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进出口动物源食品中氯霉素残留量的检测方法　液相色谱-串联质谱法

Determination of chloramphenicol residue in animal-derived food for import and export—LC-MS/MS method

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前　言

本标准的附录A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、朱晓雨、丁慧瑛、奚君阳、黄雷芳、钱艳。

本标准系首次发布的出入境检验检疫行业标准。

进出口动物源食品中氯霉素残留量的检测方法　液相色谱-串联质谱法

1　范围

本标准规定了动物源食品中氯霉素残留量的液相色谱串联质谱测定方法。

本标准适用于虾、鱼、禽肉、肠衣和蜂蜜中氯霉素残留量的检测。

2　测定方法

2.1　方法提要

用乙酸乙酯提取样品中氯霉素，正己烷脱脂，C_{18}固相萃取小柱净化，用液相色谱-串联质谱测定和确证，同位素内标法定量。

2.2　试剂和材料

除另有规定外，所有试剂均为分析纯，水为二次蒸馏水。

2.2.1　乙酸乙酯：高效液相色谱级。

2.2.2　正己烷：高效液相色谱级。

2.2.3　甲醇：高效液相色谱级。

2.2.4　氯化钠。

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

2.2.6　15%氯化钠水溶液：15 g 氯化钠溶解于 100 mL 水中。

2.2.7　甲醇：水(2：8，体积比)。

2.2.8　氯霉素标准品(chloramphenicol，CAS NO. 56-75-7，$C_{11}H_{12}Cl_2N_2O_5$)：纯度大于等于 99%。

2.2.9　氯霉素-d5 标准品：纯度大于等于 99%。

2.2.10　氯霉素标准储备溶液：称取 0.010 0 g 标准品(2.2.8)，用甲醇溶解定容至 100 mL，溶液浓度为 100 μg/mL。1℃～4℃冷藏保存。

2.2.11　氯霉素-d5 标准储备溶液：称取 0.010 0 g 标准品(2.2.9)，用甲醇溶解定容至 100 mL，溶液浓度为 100 μg/mL。1℃～4℃冷藏保存。

2.2.12　标准工作曲线工作液：用甲醇：水(1：1，体积比)稀释氯霉素标准储备溶液，5 点工作曲线的浓度分别为 0.05 ng/mL、0.1 ng/mL、0.4 ng/mL、0.8 ng/mL、1.2 ng/mL，氯霉素-d5 作为同位素内标浓度为 0.2 ng/mL。

2.3　仪器和设备

2.3.1　液相色谱-串联质谱仪：配有电喷雾离子源。

2.3.2　旋转蒸发器。

2.3.3　粉碎机。

2.3.4　均质器。

2.3.5　旋涡混合器。

2.3.6　固相萃取柱：C_{18}固相萃取小柱，500 mg，或相当者。甲醇和水各 5 mL 预洗。

2.3.7　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 50 mm 无水硫酸钠。

2.4 测定步骤

2.4.1 制样

a) 虾、鱼、禽肉、肠衣：从所取全部虾、鱼或禽肉样品中取出有代表性样品约500 g，用粉碎机粉碎；从所取肠衣样品中取出有代表性样品约300 g，用剪刀剪碎，混合均匀，均分成两份，分别装入洁净容器作为试样，密封，并标明标记。将试样于－18℃冷冻保存。

b) 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。将试样室温下保存。在抽样和制样的操作过程中，应防止样品污染或发生残留物含量的变化。

2.4.2 提取

a) 虾、鱼、禽肉：称取5 g试样(精确到0.01 g)置于50 mL具塞离心管中，准确加入0.1 mL氯霉素-d5(10 ng/mL)内标溶液，加25 mL乙酸乙酯，在均质器中以14 000 r/min均质30 s，以3 000 r/min离心5 min，将上层乙酸乙酯提取液过无水硫酸钠柱(2.3.7)，收集于浓缩瓶中，样品残渣再加入20 mL乙酸乙酯，重复上述操作，合并乙酸乙酯提取溶液，在50℃以下水浴减压浓缩至近干，用4 mL正己烷和4 mL水依次将残渣转移至10 mL具塞玻璃离心管中，于旋涡混合器上以2 000 r/min，混匀1 min，以2 000 r/min离心3 min，弃去上层正己烷溶液，再加入4 mL正己烷，重复上述操作。

b) 肠衣：称取5 g试样(精确到0.01 g)置于50 mL具塞离心管中，准确加入0.1 mL氯霉素-d5(10 ng/mL)内标溶液，加25 mL乙酸乙酯，于旋涡混合器上以2 000 r/min，混匀1 min，按上述方法操作。

c) 蜂蜜：称取5 g试样(精确到0.01 g)置于50 mL具塞离心管中，准确加入0.1 mL氯霉素-d5(10 ng/mL)内标溶液，加15 mL 15%氯化钠水溶液(2.2.6)，加25 mL乙酸乙酯，于旋涡混合器上以2 000 r/min，混匀1 min，以3 000 r/min离心5 min，将上层乙酸乙酯提取液过无水硫酸钠柱(2.3.7)，收集于浓缩瓶中，样品残渣再加入20 mL乙酸乙酯，重复上述操作，合并乙酸乙酯提取溶液，在50℃以下水浴减压浓缩至近干。用4 mL水溶解残渣。

2.4.3 净化

将水层转移至C_{18}固相萃取柱(2.3.6)，再用10 mL水分两次洗涤，洗涤液过C_{18}固相萃取柱，弃去流出液，再用5 mL甲醇水溶液(2.2.7)洗涤，负压抽干，6 mL甲醇洗脱，收集全部洗脱液，用氮气吹至约2.5 mL，加水定容至5.0 mL，混匀，供液相色谱-串联质谱仪测定。

2.5 测定

2.5.1 液相色谱条件

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

b) 流动相见表1；

c) 流速：400 μL/min；

d) 进样量：20 μL。

表1 流动相梯度洗脱

时间/min	水/(%)	甲醇/(%)
0	35	65
4	75	25
5.9	75	25
6	35	65
8	35	65

2.5.2 质谱条件

a) 离子源:电喷雾离子源;
b) 扫描方式:负离子扫描;
c) 检测方式:多反映监测;
d) 电喷雾电压(IS):−4 500 V;
e) 雾化气压力(GS1):38 Pa;
f) 气帘气压力(CUR):27 Pa;
g) 辅助气流速(GS2):45 Pa;
h) 离子源温度(TEM):525℃;
i) 去簇电压(DP)−65 V;
j) 定性离子对、定量离子对、碰撞气能量(CE)及碰撞室出口电压(CXP)见表2。

表2 氯霉素、氯霉素-d5定性离子对、定量离子对、碰撞气能量和碰撞室出口电压

名称	定性离子对 m/z	定量离子对 m/z	碰撞气能量(CE)/V	碰撞室出口电压(CXP)/V
氯霉素	321.0/256.9 321.0/152.0 321.0/194.0 321.0/175.8	321.0/256.9	−16 −25 −17 −23	−10 −10 −10 −10
氯霉素-d5	326.0/157.1	326.0/157.1	−25	−10

2.5.3 色谱测定

根据试样中被测样液的含量情况,选取响应值相近的标准工作液一起进行色谱分析。标准工作液和待测样液中氯霉素的响应值均应在仪器线性响应范围内。在上述色谱条件下氯霉素、氯霉素-d5的参考保留时间约为6 min,标准溶液的选择性离子流图参见附录A中图A.1。

2.5.4 空白试验

除不加试样外,均按上述操作步骤进行。

2.5.5 定性、定量测定

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,定量测定时采用同位素内标标准曲线法。定性时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3规定的范围,则可判断样品中存在对应的被测物。

表3 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%

2.6 结果计算和表述

用色谱数据处理机或按式(1)计算试样中氯霉素残留含量,计算结果需扣除空白值:

$$X=\frac{c\times V}{m} \qquad \cdots\cdots(1)$$

式中:

X——试样中氯霉素的残留量,单位为微克每千克(μg/kg);

c——从标准曲线上得到的氯霉素溶液浓度,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)。

3 测定低限(LOQ)和回收率

3.1 测定低限(LOQ)

测定低限为 0.1 μg/kg(LOQ)。

3.2 回收率

回收率的实验数据(在不同添加浓度范围内)见表 4。

表 4 回收率

添加浓度/(μg/kg)	回收率/(%)				
	虾	鱼	肠衣	禽肉	蜂蜜
0.1	70.0～89.0	89.0～108.0	82.0～106.0	81.0～110.0	93.0～110.0
0.4	80.0～110.0	72.5～105.0	75.0～110.0	75.0～110.0	72.5～110.0
0.8	72.5～105.0	81.2～107.5	85.0～110.0	77.5～108.7	72.5～103.7

附 录 A
（资料性附录）
氯霉素标准品选择性离子流图

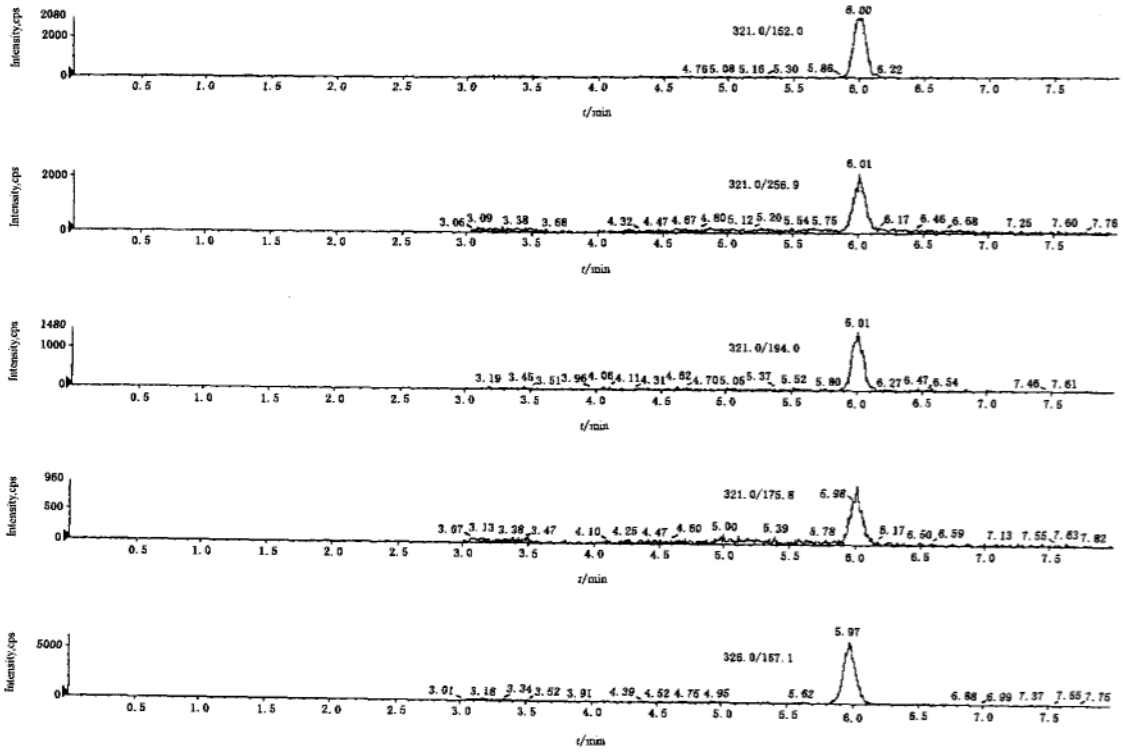

图 A.1 氯霉素(0.05 ng/mL)和氯霉素-d5(0.2 ng/mL)标准品的选择性离子流图

Foreword

Annex A of this standard is an informative annex.

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 Wen Xie, Xiao-yu Zhu, Hui-yin Ding, Jun-yang Xi, Lei-fang Huang, Yan Qian.

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.

Determination of chloramphenicol residue in animal-derived food for import and export—LC-MS/MS method

1 Scope

The standard specifies the methods of determination by LC-MS/MS of chloramphenicol in animal-derived food.

This standard is applicable to the determination of chloramphenicol in shrimp, fish, poultry meat, casing and honey samples.

2 Method of determination

2.1 Principle

Chloramphenicol is extracted from the sample by ethyl acetate. It was defatted with *n*-hexane and cleaned up with C_{18} column. Finally it is determined and confirmed by LC-MS/MS. Internal standard method is used.

2.2 Reagents and materials

Unless otherwise specified, all reagents used should be analytical grade, "water" is double distilled water.

2.2.1 Ethyl acetate: HPLC grade.

2.2.2 *n*-Hexane: HPLC grade.

2.2.3 Methanol: HPLC grade.

2.2.4 Sodium chloride.

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

2.2.6 15% sodium chloride solution: Dissolve 15 g sodium chloride in 100 mL water.

2.2.7 Methanol:water [2∶8(*V*/*V*)]

2.2.8 Chloramphenicol (CAS NO. 56-75-7, $C_{11}H_{12}Cl_2N_2O_5$): Purity≥99%.

2.2.9 Chloramphenicol-d5: Purity≥99%.

2.2.10 Stock standard solution of chloramphenicol: Accurately weigh 0.010 0 g standard (2.2.7), dissolve with methanol and quantitatively on 100 mL volumetric flask, the concentration of solution is 100 μg/mL, should be stored at 1℃～4℃ in refrigerator.

2.2.11 Stock standard solution of chloramphenicol-d5: Accurately weigh 0.010 0 g standard (2.2.8), dissolve with methanol and quantitatively on 100 mL volumetric flask, the concentration of solution is 100 μg/mL. should be stored at 1℃～4℃ in refrigerator.

2.2.12 Calibration curve standard working solutions: Working solutions of CAP were prepared in methanol:water [1∶1(*V*/*V*)] at five concentration levels, 0.05 ng/mL, 0.1 ng/mL, 0.4 ng/mL, 0.8 ng/mL, 1.2 ng/mL, with CAP-d5 as internal standard at a concentration 0.2 ng/mL.

2.3 Apparatus and equipment

2.3.1 Liquid chromatography combined with electrospray ionization mass spectrometry.

2.3.2 Rotary vacuum evaporator.

2.3.3 Blender.

2.3.4 Homogenizer.

2.3.5 Vortex mixer.

2.3.6 C_{18} column: 500 mg, or equivalent. It was conditioned with 5 mL methanol followed by 5 mL water.

2.3.7 Column of anhydrous sodium sulfate: 80 mm × 40 mm (i. d) cylinder funnel, pack with ca 5 mm absorbent cotton at the bottom of the column and fill in 50 mm anhydrous sodium sulfate.

2.4 Procedure

2.4.1 Preparation of test sample

a) Shrimp, fish, poultry meat and casing: Take the representative portions from the whole primary shrimp or fish or poultry meat sample. It is about 500 g and ground in a blender. Casing sample is about 300 g, a small cut made with scissors. Keep the prepared sample into two sample bottles,

seal and label. The test sample is stored in −18℃ refrigerator.

b) Honey: Honey sample is about 500 g. The sample that 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.

In the course of sampling and sample preparation, precautions must be taken to avoid contamination or any factors that may cause the change of residue content.

2.4.2 Extraction

a) Shrimp, fish and poultry meat: Weigh ca 5 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 0.1 mL CAP-d5(10 ng/mL) and 25 mL ethyl acetate. Homogenize for 30 s at 14 000 r/min. Centrifuge for 5 min at 3 000 r/min. The supernatant layer was passed, through anhydrous sodium sulfate column into flask. Repeat the extraction of the residues in the same way with 20 mL ethyl acetate and combined the solution. The solution evaporate to nearly dryness in a water bath below 50℃. Add 4 mL *n*-hexane and 4 mL water to dissolve residues, transfer the solution into 10 mL graduated glass tube. Blend for 1 min at 2 000 r/min, centrifuge for 3 min at 2 000 r/min, discard supernatant layer. Add 4 mL *n*-hexane and repeat the procedure.

b) Casing: Weigh ca 5 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 0.1 mL CAP-d5(10 ng/mL) and 25 mL ethyl acetate. Blend for 1 min at 2 000 r/min, operate the above procedure.

c) Honey: Weigh ca 5 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 0.1 mL CAP-d5(10 ng/mL), 15% sodium chloride solution and 25 mL ethyl acetate. Blend for 1 min at 2 000 r/min. Centrifuge for 5 min at 3 000 r/min. The supernatant layer was passed through anhydrous sodium sulfate column into flask. Repeat the extraction of the residues in the same way with 20 mL ethyl acetate and combined the solution. The solution evaporate to nearly dryness in a water bath below 50℃. Add 4 mL water to dissolve residues.

2.4.3 Clean up

Transfer the above water solution into the C_{18} column. Wash the graduated glass tube two times with 5 mL water, one time with 5 mL methanol solution (2.2.7), pass washes through C_{18} column, and discard the eluted solution. The cartridge is evacuated continuously to "dryness" under vacuum. Elute the column with 6 mL methanol and collect eluted solution. The above solution is under a gentle stream of nitrogen gas to 2.5 mL. Adjust volume of eluate to 5.0 mL with water. The solutions used for LC-MS/MS determination.

2.5 Determination

2.5.1 HPLC operating conditions

a) Column:ZORBAX Eclipse XDB-C_8,5 μm,150 mm×4.6 mm(i.d),or the equivalent;

b) Mobile phase:table 1;

c) Flow rate: 400 μL/min;

d) Injection volume:20 μL.

Table 1—Gradient of mobile phase

Time/min	Water/(%)	Methanol/(%)
0	35	65
4	75	25
5.9	75	25
6	35	65
8	35	65

2.5.2 Mass spectral acquisition

a) Source:ESI;

b) Polarity:Negative;

c) Mode:Multiple reaction monitoring;

d) IS: −4 500 V;

e) GSI:38 Pa;

f) CUR:27 Pa;

g) GS2:45 Pa;

h) TEM:525℃;

i) DP: −65 V;

j) Transitions for confirmation and quantification,CE,CXP sees table 2.

Table 2—Transitions for confirmation and quantification, CE, CXP

Compound	Transitions for confirmation m/z	Transitions for quantification m/z	CE/V	CXP/V
CAP	321.0/256.9 321.0/152.0 321.0/194.0 321.0/175.8	321.0/256.9	−16 −25 −17 −23	−10 −10 −10 −10
CAP-d5	326.0/157.1	326.0/157.1	−25	−10

2.5.3 LC-MS/MS determination

According to the approximate concentration of analyte in sample solution, select the standard working solution with similar responses to that of sample solution. The responses of the analyte in the standard working solution and the sample solution should be within the linear range of the instrument detection. Under the above LC-MS/MS operating condition, the retention time of CAP is about 6 min, selected ion chromatograms of the standards see Figure A. 1 in annex A.

2.5.4 Blank test

The operation of the blank test is the same as the described in the method of determination, but with the omission of sample addition.

2.5.5 Confirmation of LC-MS/MS

Under LC-MS/MS conditions, the working solution and sample solution is injected. If the retention times of sample chromatogram peaks are consistent with that of standard solution, calibration curve method with isotope internal standard is used for quantitative measurement. The relative intensities of sample transitions shall correspond to those of standard solution transitions for confirmation. The concentration of standard solution should be same with those of sample solution. The permitted tolerances listed in table 3, then the corresponding analyte must be present in sample.

Table 3—Maximum permitted tolerances for relative ion intensities while confirmation

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

2.6 Calculation and expression of result

Calculation the content of CAP residue in the test sample by LC-MS/MS data processor or according to the formula (1) The blank value should be subtracted from the above result of calculation.

$$X = \frac{c \times V}{m} \qquad \cdots\cdots(1)$$

Where:

X—the residue content of CAP in the test sample, μg/kg;

c—the concentration of CAP is from calibration curve, ng/mL;

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

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

3 Limit of quantification (LOQ) and recovery

3.1 Limit of quantification

The limit of quantification for CAP is 0.1 μg/kg.

3.2 Recovery

According to the experimental data, the corresponding recoveries of fortifying concentrations see table 4.

Table 4—Recovery

Spike/(μg/kg)	Recovery/(%)				
	Shrimp	Fish	Casing	Poultry meat	Honey
0.1	70.0～89.0	89.0～108.0	82.0～106.0	81.0～110.0	93.0～110.0
0.4	80.0～110.0	72.5～105.0	75.0～110.0	75.0～110.0	72.5～110.0
0.8	72.5～105.0	81.2～107.5	85.0～110.0	77.5～108.7	72.5～103.7

Annex A
(informative annex)
Selected ion chromatograms of CAP and CAP-d5 standards

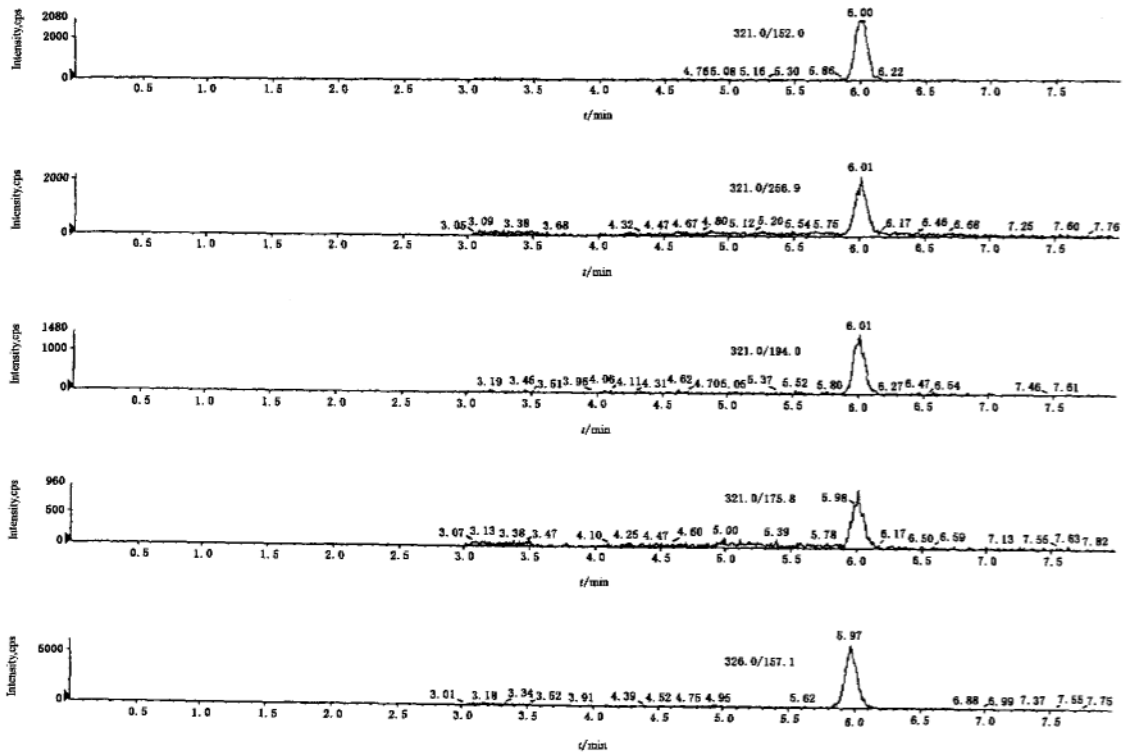

Figure A. 1—Selected ion chromatograms of CAP (0. 05 ng/mL) and CAP-d5 (0. 2 ng/mL)