SN

中华人民共和国出入境检验检疫行业标准

SN/T 2623—2010

进出口食品中吡丙醚残留量的检测方法 液相色谱-质谱/质谱法

Determination of pyriproxyfen residues in foodstuffs for import and export—HPLC-MS/MS method

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

中国标准出版社
数码防伪

食品伙伴网http://www.foodmate.net

中华人民共和国出入境检验检疫
行 业 标 准
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中国标准出版社出版
北京复兴门外三里河北街16号
邮政编码：100045
网址 www.spc.net.cn
电话：68523946 68517548
中国标准出版社秦皇岛印刷厂印刷

开本 880×1230 1/16 印张 1.25 字数 30千字
2010年11月第一版 2010年11月第一次印刷
印数 1—1 600

书号：155066·2-21234 定价 21.00 元

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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。

本标准起草单位：中华人民共和国上海出入境检验检疫局。

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进出口食品中吡丙醚残留量的检测方法 液相色谱-质谱/质谱法

1 范围

本标准规定了进出口食品中吡丙醚残留量的制样和测定方法。

本标准适用于大米、大豆、菠菜、柠檬、茶叶、蘑菇、牛肉、猪肝和牛奶中吡丙醚残留量的检测。

2 方法提要

试样中残留的吡丙醚在醋酸钠缓冲剂作用下用酸性乙腈提取，再用PSA填料净化，液相色谱-质谱进行测定，外标法定量。

3 试剂和材料

除非另有规定，均使用分析纯试剂，水为去离子水或相当纯度的水。

3.1 无水硫酸镁。

3.2 无水乙酸钠。

3.3 乙酸：色谱级。

3.4 乙腈：色谱级。

3.5 1%乙酸乙腈溶液。

3.6 N-丙基乙二胺(PSA)填料：50 μm，色谱级。

3.7 甲酸：色谱级。

3.8 乙酸铵。

3.9 0.025%甲酸水溶液(含5 mmol/L乙酸铵)：准确吸取0.25 mL甲酸(3.7)和称取0.386 g乙酸铵(3.8)于1 L容量瓶中，用水溶解并稀释定容至1 L。

3.10 吡丙醚标准品(Pyriproxyfen，$C_{20}H_{19}NO_3$，CAS编号：95737-68-1)：纯度大于99%。

3.11 吡丙醚标准储备液：准确称取适量的吡丙醚标准品，用乙腈溶解并稀释配制成100 μg/mL的标准储备液，4 ℃以下保存。

3.12 吡丙醚标准工作液：根据需要，用10%乙腈水溶液稀释成适当浓度的标准工作液，4 ℃以下保存。

3.13 微孔滤膜：0.2 μm，有机相型。

3.14 具塞聚丙稀离心管：2 mL和50 mL。

4 仪器和设备

4.1 液相色谱-质谱/质谱仪：配有电喷雾(ESI)离子源。

4.2 离心机：4 000 r/min。

4.3 分析天平：感量0.000 1 g和0.01 g。

4.4 粉碎机。

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5 试样制备与保存

5.1 大米、大豆、菠菜、柠檬、茶叶、蘑菇、牛肉、猪肝

取有代表性样品中可食部分约500 g，用组织捣碎机充分捣碎混匀，均分成两份，分别装入洁净容器内作为试样。密封，并标明标记。将试样置于—18 ℃冷冻避光保存。

5.2 牛奶

取有代表性样品约500 g，充分混匀，均分成两份，分别装入洁净容器内作为试样，密封，并标明标记。将试样置于低于4 ℃冷藏避光保存。

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。

6 测定步骤

6.1 提取

6.1.1 茶叶、大豆、蘑菇、大米样品

称取上述样品5 g(精确至0.01 g)于50 mL聚丙烯塑料离心管中，加10 mL水，混匀，静置30 min。将离心管置于冰浴中，然后加6 g无水硫酸镁和1.5 g无水乙酸钠，恢复至室温后，准确加入15.0 mL 1%乙酸乙腈溶液(3.5)，振荡提取4 min，4 000 r/min离心5 min。

6.1.2 菠菜、柠檬和牛奶样品

称取15 g(精确至0.01 g)于预先称有6 g无水硫酸镁和1.5 g无水乙酸钠的50 mL聚丙烯塑料离心管中，将离心管置于冰浴中，恢复至室温后，准确加入15.0 mL 1%乙酸乙腈溶液(3.5)，振荡提取4 min，4 000 r/min离心5 min。

6.1.3 牛肉、猪肝样品

称取15 g(精确至0.01 g)于50 mL聚丙烯塑料离心管中，准确加入15 mL 1%乙酸乙腈溶液(3.5)，振荡提取4 min，4 000 r/min离心5 min。另取称有6 g无水硫酸镁和1.5 g无水乙酸钠的50 mL聚丙烯塑料离心管，加入10 mL水，溶解，待水溶液恢复到室温后，再将乙腈提取液转移至其中，振荡4 min，4 000 r/min离心5 min。

6.2 净化

取1 mL上述乙腈提取液于预先称有50 mg PSA和150 mg无水硫酸镁的2 mL聚丙烯塑料离心管中，振荡提取1 min，在4 000 r/min离心5 min。

准确吸取200 μL上清液用水定容至1 mL，混匀后过0.2 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：CAPCELL PAK C_{18}柱，50 mm×2.0 mm(内径)，3 μm或相当者；

b) 流动相：A：0.1%甲酸乙腈溶液，B：0.025%甲酸水溶液(含5 mmol/L乙酸铵)；

c) 流速：300 μL/min，梯度洗脱程序见表1；

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表 1　梯度洗脱程序表

梯度时间/min	流动相 A/%	流动相 B/%
0	20	80
1	20	80
2	90	10
4	90	10
4.5	20	80
8.5	20	80

d)　柱温：35 ℃；

e)　进样量：20 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)　其他参数条件参见附录 A。

6.3.3　定量测定

根据试样中被测物的含量，选取响应值相近的标准工作液同时进行分析。标准工作液和待测液中吡丙醚的响应值均应在仪器线性响应范围内。在上述色谱条件下的吡丙醚的参考保留时间为1.4 min，标准溶液的多反应监测色谱图参见附录 B。

6.3.4　定性测定

按照液相色谱-质谱/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	>20～50	>10～20	≤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。

$$X=\frac{A\times c\times V}{A_s\times m\times 1\,000} \qquad \cdots\cdots(1)$$

式中：

X ——试样中吡丙醚的含量，单位为微克每千克(μg/kg)；

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A ——样液中吡丙醚的峰面积；

c ——标准溶液中吡丙醚的浓度，单位为微克每毫升(μg/mL)；

V ——样液最终定容体积，单位为毫升(mL)；

A_s——标准溶液中吡丙醚的峰面积；

m ——最终溶液所代表试样的质量，单位为克(g)。

8 测定低限、回收率

8.1 测定低限(LOQ)

本方法菠菜、柠檬、牛肉、猪肝和牛奶中的测定低限为 5.0 μg/kg。

本方法大豆、大米、蘑菇和茶叶中的测定低限为 15.0 μg/kg。

8.2 回收率

不同基质中吡丙醚添加浓度和回收率数据见表 3。

表 3 添加浓度及回收率范围

样品名称	添加浓度/(μg/kg)	回收率/%
大米	15.0	89.3～107.3
	100	83.5～101.3
	1 100	86.3～102.1
菠菜	5.0	81.4～101.8
	50.0	86.2～103.8
	2 000	79.7～101.3
柠檬	5.0	70.6～100.4
	50.0	73.8～103.2
	500	80.1～100.2
茶叶	15.0	83.3～101.3
	100	86.8～108.9
	200	79.1～104.8
大豆	15.0	83.3～104.7
	50.0	75.8～103.2
	1 100	84.0～99.0
牛肉	5.0	80.6～99.8
	10.0	83.5～106.0
	100	80.5～108.2
猪肝	5.0	77.6～101.8
	20.0	81.5～113.0
	100	77.7～107.2

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表 3（续）

样品名称	添加浓度/(μg/kg)	回收率/%
牛奶	5.0	81.4～99.4
	20.0	81.5～103.0
	100	78.2～100.8
蘑菇	15.0	84.7～104.0
	100	83.1～106.8
	600	82.9～103.1

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附 录 A
（资料性附录）
API 4000 液相色谱-四级杆串联质谱仪参数[1)]

监测离子对及电压、气压参数：

a） 离子源温度：350 ℃；

b） 电喷雾电压：5 250 V；

c） 碰撞压力：34.475 kPa(5 psi)；

d） 气帘气压力：206.85 kPa(30 psi)；

e） 雾化气压力：344.75 kPa(50 psi)；

f） 辅助气压力：413.7 kPa(60 psi)；

g） 吡丙醚定量离子对、定性离子对、去簇电压(DP)、碰撞气能量(CE)和保留时间见表 A.1。

表 A.1 吡丙醚定量离子对、定性离子对、去簇电压(DP)、碰撞气能量(CE)和保留时间

组分名称	定性离子对 m/z	定量离子对 m/z	去簇电压 DP/V	碰撞气能量 CE/V	保留时间 T_r/min
吡丙醚	322.2→185.0	322.2→185.0	110	16	1.4
	322.2→227.2			14	

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。

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附 录 B
（资料性附录）
吡丙醚多反应监测（MRM）色谱图

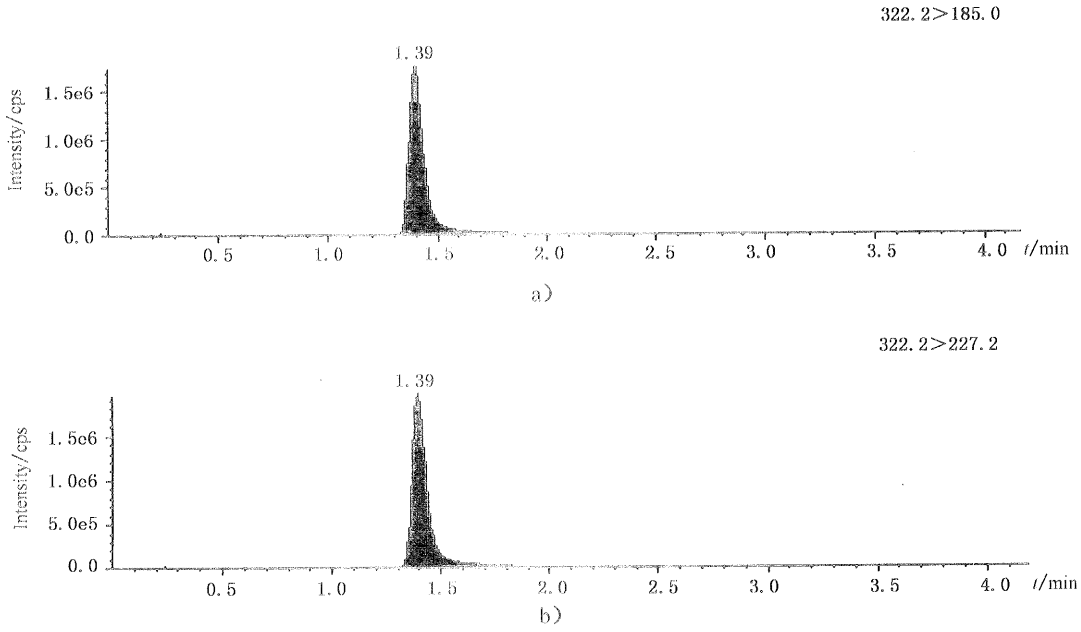


图 B.1 吡丙醚 MRM 色谱图

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

The standard was mainly drafted by Guo Dehua, Deng Xiaojun, Zhao Guangyu, Sheng Yonggang, Zhou Yao, Zhao Shanzhen.

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Determination of pyriproxyfen residues in foodstuffs for import and export—HPLC-MS/MS method

1 Scope

The standard specifies the methods of sample preparation and determination of pyriproxyfen residue in foodstuffs for import and export.

This standard is applicable to the determination of pyriproxyfen residue in rice, soybean, spinach, lemon, tea, beef, pork liver and milk.

2 Principle

The pyriproxyfen residue in sample is extracted by acidated acetonitrile with the presence of sodium acetate buffer, and cleaned up by PSA sorbent. The residue is determined by HPLC-MS/MS and quantified by external standard method.

3 Reagents and materials

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

3.1 Anhydro magnesium sulfate.

3.2 Anhydro sodium acetate.

3.3 Acetic acid: HPLC grade.

3.4 Acetonitrile: HPLC grade.

3.5 1% Acetic acid-acetonitrile.

3.6 PSA sobent, 50 μm, HPLC.

3.7 Formic acid: HPLC grade.

3.8 Ammonium acetate.

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3.9 0.025% Formic acid (containing 5 mmol/L ammonium acetate): Add 0.25 mL formic acid (3.7) and 0.386 g ammonium acetate (3.8) into 1 L volumetric flask, then dilute to 1 L with water.

3.10 Pyriproxyfen standard ($C_{20}H_{19}NO_3$ CAS:95737-68-1): Purity>99%.

3.11 Stock solution of pyriproxyfen standard: Dissolve Pyriproxyfen standard in actonitrile to final concentration of 100 μg/mL, store refrigerated at <4 ℃.

3.12 Working solutions of pyriproxyfen standard: To require as useful, dilute the stock solution (3.9) with 10% actonitrile in water, and store refrigerated at <4 ℃.

3.13 Micropore film: 0.2 μm.

3.14 Centrifuge tube conical: 2 mL, 50 mL.

4 Apparatus and equipment

4.1 Liquid chromatograhy combined with electrospray ionization mass spectrometry.

4.2 Centrifuger: 4 000 r/min.

4.3 Balances (0.000 1 mg, 0.01 g).

4.4 Homogenizer.

5 Preparation and storage of test sample

5.1 rice, soybean, spinach, lemon, tea, beef, pork liver

All primary sample is reduced to 500 g as the representative sample, which is homogenized, and then divided into two equal portions, each portion is placed into a clean vessel as a test sample, which is sealed and labeled. The test sample shall be stored below in −18 ℃ avoiding sunlight.

5.2 Milk

All primary sample is reduced to 500 g as the representative sample, which is homogenized, and then divided into two equal portions, each portion is placed into a clean vessel as a test sample, which is sealed and labeled. The test sample shall be stored below 4 ℃ avoiding sunlight.

In the course of sampling and sample preparation, precautions shall be taken to avoid contamination

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or any factor that may cause the change of residue content.

6 Procedure

6.1 Extraction

6.1.1 Tea, soybean, mushroom, rice

Weigh 5 g (accurate to 0.01 g) sample into a 50 mL plastic centrifuge tube, add 10 mL water, homogenize them thoroughly, and let the mixture stand for 30 min. Place the centrifuge tube in ice bath. And then add 6 g anhydro magnesium sulfate and 1.5 g anhydro sodium acetate into the mixture. Precisely add 15 mL 1% acetate acid in acetonirile(3.5) into the mixture at room temperature, mix them on vortex mixer for 4 min, and then centrifuge at 4 000 r/min for 5 min. The supernatant is ready for futher clean up.

6.1.2 Spinarch, lemon, milk

Weigh 15 g (accurate to 0.01 g) sample into a 50 mL plastic centrifuge tube preloaded with 6 g anhydro magnesium sulfate and 1.5 g anhydro sodium acetate. Place the plastic centrifuge into ice bath. Precisely add 15 mL 1% acetate acid in acetonirile(3.5) into the mixture at room temperature, mix them on vortex mixer for 4 min, and then centrifuge at 4 000 r/min for 5 min. The supernatant is ready for futher clean up.

6.1.3 Beef, pork liver

Weigh 15 g (accurate to 0.01 g) sample into a 50 mL plastic centrifuge tube, precisely add 15 mL 1% acetate acid in acetonirile(3.5), mix them on vortex mixer for 4 min, and then centrifuged at 4 000 r/min for 5 min. Weigh 6 g anhydro magnesium sulfate and 1.5 g anhydro sodium acetate into another centrifuge tube, dissolve them in 10 mL water. Transfer the acetonitrile extract into the mixture at room temperature, mix them on vortex mixer for 4 min, and then centrifuge at 4000 r/min for 5 min. The supernatant is ready for futher clean up.

6.2 Clean up

Add 1 mL supernatant above into a 2 mL plastic centrifuge tube which is preloaded with 50 mg PSA and 150 mg anhydro magnesium sulfate. Mix them on vortex mixer for 1 min, and then centrifuged at 4 000 r/min for 5 min.

Diulte 200 μL supernatant and dilute to 1 mL with water, filter the mixture with 0.2 μm film before HPLC-MS/MS analysis.

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6.3 Determination

6.3.1 HPLC operating conditions

a) Column: C APCELL PAK C_{18} column, 3 μm, 50 mm × 2.0 mm(i.d), or the equivalent;

b) Mobile phase: A: Acetonitrile + 0.1% formic acid, B: 0.025% formic acid (containing 5 mmol/L ammonium acetate);

c) flow rate: 300 μL/min. The aradient elution program is listed in the table 1;

Table 1—Gradient program of mobile phase

Time/min	Mobile A/%	Mobile B/%
0	20	80
1	20	80
2	90	10
4	90	10
4.5	20	80
8.5	20	80

d) Cloumn temperature: 35 ℃;

e) Injection volume: 20 μL.

6.3.2 Mass spectral acquisition

a) Source: ESI, positive mode;

b) Scanning mode: multiple reaction monitoring (MRM);

c) Other parameters are listed in the Annex A.

6.3.3 Quantitation by HPLC-MS/MS

According to the analyte concerntration of sample, choose suitable standard working solution of which the response is near that of the analyte in sample. 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 HPLC-MS/MS operating condition, the retention time of pyriproxyfen was 1.4 min, the MRM chromatogram of standard solutions is presented in the Annex B.

6.3.4 Confirmation by HPLC-MS/MS

Determine the sample under the established LC/MS-MS conditions, and calculate the intensity ratio of

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two selected ion pairs of the sample solution and the standard working solution. If the retention time of sample chromatogram peak is consistent with that of working solution, and the relative abundance ratio tolerance meets the requirments listed in table 2, it can be concluded that the analyte do exist in the sample.

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

Relative ion intensities/%	>50	>20~50	>10~>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 of the sample.

7 Calculation and expression of result

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

$$X = \frac{A \times c \times V}{A_s \times m \times 1\,000} \qquad \cdots\cdots(1)$$

Where:

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

A—the peak area of pyriproxyfen in sample solution;

c—the concentration of pyriproxyfen in standard working solution, μg/mL;

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

A_s—the peak area of pyriproxyfen in standard working solution;

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

8 Limit of quantitation(LOQ) and recovery

8.1 Limit of quantification (LOQ)

The limit of quantitation of the method for spinach, lemon, beef, pork liver and milk is 5.0 μg/kg, and for soybean, rice, mushroom and tea is 15.0 μg/kg.

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8.2 Recovery

Recovery in each matrix is listed in the table 3.

Table 3—Add Concentration and recovery

Sample	Add Concentration/ (μg/kg)	Recovery/ %
rice	15.0	89.3～107.3
	100	83.5～101.3
	1 100	86.3～102.1
spinach	5.0	81.4～101.8
	50.0	86.2～103.8
	2 000	79.7～101.3
lemon	5.0	70.6～100.4
	50.0	73.8～103.2
	500	80.1～100.2
tea	15.0	83.3～101.3
	100	86.8～108.9
	200	79.1～104.8
soybean	15.0	83.3～104.7
	50.0	75.8～103.2
	1 100	84.0～99.0
beef	5.0	80.6～99.8
	10.0	83.5～106.0
	100	80.5～108.2
pig liver	5.0	77.6～101.8
	20.0	81.5～113.0
	100	77.7～107.2
milk	5.0	81.4～99.4
	20.0	81.5～103.0
	100	78.2～100.8
mushroom	15.0	84.7～104.0
	100	83.1～106.8
	600	82.9～103.1

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Annex A
(informative)
Main Mass Parameters of API 4000[1)]

Instrumental settings:

a) TEM:350 ℃;

b) IS:5 250 V;

c) CA D:34.475 kPa(5 psi);

d) CU R:206.85 kPa(30 psi);

e) GS1:344.75 kPa(50 psi);

f) GS2:413.7 kPa(60 psi);

g) Transitions,DP,CE and retention time see table A.1.

Table A.1—Transition DP,CE and retention time

Analyte	Qualifier ion pair m/z	Quantifier ion pair m/z	DP/V	CE/V	Retention time/ min
Pyriproxyfen	322.2→185.0	322.2→185.0	110	16	1.4
	322.2→227.2			14	

1) Non-commercial statement: the parameters listed in annex A were obtained by API 4 000 mass spectrometer. 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)
MRM chromatogram of Pyriproxyfen

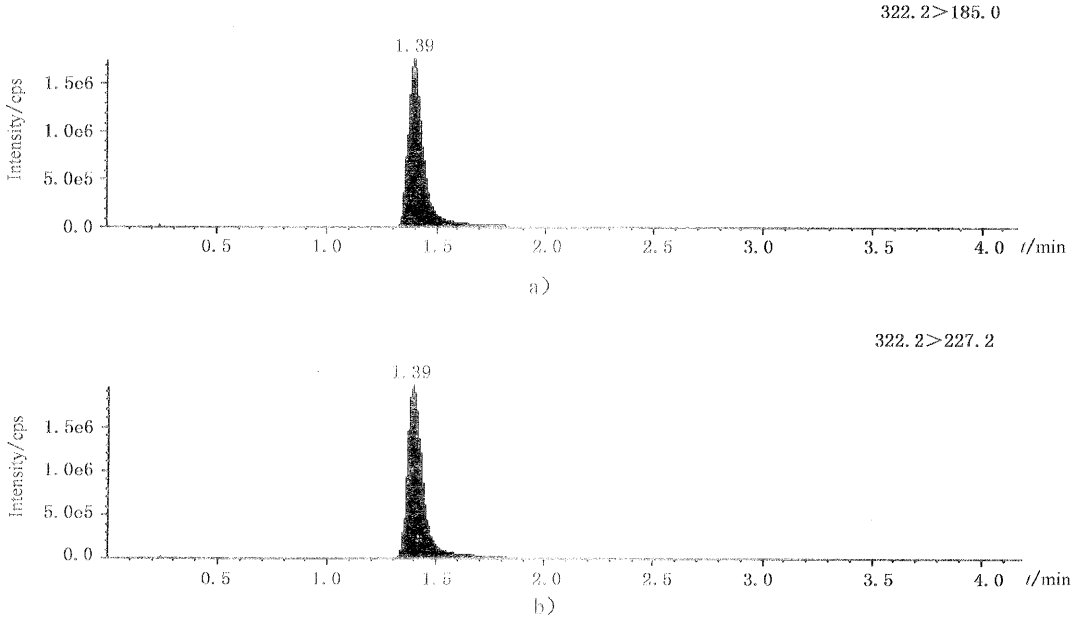


Figure B.1—MRM chromatogram of Pyriproxyfen

SN/T 2623-2010

书号:155066·2-21234
定价: 21.00 元

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