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中华人民共和国出入境检验检疫行业标准

SN/T 2216—2008

进出口动物源食品中秋水仙碱残留量的检测方法 液相色谱-质谱/质谱法

Determination of colchicine residues in foodstuffs of animal origin for import and export— LC-MS/MS method

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前　言

本标准的附录A和附录B均为资料性附录。

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、中华人民共和国深圳出入境检验检疫局负责起草。

本标准主要起草人：谢文、丁慧瑛、奚君阳、岳振峰、陈笑梅、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秋水仙碱残留量的检测方法 液相色谱-质谱/质谱法

1 范围

本标准规定了动物食品中秋水仙碱残留量检测的制样和液相色谱-质谱/质谱测定方法。

本标准适用于鱼、鸭肉、牛肉、牛肝、牛肾和牛奶中秋水仙碱残留量的检测。

2 方法提要

在磷酸盐缓冲溶液(pH8)条件下，用二氯甲烷提取样品中秋水仙碱，再用C_{18}固相萃取小柱净化，液相色谱-质谱/质谱测定和确证，外标法定量。

3 试剂和材料

除另有规定外，所有试制均为分析纯，水为二次蒸馏水。

3.1 乙腈：高效液相色谱级。

3.2 正己烷：高效液相色谱级。

3.3 二氯甲烷：高效液相色谱级。

3.4 甲醇：高效液相色谱级。

3.5 甲酸：高效液相色谱级。

3.6 磷酸二氢钠。

3.7 氢氧化钠。

3.8 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。

3.9 甲醇水溶液：甲醇-水(4+6，体积比)。

3.10 0.15%甲酸溶液：移取 0.15 mL 用水稀释至 100 mL。

3.11 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。

3.12 秋水仙碱标准品(colchicine，CAS 号为 64-86-8，$C_{22}H_{25}NO_6$)：纯度大于等于 95%。

3.13 秋水仙碱标准储备溶液：称取适量标准品(3.12)，用甲醇溶解，溶液浓度为 100 μg/mL。−18 ℃避光冷冻保存。有效期 1 个月。

3.14 标准工作溶液：根据需要用空白样品溶液将标准储备液稀释成适当浓度的秋水仙碱标准溶液。临用前配置。

3.15 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。

3.16 固相萃取柱：C_{18}，500 mg，3 mL 或相当者；使用前用 5 mL 甲醇和 5 mL 水预洗，保持柱体湿润。

3.17 有机相微孔滤膜：0.45 μm。

4 仪器和设备

4.1 高效液相色谱-质谱/质谱仪：配有电喷雾离子源。

4.2 旋转蒸发器。

4.3 粉碎机。

4.4 均质器。

4.5 旋涡混合器。

4.6 离心机:4 000 r/min。

4.7 氮吹仪。

4.8 固相萃取装置。

5 试样制备与保存

5.1 牛奶:从所取全部样品中取出有代表性样品约250 mL,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0 ℃～4 ℃保存。

5.2 鱼、鸭肉、牛肉、牛肝和牛肾:从所取全部样品中取出有代表性样品约500 g,用粉碎机粉碎,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。

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。

6 测定步骤

6.1 提取

6.1.1 鱼、鸭肉、牛肉、牛肝和牛肾

称取2 g试样(精确到0.01 g)置于50 mL具塞塑料离心管中,加入2 mL磷酸盐缓冲溶液(3.11),加入25 mL二氯甲烷,在均质器中以14 000 r/min均质30 s,以4 000 r/min离心5 min,将上层二氯甲烷提取液过无水硫酸柱,滤液收集于浓缩瓶中,样品残渣再加入20 mL二氯甲烷,重复上述操作,合并二氯甲烷提取液,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,下层溶液待净化。

6.1.2 牛奶

称取4 g试样(精确到0.01 g)置于50 mL具塞塑料离心管中,加甲醇至20 mL,以4 000 r/min离心5 min,取10.0 mL上清液加水至25 mL,加20 mL正己烷,于旋涡混合器上以2 000 r/min,混匀1 min,以4 000 r/min离心3 min,弃去上层正己烷溶液,下层溶液待净化。

6.2 净化

将下层溶液转移至C_{18}固相萃取柱(3.16),再用5 mL水洗涤两次,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(3.9)洗涤,负压抽干,6 mL甲醇洗脱,收集全部洗脱液,在50 ℃以下水浴减压浓缩至近干,用2.0 mL甲醇-水(1＋1,体积比)定容,混匀,将溶液通过0.45 μm滤膜,供液相色谱-串联质谱仪测定。

6.3 测定

6.3.1 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:乙腈-0.15%甲酸水溶液(40＋60,体积比);

c) 流速:0.4 mL/min;

d) 进样量:20 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、碰撞气均匀高纯氮气;使用前应调节各气体流量以使质谱灵敏度达

到检测要求，参考条件参见附录 A 表 A.1；

i) 监测离子对(m/z)：秋水仙碱 400.2/310.2(定量离子)、400.2/326.2、400.2/358.2。

6.3.2 高效液相色谱-串联质谱测定

根据试样中被测样液的含量情况，选取待测物的响应值在仪器线性响应范围内的浓度进行测定，如超出仪器线性响应范围应进行稀释。在上述色谱条件下秋水仙碱的参考保留时间约为 4.8 min，标准溶液的选择性离子流图参见附录 B。

6.3.3 液相色谱-串联质谱确证

按照液相色谱-串联质谱条件测定样品和标准工作溶液，样品中待测物质的保留时间与标准溶液中待测物质的保留时间偏差在±2.5%之内。定量测定时采用标准曲线法。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 1 规定的范围，则可判断样品中存在对应的被测物。

表 1 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

6.3.4 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)计算试样中秋水仙碱残留含量，计算结果需扣除空白值：

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

式中：

X——试样中秋水仙碱的残留量，单位为微克每千克(μg/kg)；

c_i——从标准曲线上得到的秋水仙碱溶液浓度，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)。

8 测定低限(LOQ)和回收率

8.1 测定低限(LOQ)

方法测定低限为 1.0 μg/kg。

8.2 回收率

在不同添加浓度回收率数据见表 2。

表 2 秋水仙碱在不同基质中回收率范围

基　质	添加浓度/(μg/kg)	回收率/%
鱼	1.0	70.4～98.7
	5.0	70.8～100.8
	10.0	70.3～106.0
鸭肉	1.0	74.0～97.4
	5.0	74.0～97.5
	10.0	75.2～103.6

表 2（续）

基　质	添加浓度/(μg/kg)	回收率/%
牛肉	1.0	71.6～99.3
	5.0	73.8～102.8
	10.0	71.6～103.0
牛肝	1.0	70.4～109.0
	5.0	73.3～102.2
	10.0	73.7～101.0
牛肾	1.0	70.2～98.3
	5.0	70.3～98.1
	10.0	70.0～102.0
牛奶	1.0	78.4～105.0
	5.0	70.9～102.3
	10.0	75.4～103.0

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

监测离子对及电压参数：

a) 电喷雾电压(IS)：4 800 V；
b) 雾化气压力(GS1)：289.59 kPa(42 psi)；
c) 气帘气压力(CUP)：172.375 kPa(25 psi)；
d) 辅助气流速(GS2)：310.275 kPa(45 psi)；
e) 离子源温度(TEM)：540 ℃；
f) 碰撞气(CAD)：34.475 kPa(5 psi)；
g) 去簇电压(DP)：90 V；
h) 碰撞室出口电压(CXP)：10 V；
i) 离子对、碰撞能量(CE)见表 A.1。

表 A.1 离子对、碰撞能量(CE)

待测物	离子对 m/z	碰撞能量(CE)/V
秋水仙碱	400.2/310.2[a]	38
	400.2/326.2	35
	400.2/358.2	31
[a] 为定量离子对。		

1) 非商业性声明：附录 A 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。

附 录 B
（资料性附录）
秋水仙碱标准品选择性离子流图

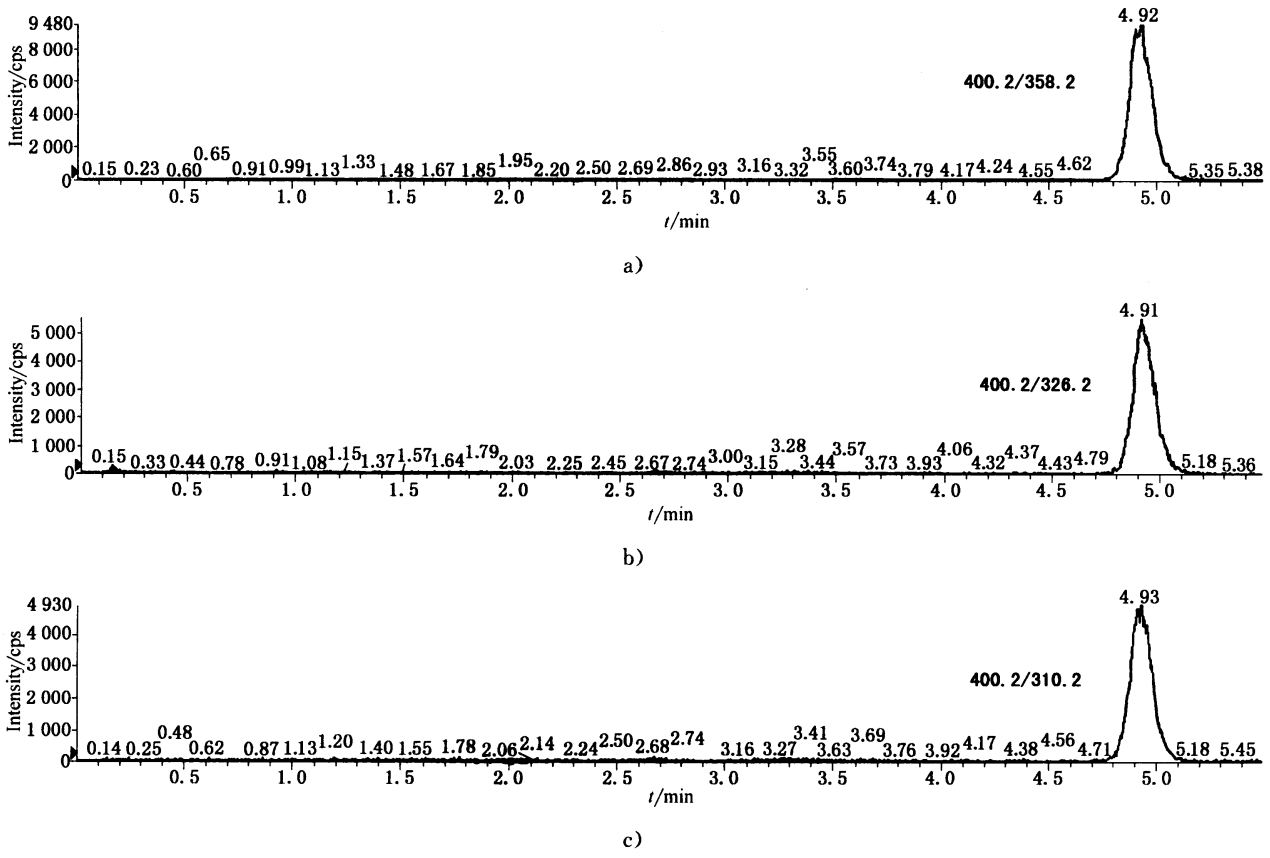

图 B.1 秋水仙碱(1 ng/mL)标准品的选择性离子流图

Foreword

Annex A and Annex B of this standard are 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 and Shenzhen Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The standard was mainly drafted by Wen Xie, Hui-yin Ding, Jun-yang Xi, Zhen-feng Yue, Xiao-mei Chen, Yan Qian.

This standard is a professional standard for entry-exit inspection and quarantine promulgated for the first time[1)].

1)Note: This English Version, a translation from the Chinese text, is solely for guidance.

Determination of colchicine residues in foodstuffs of animal origin for import and export— LC-MS/MS method

1 Scope

The standard specifies the methods of sample preparation and determination by LC-MS/MS of colchicine residues in foodstuffs of animal origin.

This standard is applicable to the determination of colchicine in fish, duck meat, cattle meat, cattle liver, cattle kidney and milk.

2 Principle

Sample is added with phosphate buffer(pH8). Then it is extracted from the sample by dichloromethane. It is cleaned up with C_{18} column. It is determined by LC-MS/MS and quantified by external method.

3 Reagents and materials

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

3.1 Acetonitrile: HPLC grade.

3.2 n-hexane: HPLC grade.

3.3 Dichloromethane: HPLC grade.

3.4 Methanol: HPLC grade.

3.5 Formic acid: HPLC grade.

3.6 Sodium dihydrogen phosphate.

3.7 Sodium hydroxide.

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

3.9 Methanol-water(4+6, *V*/*V*).

3.10 0.15% formic acid water solution: Dissolve 0.15 mL formic acid in 100 mL water.

3.11 Sodium dihydrogen phosphate buffer: Dissolve 13.8 g sodium dihydrogen phosphate in 950 mL water, adjust to pH 8.0 with 0.1 mol/L sodium hydroxide and dilute to 1 L.

3.12 Colchicine(CAS No. 64-86-8): Purity≥95%.

3.13 Stock standard solution: Accurately weigh appropriate standard(3.12), dissolve with methanol, the concentration of solution is 100 μg/mL. It should be stored in brown volumtric flask at −18 ℃ in refrigerator. Stock standard solution is stable for a month.

3.14 Calibration curve standard working solutions: Working solutions were prepared in blank sample solution. It is prepared before using.

3.15 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 40 mm anhydrous sodium sulfate.

3.16 Column: C_{18} 500 mg, 3 mL or equivalent. It is conditioned with 5 mL methanol followed by 5 mL water.

3.17 Filter: 0.45 μm.

4 Apparatus and equipment

4.1 Liquid chromatograhy with electrospray ionization mass spectrometry.

4.2 Rotary vacuum evaporator.

4.3 Blend.

4.4 Homogenizer.

4.5 Vortex mixer.

4.6 Centrifuge: 4 000 r/min.

4.7 Nitrogen evaporator.

4.8 SPE-12G Column Processor.

5 Preparation of test sample

5.1 Milk: Take the representative portions from the whole sample. It is about 250 mL. Keep the

prepared sample into two sample bottles, seal and label. The rest sample is stored at 0 ℃～4 ℃ refrigerator.

5.2 Duck meat, cattle meat, cattle liver, cattle kidney and milk.

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.

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

6 Analytical Procedure

6.1 Extraction

6.1.1 Fish, duck meat, cattle meat, cattle liver and cattle kidney

Weigh ca 2 g of the test sample(accurate to 0.01 g) into a 50 mL centrifuge tube. Add 2 mL phosphate buffer(3.11). Add 25 mL dichloromethane and Homogenize for 30 s at 14 000 r/min. Centrifuge for 5 min under 4 000 r/min. The supernatant layer was passed through anhydrous sodium sulfate column into flask. Repeat the extraction in the same way with 20 mL dichloromethane, combined the solution. The solution is evaporated 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.

6.1.2 Milk

Weigh ca 4 g of the test sample(accurate to 0.01 g) into a 50 mL centrifuge tube, adjust volume to 20 mL with methanol. Centrifuge for 5 min under 4 000 r/min. Transfer 10.0 mL supernatant layer and adjust volume to 25 mL with water, add 20 mL *n*-hexane and vortex for 1 min at 2 000 r/min. Centrifuge for 5 min at 4 000 r/min, discard supernatant layer. The down layer is ready for cleaning up.

6.2 Clean up

Transfer the above solution into the C_{18} column(3.16). Rinse the tube and C_{18} column with 5 mL water twice times, discard the eluate. Rinse the column with 5 mL methanol-water(3.9), discard the eluate. The cartridge is evacuated continuously to"dryness". Elute the column with 6 mL methanol. The solution is evaporated to nearly dryness in a water bath below 50 ℃. Add exactly 2.0 mL methanol-water(1+1, *V*/*V*) to dissolve the residues. The solution is passed through 0.45 μm filter. It is

ready for LC-MS/MS determination.

6.3 Determination

6.3.1 LC-MS/MS operating conditions

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

b) Mobile phase: acetonitrile − 0.15% formic acid solution(40 + 60, *V*/*V*);

c) Flow rate: 0.4 mL/min;

d) Injection volume: 20 μL;

e) Source: ESI;

f) Polarity: Positive;

g) Mode: Multiple reaction monitoring;

h) Carrier gas: Nitrogen(purity > 99.999%). Instrumental settings may be optimized. See table A.1 in annex A;

i) Transitions(m/z): Colchicine 400.2/310.2(quantification), 400.2/326.2, 400.2/358.2.

6.3.2 LC-MS/MS determination

According to the concentrations of analyte in sample solution, content should be within the linear range of the calibration curve. If it is over the range, the solution should be diluted. Under the above LC-MS/MS operating condition, the retention time of colchicine is 4.8 min. Selected ion chromatograms of the standards see Figure B.1 in annex B.

6.3.3 LC-MS/MS confirmation

Under LC-MS/MS conditions, the working solution and sample solution are injected. The retention time of the analyte in sample solution shall correspond to that of the analyte in standard solution. Tolerance is within ±2.5%. Calibration curve method 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 1, and then the corresponding analyte must be present in sample.

Table 1—Maximum permitted tolerances for relative ion intensities while confirmation

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

6.3.4 Blank test

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

7 Calculation and expression of result

Calculation the content of colchicine residues 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.

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

Where:

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

c_i—the concentration of colchicine residue 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.

8 Limit of quantification(LOQ) and recovery

8.1 Limit of quantification

The limit of quantification is 1.0 μg/kg.

8.2 Recovery

According to the experimental data, the corresponding recoveries of fortifying concentrations see table 2.

Table 2—The recoveries of colchicine in different matrix

Matrix	Spike level/(μg/kg)	Recovery/%
Fish	1.0	70.4～98.7
	5.0	70.8～100.8
	10.0	70.3～106.0
Duck meat	1.0	74.0～97.4
	5.0	74.0～97.5
	10.0	75.2～103.6
Cattle meat	1.0	71.6～99.3
	5.0	73.8～102.8
	10.0	71.6～103.0
Cattle liver	1.0	70.4～109.0
	5.0	73.3～102.2
	10.0	73.7～101.0
Cattle kidney	1.0	70.2～98.3
	5.0	70.3～98.1
	10.0	70.0～102.0
Milk	1.0	78.4～105.0
	5.0	70.9～102.3
	10.0	75.4～103.0

Annex A
(informative annex)
API 4000 LC-MS/MS conditions[1)]

Instrumental settings:

a) IS:4 800 V;

b) GS1:289.59 kPa(42 psi);

c) CUR:172.375 kPa(25 psi);

d) GS2:310.275 kPa(45 psi);

e) TEM:540 ℃;

f) CAD:34.475 kPa(5 psi);

g) DP:90 V;

h) CXP:10 V;

i) Transitions and CE see table A.1.

Table A.1—Transitions and CE

Compound	Transitions m/z	CE/V
colchicine	400.2/310.2[a]	38
	400.2/326.2	35
	400.2/358.2	31
[a] quantification.		

1) Non-commercial statement: the equipments and their models involved in the standard method are not related to commercial motive. The analysts are encouraged to use different equipments and models.

Annex B

(informative annex)

Selected ion chromatograms of colchicine standard

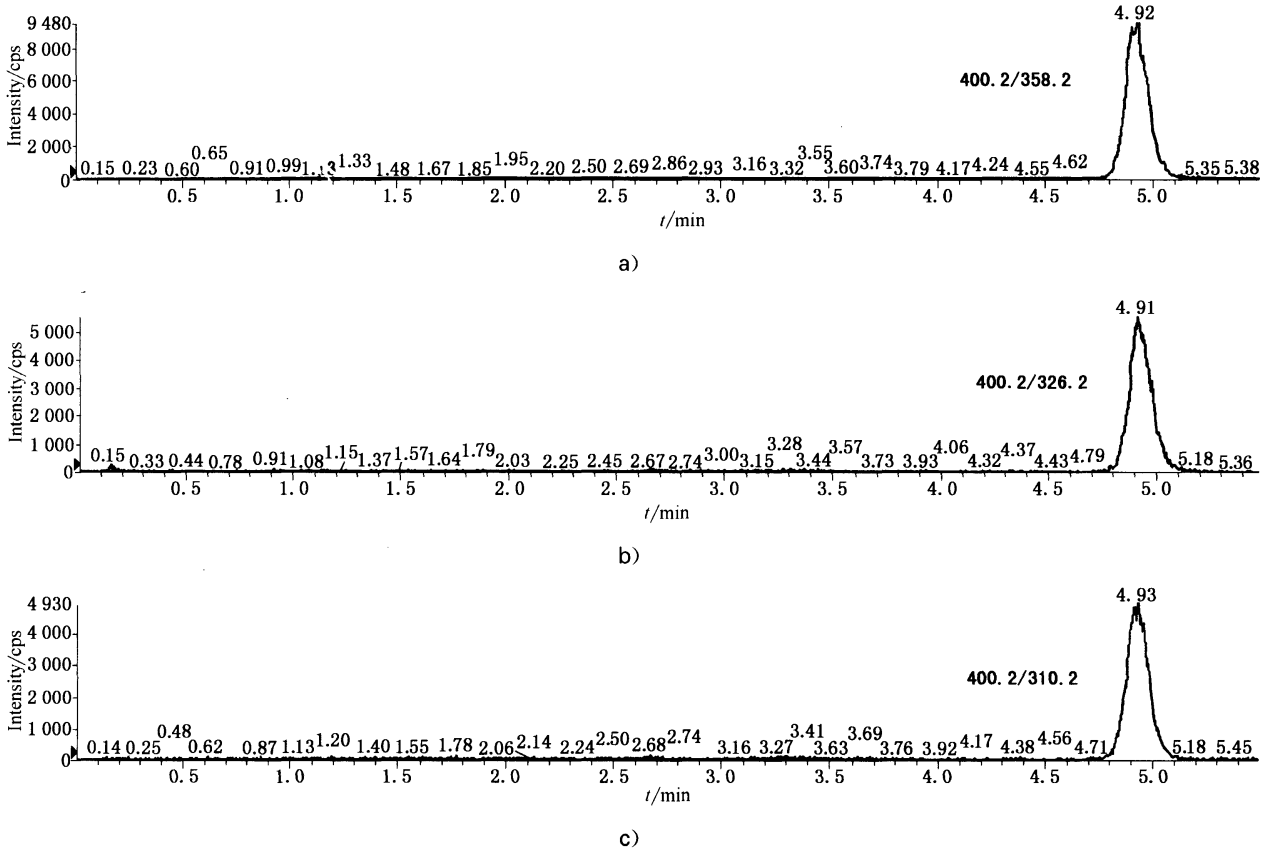

Figure B.1—Selected ion chromatograms of colchicine standard(1 ng/mL)