SN

中华人民共和国出入境检验检疫行业标准

SN/T 2580—2010

进出口蜂王浆中16种磺胺类药物残留量的测定 液相色谱-质谱/质谱法

Determination of sixteen sulfonamides residues in royal jelly for import and export—LC-MS/MS

2010-05-27 发布　　2010-12-01 实施

中华人民共和国
国家质量监督检验检疫总局　发布

前　言

本标准按照 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。

本标准主要起草人：谢文、陈笑梅、丁慧瑛、奚君阳、韩超、张慧敏。

进出口蜂王浆中16种磺胺类药物残留量的测定 液相色谱-质谱/质谱法

1 范围

本标准规定了蜂王浆中16种磺胺类药物残留测定的制样和液相色谱-质谱/质谱测定方法。

本标准适用于蜂王浆中磺胺嘧啶、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，上清液用乙酸乙酯提取，再用MCX和C_{18}固相萃取小柱净化，液相色谱-质谱/质谱测定，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 乙酸乙酯：高效液相色谱级。

4.4 甲酸：高效液相色谱级。

4.5 氯化钠。

4.6 氨水：25%～28%。

4.7 5%氨水甲醇溶液：氨水-甲醇(5+95，体积比)。

4.8 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、磺胺嘧啶-D4、磺胺二甲基嘧啶-D4、磺胺二甲氧嘧啶-D4标准品：纯度大于等于98%，见附录A表A.1。

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

4.10 磺胺嘧啶-D4、磺胺二甲基嘧啶-D4、磺胺二甲氧嘧啶-D4标准储备溶液：分别称取标准品(4.8)，

分别用甲醇溶解定容至100 mL，溶液浓度为100 μg/mL，1 ℃～4 ℃冰箱保存。

4.11 空白样品提取液：用不含16种磺胺药物的蜂王浆样品，按照第7章制备空白样品溶液。

4.12 标准工作溶液：根据需要用空白样品提取液(4.11)将标准储备液稀释成浓度为1 ng/mL、1.25 ng/mL、2.5 ng/mL、5 ng/mL、12.5 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、50 μg/kg磺胺类药物。内标溶液浓度均为1 ng/mL。

4.13 MCX固相萃取小柱，500 mg，3 mL或相当者。使用前依次用5 mL甲醇，5 mL水预洗。

4.14 C_{18}固相萃取小柱，500 mg，3 mL或相当者。使用前依次用5 mL甲醇预洗。

4.15 微孔滤膜：0.45 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。

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，待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0.1 mL同位素内标溶液(40 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，移取10.0 mL上清液，加10 mL水，混匀，加2 g氯化钠和20 mL乙酸乙酯提取，下层溶液再用20 mL乙酸乙酯提取一次，合并乙酸乙酯提取溶液，在45 ℃以下水浴中减压浓缩至近干。

7.2 净化

用20 mL水溶解残渣，将溶液转移至MCX固相萃取小柱中(4.13)，弃去流出液，加5 mL甲醇-水(1＋1，体积比)淋洗，抽干，用8 mL5%氨水甲醇(4.7)洗脱，控制流速1 mL/min～2 mL/min，收集全部洗脱液，在50 ℃以下水浴下减压浓缩至近干，10 mL甲醇将残渣溶解并全部转移至C_{18}固相萃取小柱中(4.14)，控制流速1 mL/min～2 mL/min，收集全部流出液，50 ℃以下水浴中减压浓缩至近干，用4.0 mL甲醇-0.15%甲酸(3＋7，体积比)溶解残渣，混匀，溶液过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
14.5	2	20	78
20	2	20	78

c) 流速：0.8 mL/min；

d) 进样量：30 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、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、磺胺喹噁啉的参考保留时间分别约为 4.1 min、4.6 min、5.0 min、5.7 min、7.4 min、7.6 min、7.9 min、8.6 min、10.6 min、11.3 min、11.7 min、11.7 min、12.2 min、12.6 min、12.9 min、13.0 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)计算试样中磺胺类药物的残留含量，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 ——试样中磺胺类药物残留量，单位为微克每千克(μg/kg)；

c_i ——基质标准溶液中磺胺类药物的浓度，单位为纳克每毫升(ng/mL)；

V ——样液最终定容体积，单位为毫升(mL)；

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

磺胺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 16 种磺胺类药物添加回收率范围(n=6)

化合物	添加浓度/(μg/kg)	回收率/%
磺胺嘧啶	5	85.8～95.0
	10	93.3～102.0
	20	89.5～95.0
磺胺噻唑	5	83.4～87.2
	10	81.3～90.2
	20	81.5～90.0
磺胺吡啶	5	87.6～97.4
	10	94.6～104.0
	20	89.0～94.0
磺胺甲基嘧啶	5	89.6～95.0
	10	96.9～105
	20	93.5～98.5
磺胺二甲基嘧啶	5	93.8～100.2
	10	91.9～96.1
	20	94.5～101.0

表 3（续）

化合物	添加浓度/(μg/kg)	回收率/%
磺胺-5-(对)甲氧嘧啶	5	90.8～100.2
	10	87.4～93.4
	20	92.0～97.5
磺胺甲噻二唑	5	81.6～86.4
	10	74.3～81.4
	20	84.5～91.0
磺胺甲氧哒嗪	5	84.2～94.6
	10	78.7～81.3
	20	87.0～92.0
磺胺氯哒嗪	5	96.8～106.0
	10	83.0～91.2
	20	84.0～90.0
嘧啶磺胺-6-(间)甲氧嘧啶	5	90.4～94.6
	10	86.9～93.1
	20	91.5～97.0
磺胺邻二甲氧嘧啶	5	97.6～109.2
	10	89.2～94.2
	20	91.5～96.0
磺胺甲基异恶唑	5	97.6～105.0
	10	94.8～98.3
	20	94.0～98.0
磺胺二甲异恶唑	5	97.6～107.2
	10	83.4～88.6
	20	89.5～96.5
苯酰磺胺	5	88.4～96.0
	10	79.5～83.4
	20	77.0～83.5
磺胺二甲氧嘧啶	5	95.8～108.2
	10	87.9～96.8
	20	92.0～96.0
磺胺喹噁啉	5	95.6～107.4
	10	87.8～91.8
	20	96.5～102.0

附 录 A
（规范性附录）
磺胺类药物标准品信息

表 A.1 磺胺类药物标准品信息

中文名称	英文名称	CAS 编号	分子式	相对分子质量
磺胺嘧啶	sulfadiazine	68-35-9	$C_{10}H_{10}N_4O_2S$	250.27
磺胺噻唑	sulfathiazole	72-14-0	$C_9H_9N_3O_2S_2$	255.31
磺胺吡啶	sulfapyridine	144-83-2	$C_{11}H_{11}N_3O_2S$	249.28
磺胺甲基嘧啶	sulfamerazine	127-79-7	$C_{11}H_{12}N_4O_2S$	264.30
磺胺二甲基嘧啶	sulfamethazine	57-68-1	$C_{12}H_{14}N_4O_2S$	278.32
磺胺-5-(对)甲氧嘧啶	sulfameter	651-06-9	$C_{11}H_{12}N_4O_3S$	280.30
磺胺甲噻二唑	sulfamethizole	144-82-1	$C_9H_{10}N_4O_2S_2$	270.32
磺胺甲氧哒嗪	sulfamethoxypyridazine	80-35-3	$C_{11}H_{12}N_4O_3S$	280.30
磺胺氯哒嗪	sulfachloropyridazine	80-32-0	$C_{10}H_9ClN_4O_2S$	284.72
磺胺-6-(间)甲氧嘧啶	sulfamonomethoxine	1220-83-3	$C_{11}H_{12}N_4O_3S$	280.30
磺胺邻二甲氧嘧啶	sulfadoxine	2447-57-6	$C_{12}H_{14}N_4O_4S$	310.32
磺胺甲基异恶唑	sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253.27
磺胺二甲异恶唑	sulfafurazole	127-69-5	$C_{11}H_{13}N_3O_3S$	267.30
苯酰磺胺	sulfabenzamide	127-71-9	$C_{13}H_{12}N_2O_3S$	276.31
磺胺二甲氧嘧啶	sulfadimethoxine	122-11-2	$C_{12}H_{14}N_4O_4S$	310.32
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	—	$C_{10}H_6D_4N_4O_2S$	254.30
磺胺二甲基嘧啶-D4	sulfamethazine-D4	—	$C_{12}H_{10}D_4N_4O_2S$	282.36
磺胺二甲氧嘧啶-D4	sulfadimethoxine-D4	—	$C_{12}H_{10}D_4N_4O_4S$	314.35

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)]

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 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

化合物名称	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
磺胺嘧啶	251.1/156.3[a] 251.1/108.2	65	22 33	10	磺胺嘧啶-D4
磺胺噻唑	255.8/156.3[a] 255.8/108.2	70	22 30	10	磺胺嘧啶-D4
磺胺吡啶	249.9/156.2[a] 249.9/184.1	70	24	10	磺胺嘧啶-D4
磺胺甲基嘧啶	265.1/156.3[a] 265.1/172.2	65	25 23	10	磺胺嘧啶-D4
磺胺二甲基嘧啶	279.2/156.3[a] 279.2/186.1	70	26	10	磺胺二甲基嘧啶-D4
磺胺-5-(对)甲氧嘧啶	281.2/156.3a[a] 281.2/215.4b	70	25 26	10	磺胺二甲基嘧啶-D4
磺胺甲噻二唑	271.0/156.2[a] 271.0/108.2	70	24 32	10	磺胺二甲基嘧啶-D4
磺胺甲氧哒嗪	281.2/156.3b[a] 281.2/215.4b	70	25 26	10	磺胺二甲基嘧啶-D4
磺胺氯哒嗪	285.0/156.3[a] 285.0/108.2	70	22 35	10	磺胺二甲氧嘧啶-D4
磺胺-6-(间)甲氧嘧啶	281.2/156.3c[a] 281.2/215.4c	70	25 26	10	磺胺二甲基嘧啶-D4

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）

化合物名称	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
磺胺邻二甲氧嘧啶	311.2/156.3a[a] 311.2/108.2a	70	30 37	10	磺胺二甲氧嘧啶-D4
磺胺甲基异恶唑	254.1/156.3[a] 254.1/108.2	65	22 36	10	磺胺二甲氧嘧啶-D4
磺胺二甲异恶唑	268.0/156.3[a] 268.0/113.2	70	22	10	磺胺二甲氧嘧啶-D4
苯酰磺胺	277.0/156.2[a] 277.0/92.0	68	19 41	7 8	磺胺二甲氧嘧啶-D4
磺胺二甲氧嘧啶	311.2/156.3b[a] 311.2/108.2b	70	30 40	10	磺胺二甲氧嘧啶-D4
磺胺喹噁啉	301.2/156.3[a] 301.2/108.2	55	24 37	13 9	磺胺二甲氧嘧啶-D4
磺胺嘧啶-D4	255.2/160.3	56	22	12	
磺胺二甲基嘧啶-D4	283.1/186.3	63	29	10	
磺胺二甲氧嘧啶-D4	315.3/156.3	65	29	10	

[a] 该离子对为定量离子对。

附 录 C
（资料性附录）
磺胺类药物标准品选择性离子流图

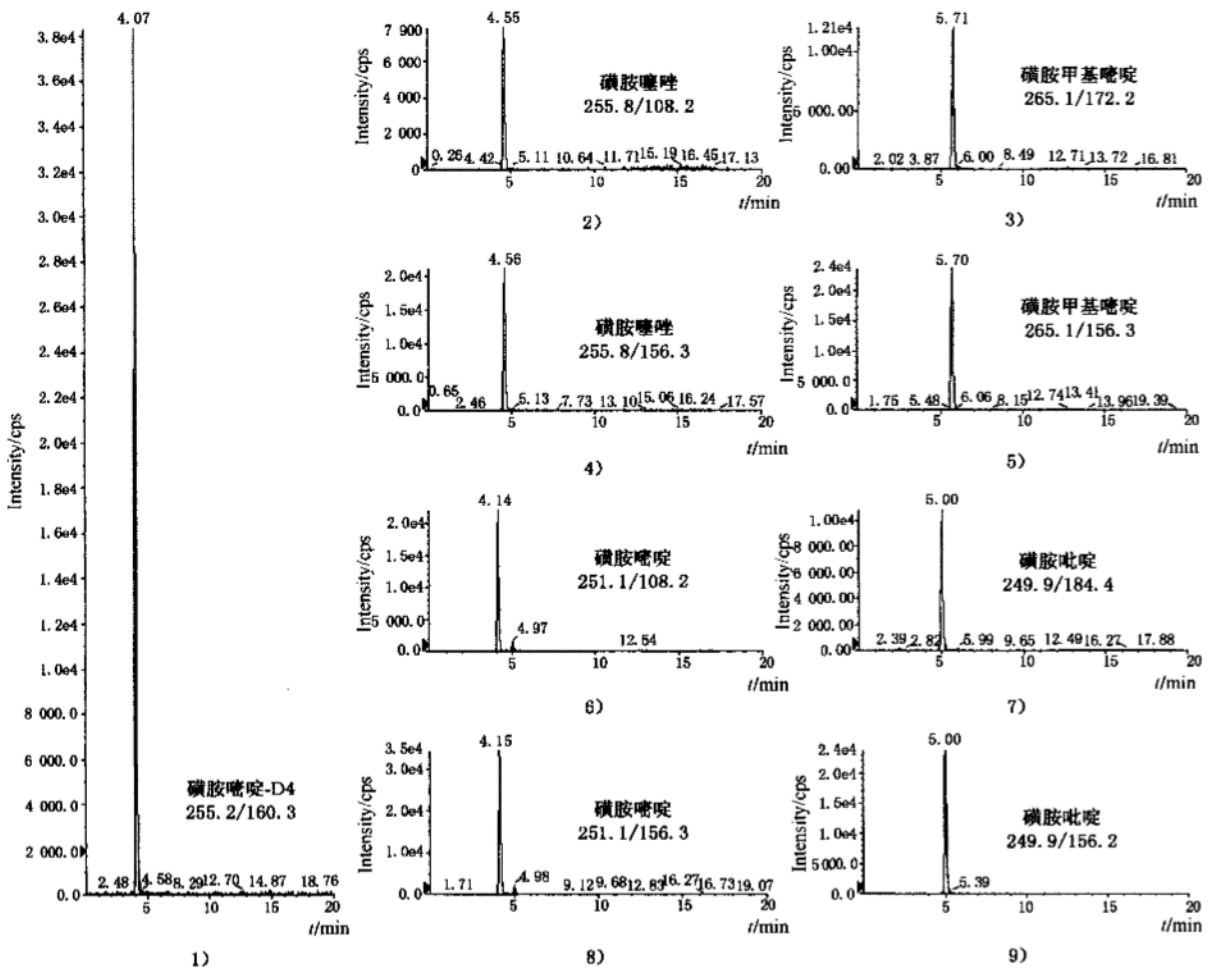

图 C.1 磺胺类药物(1.25 ng/mL)的标准品的选择性离子流图

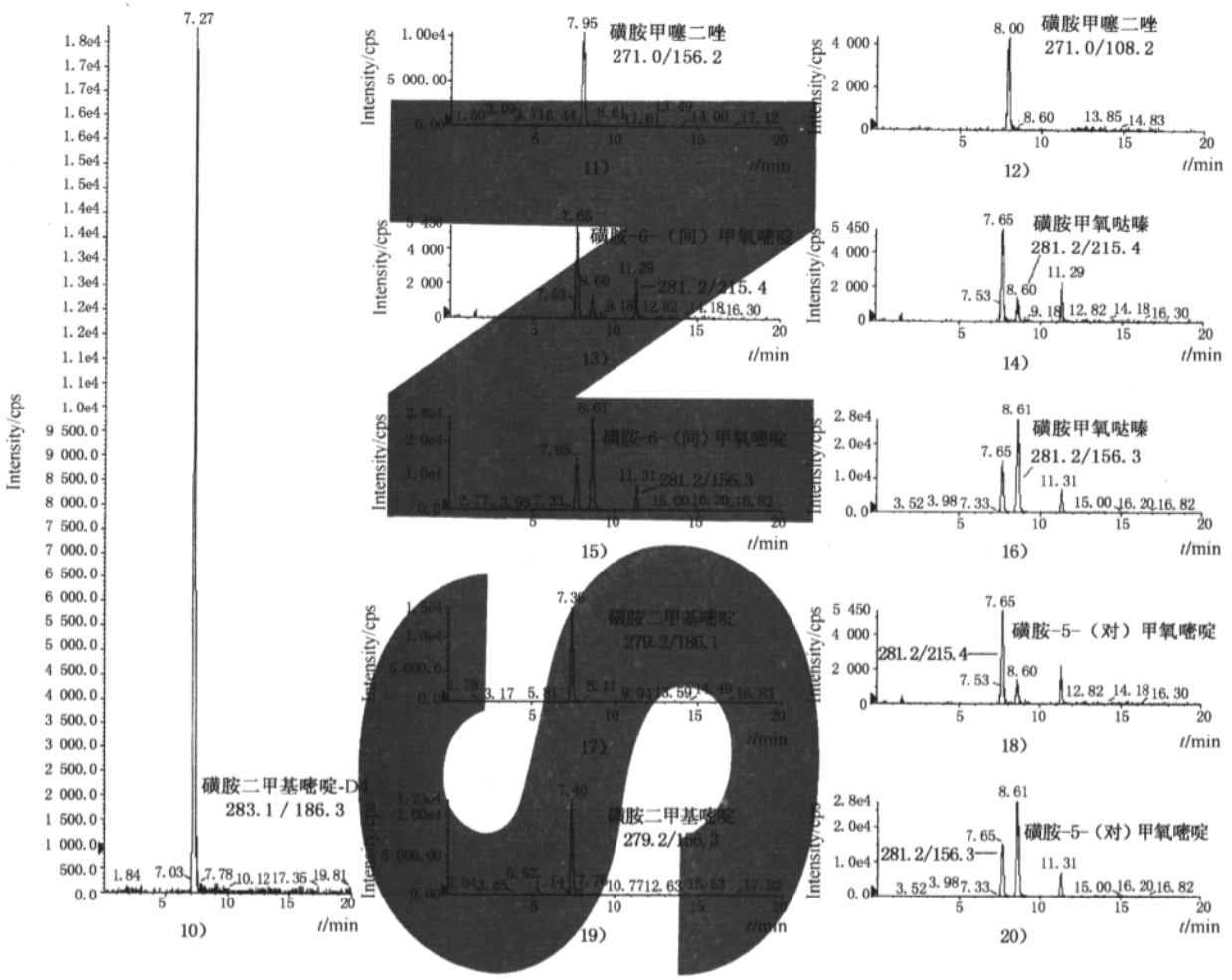
磺胺二甲基嘧啶-D4
283.1 / 186.3
磺胺甲噻二唑
271.0/156.2
磺胺甲噻二唑
271.0/108.2
磺胺-6-（间）甲氧嘧啶
281.2/215.4
磺胺甲氧哒嗪
281.2/215.4
磺胺-6-（间）甲氧嘧啶
281.2/156.3
磺胺甲氧哒嗪
281.2/156.3
磺胺二甲基嘧啶
279.2/186.1
磺胺-5-（对）甲氧嘧啶
281.2/215.4
磺胺二甲基嘧啶
279.2/186.3
磺胺-5-（对）甲氧嘧啶
281.2/156.3
Intensity/cps
t/min
10)
11)
12)
13)
14)
15)
16)
17)
18)
19)
20)

图 C.1（续）

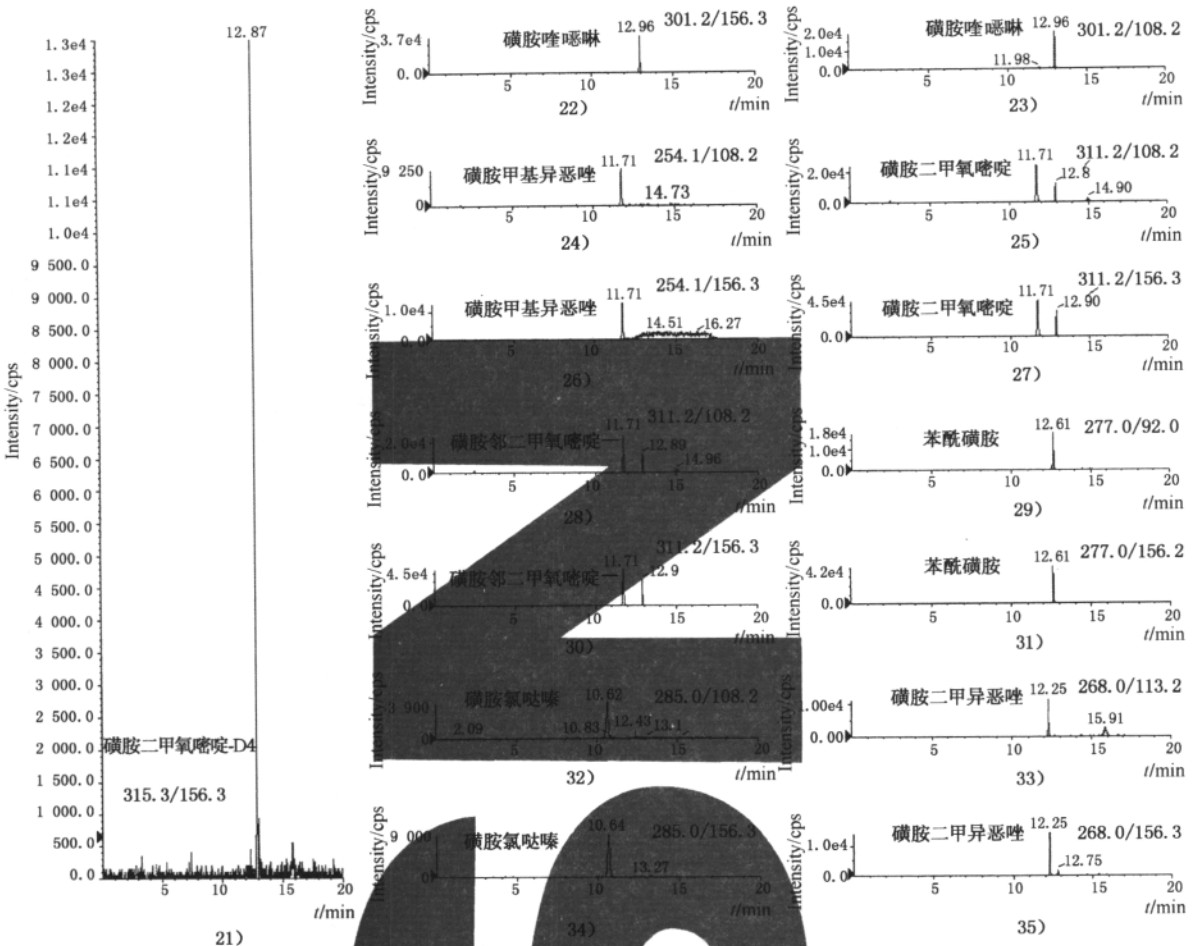
磺胺二甲氧嘧啶-D4
315.3/156.3
12.87
Intensity/cps
t/min
21)
磺胺喹噁啉
12.96
301.2/156.3
22)
磺胺喹噁啉
12.96
301.2/108.2
11.98
23)
磺胺甲基异恶唑
11.71
254.1/108.2
14.73
24)
磺胺二甲氧嘧啶
11.71
311.2/108.2
12.8
14.90
25)
磺胺甲基异恶唑
11.71
254.1/156.3
14.51
16.27
26)
磺胺二甲氧嘧啶
11.71
311.2/156.3
12.90
27)
磺胺邻二甲氧嘧啶一
11.71
311.2/108.2
12.89
14.96
28)
苯酰磺胺
12.61
277.0/92.0
29)
磺胺邻二甲氧嘧啶一
11.71
311.2/156.3
12.9
30)
苯酰磺胺
12.61
277.0/156.2
31)
磺胺氯哒嗪
10.62
285.0/108.2
2.09
10.83
12.43
32)
磺胺二甲异恶唑
12.25
268.0/113.2
15.91
33)
磺胺氯哒嗪
10.64
285.0/156.3
13.27
34)
磺胺二甲异恶唑
12.25
268.0/156.3
12.75
35)

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, Xi Junyang, Han Chao, Zhang Huimin.

Determination of sixteen sulfonamides residues in royal jelly for import and export—LC-MS/MS

1 Scope

This standard specifies the method of sample preparation and determination of sixteen sulfonamides residues in royal jelly by LC-MS/MS.

This standard is applicable to the determination of residues of sulfadiazine, sulfathiazloe, sulfapyridine, sulfamerazine, sulfamethazine, sulfameter, sulfamethizole, sulfamethoxypyridazine, sulfachloropyridazine, sulfamonomethoxine, sulfadoxine, sulfamethoxazole, sulfafurazole, sulfabenzamide, sulfadimethoxine, sulfaquinoxaline 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 extracted with ethyl acetate. After MCX and C_{18} solid phase extraction cartridge purification, the residues are determined by LC-MS/MS, and quantified by internal standard method.

4 Reagents and materials

Unless otherwise specified, all regents 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 Ethyl acetate: HPLC grade.

4.4 Formic acid: HPLC grade.

4.5 Sodium chloride.

4.6 Ammonia water: 25%～28%.

4.7 5% Ammonia methanol solution: Ammonia water-methanol(5+95, *V*/*V*).

4.8 Sulfadiazine, sulfathiazloe, sulfapyridine, sulfamerazine, sulfamethazine, sulfameter, sulfamethizole, sulfamethoxypyridazine, sulfachloropyridazine, sulfamonomethoxine, sulfadoxine, sulfamethoxazole, sulfafurazole, sulfabenzamide, sulfadimethoxine, sulfaquinoxaline, sulfadiazine-D4, sulfamethazine-D4, sulfadimethoxine-D4 standards: Purity ≥ 98%. Other information see annex A table A.1.

4.9 Standard stock solution: Accurately weigh an adequate amount of each standard (4.8) (accurate to 0.1 mg), dissolve in methanol and prepare a solution of 100 μg/mL as the standard stock solution respectively, stored at 1℃～4 ℃, They are stable for three months.

4.10 Isotope internal standard stock solution: Weigh an adequate amount of each isotope internal standard (4.8) (accurate to 0.1 mg), dissolve in methanol respectively, the concentration of solution is 100 μg/mL as the standard stock solution, stored at 1 ℃～4 ℃.

4.11 Blank solution of sample: According to section 7, blank solution is prepared with royal jelly without sulfonamides.

4.12 Calibration curve standard working solution: According to the requirement, dilute the standard stock solution to 1 ng/mL, 1.25 ng/mL, 2.5 ng/mL, 5 ng/mL, and 12.5 ng/mL, mix standard working solution with blank solution of sample (4.11) before using. It is same as 4 μg/kg, 5 μg/kg, 10 μg/kg, 20 μg/kg, and 50 μg/kg sulfonamides in sample. The concentrations of isotope internal standard solution are 1 ng/mL.

4.13 MCX solid-phase extraction (SPE) cartridge: 500 mg, 3 mL or equivalent. It should be conditioned with 5 mL methanol followed by 5 mL water before use.

4.14 C_{18} solid-phase extraction (SPE) cartridge: 500 mg, 3 mL or equivalent. It should be conditioned with 5 mL methanol before use.

4.15 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 1g and 0.01g.

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 Procedure

7.1 Extraction

Weigh 2 g test sample (accurate to 0.01g) into a 50 mL centrifuge tube. Add 0.1 mL isotope internal standard solution (40 ng/mL) and 10 mL water, mix it. Wait 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. Transfer 10.0 mL supernatant layer into a container, add 10 mL water, mix it. Add 2 g sodium chloride and 20 mL ethyl acetate, vortex for 1 min under 2 000 r/min, centrifuge for 5 min under 6 000 r/min. The supernatant layer was transferred into flask. Repeat the extraction in the same way with 20 mL ethyl acetate and combined the solution. Ethyl acetate is evaporated to nearly dryness in a water bath below 45 ℃.

7.2 Clean up

The residues are redissolved in 20 mL water. Transfer the solution into MCX cartridge (4.13),

discard the eluate. The cartridge is rinsed with 5 mL methanol-water (1 + 1, *V/V*). The cartridge is dried to "dryness". Elute the cartridge with 8 mL 5% ammonium methanol (4.7), flow rate is 1 mL/min～2 mL/min. The solution is evaporated to nearly dryness in a water bath below 50 ℃. The residues are redissolved with 10 mL methanol. Transfer the solution into C_{18} cartridge (4.14), flow rate is 1 mL/min～2 mL/min, the eluate is evaporated to nearly dryness in a water bath below 50 ℃. The residues is reconstituted in 4.0 mL methanol-0.15% formic acid (3 + 7, *V/V*), mix it. The solution is passed through a 0.45 μm filter. The filtrate is ready for LC-MS/MS determination.

7.3 Determination

7.3.1 LC-MS/MS operating condition

a) LC column: C_8 column, 150 mm × 4.6 mm (i.d), 5 μm, or 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
14.5	2	20	78
20	2	20	78

c) Flow rate: 0.8 mL/min;

d) Injection volume: 30 μL;

e) Ion source: electrospray ionization;

f) Polarity: positive modes;

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 sulfonamides in sample solution, select the standard working solution of similar concentration to that of sample solution. The responses of sulfonamides 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 sulfadiazine, sulfathiazloe, sulfapyridine, sulfamerazine, sulfamethazine, sulfameter, sulfamethizole, sulfamethoxypyridazine, sulfachloropyridazine, sulfamonomethoxine, sulfadoxine, sulfamethoxazole, sulfafurazole, sulfabenzamide, sulfadimethoxine, sulfaquinoxaline is 4.1 min, 4.6 min, 5.0 min, 5.7 min, 7.4 min, 7.6 min, 7.9 min, 8.6 min, 10.6 min, 11.3 min, 11.7 min, 11.7 min, 12.2 min, 12.6 min, 12.9 min, and 13.0 min. Selected ion chromatograms of the standards are shown in Figure C.1 of annex C.

7.3.3 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 that described in the method of determination, but with omission of sample addition.

8 Calculation and expression of result

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

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

Where

X_i —the residue content of sulfonamides residues in the test samples, μg/kg;

c_i —the concentration of sulfonamides residues 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 quantifications of sulfonamides are 5 μg/kg.

9.2 Recovery

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

Table 3—Recoveries of sulfonamides(n=6)

Compound	Spiked level/(μg/kg)	Recovery/%
sulfadiazine	5	85.8～95.0
	10	93.3～102.0
	20	89.5～95.0
sulfathiazloe	5	83.4～87.2
	10	81.3～90.2
	20	81.5～90.0
sulfapyridine	5	87.6～97.4
	10	94.6～104.0
	20	89.0～94.0
sulfamerazine	5	89.6～95.0
	10	96.9～105
	20	93.5～98.5
sulfamethazine	5	93.8～100.2
	10	91.9～96.1
	20	94.5～101.0

Table 3 (continued)

Compound	Spiked level/(μg/kg)	Recovery/%
sulfameter	5	90.8～100.2
	10	87.4～93.4
	20	92.0～97.5
sulfamethizole	5	81.6～86.4
	10	74.3～81.4
	20	84.5～91.0
sulfamethoxypyridazine	5	84.2～94.6
	10	78.7～81.3
	20	87.0～92.0
sulfachloropyridazine	5	96.8～106.0
	10	83.0～91.2
	20	84.0～90.0
sulfamonomethoxine	5	90.4～94.6
	10	86.9～93.1
	20	91.5～97.0
sulfadoxine	5	97.6～109.2
	10	89.2～94.2
	20	91.5～96.0
sulfamethoxazole	5	97.6～105.0
	10	94.8～98.3
	20	94.0～98.0
sulfafurazole	5	97.6～107.2
	10	83.4～88.6
	20	89.5～96.5
sulfabenzamide	5	88.4～96.0
	10	79.5～83.4
	20	77.0～83.5
sulfadimethoxine	5	95.8～108.2
	10	87.9～96.8
	20	92.0～96.0
sulfaquinoxaline	5	95.6～107.4
	10	87.8～91.8
	20	96.5～102.0

Annex A
(Normative annex)
Standard information

Table A. 1—Information of sulfonamides standards

Compound	CAS No	Molecular	Molecular weight
sulfadiazine	68-35-9	$C_{10}H_{10}N_4O_2S$	250. 27
sulfathiazloe	72-14-0	$C_9H_9N_3O_2S_2$	255. 31
sulfapyridine	144-83-2	$C_{11}H_{11}N_3O_2S$	249. 28
sulfamerazine	127-79-7	$C_{11}H_{12}N_4O_2S$	264. 30
sulfamethazine	57-68-1	$C_{12}H_{14}N_4O_2S$	278. 32
sulfameter	651-06-9	$C_{11}H_{12}N_4O_3S$	280. 30
sulfamethizole	144-82-1	$C_9H_{10}N_4O_2S_2$	270. 32
sulfamethoxypyridazine	80-35-3	$C_{11}H_{12}N_4O_3S$	280. 30
sulfachloropyridazine	80-32-0	$C_{10}H_9ClN_4O_2S$	284. 72
sulfamonomethoxine	1220-83-3	$C_{11}H_{12}N_4O_3S$	280. 30
sulfadoxine	2447-57-6	$C_{12}H_{14}N_4O_4S$	310. 32
sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253. 27
sulfafurazole	127-69-5	$C_{11}H_{13}N_3O_3S$	267. 30
sulfabenzamide	127-71-9	$C_{13}H_{12}N_2O_3S$	276. 31
sulfadimethoxine	122-11-2	$C_{12}H_{14}N_4O_4S$	310. 32
sulfaquinoxaline	59-40-5	$C_{14}H_{12}N_4O_2S$	300. 33
sulfadiazine-D4	—	$C_{10}H_6D_4N_4O_2S$	254. 30
sulfamethazine-D4	—	$C_{12}H_{10}D_4N_4O_2S$	282. 36
sulfadimethoxine-D4	—	$C_{12}H_{10}D_4N_4O_4S$	314. 35

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

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, CXP

Compound	Transitions m/z	DP/ V	CE/ V	CXP/ V	Internal compound
sulfadiazine	251.1/156.3[a] 251.1/108.2	65	22 33	10	sulfadiazine-D4
sulfathiazloe	255.8/156.3[a] 255.8/108.2	70	22 30	10	sulfadiazine-D4
sulfapyridine	249.9/156.2[a] 249.9/184.4	70	24	10	sulfadiazine-D4
sulfamerazine	265.1/156.3[a] 265.1/172.2	65	25 23	10	sulfadiazine-D4
sulfamethazine	279.2/156.3[a] 279.2/186.1	70	26	10	sulfamethazine-D4

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 (contiuned)

Compound	Transitions m/z	DP/ V	CE/ V	CXP/ V	Internal compound
sulfameter	281. 2/156. 3a[a] 281. 2/215. 4a[a]	70	25 26	10	sulfamethazine-D4
sulfamethizole	271. 0/156. 2[a] 271. 0/108. 2	70	24 32	10	sulfamethazine-D4
sulfamethoxypyridazine	281. 2/156. 3b[a] 281. 2/215. 4b	70	25 26	10	ulfamethazine-D4
sulfachloropyridazine	285. 0/156. 3[a] 285. 0/108. 2	70	22 35	10	sulfadimethoxine-D4
sulfamonomethoxine	281. 2/156. 3c[a] 281. 2/215. 4c	70	25 26	10	sulfamethazine-D4
sulfadoxine	311. 2/156. 3a[a] 311. 2/108. 2a	70	30 37	10	sulfadimethoxine-D4
sulfamethoxazole	254. 1/156. 3[a] 254. 1/108. 2	65	22 36	10	sulfadimethoxine-D4
sulfafurazole	268. 0/156. 3[a] 268. 0/113. 2	70	22	10	sulfadimethoxine-D4
sulfabenzamide	277. 0/156. 2[a] 277. 0/92. 0	68	19 41	7 8	sulfadimethoxine-D4
sulfadimethoxine	311. 2/156. 3b[a] 311. 2/108. 2b	70	30 40	10	sulfadimethoxine-D4
sulfaquinoxaline	301. 2/156. 3[a] 301. 2/108. 2	55	24 37	13 9	sulfadimethoxine-D4
sulfadiazine-D4	255. 2/160. 3	56	22	12	
sulfamethazine-D4	283. 1/186. 3	63	29	10	
sulfadimethoxine-D4	315. 3/156. 3	65	29	10	
[a] Product ion is used for quantification.					

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

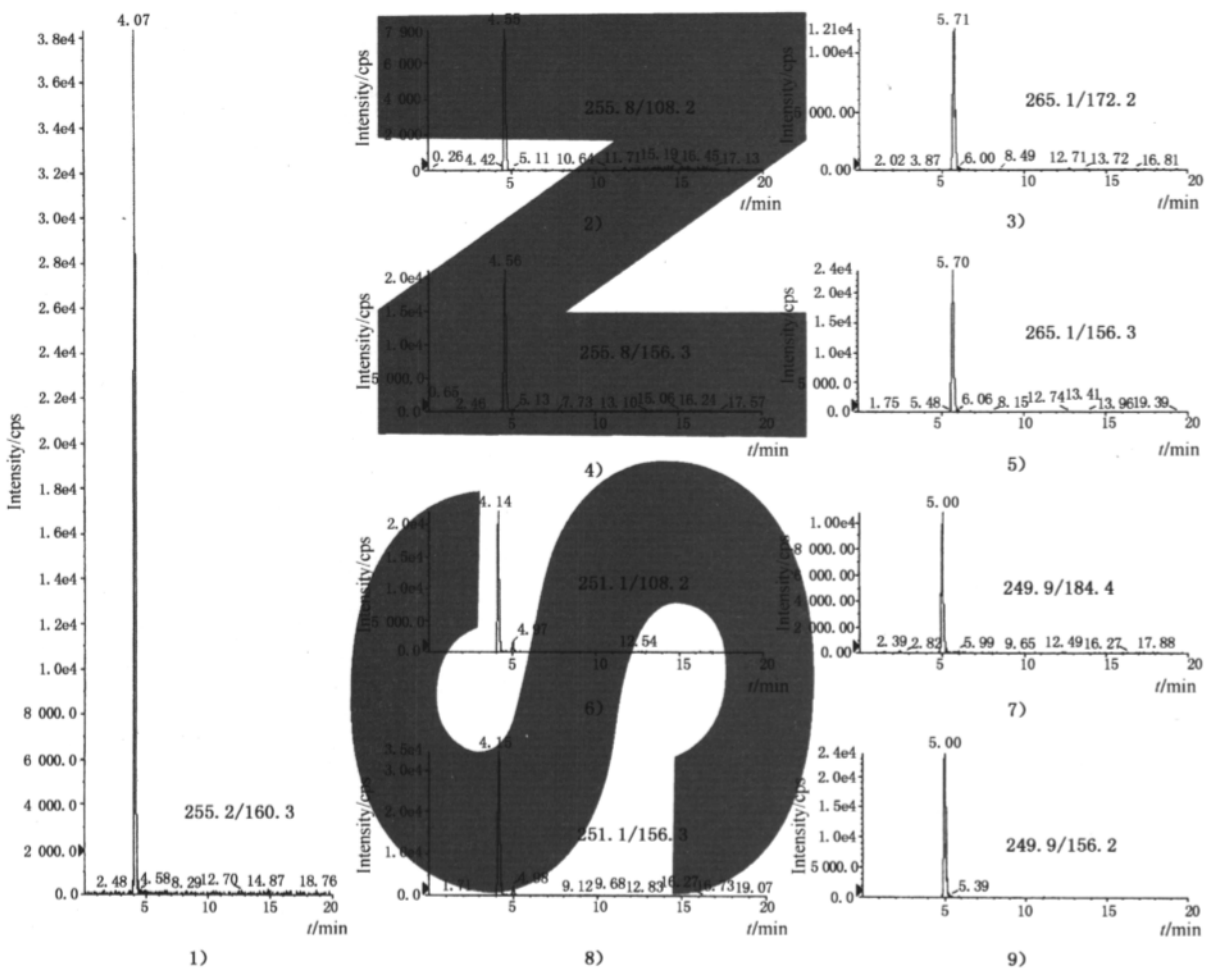

Figure C. 1—Selected ion chromatograms of sulfonamides standards (1. 25 ng/mL)

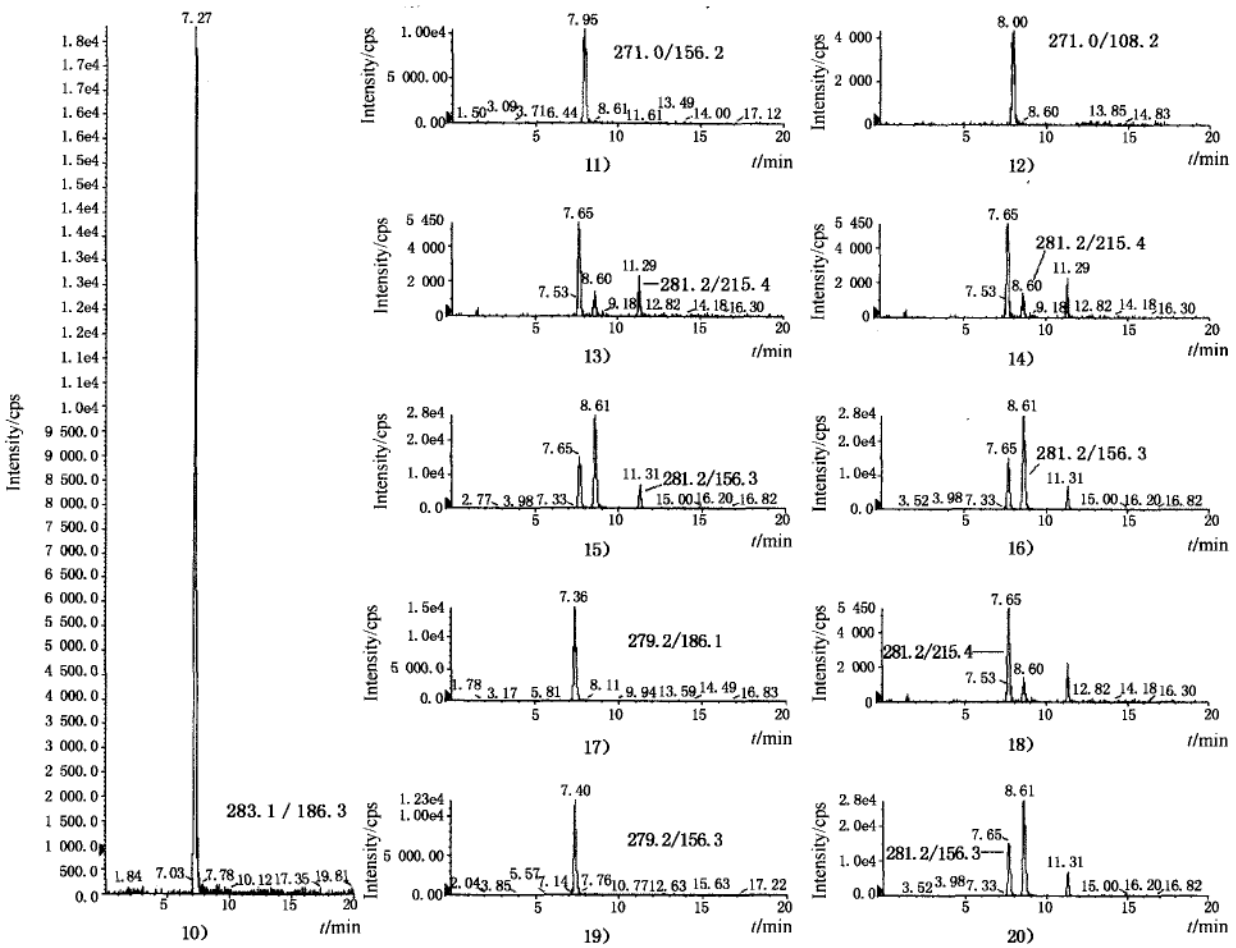

Figure C. 1（continued）

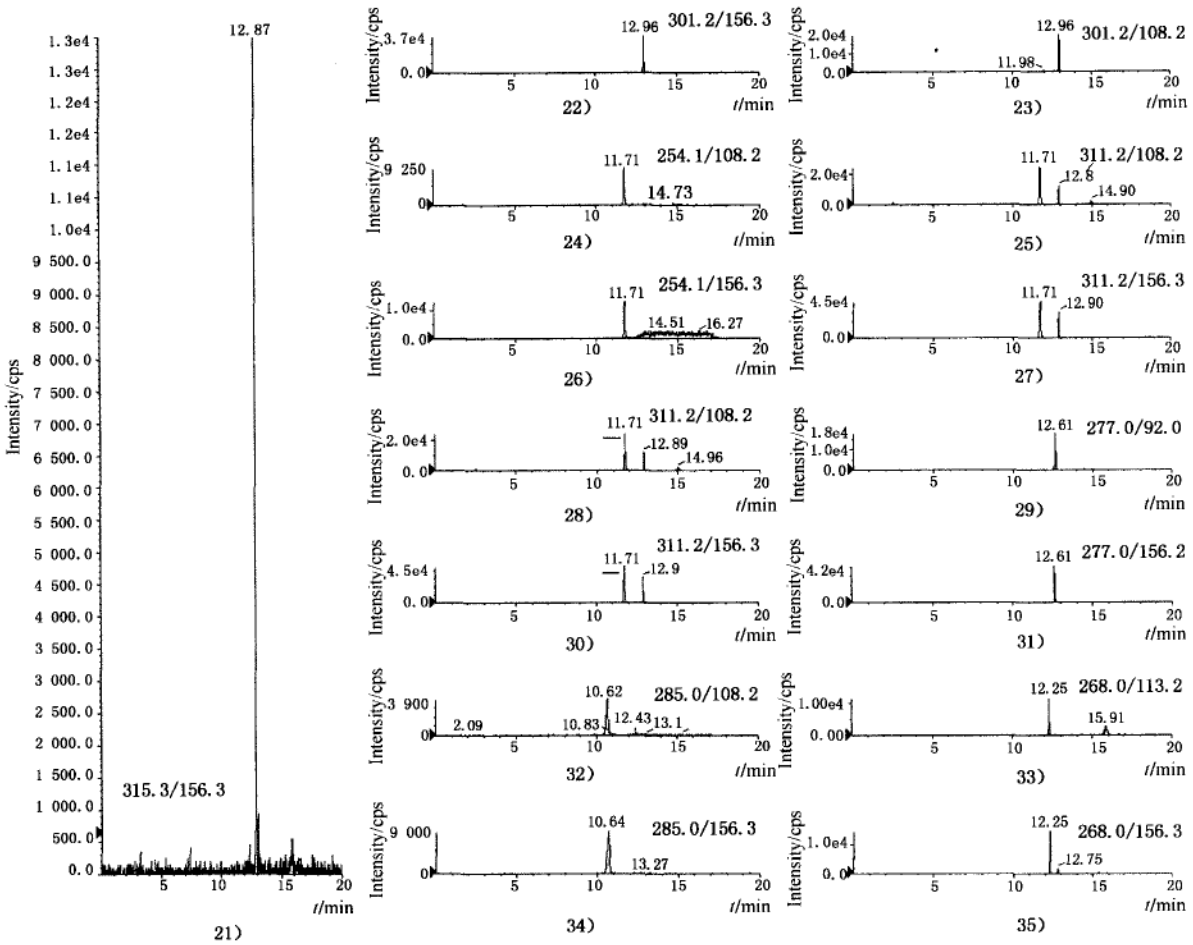

Figure C.1 (continued)