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中华人民共和国出入境检验检疫行业标准

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进出口动物源食品中克伦特罗、莱克多巴胺、沙丁胺醇、特布他林残留量的检测方法　液相色谱-质谱/质谱法

Determination of clenbuterol, ractopamine, salbutamol and terbutalin residues in foodstuffs of animal origin for import and export—HPLC-MS/MS method

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前　言

本标准的附录A和附录B为资料性附录。

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：中华人民共和国上海出入境检验检疫局、中华人民共和国河南出入境检验检疫局、中华人民共和国湖南出入境检验检疫局、中华人民共和国江苏出入境检验检疫局。

本标准主要起草人：邓晓军、朱坚、郭德华、李波、杨冀州、戴华、陈惠兰、郭俊峰、王美玲、陈墨莲。

本标准系首次发布的出入境检验检疫行业标准。

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进出口动物源食品中克伦特罗、莱克多巴胺、沙丁胺醇、特布他林残留量的检测方法　液相色谱-质谱/质谱法

1　范围

本标准规定了动物源性食品中克伦特罗、莱克多巴胺、沙丁胺醇、特布他林残留量的制样和测定方法。

本标准适用于动物源性食品肌肉和内脏中克伦特罗、莱克多巴胺、沙丁胺醇、特布他林残留量的检测。

2　试样制备与保存

2.1　试样制备

从所取全部样品中取出有代表性样品约500 g，充分绞碎，混匀，均分成两份，分别装入洁净容器内。密封作为试样，标明标记。在制样的操作过程中，应防止样品受到污染或发生残留物含量的变化。

2.2　试样保存

将试样于－18℃保存。

3　方法提要

试样中的药物残留采用pH5.2的乙酸铵缓冲溶液提取，同时加入β-盐酸葡萄糖醛甙酶-芳基硫酯酶进行酶解后，提取液经C_{18}和SCX双SPE柱净化，液相色谱-质谱进行测定，内标法定量。

4　试剂和材料

除非另有规定，均使用分析纯试剂，水为超纯水。

4.1　甲醇：液相色谱级。

4.2　乙腈：液相色谱级。

4.3　乙酸铵。

4.4　乙酸乙酯。

4.5　氢氧化铵。

4.6　盐酸。

4.7　甲酸。

4.8　冰乙酸。

4.9　β-盐酸葡萄糖醛甙酶-芳基硫酸酯酶：含β-盐酸葡萄糖醛甙酶134 600 U/mL，芳基硫酸酯酶5 200 U/mL。

4.10　乙酸铵缓冲溶液：0.02 mol/L，pH＝5.2。溶解1.54 g乙酸铵于900 mL水中，用冰乙酸调节pH到5.2±0.1，最后用水稀释到1 L，于＋4℃贮存，使用期为1个月。

4.11　乙酸乙酯-氢氧化铵(97＋3，体积比)。使用前搅拌，同时用超声波水浴超声使成均相。

4.12　盐酸溶液：0.03 mol/L。取30 mL，1 mol/L的盐酸溶液用水稀释到1 L。

4.13　标准品：特布他林(分子式：$C_{12}H_{19}NO_3$，CAS号：23031-25-6)、克伦特罗(分子式：$C_{12}H_{18}Cl_2N_2O$ CAS号：37148-27-9)、D_9-克伦特罗、沙丁胺醇(分子式：$C_{13}H_{21}NO_3$ CAS号：18559-94-9)、D_3-沙丁胺醇、

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莱克多巴胺(分子式 $C_{18}H_{23}NO_3$ CAS 号:97825-25-7)、D_5-莱克多巴胺纯度≥98%。

4.14 标准储备液的配制(1.0 mg/mL):分别称取约 50 mg(±0.1 mg)的特布他林、莱克多巴胺、克伦特罗、沙丁胺醇标准品于 50 mL 容量瓶中,用少量甲醇溶解,然后用甲醇配成 1.0 mg/mL 标准储备溶液。低于 5℃保存,有效期为 1 年。

4.15 同位素内标标准储备液的配制(1.0 mg/mL):分别称取约 50 mg(±0.1 mg)的 D_9-克伦特罗、D_3-沙丁胺醇、D_5-莱克多巴胺标准品于 50 mL 容量瓶中,用少量甲醇溶解,然后用甲醇配成 1.0 mg/mL 标准储备溶液。低于 5℃保存,有效期为 1 年。

4.16 混合标准中间溶液的配置:用甲醇分别稀释标准储备液至终浓度约为 1.0 μg/mL 和 100.0 ng/mL,低于 5℃保存,有效期为 6 个月。

4.17 混合同位素内标标准中间溶液的配置:用甲醇分别稀释同位素内标标准储备液至终浓度约为 100.0 ng/mL,低于 5℃保存,有效期为 6 个月。

4.18 标准工作液的配置:根据需要,临用时吸取一定量的混合标准中间溶液(4.16)和混合同位素内标中间标准溶液(4.17),用水稀释配制成适当浓度的混合标准工作溶液(参考线性浓度范围为 0.5 ng/mL~10 ng/mL),每毫升该混合标准工作溶液含有同位素内标各 1.0 ng。低于 5℃保存,有效期为 6 个月。

4.19 C_{18} SPE 柱:LC-18 Sep Pak 500 mg,3 mL 或相当者。

4.20 SCX SPE 柱:LC-SCX Sep Pak 500 mg,3 mL 或相当者。

4.21 水相滤膜:0.45 μm。

5 仪器和设备

5.1 液相色谱-串联质谱仪:配有电喷雾(ESI)离子源。

5.2 涡旋混合器。

5.3 离心机:转速≥4 000 r/min。

5.4 旋转蒸发器。

5.5 感量天平(0.1 mg 和 0.01 g)。

5.6 超声波发生器。

5.7 恒温箱。

5.8 真空过柱装置。

5.9 吹氮浓缩装置。

5.10 粉碎机。

6 测定步骤

6.1 提取

称取 10 g(精确至 0.1 g)试样,于 50 mL 塑料离心管中,加入 20 mL pH 为 5.2 的乙酸铵缓冲溶液(4.10),在 2 000 r/min 下涡旋混合 2 min。添加 200.0 μL 同位素内标物(4.17)于待测样品中。加 50 μL 的β-盐酸葡萄糖醛甙酶-芳基硫酯酶,加盖后超声提取 15 min。在恒温箱中于 37℃酶解 16 h。以 3 800 r/min 离心 10 min 后,将上清液过 0.45 μm 滤膜(4.21)取 5 mL 滤液于 10 mL 带刻度的玻璃试管。

6.2 净化

将 C_{18} 小柱(4.19)和 SCX(4.20)小柱按从上到下的次序装好。依次用 5 mL 水、5 mL 甲醇和 5 mL 0.03 mol/L 盐酸(4.12)溶液以 1.0 mL/min 的流速过柱活化。转移过滤后的提取液至固相层析柱的顶部,以 0.5 mL/min 流速过固相层析柱,此步操作至少需要 10 min。用 5 mL 水、5 mL 甲醇淋洗小柱,在较强的负压下抽 10 min。取下 C_{18} 小柱。用 12 mL 混合均匀的乙酸乙酯-氢氧化铵溶液(4.11),洗脱 SCX 柱子上的待分析成分并收集洗脱液,并在吹氮浓缩装置上于 40℃±5℃的温度下蒸干。加入

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1.0 mL 乙腈＋水(1＋9)振荡溶解残渣后过 0.45 μm 滤膜。滤液供液相色谱-质谱测定。

6.3 测定

6.3.1 高效液相色谱条件

a) 色谱柱：C_{18}柱，150 mm(柱长)×2.1 mm(内径)，粒度 5 μm；

b) 流动相：A：乙腈＋0.1%甲酸，B：2 mmol/L 乙酸铵水溶液＋0.1%甲酸；流速：200 μL/min，梯度洗脱程序见表 1。

表 1 梯度洗脱程序表

梯度时间/min	流动相比例/(%)	
	流动相 A	流动相 B
0	10	90
5	50	50
7	50	50
8	10	90
12	10	90

c) 柱温：35℃；

d) 进样量：30 μL。

6.3.2 质谱条件

a) 离子源：电喷雾 ESI，正离子；

b) 扫描方式：多反应监测 MRM；

c) 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，详细条件参考附录 A；

d) 电喷雾电压(IS)、碰撞电压(CE)、去簇电压(DP)、碰撞室入口电压(EP)、碰撞室出口电压(CXP)应优化至最佳灵敏度，监测离子对和定量离子等详细条件参考附录 A。

6.3.3 定量测定

根据试样中被测物的药物含量，选取响应值相近的标准工作液(4.18)同时进行分析。标准工作液和待测液中四种药物的响应值均应在仪器线性响应范围内。如果含量超过标准曲线范围，应用乙腈＋水(1＋9)稀释到合适浓度后分析。在上述色谱条件下的待测药物的参考保留时间分别为 3.0 min(沙丁胺醇)、3.1 min(特布它林)、9.4 min(莱克多巴胺)和 9.9 min(克伦特罗)，标准溶液的选择性离子流图参见附录 B。

6.3.4 定性测定

按照液相色谱-串联质谱条件测定样品和标准工作溶液，如果检测的质量色谱峰保留时间与标准品一致，定性离子对的相对丰度，是用相对于最强离子丰度的强度百分比表示，应当与浓度相当标准工作溶液的相对丰度一致，相对丰度允许偏差不超过表 2 规定的范围，则可判断样品中存在对应的被测物。

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 至 50	＞10 至 20	≤10
允许的相对偏差/(%)	±20	±25	±30	±50

6.4 空白试验

除不加试样外，均按上述操作步骤进行。

7 结果计算和表述

用色谱数据处理机或按式(1)计算样品中待测药物残留量。计算结果需扣除空白值。

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

式中：

X——样品中待测组分残留量，单位为毫克每千克(mg/kg)；

c——标准工作溶液中药物的浓度，单位为纳克每毫升(ng/mL)；

c_i——样液中内标物的浓度，单位为纳克每毫升(ng/mL)；

A——样液中药物的峰面积；

A_{si}——标准工作溶液中内标物的峰面积；

V——样品溶液最终定容体积，单位为毫升(mL)；

c_{si}——标准工作溶液中内标物的浓度，单位为纳克每毫升(ng/mL)；

A_i——样液中内标物的峰面积；

A_s——标准工作溶液中药物的峰面积；

m——最终样液代表的试样质量，单位为克(g)。

注：待测药物采用相对应的同位素作为内标，特布他林采用同位素标记的沙丁胺醇作为内标。

8 测定低限、回收率

8.1 测定低限(LOQ)

本方法肉和肝的测定低限为0.000 5 mg/kg。

8.2 回收率

8.2.1 肉中克伦特罗、莱克多巴胺、沙丁胺醇、特布他林添加浓度及回收率试验数据见表3。

表3 肉中克伦特罗、莱克多巴胺、沙丁胺醇、特布他林回收率数据表

药物名称	添加浓度/(μg/kg)	回收率范围/(%)	药物名称	添加浓度/(μg/kg)	回收率范围/(%)
克伦特罗	0.5	73.4～96.4	沙丁胺醇	0.5	80.8～91.6
	1.0	98.5～126.5		1.0	104.7～110.9
	2.0	86.5～102.0		2.0	73.5～78.5
莱克多巴胺	0.5	90.6～98.8	特布他林	0.5	80.6～108.0
	1.0	91.7～108.9		1.0	90.0～97.3
	2.0	90.6～98.8		2.0	80.6～108.0

8.2.2 肝中克伦特罗、莱克多巴胺、沙丁胺醇、特布他林添加水平及回收率试验数据见表4。

表4 肝中克伦特罗、莱克多巴胺、沙丁胺醇、特布他林回收率数据表

药物名称	添加浓度/(μg/kg)	回收率范围/(%)	药物名称	添加浓度/(μg/kg)	回收率范围/(%)
克伦特罗	0.5	94.6～100.8	沙丁胺醇	0.5	77.2～89.8
	1.0	78.3～98.4		1.0	77.3～92.0
	2.0	90.5～99.0		2.0	73.5～80.0
莱克多巴胺	0.5	96.4～100.4	特布他林	0.5	77.2～90.6
	1.0	95.5～122.0		1.0	80.5～94.9
	2.0	92.0～108.5		2.0	73.0～79.5

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附　录　A[1)]
（资料性附录）
质谱参数

a）　电喷雾电压(IS)：5 500 V；
b）　雾化气压力(GS1)：50 Pa；
c）　气帘气压力(CUR)：20 Pa；
d）　辅助气流速(GS2)：30 Pa；
e）　离子源温度(TEM)：550℃；
f）　碰撞电压(CE)、去簇电压(DP)见表 A.1 所示。碰撞室入口电压(EP)为 10 V、碰撞室出口电压(CXP)为 13 V。

表 A.1　β-兴奋剂及内标碰撞电压、去簇电压参数表

	母离子(Q1)m/z	子离子(Q3)m/z	碰撞电压/V	去簇电压/V
克伦特罗	277.3	203.0[a]	18	45
		259.2	20	50
莱克多巴胺	302.3	164.2[a]	12	50
		284.3	22	50
沙丁胺醇	240.2	148.1[a]	25	55
		222.1	17	75
特布它林	226.2	152.1[a]	23	48
		107.1	43	45
D_9-克伦特罗	286.3	204.0	16	75
D_5-莱克多巴胺	307.2	167.2	15	45
D_3-沙丁胺醇	243.2	151.1	22	55
[a] 为定量离子对。				

1）　附录所列参数是在 API4000 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。

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附 录 B
（资料性附录）
标准品 MRM 图谱

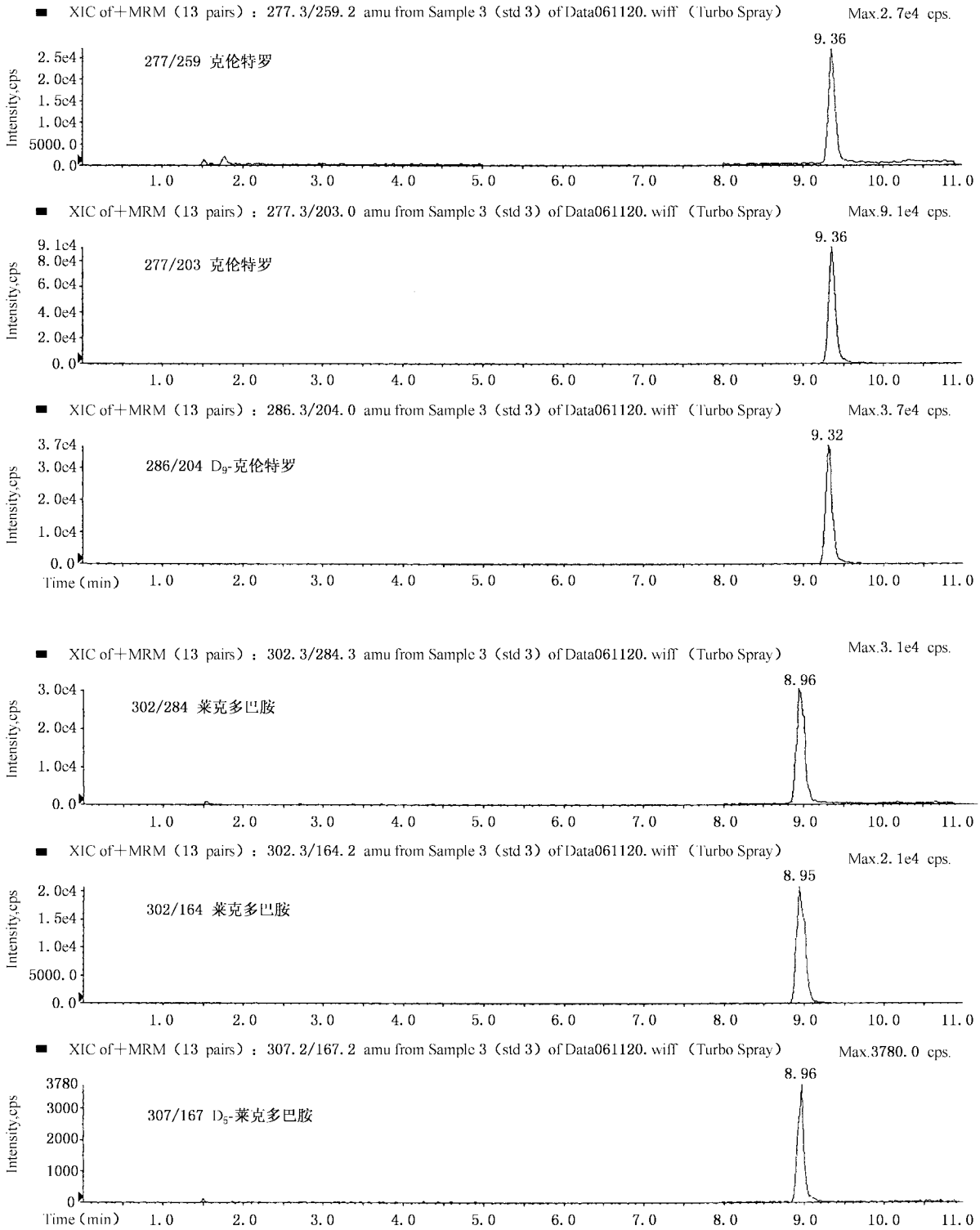


图 B.1 克仑特罗、莱克多巴胺标准品的多反应监测(MRM)图谱

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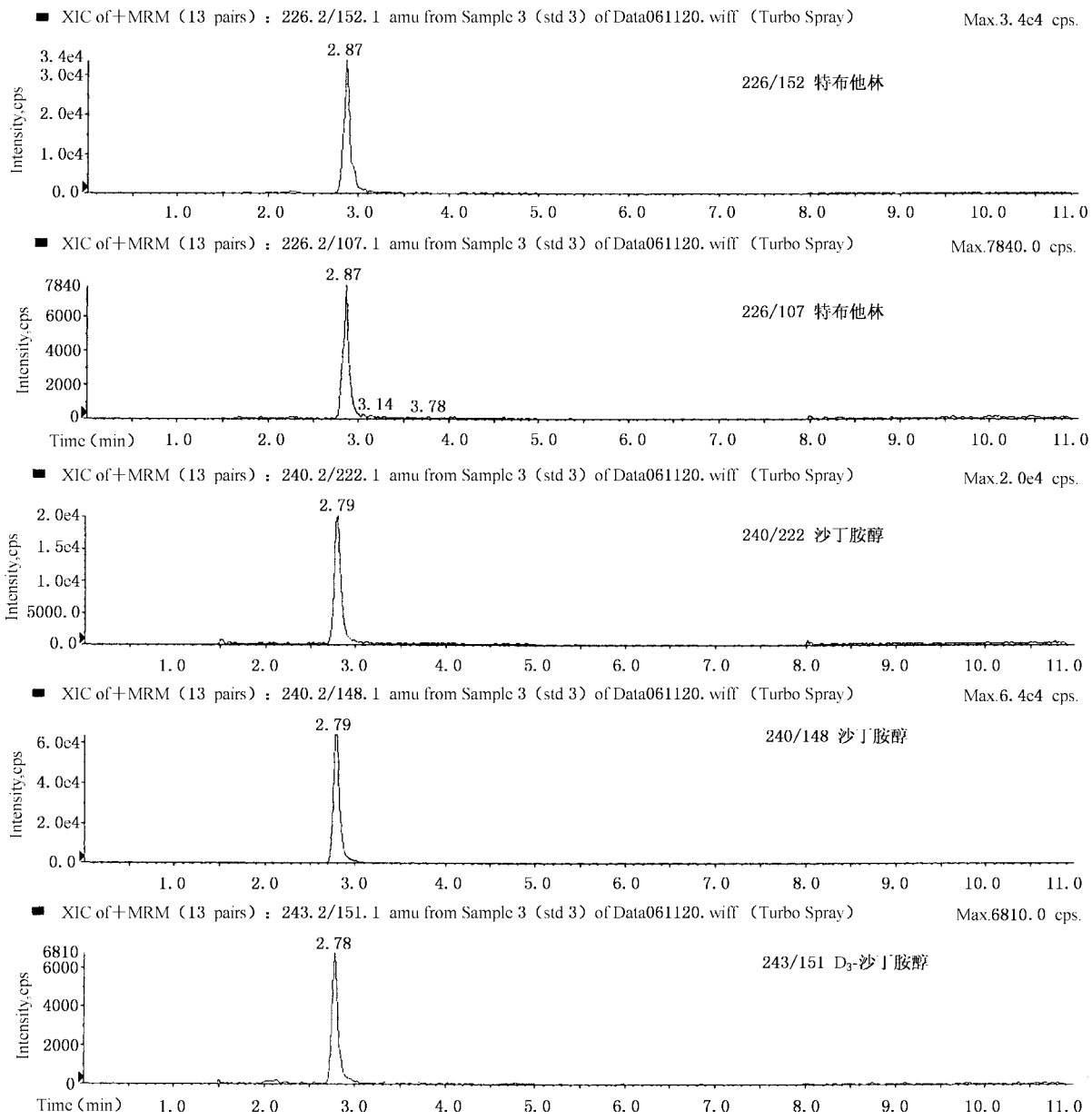


图 B.2　特布他林、沙丁胺醇标准品的多反应监测(MRM)图谱

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Foreword

Annex A and B of this standard are informative annexes.

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 Shanghai Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Henan Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Hunan Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Jiangsu Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The standard was mainly drafted by Deng Xiaojun, Zhu Jian, Guo Dehua, Li Bo, Yang Jizhou, Dai Hua, Chen Huilan, Guo Junfeng, Wang Meilin, Chen Molian.

This standard is a professional standard for entry-exit inspection and quarantine promulgated for the first time.

Note: this English version, a translation from the Chinese text, is solely for guidance.

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Determination of clenbuterol, ractopamine, salbutamol and terbutalin residues in foodstuffs of animal origin for import and export— HPLC-MS/MS method

1 Scope

The standard specifies the methods of sample prepafation and determination of clenbuterol, ractopamine, salbutamol and terbutalin residues in foodstuffs of animal origin by HPLC-MS/MS.

This standard is applicable to the determination of clenbuterol, ractopamine, salbutamol and terbutalin residues in meat and viscera of animal oringin by HPLC-MS/MS.

2 Preparation and storage of test sample

2.1 Preparation of sample

The combined primary sample is reduced to 500 g which is totally minced and divided into two equal portions, each portion is placed into a clean vessel as a test sample, which is then sealed. In the course of sampling and sample preparation, precaution should be taken to avoid contamination or any factor that may causes the change of residue content.

2.2 storage of sample

The test sample should be stored at −18℃.

3 Principle

Clenbuterol, ractopamine, salbutamol and terbutalin residues are extracted from the test sample with pH5.2 ammonium acetate buffers followed by hydrolysis with glucosidase. The extract is cleaned up by SCX and C_{18} solid phase column. Four residues are determined by HPLC-MS/MS, and quantified by isotope internal standard method.

4 Reagents and materials

Unless otherwise specified, all the reagent used should be analytical grade, water is deionized water.

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4.1 Methanol.

4.2 Acetonitrile:HPLC grade.

4.3 Ammonium acetate.

4.4 Acetic ether.

4.5 Ammonium hydroxide.

4.6 Hydrochloric acid.

4.7 Formic acid.

4.8 Acetic acid.

4.9 β-glucuronidase:100 U/mL.

4.10 Ammonium acetate buffer:0.02 mol/L,pH=5.2. Weigh 1.54 g Ammonium Acetate in 900 mL water,adjust pH to 5.2±0.1 with acetic acid and add water to final volume of 1 L.

4.11 Acetic ether-ammonium hydroxide solution (97+3, V/V). Mix to homogeneous phase before use.

4.12 0.03 mol/L HCl. Dilute 30 mL 1 mol/L HCl to final volume of 1 L with water.

4.13 Clenbuterol ($C_{12}H_{18}Cl_2N_2O$ CAS:37148-27-9),ractopamine ($C_{18}H_{23}NO_3$ CAS:97825-25-7),salbutamol ($C_{13}H_{21}NO_3$ CAS:18559-94-9) and terbutalin ($C_{12}H_{19}NO_3$,CAS:23031-25-6),D_9-Clenbuterol,D_5-ractopamine,D_3-Salbutamol standards: purity>98%.

4.14 Stock standard solution of clenbuterol,ractopamine,salbutamol,terbutalin (1.0 mg/mL): accurately weigh 50 mg (±0.1 mg) standard in volumic flask,add to the bottles a known amount of water to produce a solution containing compounds all at 1.0 mg/mL and store refrigerated at <5℃, assign a shelflife of 12 months.

4.15 Stock internal standards solution of D_9-clenbuterol,D_5-ractopamine,D_3-salbutamol (1.0 mg/mL):accurately weigh 50 mg (±0.1 mg) standard in volumic flask,add to the bottles a known amount of water to produce a solution containing compounds all at 1.0 mg/mL and store refrigerated at <5℃,assign a shelflife of 12 months.

4.16 Mixture intermediate standard solution: dilute stock standard solution of clenbuterol,ractopamine,salbutamol,terbutalin(1.0 mg/mL) to 1.0 μg/mL and 100.0 ng/mL in methanol,store refrigerated at <5℃,assign a shelflife of 6 months.

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4.17 Mixture intermediate internal standard solution: dilute stock standard solution of D_9-clenbuterol, D_5-ractopamine, D_3-salbutamol (1.0 mg/mL) to 100.0 ng/mL in methanol, store refrigerated at <5℃, assign a shelflife of 6 months.

4.18 Working standard solutions: To require as useful, dilute the mixture intermediate standard solutions(4.16) and mixture intermediate internal standard solutions(4.17) with water freshly (The reference calibration region is 0.5 ng/mL~10 ng/mL). And the concentration of internal standards in per working standard solution is 1.0 ng/mL and store refrigerated at <5℃, assign a shelflife of 6 months.

4.19 SCX columns: LC-SCX Sep Pak, 500 mg, 3 mL or equivalent.

4.20 C_{18} columns: LC-18, 500 mg, 3 mL or equivalent.

4.21 0.45 μm hydrophilic film.

5 Apparatus and equipment

5.1 Liquid chromatograhy combined with electrospray ionization mass spectrometry.

5.2 Vortex mixer.

5.3 Centrifuger: 4 000 r/min, and equipped with 250 mL polyethylene or polypropylene bottles.

5.4 Rotary vacuum evaporator.

5.5 Balances (0.1 mg, 0.01 g).

5.6 Ultrasonic apparatus.

5.7 Incubator.

5.8 Apparatus of SPE.

5.9 Nitrogen evaporator.

5.10 Pulverizer.

6 Procedure

6.1 Extraction

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Weigh 10 (0.1 g) sample in 50 mL plastic centrifuge tubes, add 20 mL pH5.2 ammonium acetate buffer(4.10) and 20.0 ng internal standard of D_6-clenbuterol, D_5-ractopamine, D_3-Salbutamol 50 μL and β-glucuronidase respectively to sample matrix and vibratefor 15 min followed by hydrolysis for 16h at 37℃. The tubes is centrifuged at 3 800 r/min for 10 min. Transfer supernatant through 0.45 μm film(4.21) to get 5 mL sample solution for SPE cleanup.

6.2 Cleaning up

Load 5 mL filtered solution to C_{18}(4.19) and SCX(4.20) SPE cartridge(C_{18} and SCX columns were placed up-bottom and conditioned with 5 mL methanol, 5 mL water and 5 mL 0.03 mol/L HCl(4.12) at the flow rate of 1.0 mL/min). Then, the column was rinsed with 5 mL water and 5 mL methanol and remove the C_{18} SPE column. After purge the cartridges at vacuum, elute with 12 mL acetic ether/ammonium hydroxide. (4.11), The eluates was concentrated to nearly dryness by gentle nitrogen and added 1 mL acetonitrile/water(1 + 9) to resolve the residence and filter by 0.45 μm film before HPLC-MS analysis.

6.3 Determination

6.3.1 HPLC operating conditions

a) Column: C_{18}, 5 μm, 150 mm × 4.6 mm(i.d), or the equivalent.

b) Mobile phase: A: Acetonitrile + 0.1% formic acid, B: 2 mmol/L Ammonium acetate solution + 0.1% formic acid. Flow rate: 200 μL/min. Gradient program is listed as table 1.

Table 1—Gradient program of mobile phase

Time/min	Ratio/(%)	
	Mobile phase A	Mobile phase B
0	10	90
5	50	50
7	50	50
8	10	90
12	10	90

c) Column temperature: 35℃.

d) Injection volume: 30 μL.

6.3.2 Mass spectral acquisition

a) Source: ESI, positive mode;

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b) Monitor mode: multiple reaction monitoring, MRM;

c) Nebulizer gas(GS1), curtain gas(CUR), auxiliary heater gas(GS2) and collision gas are high purity nitrogen 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 are listed as annex A;

d) Collision energy(CE), deculstering potential(DP), collision cell exit Potential (CXP), collision cell entrance potential (EP) and electrospray capillary voltage(IS) should be optimized to the best sensitivity. Related parameters and qualifier and quantifier MRM are listed as annex A.

6.3.3 Quantitation of LC-MS/MS

According to the HPLC-MS/MS operating condition above, determine the sample solution and the standard working solution (4.16) simultaneously. The responses of the analyte in the standard working solution and the sample solution should both be within the linear range of the instrument detection. Quantified by internal standard. Under the above HPLC-MS/MS operating condition, the retention time of salbutamol is about 3.0 min, terbutalin is about 3.1 min, ractopamine is about 9.4 min, and clenbuterol is about 9.9 min respectively. Selected ion chromatograms of the standards see Figure B.1 and B.2 in annex B.

6.3.4 Confirmation of LC-MS/MS

Determinated under the established LC/MS-MS conditions, and calculated the intensity ratio of two selected ion pairs of the sample solution and the standard working solution. If the retention times of sample chromatogram peaks are consistent with that of working solution, And the relative abundance ratio tolerance is same as listed (table 2) it is safe to conclude that this compound do exit in the sample.

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

Relative ion intensities/(%)	>50	>20 to 50	>10 to 20	≤10
Permitted relative tolerances/(%)	±20	±25	±30	±50

6.4 Blank test

The operation of the blank test is the same as the described in the method of determination, but without addition the sample.

7 Calculation and expression of result

Calculation the content of clenbuterol, ractopamine, salbutamol and terbutalin residue in the test sample by HPLC-MS/MS data processor or according to the formula (1). The blank value should be sub-

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tracted from the above result of calculation.

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

where

X—the residue content of β-agonist in the test sample, mg/kg;

c—the concentration of β-agonist in standard working solution, ng/mL;

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

A—the peak area of β-agonist in sample solution;

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

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

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

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

A_s—the peak area of β-agonist in standard working solution;

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

(Annotation: All β-agonist employ the corresponding isotope as the internal standard respectively except that terbutalin uses D_3-salbutamol as the internal standard)

8 Limit of quantitation(LOQ) and recovery

8.1 Limit of quantification (LOQ)

The limit of determination of the method for liver and meat is 0.000 5 mg/kg.

8.2 Recovery

8.2.1 According to the experimental data, the fortified concentration of clenbuterol, ractopamine, salbutamol and terbutalin in meat and its corresponding recovery are showed in Table 3.

Table 3—Recovery range of clenbuterol, ractopamine, salbutamol and terbutalin in meat

Chemicals	Concentration/ (μg/kg)	Recovery range/ (%)	Chemicals	Concentration/ (μg/kg)	Recovery range/ (%)
Clenbuterol	0.5	73.4～96.4	Salbutamol	0.5	80.8～91.6
	1.0	98.5～126.5		1.0	104.7～110.9
	2.0	86.5～102.0		2.0	73.5～78.5
Ractopamine	0.5	90.6～98.8	Terbutalin	0.5	80.6～108.0
	1.0	91.7～108.9		1.0	90.0～97.3
	2.0	90.6～98.8		2.0	80.6～108.0

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8.2.2 According to the experimental data, the fortified concentration of clenbuterol, ractopamine, salbutamol and terbutalin in liver its corresponding recovery are showed in Table 4.

Table 4—Recovery range of clenbuterol, ractopamine, salbutamol and terbutalin in liver

Chemicals	Concentration/ (μg/kg)	Recovery range/ (%)	Chemicals	Concentration/ (μg/kg)	Recovery range/ (%)
Clenbuterol	0.5	94.6～100.8	Salbutamol	0.5	77.2～89.8
	1.0	78.3～98.4		1.0	77.3～92.0
	2.0	90.5～99.0		2.0	73.5～80.0
Ractopamine	0.5	96.4～100.4	Terbutalin	0.5	77.2～90.6
	1.0	95.5～122.0		1.0	80.5～94.9
	2.0	92.0～108.5		2.0	73.0～79.5

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Annex A[1)]
(informative annex)
Main Mass Parameters

a) Electrospray capillary voltage:5 500 V;

b) GS1:50 Pa;

c) CUR:20 Pa;

d) GS2:30 Pa;

e) Ion source tempeture:550℃;

f) Qualifier and Quantifier MRM, Collision Energy(CE), Declustering Potential(DP) are showed in Table A. 1. Collision Cell Exit Potential (CXP) and Collision Cell Entrance potential (EP) are 10 V and 13 V respectively.

Table A. 1—Transitions for confirmation and quantititation, CE, CXP

	Precusor ion (Q1) m/z	Product ion(Q3) m/z	CE/V	DP/V
Clenbuterol	277.3	203.0[a]	18	45
		259.2	20	50
Ractopamine	302.3	164.2[a]	12	50
		284.3	22	50
Salbutamol	240.2	148.1[a]	25	55
		222.1	17	75
Terbutalin	226.2	152.1[a]	23	48
		107.1	43	45
D_9-clenbuterol	286.3	204.0	16	75
D_5-ractopamine	307.2	167.2	15	45
D_3-salbutamol	243.2	151.1	22	55
[a] the symbol represents the quantitative transition.				

1) the equipments and their types involved in the standard method are not related to commercial aims, and it is encouraged to use equipments of different corporation or different type.

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Annex B
(informative annex)
MRM chromatograms

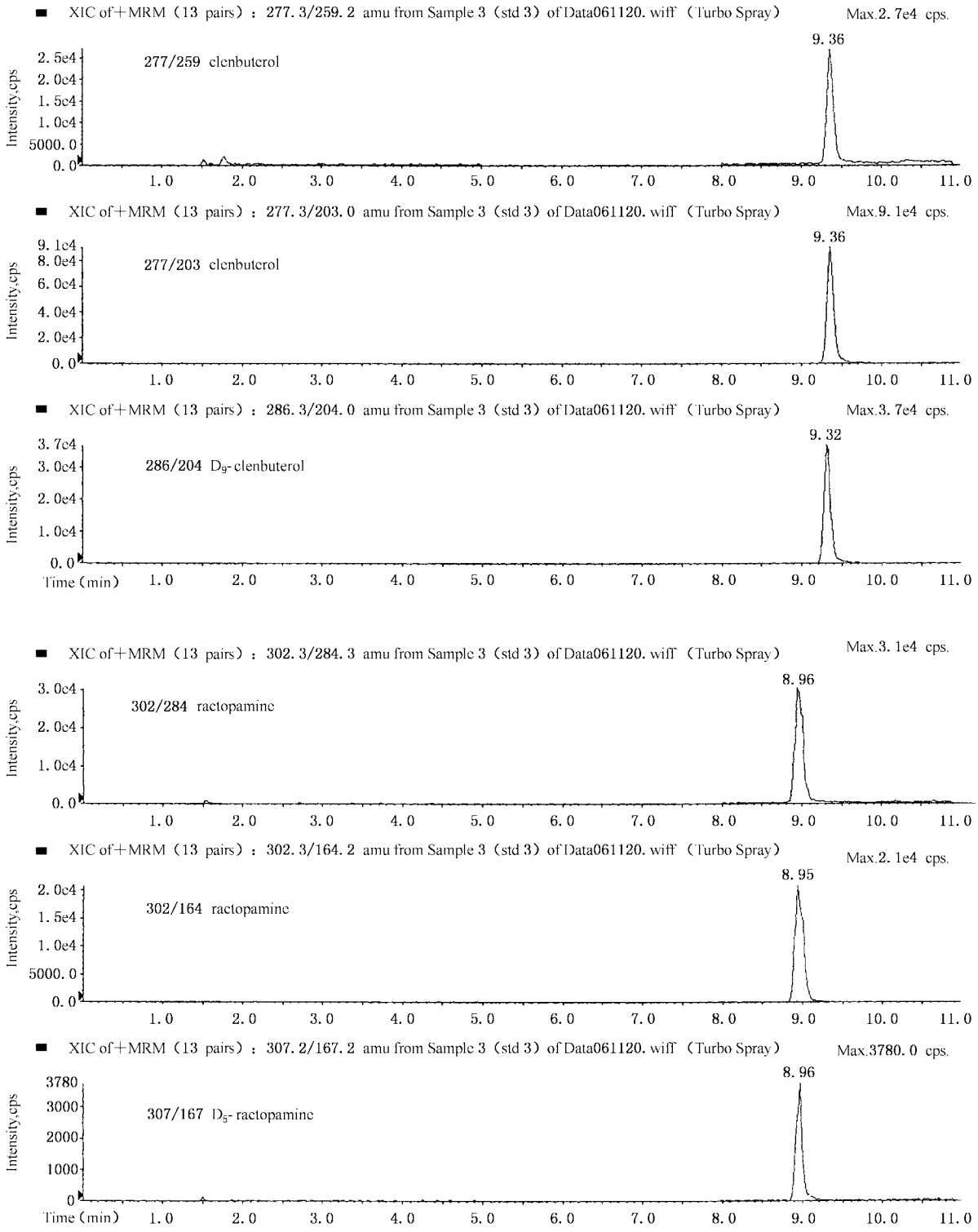


Figure B.1—MRM chromatograms of clenbuterol and ractopamine standards

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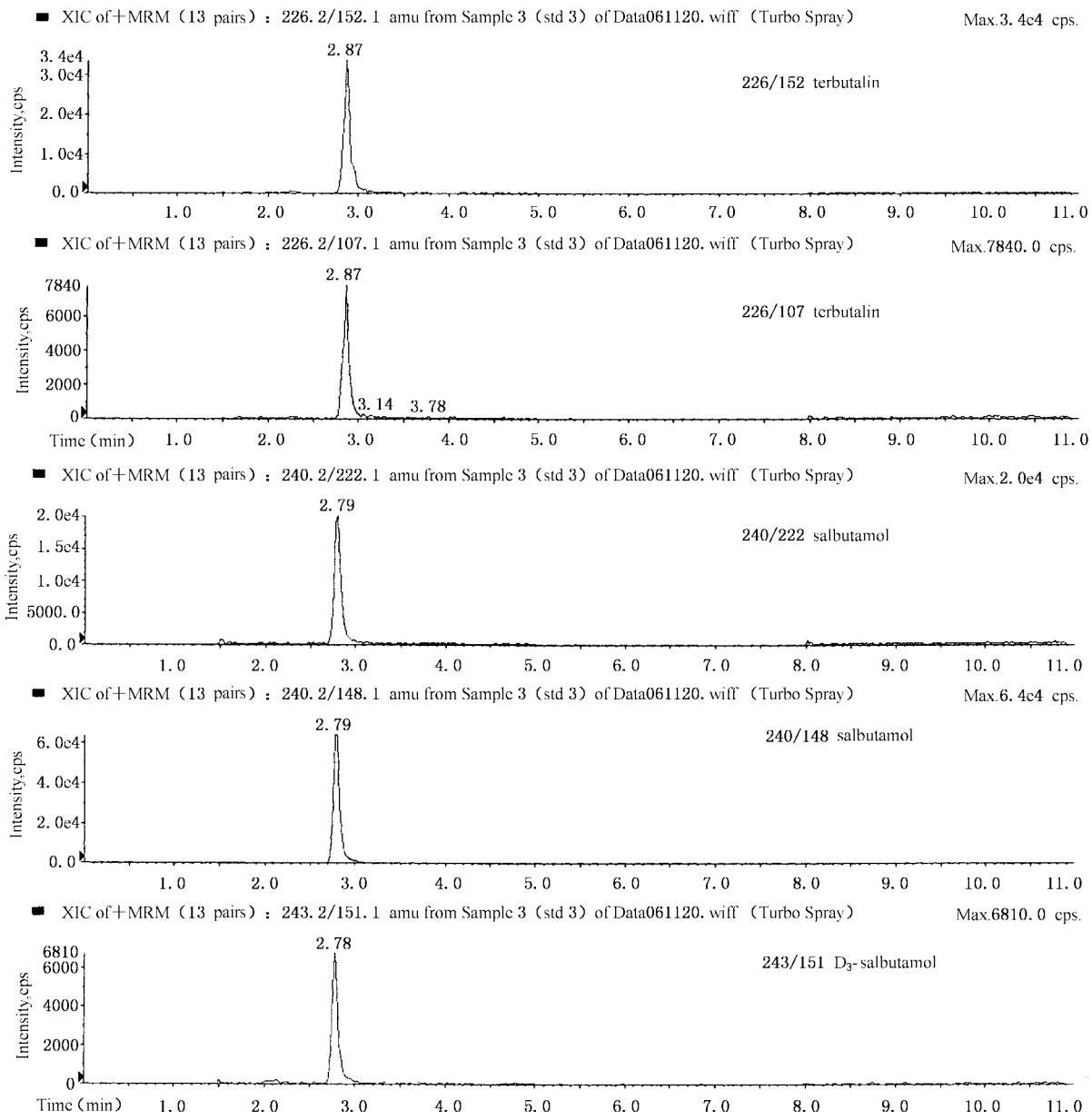


Figure B.2—MRM chromatograms of terbutalin and salbutamol standards

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