2* 277. 0/92. 0	68	19 41	7 8	Sulfadimethoxine-D4	12. 8
15	Sulfadimethoxine	311. 2/156. 3* 311. 2/108. 2	70	30 37	10	Sulfadimethoxine-D4	13. 1
16	Sulfaquinoxaline	301. 2/156. 3* 301. 2/108. 2	55	24 37	13 9	Sulfaquinoxaline-D6	13. 1
	Sulfadiazine-D4	255. 2/160. 3	56	22	12		
	Sulfathiazole-D6	262. 0/97. 9	72	39	8		
	Sulfapyridine-D6	256. 2/97. 9	70	38	7		
	Sulfamethazine-D4	283. 1/186. 3	63	29	10		
	Sulfamethoxazole-D6	260. 2/162. 3	75	22	20		
	Sulfadimethoxine-D4	315. 3/156. 3	65	29	10		
	Sulfaqumoxaline-D6	307. 3/162. 3	80	24	12		
17	1-(2-Hydroxyethyl)-2-hydroxy-methyl-5-nitroimidazol (MNZOH)	188. 1/123. 0* 188. 1/126. 0	53	19 26	10	DMZOH-D3	3. 34
18	2-Methyl-5-nitroimidazole	128. 0/82. 0* 128. 0/42. 0	60	26 51	10		3. 59

Table B. 1 (continued)

NO.	Compound	Ion pairs *m/z*	Declustering Potential (DP) V	Collision Energy (CE) V	Collision Cell Exit Potential (CXP) V	Isotope internal compound	Retetion time min
19	2-Hydroxymethyl-1-methyl-5-nitroimidazole (DMZOH)	158. 0/140. 1* 158. 0/55. 0	48	16 31	10	DMZOH-D3	4. 14
20	Metronidazole (MNZ)	172. 0/128. 2* 172. 0/82. 1	62	21 35	11 14	DMZOH-D3	4. 07
21	Dimetridazole (DMZ)	142. 0/96. 0* 142. 0/81. 0	62	23 36	8 14	DMZ-D3	4. 83
22	Ronidazole (RNZ)	201. 1/140. 2* 201. 1/110. 2	37	17 25	8	RNZ-D3	4. 65
23	5-Chloro-1-methyl-4-nitroimidazole	162. 0/116. 0* 162. 0/145. 0	68	26 24	10		7. 09
24	5-Nitrobenzimidazole	164. 0/118. 0* 164. 0/91. 0	64	32 52	10		7. 51
25	Ipronidazole-OH (IPZOH)	186. 3/168. 1* 186. 3/122. 3	45	19 29	9 5	IPZOH-D3	10. 5
26	Ipronidazole (IPZ)	170. 2/109. 0* 170. 2/124. 1	63	37 26	10	IPZ-D3	12. 5
	DMZOH-D3	161. 2/143. 1	51	18	6		
	DMZ-D3	145. 2/99. 0	85	25	10		
	RNZ-D3	204. 2/143. 3	50	17	10		
	IPZOH-D3	189. 2/171. 2	60	20	9		
	IPZ-D3	173. 1/127. 2	67	28	6		
27	Marbofloxacin	363. 2/320. 2* 363. 2/345. 3	63	23 25	10		5. 58
28	Enoxacin	321. 2/303. 1* 321. 2/234. 4	67	29 32	10	Enoxacin-D8	6. 38
29	Ofloxacin	362. 2/318. 3* 362. 2/261. 2	75	27 39	8 6	Ofloxacin-D3	6. 76
30	Norfloxacin	320. 0/233. 3* 320. 0/276. 3	74	35 25	11 14	Norfloxacin-D5	7. 51
31	*Ciprofloxacin*	332. 1/288. 3* 332. 1/230. 9	85	27 50	7 12	*Ciprofloxacin-D8*	8. 00
32	Danofloxacin	358. 2/340. 3* 358. 2/283. 1	65	35 33	9 9		8. 17
33	Enrofloxacine	360. 2/316. 2* 360. 2/245. 4	76	29 35	7 9	Enrofloxacine-D5	8. 38

Table B. 1 (continued)

NO.	Compound	Ion pairs *m/z*	Declustering Potential (DP) V	Collision Energy (CE) V	Collision Cell Exit Potential (CXP) V	Isotope internal compound	Retetion time min
34	Lomefloxacin	352. 3/265. 1* 352. 3/308. 2	80	35 24	10 10		8. 70
35	Orbifloxacin	396. 0/352. 0* 396. 0/295. 0	76	27 35	9 9		8. 92
36	Difloxacin	400. 0/356. 0* 400. 0/299. 0	76	29 42	9	Difloxacin-D4	10. 6
37	Sarafloxacin	386. 1/342. 4* 386. 1/299. 2	87	27 39	8 7	Sarafloxacin-D8	11. 2
38	Sparfloxacin	393. 2/292. 2* 393. 2/349. 2	94	36 28	10 10		11. 7
39	Oxolinic acid	262. 0/244. 4* 262. 0/216. 3	75	25 40	18 18		12. 9
40	Nalidixic acid	233. 2/215. 0* 233. 2/187. 0	53	21 35	10 10		14. 1
41	Flumequine	262. 1/244. 0* 262. 1/202. 1	73	27 49	10 10		14. 3
	Enoxacin-D8	329. 4/311. 2	66	28	7		
	Ofloxacin-D3	365. 2/321. 4	65	26	8		
	Norfloxacin-D5	325. 4/281. 2	65	25	9		
	Ciprofloxacin-D8	340. 1/296. 5	90	27	10		
	Enrofloxacine-D5	365. 1/321. 5	70	28	8		
	Difloxacin-D4	404. 3/360. 2	76	28	9		
	Sarafloxacin-D8	394. 1/376. 5	70	30	8		
42	Spiramycin	843. 6/174. 3* 843. 6/318. 5	110	50 40	14 9		11. 8
43	Tilmicosin	869. 7/174. 4* 869. 7/156. 6	120	58 68	15 12		12. 1
44	Oleandomycin	688. 6/158. 4* 688. 6/544. 6	65	40 23	13 19		12. 6
45	Tylosin	916. 5/174. 2* 916. 5/772. 7	96	53 41	15		12. 7
46	Erythromycin	734. 6/158. 5* 734. 6/576. 5	80	40 27	13 17		12. 7
47	Roxithromycin	837. 6/679. 6* 837. 6/158. 4	90	48 30	14 22		13. 3

Table B. 1 (continued)

NO.	Compound	Ion pairs *m/z*	Declustering Potential (DP) V	Collision Energy (CE) V	Collision Cell Exit Potential (CXP) V	Isotope internal compound	Retetion time min
48	Josamycin	828.8/174.3* 828.8/229.3	105	45 42	14		13.6
49	Kitasamycin	772.7/174.3* 772.7/109.0	115	47 63	8 10		13.0
50	Tiamulin	494.3/192.5* 494.3/119.2	60	26 54	10		12.9
51	Lincomycin	407.2/126.3* 407.2/359.4	90	26 28	10 9		5.15
52	Clindamycin	425.9/126.1* 425.9/378.2	90	48 28	10		12.4
53	Pirlimycin	411.2/112.1* 411.2/363.1	90	44 24	10		12.3
54	Praziquantel	313.5/203.2* 313.5/83.2	74	25 39	4 6		17.1
Note: "*" The product ion is used for quantification.							

Annex C
(Informative annex)
Multiple reaction monitoring chromatogram of the multi-veterinary drugs standard

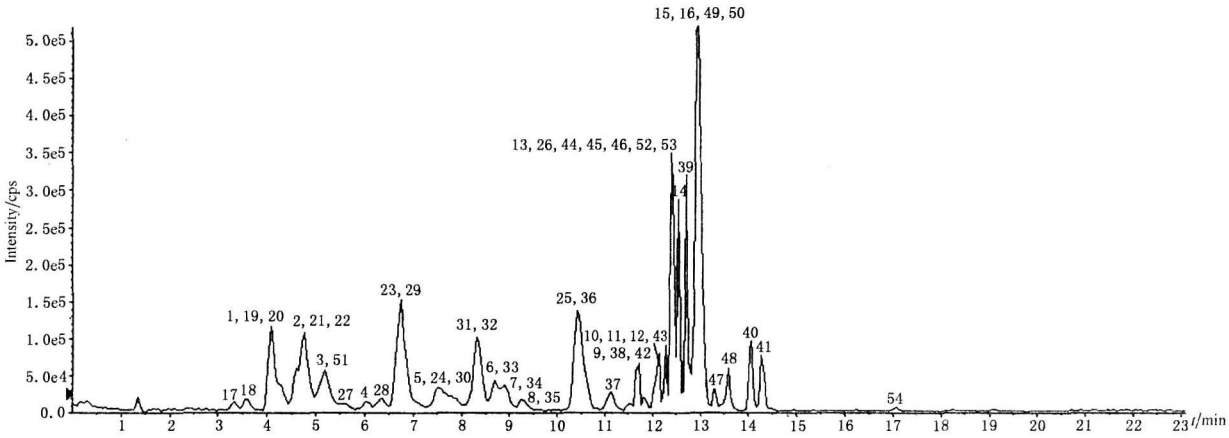

Figure C. 1—TIC chromatograph of veterinary drugs standard solution(sulfanilamides and nitroimidazoles are 1.0 μg/kg, qluinolones are 2.0 μg/kg, macrolide antibiotics are 3.0 μg/kg, lincosamides are 2.0 μg/kg and praziquantel is 0.3 μg/kg)

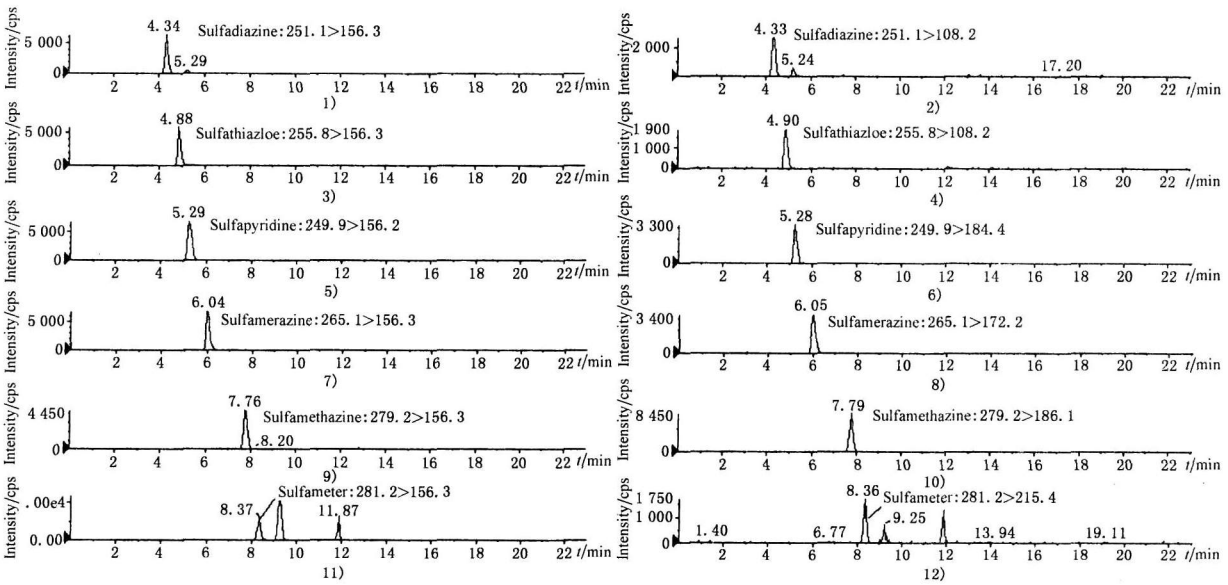

Figure C. 2—MRM chromatograph of sulfanilamides standard solution(1.0 μg/kg)

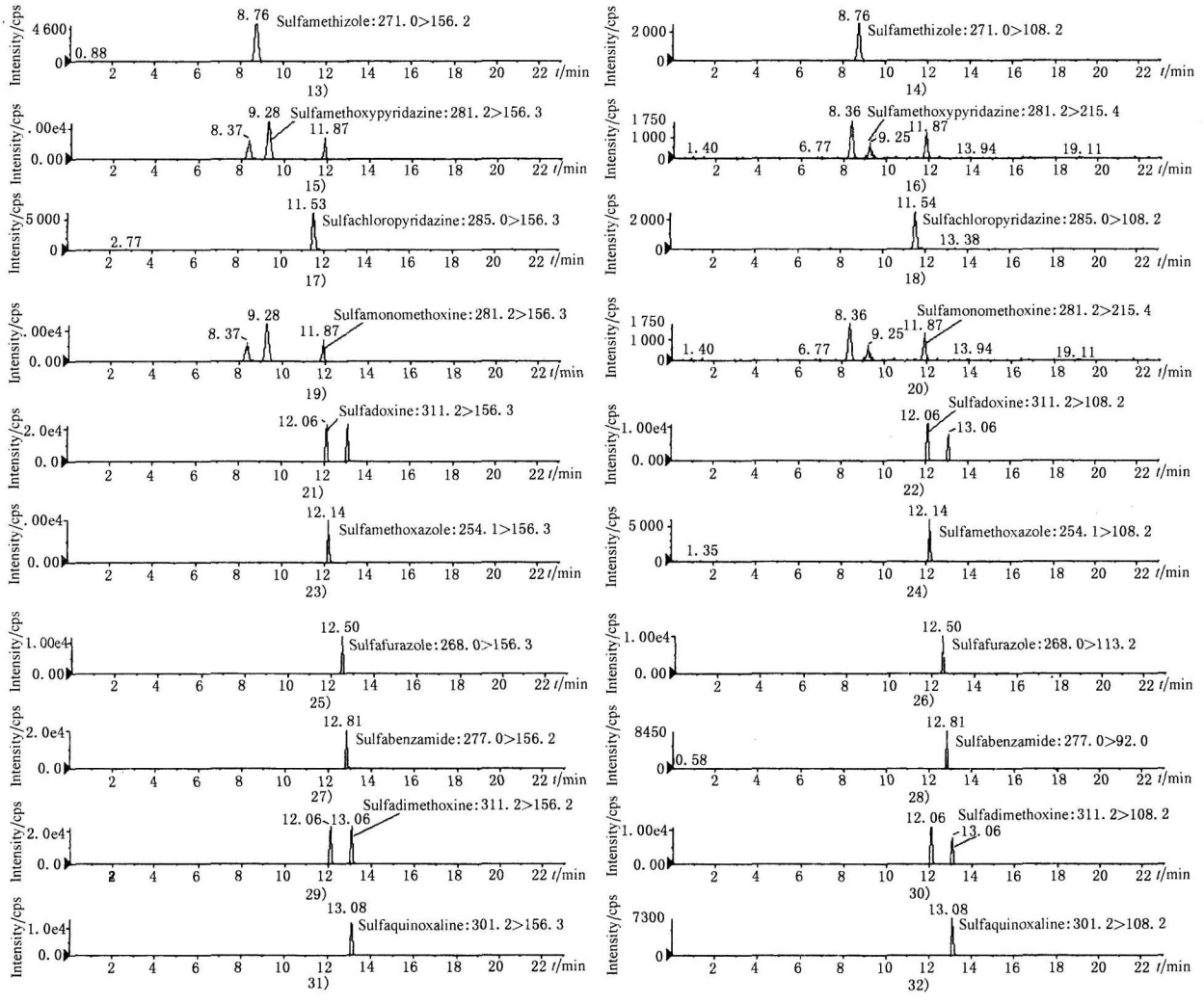

Figure C. 2 (continued)

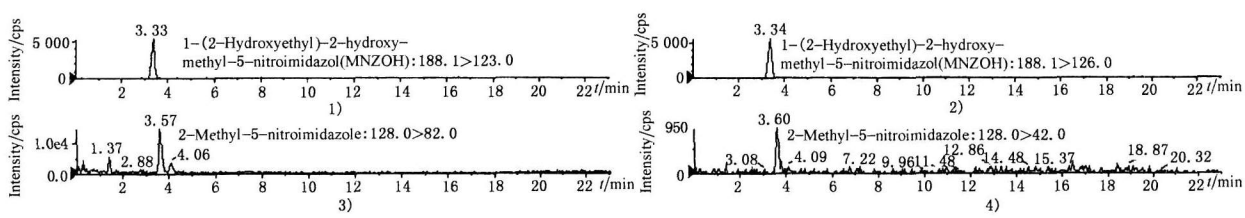

Figure C. 3—MRM chromatograph of nitroimidazoles standard solution(1.0 μg/kg)

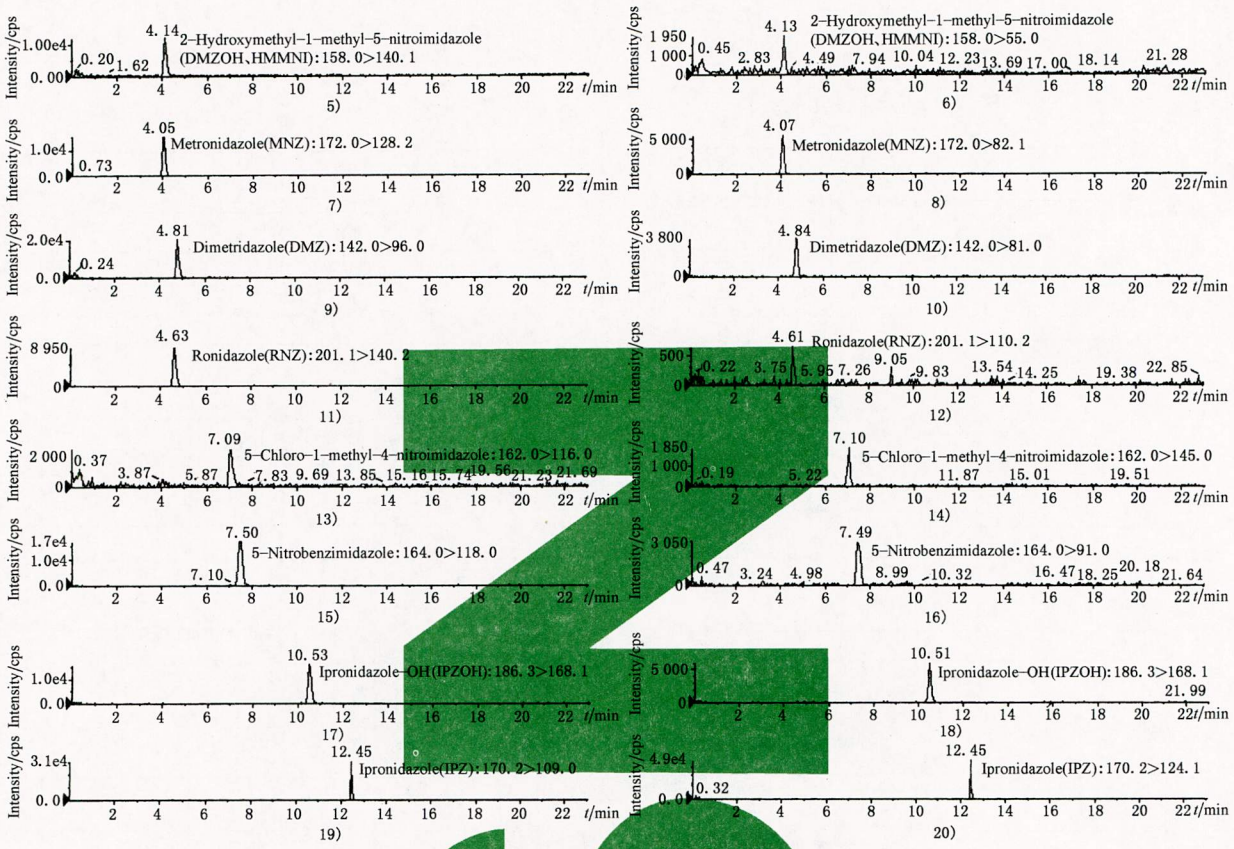

Figure C.3 (continued)

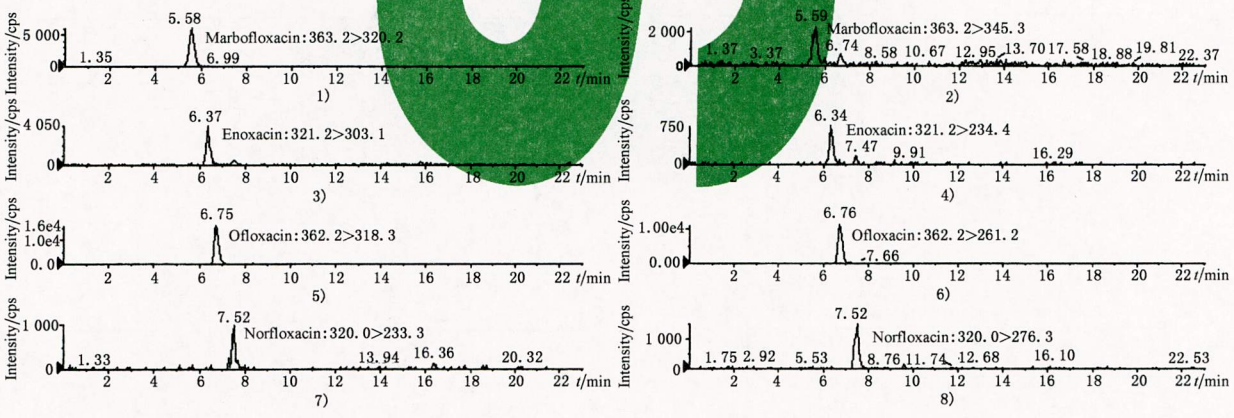

Figure C.4—MRM chromatograph of qluinolones standard solution(2.0 μg/kg)

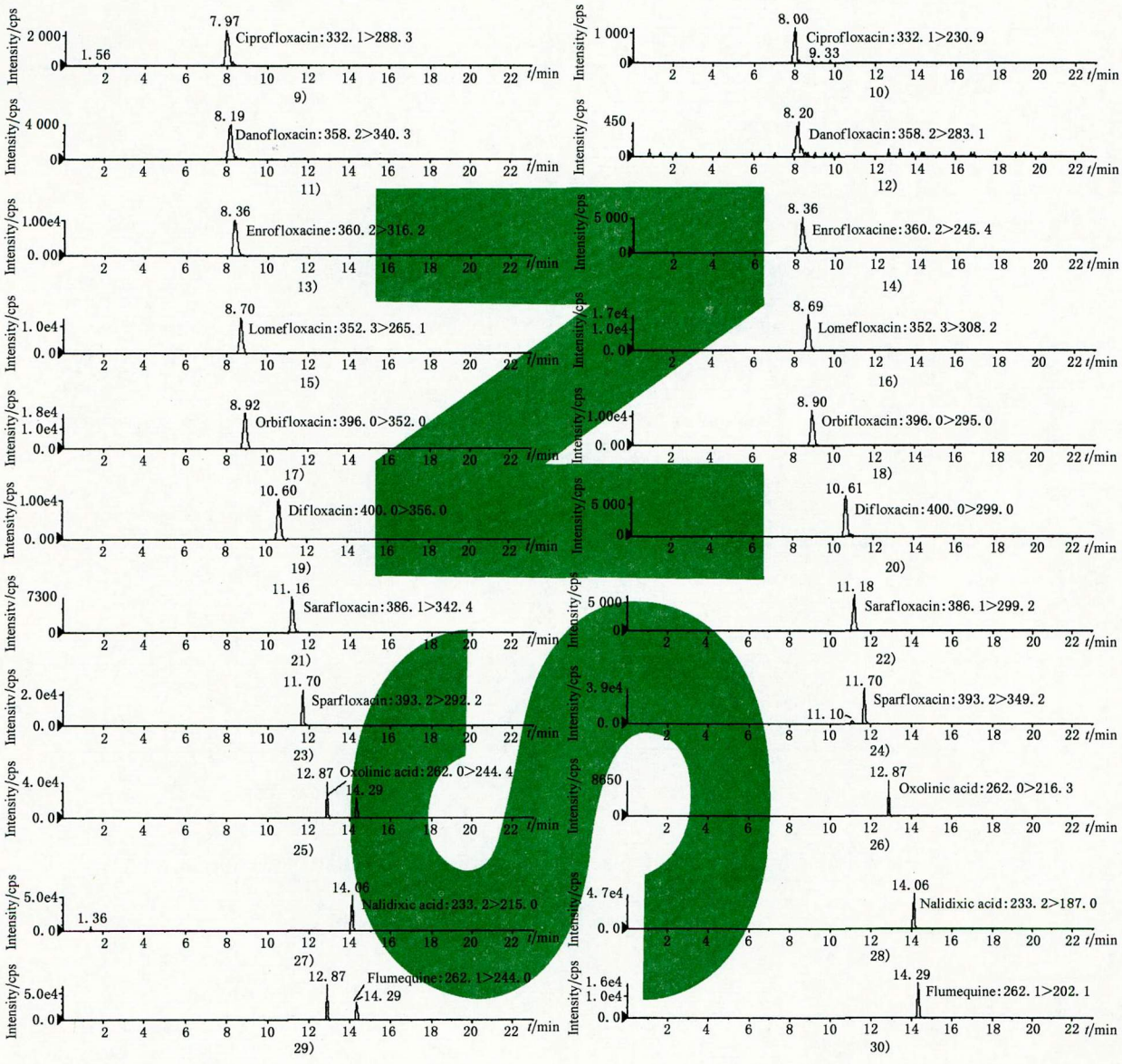

Figure C. 4 (continued)

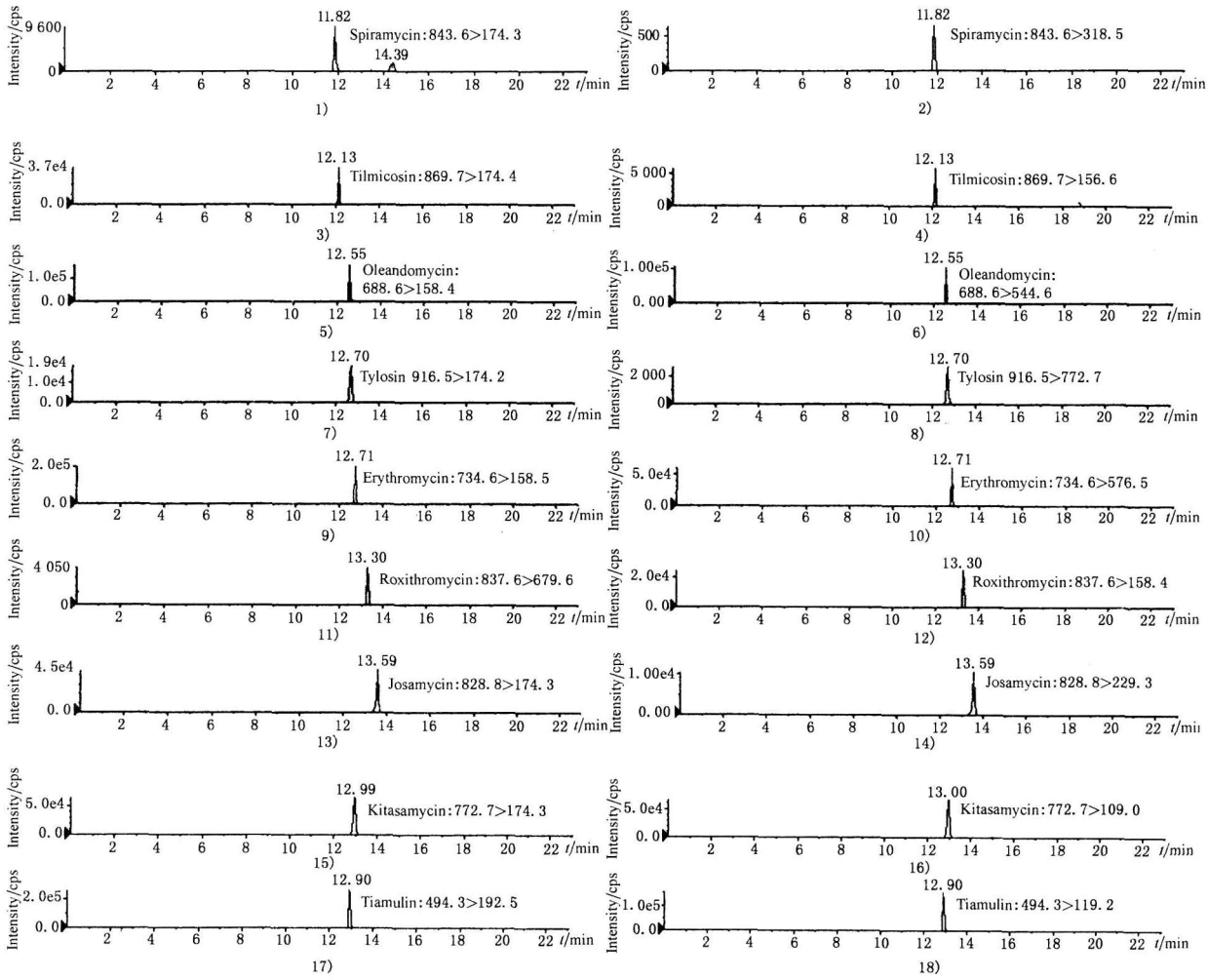

Figure C.5—MRM chromatograph of macrolide antibiotics standard solution(3.0 μg/kg)

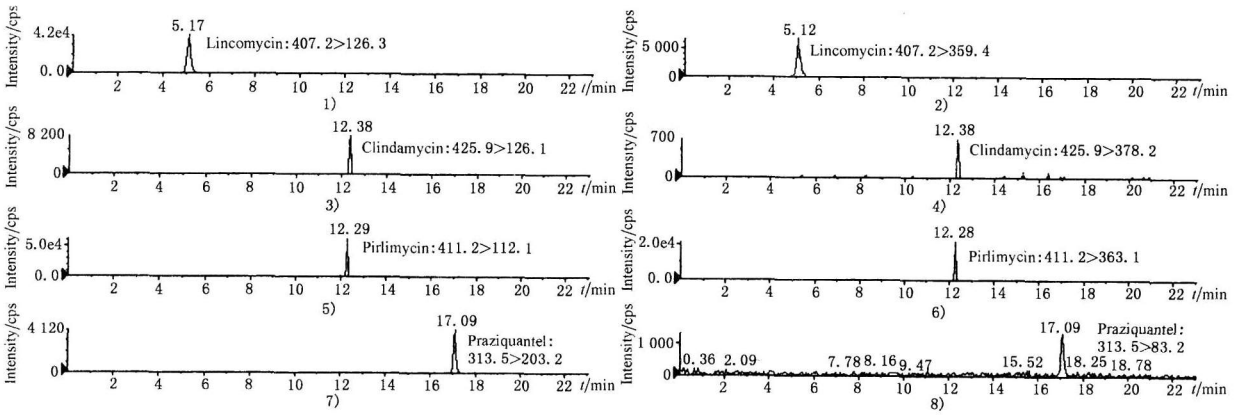

Figure C.6—MRM chromatograph of lincosamides standard solution
(2.0 μg/kg)and praziquantel(0.3 μg/kg)

Annex D
(Informative annex)
The recovery of multi-veterinary drugs

Table D. 1—Spiked concentration and recovery of multi -veterinary drugs in pork, shrimp and honey($n = 6$)

Compound	Spiked conc. μg/kg	Recovery(Pork) %	Recovery(shrimp) %	Recovery(honey) %
Sulfadiazine	1.0	88.9～106	76.3～102	96.5～109
	2.0	93.0～109	81.1～107	95.0～98.6
	4.0	97.9～111	76.5～107	96.1～106
Sulfathiazloe	1.0	86.7～107	77.7～113	98.9～110
	2.0	93.3～110	90.5～106	89.7～104
	4.0	88.5～106	90.7～109	84.4～110
Sulfapyridine	1.0	84.5～112	86.5～106	86.5～105
	2.0	87.7～107	78.9～106	80.0～95.8
	4.0	79.4～109	81.1～109	85.0～108
Sulfamerazine	1.0	86.8～105	98.6～110	90.8～106
	2.0	85.5～109	77.7～108	86.8～94.2
	4.0	85.6～108	90.4～100	93.0～105
Sulfamethazine	1.0	79.2～103	95.0～114	86.5～108
	2.0	90.0～109	102～114	91.4～101
	4.0	95.1～108	103～111	92.2～110
Sulfameter	1.0	89.4～105	92.4～112	94.5～107
	2.0	89.7～110	98.6～109	91.1～106
	4.0	90.5～107	87.9～111	92.6～110
Sulfamethizole	1.0	73.2～97.7	81.9～103	85.0～94.3
	2.0	85.4～106	71.4～99.1	86.1～99.1
	4.0	84.6～100	77.5～95.8	84.7～96.1
Sulfamethoxypyridazine	1.0	71.9～101	79.8～118	80.9～94.8
	2.0	75.1～106	95.7～114	78.8～94.0
	4.0	78.0～93.2	97.4～118	84.5～94.7
Sulfachloropyridazine	1.0	84.7～109	66.2～97.3	64.7～76.4
	2.0	79.0～103	74.3～99.2	82.5～97.0
	4.0	83.0～107	72.6～98.0	81.1～94.5

Table D. 1 (continued)

Compound	Spiked conc. μg/kg	Recovery(Pork) %	Recovery(shrimp) %	Recovery(honey) %
Sulfamonomethoxine	1. 0	98. 1～109	73. 5～104	86. 4～99. 8
	2. 0	87. 8～110	91. 6～109	88. 0～110
	4. 0	83. 4～107	93. 3～116	90. 4～114
Sulfadoxine	1. 0	86. 7～107	81. 7～109	77. 2～104
	2. 0	88. 1～104	92. 0～103	69. 0～80. 4
	4. 0	88. 2～108	86. 7～105	78. 9～98. 1
Sulfamethoxazole	1. 0	85. 9～108	88. 4～105	79. 2～95. 2
	2. 0	86. 8～102	72. 3～99. 2	81. 8～108
	4. 0	91. 7～109	78. 3～94. 2	83. 4～99. 4
Sulfafurazole	1. 0	88. 7～106	79. 3～107	54. 5～78. 0
	2. 0	78. 8～107	78. 6～92. 9	55. 8～75. 1
	4. 0	80. 0～108	77. 8～97. 2	60. 9～88. 2
Sulfabenzamide	1. 0	82. 8～111	73. 8～106	52. 5～73. 7
	2. 0	77. 8～97. 7	82. 7～105	54. 2～72. 2
	4. 0	78. 4～107	82. 9～105	53. 6～65. 4
Sulfadimethoxine	1. 0	95. 7～106	91. 0～107	81. 5～94. 8
	2. 0	77. 4～111	106～112	81. 4～104
	4. 0	81. 3～107	101～111	81. 5～109
Sulfaquinoxaline	1. 0	91. 5～107	75. 6～109	78. 5～100
	2. 0	90. 9～113	65. 0～79. 5	71. 1～90. 3
	4. 0	92. 3～111	73. 5～106	83. 8～104
1-(2-Hydroxyethyl)-2-hydroxy-methyl-5-nitroimidazol(MNZOH)	1. 0	88. 8～109	92. 2～114	99. 5～108
	2. 0	88. 1～114	91. 0～109	95. 5～108
	4. 0	78. 8～105	98. 4～115	97. 7～107
2-Methyl-5-nitroimidazole	1. 0	75. 3～99. 8	74. 9～103	64. 4～82. 3
	2. 0	78. 2～99. 9	78. 2～109	74. 8～99. 6
	4. 0	74. 7～89. 0	75. 3～90. 5	74. 2～94. 2
2-Hydroxymethyl-1-met hyl-5-nitroimidazole(DMZOH)	1. 0	85. 2～111	75. 4～103	83. 2～104
	2. 0	91. 1～101	83. 7～106	92. 4～110
	4. 0	85. 8～101	86. 1～112	85. 7～107
Metronidazole(MNZ)	1. 0	97. 3～111	94. 8～109	99. 7～107
	2. 0	98. 5～104	85. 6～107	95. 8～110
	4. 0	99. 1～107	89. 2～104	94. 6～106

Table D. 1 (continued)

Compound	Spiked conc. μg/kg	Recovery(Pork) %	Recovery(shrimp) %	Recovery(honey) %
Dimetridazole(DMZ)	1.0	77.7～104	94.3～109	90.2～103
	2.0	96.5～102	88.3～107	85.8～96.9
	4.0	91.8～106	90.5～106	91.1～102
Ronidazole(RNZ)	1.0	86.4～101	93.0～113	85.6～98.2
	2.0	87.4～96.3	95.9～113	90.5～102
	4.0	88.7～102	89.8～104	87.3～105
5-Chloro-1-methyl-4-nitroimidazole	1.0	72.5～94.1	72.5～100	72.4～99.7
	2.0	71.2～97.4	70.7～88.3	80.9～94.1
	4.0	72.5～87.0	78.8～97.8	84.2～97.5
5-Nitrobenzimidazole	1.0	65.0～74.2	60.9～80.1	62.8～85.4
	2.0	60.5～69.1	53.7～74.4	72.0～84.3
	4.0	60.9～78.7	53.0～70.7	83.2～89.2
Ipronidazole-OH(IPZOH)	1.0	89.1～105	98.1～113	85.6～105
	2.0	91.8～103	104～113	82.4～102
	4.0	86.6～110	90.6～109	87.8～101
Ipronidazole(IPZ)	1.0	90.6～106	96.3～105	94.9～106
	2.0	97.6～105	99.0～111	82.9～104
	4.0	90.3～109	90.1～113	92.9～109
Marbofiloxacin	2.0	73.8～92.7	65.2～90.5	84.8～102
	4.0	63.4～78.4	59.2～83.4	74.9～96.2
	8.0	60.2～74.9	57.9～72.5	74.3～86.0
Enoxacin	2.0	79.8～95.9	90.1～105	73.2～79.0
	4.0	85.3～104	88.6～115	74.8～90.6
	8.0	92.7～101	88.2～118	85.5～98.3
Ofloxacin	2.0	84.7～105	93.1～117	95.7～104
	4.0	92.7～112	93.8～107	94.8～100
	8.0	92.8～109	97.4～116	93.5～110
Norfloxacin	2.0	60.4～82.7	90.3～110	70.3～95.2
	4.0	72.7～99.4	72.8～109	74.3～89.0
	8.0	76.7～86.4	82.2～116	77.5～89.0
Ciprofloxacin	2.0	65.4～88.2	69.5～103	81.3～98.1
	4.0	64.3～85.8	84.0～115	80.7～102
	8.0	69.3～100	82.6～112	92.8～101

Table D. 1 (continued)

Compound	Spiked conc. μg/kg	Recovery(Pork) %	Recovery(shrimp) %	Recovery(honey) %
Danofloxacin	2.0	52.9～79.1	75.0～102	80.1～110
	4.0	49.0～65.9	67.0～95.6	76.7～100
	8.0	51.8～70.3	61.8～88.6	71.6～96.7
Enrofloxacine	2.0	86.6～102	97.8～105	92.0～108
	4.0	95.6～103	92.2～118	93.7～105
	8.0	95.6～110	102～113	96.6～105
Lomefloxacin	2.0	36.1～51.1	50.1～76.0	67.5～80.6
	4.0	45.8～62.2	49.0～68.8	63.1～75.4
	8.0	53.6～72.7	53.8～63.1	71.5～76.4
Orbifloxacin	2.0	52.5～79.8	57.0～83.9	71.7～87.5
	4.0	56.8～81.2	50.5～70.9	80.1～89.6
	8.0	64.0～81.7	61.9～79.6	81.6～99.6
Difloxacin	2.0	85.3～118	77.2～110	97.9～113
	4.0	89.0～121	79.7～98.2	93.2～110
	8.0	85.5～109	79.4～107	95.7～108
Sarafloxacin	2.0	87.0～111	72.7～113	76.6～98.4
	4.0	88.5～107	69.6～95.5	72.0～102
	8.0	87.3～105	77.2～101	75.9～109
Sparfloxacin	2.0	48.8～72.0	62.2～83.5	80.7～107
	4.0	51.0～73.3	53.6～71.8	86.4～107
	8.0	56.4～71.8	57.3～76.0	82.0～103
Oxklinic acid	2.0	60.6～88.5	62.8～92.2	68.0～101
	4.0	74.2～93.9	70.0～95.0	75.6～109
	8.0	75.1～93.1	74.7～85.1	84.7～98.7
Nalidixic acid	2.0	68.2～79.4	51.5～70.6	83.1～98.5
	4.0	63.0～85.3	60.0～87.4	87.2～100
	8.0	66.4～82.6	64.2～76.8	93.6～109
Flumequine	2.0	68.7～76.0	63.6～88.7	71.1～99.4
	4.0	62.2～85.1	65.5～82.1	73.1～101
	8.0	57.5～79.3	62.2～70.7	80.9～102
Spiramycin	3.0	21.1～29.8	41.0～57.0	62.6～83.5
	6.0	24.5～28.9	33.3～45.6	48.9～66.4
	12	31.1～43.2	44.3～53.8	67.7～85.5

Table D. 1 (continued)

Compound	Spiked conc. μg/kg	Recovery(Pork) %	Recovery(shrimp) %	Recovery(honey) %
Tilmicosin	3.0	30.2～47.3	44.1～59.7	73.2～105
	6.0	42.5～51.5	40.2～50.4	82.0～97.6
	12	57.6～78.6	60.4～87.5	91.4～111
Oleandomycin	3.0	37.8～59.6	35.7～53.0	82.0～106
	6.0	47.9～59.1	37.2～55.8	82.3～107
	12	69.8～87.6	58.7～84.6	81.3～105
Tylosin	3.0	34.1～42.7	40.0～50.0	74.2～93.3
	6.0	35.3～43.1	32.4～40.7	83.1～108
	12	41.2～52.9	44.5～62.7	85.3～109
Erythromycin	3.0	33.9～53.6	33.7～49.8	32.6～70.9
	6.0	41.9～63.5	45.4～63.7	37.1～69.1
	12	47.5～66.4	45.2～61.2	42.7～72.4
Roxithromycin	3.0	51.0～78.0	48.9～71.1	85.8～108
	6.0	53.4～75.7	58.3～71.7	93.3～114
	12	66.1～88.3	54.7～76.2	96.4～110
Josamycin	3.0	41.4～59.1	34.0～44.0	88.8～110
	6.0	41.2～59.7	30.8～40.0	88.0～111
	12	44.2～60.3	36.1～50.1	91.6～109
Kitasamycin	3.0	28.3～38.9	35.1～51.1	42.0～58.6
	6.0	26.1～35.4	31.3～45.4	47.3～63.8
	12	31.1～42.9	33.0～47.9	50.8～61.4
Tiamulin	3.0	46.1～59.8	41.4～54.0	53.3～68.7
	6.0	50.3～62.5	32.2～41.5	70.7～86.9
	12	55.4～69.1	38.6～52.8	58.8～75.4
Lincomycin	2.0	32.7～50.3	46.2～63.8	78.3～89.4
	4.0	41.4～62.6	50.5～69.5	81.2～86.5
	8.0	46.2～57.8	45.3～60.4	78.3～89.6
Clindamycin	2.0	34.1～47.8	37.8～52.6	90.2～107
	4.0	38.7～53.3	35.4～48.7	76.0～93.9
	8.0	44.2～58.4	32.3～48.1	76.6～107
Pirlimycin	2.0	20.9～37.8	36.2～42.6	64.1～94.1
	4.0	26.6～43.7	39.5～56.1	61.3～75.9
	8.0	27.3～38.4	38.5～47.9	60.0～84.7

Table D. 1 (continued)

Compound	Spiked conc. μg/kg	Recovery(Pork) %	Recovery(shrimp) %	Recovery(honey) %
Praziquantel	0. 3	41. 8～55. 9	35. 9～52. 9	62. 3～78. 5
	0. 6	37. 5～47. 5	37. 1～49. 9	69. 3～79. 5
	1. 2	39. 7～47. 3	39. 5～50. 8	67. 2～85. 6
[a] the matrix is pork. [b] the matrix is shrimp. [c] the matrix is honey.				