SN

中华人民共和国出入境检验检疫行业标准

SN/T 2073—2008

进出口植物性产品中吡虫啉残留量的检测方法　液相色谱串联质谱法

Determination of imidacloprid residue in plant products for import and export—LC-MS/MS method

2008-04-29 发布　　2008-11-01 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负责起草。

本标准主要起草人：谢文、丁慧瑛、蒋晓英、奚君阳、钱艳、黄雷芳。

本标准系首次发布的出入境检验检疫行业标准。

进出口植物性产品中吡虫啉残留量的检测方法　液相色谱串联质谱法

1　范围

本标准规定了植物性产品中吡虫啉残留量的制样和液相色谱串联质谱测定方法。

本标准适用于毛豆、西兰花、萝卜、板栗、大米、辣椒和茶叶中吡虫啉残留量的检测。

2　方法提要

用乙腈提取样品中吡虫啉，提取液再用弗罗里硅土和活性炭混合柱净化，液相色谱串联质谱测定和确证，外标法定量。

3　试剂和材料

除另有规定外，所有试剂均为分析纯，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　丙酮：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　氯化钠。

3.7　活性炭：粒度 0.038 mm～0.124 mm(120 目～400 目)。

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　弗罗里硅土：粒度 0.15 mm～0.25 mm(60 目～100 目)。650℃灼烧 4 h，使用前 130℃活化 4 h，在干燥器内冷却至室温，加 1%的水脱活，备用。

3.10　0.15%甲酸水溶液：0.15 mL 甲酸溶解于 100 mL 水中。

3.11　丙酮-正己烷(1＋1，体积比)。

3.12　甲醇-水(1＋1，体积比)。

3.13　吡虫啉标准品(imidacloprid，CAS No. 138261-41-3，$C_9H_{10}ClN_5O_2$)：纯度大于等于 98.5%。

3.14　吡虫啉标准储备溶液：称取适量标准品(3.13)，用甲醇溶解，溶液浓度为 100 μm/mL。1℃～4℃冷藏保存。有效期 6 个月。

3.15　标准工作曲线工作液：根据需要用空白样品溶液将标准储备液稀释成 5 ng/mL、10 ng/mL、50 ng/mL、100 ng/mL、250 ng/mL 的混合标准工作溶液，当样品取样量为 2 g 时，相当于样品中含有 5 μg/kg、10 μg/kg、50 μg/kg、100 μg/kg、250 μg/kg 吡虫啉。

3.16　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。

3.17　净化柱：200 mm×15 mm(内径)玻璃柱，底部垫约 5 mm 脱脂棉和约 20 mm 无水硫酸钠，5 g 弗罗里硅土和 0.2 g 活性炭混匀装柱，顶端加约 20 mm 无水硫酸钠，使用前用 20 mL 正己烷预洗。

4　仪器和设备

4.1　高效液相色谱-串联质谱仪：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。

5 试样制备与保存

5.1 蔬菜、坚果

从所取全部样品中取出有代表性样品约200 g，取可食部分经捣碎机充分捣碎均匀，均分成两份，装入洁净容器内，一份作为试样，另一份作为留样，密封，标明标记，4℃冰箱存放。

5.2 茶叶、粮谷

将所取全部样品缩分成100 g，磨碎，通过孔径为0.85 mm的筛(20目)，均分成两份，装入洁净的容器内，一份作为试样，另一份作为留样，密封，标明标记，室温存放。

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。

6 测定步骤

6.1 提取

6.1.1 蔬菜、坚果、粮谷

称取2 g样品(精确到0.01 g)置于50 mL具塞离心管中，脱水蔬菜、粮谷加3 mL水浸泡0.5 h，加入15 mL乙腈，以14 000 r/min均质30 s，以3 000 r/min离心5 min，将上层乙腈置另一个50 mL离心管中，再加入15 mL乙腈，重复上述操作，合并乙腈提取液，加入过量氯化钠振摇，以3 000 r/min离心5 min，将上层乙腈提取液过无水硫酸钠柱(3.16)，收集于浓缩瓶中，再用5 mL乙腈洗涤无水硫酸钠柱，在45℃下旋转蒸发至近干。

6.1.2 茶叶

称取1 g试样(精确到0.01 g)置于50 mL具塞离心管中，加3 mL水浸泡0.5 h，加入15 mL乙腈，以14 000 r/min均质30 s，按6.1.1方法操作。

6.2 净化

用5 mL丙酮分两次将残渣转移至净化柱中(3.17)，用80 mL正己烷-丙酮(1+1，体积比)洗脱。收集全部洗脱液，在45℃以下水浴上减压浓缩至干，用甲醇-水(1+1，体积比)定容至2.0 mL，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，5 μm，150 mm×4.6 mm(内径)或相当者；

b) 流动相见表1；

表1 流动相梯度洗脱

时间/min	乙腈/%	0.15%甲酸水溶液/%
0	30	70
3.5	70	30
8	70	30
10	30	70
16	30	70

c) 流速：300 μL/min；

d) 进样量：10 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;

i) 监测离子对(m/z):吡虫啉 256.0/209.3(定量离子)、256.0/175.2。

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。在上述色谱条件下吡虫啉的参考保留时间约为 9.5 min,标准溶液的选择性离子流图参见附录 B 中图 B.1。

6.3.3 空白试验

除不加试样外,均按上述操作步骤进行。

6.3.4 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 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	>20～50	>10～20	≤10
允许的相对偏差/%	±20	±25	±30	±50

7 结果计算和表述

用色谱数据处理机或按式(1)计算试样中吡虫啉残留含量,计算结果需扣除空白值:

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

式中:

X——试样中吡虫啉的残留量,单位为毫克每千克(mg/kg);

c_i——从标准曲线上得到的吡虫啉溶液浓度,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)

茶叶测定低限为 0.02 mg/kg,毛豆、西兰花、萝卜、板栗、大米、辣椒测定低限为 0.01 mg/kg。

8.2 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 吡虫啉在不同基质中回收率范围

基质	添加浓度/(mg/kg)	回收率范围/%
毛豆	0.01	74.1～95.1
	0.05	70.4～95.6
	0.10	74.3～101.6

表 3（续）

基质	添加浓度/(mg/kg)	回收率范围/%
西兰花	0.01	70.5～92.4
	0.05	71.8～85.0
	0.10	74.5～91.5
萝卜	0.01	73.0～91.8
	0.05	71.2～93.8
	0.10	70.3～97.6
板栗	0.01	71.4～91.6
	0.05	71.8～93.0
	0.10	70.5～92.4
大米	0.01	71.0～94.6
	0.05	71.6～98.2
	0.10	70.8～91.2
辣椒	0.01	72.6～94.5
	0.05	71.2～96.4
	0.10	71.5～94.1
茶叶	0.02	70.0～93.0
	0.1	70.5～89.1
	0.20	70.0～85.1

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(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)：540℃；

f） 碰撞气(CAD)41.37 kPa(6 Psi)；

g） 离子对、去簇电压(DP)、碰撞气能量(CE)及碰撞室出口电压(CXP)见表 A.1。

表 A.1 离子对、去簇电压(DP)、碰撞气能量(CE)及碰撞室出口电压(CXP)

待测物	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
吡虫啉	256.0/209.3[a]	62	22	18
	256.0/175.2	62	22	12
[a] 为定量离子对。				

1） 非商业性声明：附录表 A.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。

附　录　B
（资料性附录）
吡虫啉标准品选择性离子流图

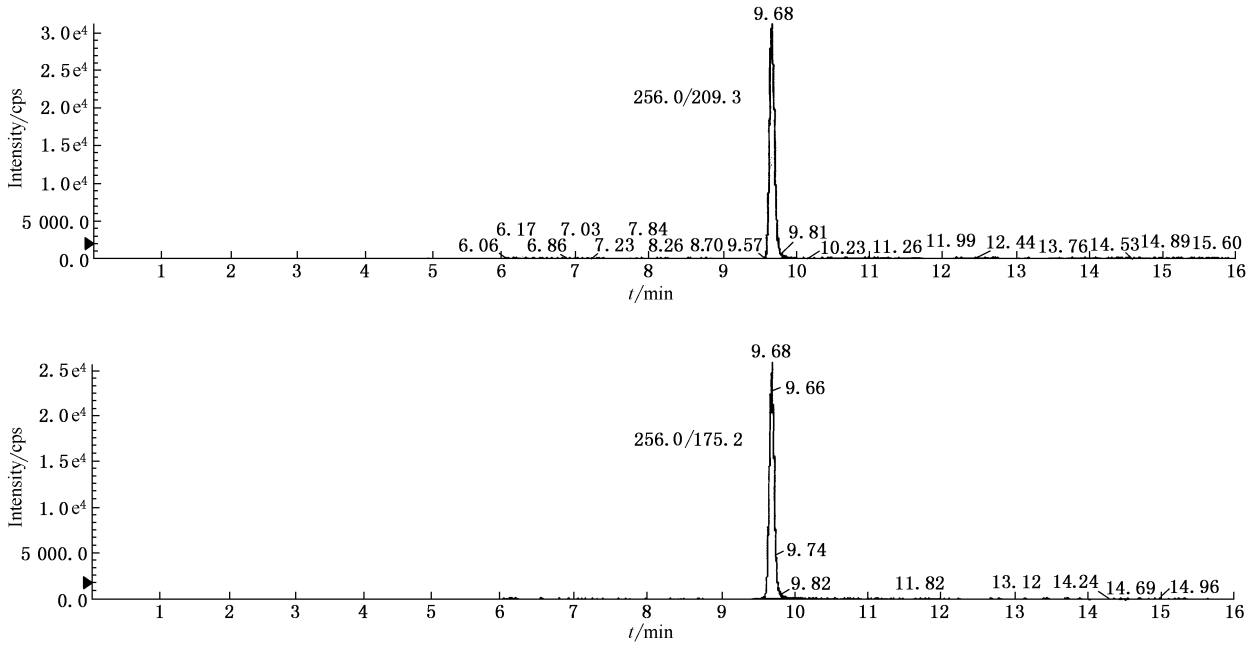

图 B.1　吡虫啉(5 ng/mL)标准品的选择性离子流图

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.

The standard was mainly drafted by Wen Xie，Hui-yin Ding，Xiao-yin Jiao，Jun-yang Xi，Yan Qian，Lei-fang Huang.

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 imidacloprid residue in plant products 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 imidacloprid residue in plant products.

This standard is applicable to the determination of imidacloprid in green soybeans, broccoli, radish, chestnut, rice, pimiento and tea.

2 Principle

Imidacloprid is extracted from the sample by acetonitrile. It is cleaned up with mixture florisil and active charcoal column. Finally it is determined and confirmed by LC-MS/MS. External standard method is used.

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 Acetone: HPLC grade.

3.4 Methanol: HPLC grade.

3.5 Formic acid: HPLC grade.

3.6 Sodium chloride.

3.7 Active charcoal: granule size 0.038 mm～0.124 0 mm(120 mesh～400 mesh).

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 Florisil: granule size 0.15 mm～0.25 mm (60 mesh～100 mesh), ignite for 4 h at 650℃, then heat for 4 h at 130℃ in an oven, cool to room temperature in desiccator and add 1% of water before use.

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

3.11 Acetone-*n*-hexane (1+1, *V*/*V*).

3.12 Methanol-water (1+1, *V*/*V*).

3.13 Imidacloprid (CAS No. 138261-41-3, $C_9H_{10}ClN_5O_2$): Purity≥98.5%.

3.14 Stock standard solution: accurately weigh appropriate standard (3.13), dissolve with methanol, the concentration of solution is 100 μg/mL. It should be stored at 1℃～4℃ in refrigerator. Stock standard solution is stable for 6 months.

3.15 Calibration curve standard working solutions: Working solutions were prepared in methanol at five concentration levels, 5 ng/mL, 10 ng/mL, 50 ng/mL, 100 ng/mL, 250 ng/mL. It is same as 5 μg/kg, 10 μg/kg, 50 μg/kg, 100 μg/kg, 250 μg/kg imidacloprid in 2 g sample.

3.16 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.

3.17 Column for clean-up: 200 mm × 15mm (i.d.) glass cloumn, pack with 5 mm absorbent cotton at the bottom of the column and fill in 20 mm anhydrous sodium sulfate, mixture 5 g florisil and 0.2 g active charcoal, and 20 mm anhydrous sodium sulfate at top. Add 20 mL of *n*-hexane wash before use.

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.

5 Preparation of test sample

5.1 Vegetable and nut

Take the representative portions from the whole sample. It is about 200 g and ground in a blender. Keep the prepared sample into two sample bottles,seal and label. The rest sample is stored in 4℃ refrigerator.

5.2 Tea and grain

Take the representative portions from the whole sample. It is about 100g and ground in a blender. Granule size 0.85 mm (20 mesh). Keep the prepared sample into two sample bottles,seal and label. The rest sample is stored in room temperature.

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 Vegetable,nut and grain

Weigh ca 2 g of the test sample(accurate to 0.01 g) into a 50 mL centrifuge tube. Dry vegetable and grain add 3 mL water for 0.5 h. Add 15 mL acetonitrile and Homogenize for 30 s at 14 000 r/min. Centrifuge for 5 min under 3 000 r/min. Transfer the supernatant layer into centrifuge tube. Repeat the extraction in the same way with 15 mL acetonitrile,combined the solution. Add excess sodium chloride,and shake it,centrifuge for 5 min under 3 000 r/min. The supernatant layer was passed through anhydrous sodium sulfate column into flask (3.16). Add 5 mL acetonitrile to rinse anhydrous sodium sulfate column. The solution is evaporated in a water bath below 45℃.

6.1.2 Tea

Weigh ca 1 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube,add 3 mL water for 0.5 h. Add 15 mL acetonitrile and homogenize for 30 s at 14 000 r/min. Operate the above procedure 6.1.1.

6.2 Clean up

Add 5 mL acetone to dissolve residues. Transfer the above solution into the column(3.17). Elute the column with 80 mL *n*-hexane-acetone (1+1, *V*/*V*)(3.11). The solution is evaporated to nearly dryness in a water bath below 45℃. Add exactly 2.0 mL methanol-water (1+1, *V*/*V*)(3.12) to dissolve the residue. 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, 5 μm, 150 mm×4.6 mm(i.d), or the equivalent;

b) Mobile phase: table 1;

Table 1—Gradient of mobile phase

Time/min	acetonitrile/%	0.15% formic acid solution/%
0	30	70
3.5	70	30
8	70	30
10	30	70
16	30	70

c) Flow rate: 300 μL/min;

d) Injection volume: 10 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): Imidacloprid 256.0/209.3 (quantification), 256.0/175.2.

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 imidacloprid is 9.5 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 2, and then the corresponding analyte must be present in 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

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 imidacloprid 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.

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

Where

X—the residue content of imidacloprid residue in the test sample, mg/kg;

c_i—the concentration of imidacloprid 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 for imidacloprid in tea sample is 0.02 mg/kg. The limit of quantifications for imidacloprid in green soybeans, broccoli, radish, chestnut, rice, pimiento are 0.01 mg/kg

8.2 Recovery

According to the experimental data, the corresponding recoveries of fortifying concentrations see table 3.

Table 3—The recoveries of imidacloprid in different matrix

Matrix	Spike level/(mg/kg)	Recovery/%
Green soybeans	0.01	74.1～95.1
	0.05	70.4～95.6
	0.10	74.3～101.6
Broccoli	0.01	70.5～92.4
	0.05	71.8～85.0
	0.10	74.5～91.5
Radish	0.01	73.0～91.8
	0.05	71.2～93.8
	0.10	70.3～97.6
Chestnut	0.01	71.4～91.6
	0.05	71.8～93.0
	0.10	70.5～92.4
Rice	0.01	71.0～94.6
	0.05	71.6～98.2
	0.10	70.8～91.2
Pimiento	0.01	72.6～94.5
	0.05	71.2～96.4
	0.10	71.5～94.1
Tea	0.02	70.0～93.0
	0.1	70.5～89.1
	0.20	70.0～85.1

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:41.37 kPa(6 Psi);

g) Transitions,DP,CE,CXP see table A.1.

Table A.1—Transitions,DP,CE,CXP

Compound	Transitions m/z	DP/V	EC/V	CXP/V
imidacloprid	256.0/209.3[a]	62	22	18
	256.0/175.2	62	22	12
[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 imidacloprid standard

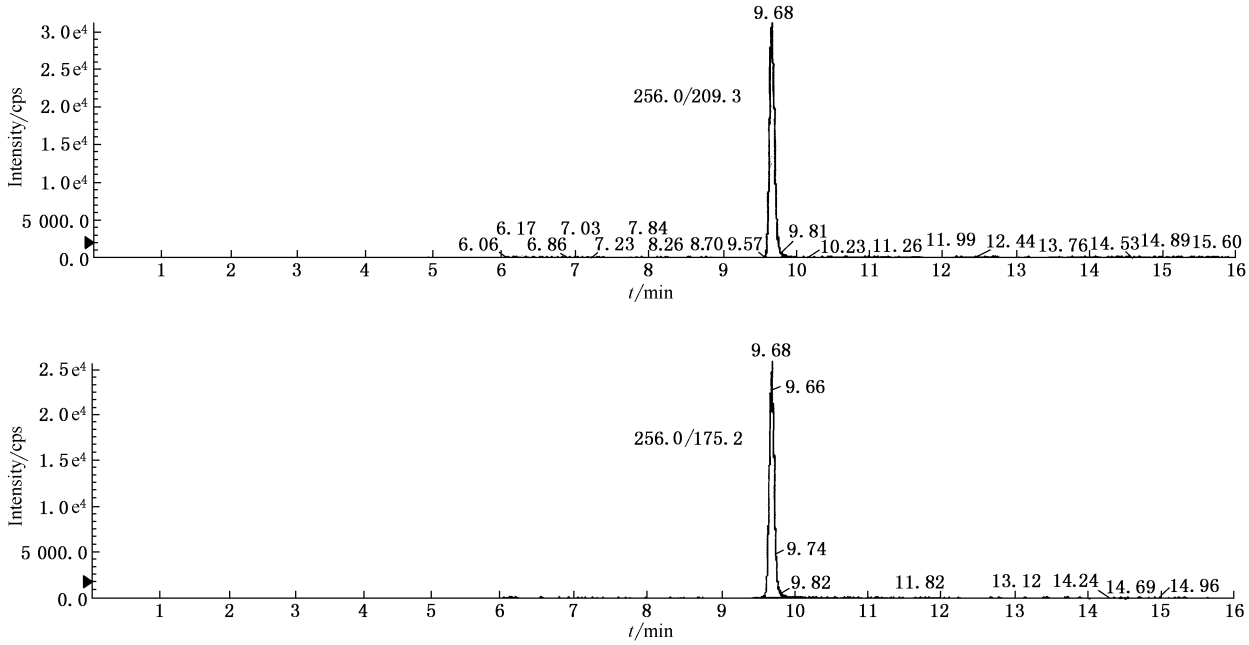

Figure B.1—Selected ion chromatograms of imidacloprid standard（5 ng/mL）