SN

中华人民共和国出入境检验检疫行业标准

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进出口食品中氟虫腈残留量检测方法 气相色谱-质谱法

Determination of fipronil residues in food for import and export—GC-MS method

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前　言

本标准附录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。

本标准主要起草单位：中华人民共和国山东出入境检验检疫局、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：王建华、李杰、黄志强、沈崇钰、蔡发、孙忠松、王敬塘。

本标准系首次发布的出入境检验检疫行业标准。

进出口食品中氟虫腈残留量检测方法 气相色谱-质谱法

1 范围

本标准规定了食品中氟虫腈残留量检测和制样、气相色谱-质谱检测和确证方法。

本标准适用于菠菜、藕、草莓、花生、鸡肉、猪肉、鳕鱼、蜂蜜、板栗、茶叶和酱油中氟虫腈残留量的测定和确证。

2 方法提要

试样经乙腈提取，以正己烷液液分配和初级次级胺(PSA)固相萃取柱净化，用气相色谱-负化学源质谱测定，外标法定量。

3 试剂和材料

除另有规定外，所用试剂均为分析纯，水为二次蒸馏水。

3.1 丙酮：残留级。

3.2 乙腈：残留级。

3.3 正己烷：残留级。

3.4 氯化钠：650℃灼烧 4 h，置入干燥器中冷却，备用。

3.5 丙酮＋正己烷(3＋7)：取 300 mL 丙酮，加入 700 mL 正己烷，摇匀备用。

3.6 氟虫腈标准品(Fipronil，$C_{12}H_4Cl_2F_6N_4OS$，CAS 120067-37-3)：纯度大于等于 96.5%。

3.7 氟虫腈标准溶液的配制：准确称取适量标准品，用少量的丙酮溶解，并以丙酮配制成浓度为 1.0 mg/mL的标准储备液。根据需要再用丙酮＋正己烷(3.5)稀释成适当浓度的标准工作溶液。保存于－18℃冰箱中。

3.8 丙基乙二胺键合硅胶(Primary Secondary amine，PSA)固相萃取柱：500 mg，3 mL 或相当者。

4 仪器与设备

4.1 气相色谱-质谱仪：配有负化学源。

4.2 固相萃取装置。

4.3 均质器。

4.4 旋转蒸发器。

4.5 氮气浓缩仪。

4.6 具塞离心管：50 mL、100 mL。

4.7 浓缩瓶：50 mL、100 mL。

4.8 移液管：1 mL、2 mL、5 mL、10 mL。

5 试样制备与保存

5.1 试样制备

5.1.1 粮谷

取有代表性样品量 500 g，用磨碎机全部磨碎并通过 2.0 mm 圆孔筛。混匀，均分成两份作为试样，

分装入洁净的的容器内，密闭，标明标记。

5.1.2 水果或蔬菜

取有代表性样品500 g，将其可食用部分切碎后，依次用食品捣碎机将样品加工成浆状。混匀，装入洁净的容器内，密闭，标明标记。

5.1.3 肉及肉制品

取有代表性样品500 g，取可食部分经捣碎机充分捣碎均匀，装入洁净容器内作为试样。密闭，标明标记。

5.1.4 蜂蜜及蜂制品

取有代表性样品量500 g，对无结晶的蜂蜜样品将其搅拌均匀；对有结晶析出的蜂蜜样品，在密闭情况下，将样品瓶置于不超过60℃的水浴中温热，振荡，待样品全部融化后搅匀，迅速冷却至室温，在融化时应注意防止水分挥发。装入洁净的容器，密闭，标明标记。

5.1.5 茶叶

取有代表性样品500 g，用磨碎机全部磨碎并通过2.0 mm圆孔筛。混匀，装入洁净的洁净容器内，密闭，标明标记。

5.1.6 坚果

取有代表性样品500 g，用磨碎机全部磨碎并通过20 mm圆孔筛。混匀，均分成两份作为试样，分装入洁净的洁净容器内，密闭，标明标记。

5.1.7 水产品

取有代表性样品500 g，从所取样品中取出约1 kg，取可食部分经捣碎机充分捣碎均匀，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。

5.2 试样保存

粮谷类试样于0℃～4℃保存；水果和蔬菜及其他类试样于－18℃以下冷冻保存。在抽样及制样的操作过程中，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

称取10 g试样(精确至0.1 g)于100 mL具塞离心管中，加入10 mL水，准确加入40 mL乙腈(3.2)，用均质器高速匀浆提取2 min(酱油和蜂蜜仅需剧烈振荡10 min)，再加入5 g氯化钠(3.4)，剧烈振荡10 min，3 000r/ min离心10 min。

6.2 净化

6.2.1 液-液分配净化

取上层提取液20 mL(菠菜、藕、草莓)或10 mL(花生、鸡肉、猪肉、鳕鱼、蜂蜜、板栗、茶叶和酱油)转移至50 mL具塞离心管(4.5)中，加入10 mL正己烷(3.3)，振摇3 min，静置分层，弃去上层正己烷相，再用10 mL正己烷重复操作一次，弃去正己烷相；下层乙腈相收集于100 mL浓缩瓶中，于40℃水浴中浓缩至近干，加入1.0 mL丙酮＋正己烷(3.5)溶解残渣。

6.2.2 固相萃取(SPE)净化

使用前用5 mL丙酮＋正己烷(3.5)预淋PSA柱。将样液(6.2.1)倾入柱中，用10 mL丙酮＋正己烷(3.5)进行洗脱，控制流速小于等于2 mL/min。收集全部洗脱液于50 mL浓缩瓶中，于40℃水浴中浓缩至近干。用丙酮＋正己烷(3.5)溶解并定容至1.0 mL，供气相色谱-质谱仪测定。

6.3 测定

6.3.1 气相色谱-质谱条件

a) 色谱柱：HP-5MS石英毛细管柱，30 m×0.25 mm(内径)，膜厚0.25 μm，或相当者；

b) 色谱柱温度：初始温度为70℃，以30℃/ min程序升温至200℃，保持10 min，再以50℃/min

程序升温至270℃，保持4 min；

c) 进样口温度：250℃；

d) 色谱-质谱接口温度：280℃；

e) 载气：氦气，纯度大于等于99.999%，恒压模式，柱头压1.45 MPa；

f) 进样量：1 μL；

g) 进样方式：无分流进样，0.65 min后开阀；

h) 电离方式：负化学电离；

i) 离子源温度：150℃；

j) 四极杆温度：150℃；

k) 反应气：甲烷，纯度大于等于99.99%；

l) 测定方式：选择离子监测方式；

m) 选择监测离子(m/z)：定量366，定性333、368、400；

n) 溶剂延迟：4.0 min。

6.3.2 气相色谱-质谱检测及确证

根据样液中被测物含量情况，选定浓度相近的标准工作溶液，对标准工作溶液与样液等体积参插进样测定，标准工作溶液和待测样液中氟虫腈的响应值均应在仪器检测的线性范围内。

如果样液与标准工作溶液的选择离子色谱图中，在相同保留时间有色谱峰出现，并且在扣除背景后的样品质谱图中，所选离子均出现，所选择离子的丰度比与标准品对应离子的丰度比，其值在允许范围内(允许范围见表1)。在6.3.1条件下，氟虫腈保留时间是11.1 min，其监测离子(m/z)为m/z 366、333、368、400(其丰度比为100∶27∶70∶35)对其进行确证；根据定量离子m/z 366对其进行外标法定量。在6.3.1条件下，氟虫腈标准物的气相色谱-质谱总离子流色谱图和全扫描质谱图参见附录A中图A.1和附录B中图B.1。

表1 使用定性气相色谱-质谱时相对离子丰度最大容许误差

相对丰度(基峰)/%	>50	>20～50	>10～20	≤10
GC-MS/NCI相对离子丰度最大允许误差/%	±20	±25	±30	±50

6.4 结果计算和表述

用色谱数据处理机或按式(1)计算试样中氟虫腈残留量：

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

式中：

X——试样中氟虫腈残留量，单位为毫克每千克(mg/kg)；

h——样液中氟虫腈的色谱峰高，单位为毫米(mm)；

h_S——标准工作液中氟虫腈的色谱峰高，单位为毫米(mm)；

c——标准工作液中氟虫腈的浓度，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)；

m——最终样液所代表的试样质量，单位为克(g)。

7 测定低限、回收率

7.1 测定低限

本方法的测定低限为0.002 mg/kg。

7.2 回收率

样品的添加浓度及回收率的实验数据见表2。

表 2 样品的添加浓度及回收率的实验数据

样品	添加浓度/(μg/kg)	回收率范围/%	样品	添加浓度/(μg/kg)	回收率范围/%
菠菜	2	90.0～110.0	鳕鱼	2	90.0～110.0
	4	95.0～107.5		4	92.5～107.5
	10	97.0～112.0		10	97.0～119.0
藕	2	95.0～110.0	蜂蜜	2	90.0～110.0
	4	97.5～110.0		4	92.5～107.5
	10	96.0～112.0		10	96.0～112.0
草莓	2	81.5～100.8	板栗	2	95.0～115.0
	4	92.5～107.5		4	92.5～107.5
	10	96.0～112.0		10	95.0～114.0
花生	2	90.0～110.0	茶叶	2	90.0～110.0
	4	90.0～112.5		4	97.5～112.5
	10	94.0～117.0		10	97.0～113.0
鸡肉	2	90.0～110.0	酱油	2	95.0～110.0
	4	95.0～107.5		4	92.5～115.0
	10	94.0～112.0		10	94.0～115.0
猪肉	2	90.0～ 110.0			
	4	92.5～112.5			
	10	94.0～112.0			

附 录 A
（资料性附录）
氟虫腈标准物质的气相色谱-质谱总离子流色谱图

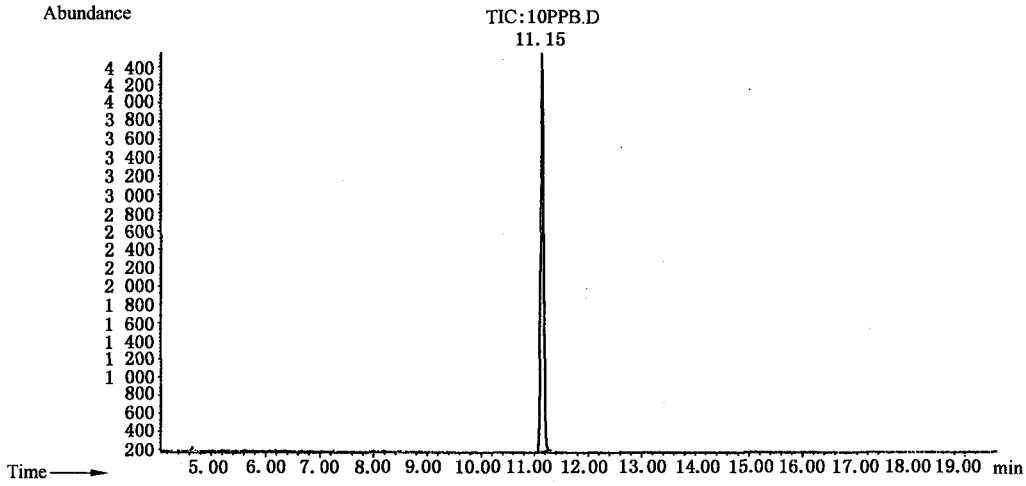

图 A.1 氟虫腈标准物的气相色谱-质谱总离子流色谱图

附 录 B
（资料性附录）
氟虫腈标准物质的气相色谱-质谱图

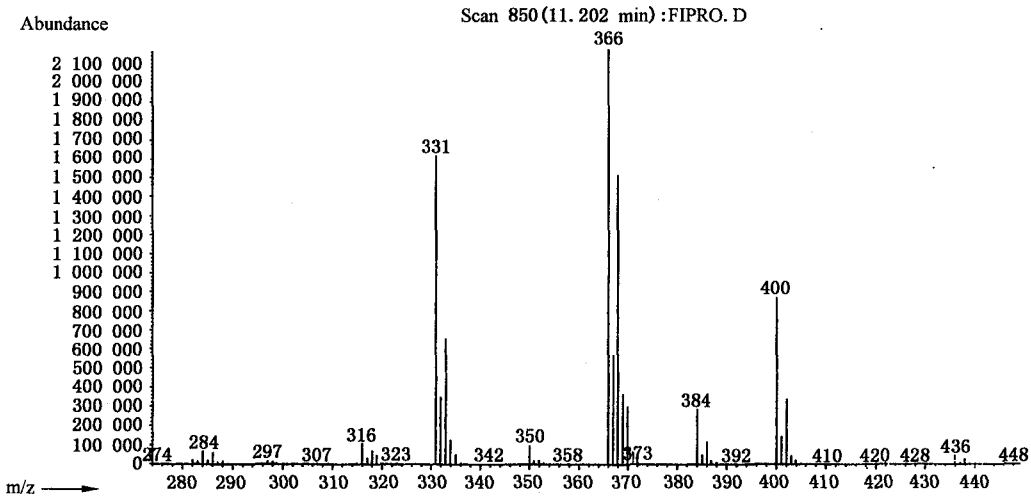

图 B.1 氟虫腈标准物质气相色谱-质谱图

Foreword

Annex A and Annex B of this standard are informative annexes.

This standard was proposed by and is under the charge of the Certification and Accreditation Administration of the People's Republic of China.

This standard was drafted by the Shandong Entry-Exit Inspection and Quarantine Bureau、Hunan Entry-Exit Inspection and Quarantine Bureau and Jiangsu Extry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

This main drafters of this standard are: Wang Jianhua, Li Jie, Huang Zhi qiang, Shen Cheng yu, Caifa Sun zhongsong Wang Jingtang

This standard is a professional one which is promulgated for the first time.

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

Determination of fipronil residues in food for import and export—GC-MS method

1 Scope

This standard specifies the method of sample preparation and determination of fipronil residue in foodstuffs by gas chromatography-mass spectrometry.

This standard is applicable to the determination and confirmation of fipronil residue in spinach, lotus, strawberry, peanut, chicken, pork, ling, honey, chestnut, tea and soy.

2 Principle

The test sample is extracted with acetonitrile, then the extract is partitioned with *n*-hexane before cleaning up procedure by passing through a PSA solid phase extraction (SPE) column. The determined by GC-NCI-MS, and quantitated by external standard method.

3 Reagents and materials

All the reagents used should be analytically pure unless otherwise specified. "Water" is redistilled water.

3.1 Acetone: residual grade.

3.2 Acetonitrile: residual grade.

3.3 *n*-Hexane. residual grade.

3.4 Sodium chloride: heated at 650℃ for 4 h, and stored in a sealed container.

3.5 Acetone-*n*-Hexane (3+7): Dilute 300 mL acetone with *n*-Hexane to the volume of 1 000 mL.

3.6 Fipronil standard (Fipronil, $C_{12}H_4Cl_2F_6N_4OS$, CAS 120067-37-3): Purity≥96.5%.

3.7 Standard stock solution: Accurately weigh appropriate amount of fipronil standard and dissolve with a little volume of acetone followed by a further dilution to the final concentration of 1.0 mg/mL. Then dilute the standard stock solution with acetone to make standard working solution. of required con-

centration and stored at −18 ℃.

3.8 SPE column:500 mg,3 mL or equivalent.

4 Apparatus and equipment

4.1 Gas chromatography equipped with negative chemical ionization mass spectrometry.

4.2 SPE-12G Column Processor.

4.3 Homogenizer.

4.4 Rotary vacuum evaporator.

4.5 Nitrogen evaporator.

4.6 Centrifuge tube:50 mL,100 mL with stopper.

4.7 Concentrating bottle:50 mL,100 mL.

4.8 Graduated pipettes:1 mL,2 mL,5 mL,10 mL.

5 Preparation and storage of test sample

5.1 Preparation of test sample

5.1.1 Cereals

Take approximately 500 g of representative sample. Grind with a grinder to pass through a 2.0 mm round-hole sieve. Mix thoroughly and divide into two equal portions. Each portion is placed into a clean container,as test sample,sealed and labelled.

5.1.2 Fruits and vegetables

Take approximately 500 g of representative sample(without wash by water). The edible parts are blended and homogenized in a high speed blender. Divide into two equal portions. Each portion is placed into a clean container as test sample,sealed and labelled.

5.1.3 Meat and meat product

Take approximately 500 g of representative sample. The edible parts are blended and homogenized in

a high speed blender. Divide into two equal portions. Each portion is placed into a clean container as the test sample, sealed and labeled.

5.1.4 Honey and honey product

Take approximately 500 g of representative sample. Homoenize the non- crystallization honey and the crystallized honey in the sample bottle should be warmed under water bath at the temperature of no more than 60℃. Shake the bottel until the sample is completely dissolved. Sample should be homogenized and cooled down to room temperature rapidly. Do prevent the evaporating of water during the heating procedure. Then place into a clean container as the test sample, sealed and labeled.

5.1.5 Tea

Take approximately 500 g of representative sample. Grind and pass through a 2.0 mm round-hole sieve. Mix thoroughly and place into a clean container as the test sample, sealed and labeled.

5.1.6 Nut

Take approximately 500 g of representative sample, Grind and pass through a 2.0 mm round-hole sieve. Mix thoroughly and place into a clean container as the test sample, sealed and labeled.

5.1.7 Aquatic product

Take approximately 500 g of representative sample. The edible parts are blended and homogenized in a high speed blender. Divide into two equal portions. Each portion is placed into a clean container as the test sample, sealed and labeled.

5.2 Storage of test sample

The test samples of cereals and oil seeds should be stored at the range of 0℃～4℃. The test samples of fruits and vegetables should be stored below －18℃. During sampling and sample preparation, precaution should be taken to avoid contamination or any factors which may cause the change of residue content.

6 Procedure

6.1 Extraction

Weigh ca. 10 g of the test sample into a 100 mL centrifuge tube equipped with a stopper. And accurately add 10 mL of water, 40mL acetonitrile(3.2) into the flask. Extract for 2 min in a high speed homogenizer(shake for 10 min for sauce and honey only). Add 5 g Sodium chloride(3.4), shake for 10 min, centrifuge for 10 min at 3 000 r/min.

6.2 Cleaning up

6.2.1 Liquid/liquid partition

Transfer the 20 mL (spinach, lotus, strawberry) or 10 mL (peanut, chicken, pork, ling, honey, chestnut, tea and sauce) of supematant into a 50 mL centrifuge tube(4.5), add 10 mL of hexane, shake for 3 min and place aside for separation. Discard the hexane phase. Repeat above procedure and condense acetonitrile to nearly dryness by a rotary evaporator at 40 ℃. Dissolve the residue with 1.0 mL of acetone-hexane (3.5) for SPE purification.

6.2.2 SPE cleaning up

Rinse a PSA column with 5 mL of acetone-hexane (3.5). Load the above solution to column. Elute the column with 10 mL of acetone-hexane (3.5), and flow rate below 2 mL/min. all eluates are transfered into a 50 mL concentrate bottle and evaporated to dryness by a rotary evaporator at 40 ℃. Dissolve the residue with acetone-hexane(3.5) to exact volume of 1.0 mL for determination by GC-MS.

6.3 Determination

6.3.1 GC-MS operating condition

a) Chromatographic column: HP-5MS silica capillary column 30 m × 0.25 mm (i.d.), 0.25 μm film thickness, and or equivalent;

b) Column temperature: 70℃, ramp at 30℃/min to 200℃, hold for 10 min, and then increase at 50℃/min to 270℃, hold for 4 min;

c) Injector temperature: 250℃;

d) Interface temperature: 280℃;

e) Carrier gas: Helium, purity≥99.999 %, constant pressure mode: 1.45 MPa;

f) Injection volume: 1 μL;

g) Injection mode Splitless, open the valve after 0.65 min;

h) Electrical ionization mode: NCI;

i) Quadropole temperature: 150℃;

j) Ion source temperature: 150℃;

k) Methane ≥99.99%;

l) Selected ion monitoring mode;

m) Monitoring ions (m/z):quantitied by 366,qualified by 333,368,400;

n) Solvent delay:4.0 min.

6.3.2 Quantitation and qualification by GC-MS

Select appropriate standard working solution with similar concentration level to that in sample solution,The standard working solution should be injected before and between the injections of the sample solutions with same injection volume. The response value of fipronil in the standard working solution and sample solution should be within the linear range of the instrumental detection.

Permitted tolerance for similarity of relative abundance ratio is listed as table 1. When retention time of target peak is according with that of standard solution,postitive samle will be proved based on selected monitoring ions (m/z) 366,333,368,400 (relative abundance ratio:100 : 27 : 70 : 35),with (m/z) 366 for quantitation. Under chromatographic condition above(6.3.1),rention time of fipronil standard is 11.1 min,GC-MS selected ion chromatogram and mass spectrum of the fipronil standard are shown respectively as figure A.1 in annex A and figure B.1 in annex B.

Table 1—Maximum permitted tolerance for relative ion intensities using a range of mass spectrometric techniques

Relative intensity(base peak)/%	>50	>20~50	>10~20	≤10
GC-MS/NCI(relative)/%	±20	±25	±30	±50

6.4 Calculation and expression of the result

Calculate the content of fipronil residue in the test sample by GC-MS data processor or according to the followed formula(1):

$$X = \frac{h \times c \times V}{h_s \times m} \quad \cdots\cdots(1)$$

where

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

h—the peak height of fipronil in the sample solution,mm;

h_s—the peak height of fipronil in the standard working solution,mm;

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

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

m—the corresponding mass of the test sample representing the final sample solution, g.

7 Limit of determination and recovery

7.1 Limit of determination

The limit of determination of this method is 0.002 mg/kg.

7.2 Recovery

The experimental data of the concentrations of fipronil in the fortified sample and its corresponding recoveries are: seen in table 2.

Table 2—The experimental data of the concentrations of fipronil in the fortified sample and its corresponding recoveries

sample	Added concentrations/ (μg/kg)	Recovery range/ %	sample	Added concentrations/ (μg/kg)	Recovery range/ %
spinach	2	90.0～110.0	ling	2	90.0～110.0
	4	95.0～107.5		4	92.5～107.5
	10	97.0～112.0		10	97.0～119.0
lotus	2	95.0～110.0	honey	2	90.0～110.0
	4	97.5～110.0		4	92.5～107.5
	10	96.0～112.0		10	96.0～112.0
strawberry	2	81.5～100.8	chestnut	2	95.0～115.0
	4	92.5～107.5		4	92.5～107.5
	10	96.0～112.0		10	95.0～114.0
peanut	2	90.0～110.0	tea	2	90.0～110.0
	4	90.0～112.5		4	97.5～112.5
	10	94.0～117.0		10	97.0～113.0
chicken	2	90.0～110.0	soy	2	95.0～110.0
	4	95.0～107.5		4	92.5～115.0
	10	94.0～112.0		10	94.0～115.0
pork	2	90.0～ 110.0			
	4	92.5～112.5			
	10	94.0～112.0			

Annex A
(informative)
GC-MS total ion chromatogram of the fipronil standard

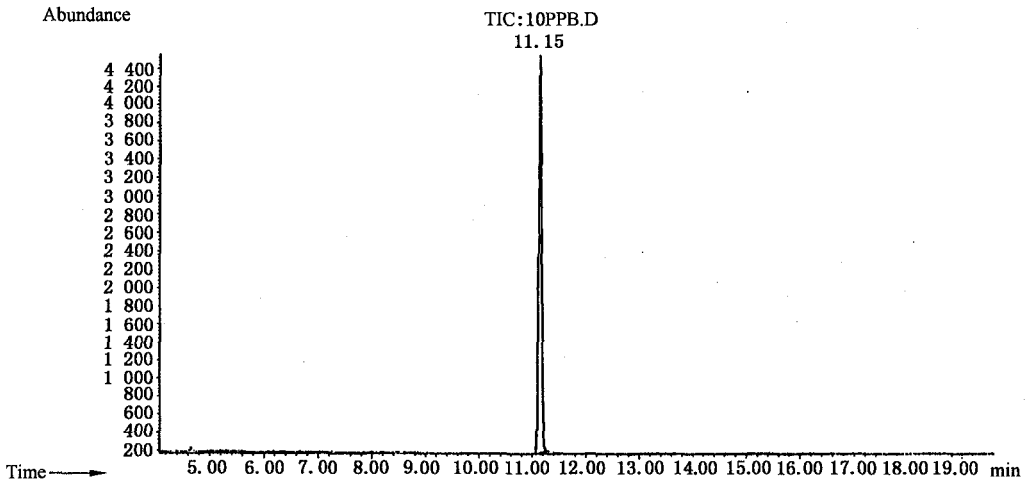

Figure A. 1—GC-MS total ion chromatogram of the fipronil standard

Annex B
(informative)
Mass spectrum of the fipronil standard

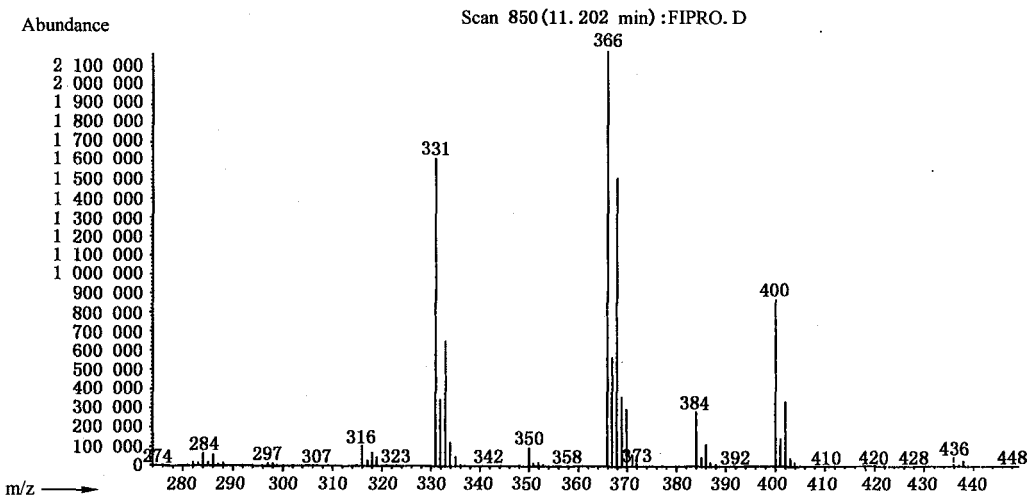

Figure B. 1—Mass spectrum of the fipronil standard