SN

中华人民共和国出入境检验检疫行业标准

SN/T 1740—2006

进出口食品中四螨嗪残留量的检测方法 气相色谱串联质谱法

Determination of clofentezine residues in foods for import and export—Gas chromatography mass spectrometry 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委员会提出并归口。

本标准起草单位：中华人民共和国吉林出入境检验检疫局。

本标准主要起草人：王明泰、牟峻、刘志研、宋立国、李爱军、孟杰。

本标准系首次发布的出入境检验检疫行业标准。

进出口食品中四螨嗪残留量的检测方法 气相色谱串联质谱法

1 范围

本标准规定了进出口食品中四螨嗪残留量的气相色谱-质谱检测方法。

本标准适用于进出口柑桔、苹果、菠菜、西兰花、牛肝、鸡肾中四螨嗪残留量的测定和确证。

2 原理

试样用水+丙酮(1+4)振荡提取，经二氯甲烷液-液分配，以凝胶色谱柱净化，再经氟罗里硅土固相柱净化，洗脱液浓缩并溶解定容后，供气相色谱-质谱仪检测，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。

3.2 二氯甲烷。

3.3 环己烷。

3.4 乙酸乙酯。

3.5 正己烷。

3.6 无水硫酸钠：650℃灼烧 4 h，贮于密封容器中备用。

3.7 硫酸钠水溶液：20 g/L。

3.8 氟罗里硅土固相萃取柱：1.0 g，或相当者。

3.9 四螨嗪标准品(Clofentezine，$C_{14}H_8Cl_2N_4$，74115-24-5)：纯度≥99%。

3.10 标准储备溶液：准确称取适量的四螨嗪标准品，用丙酮配制成浓度为 100 μg/mL 的标准储备溶液。

3.11 标准工作溶液：根据需要再用丙酮稀释成适用浓度的标准工作溶液。

4 仪器与设备

4.1 气相色谱-质谱仪：配有质量选择检测器。

4.2 凝胶色谱仪：配有单元泵和馏分收集器。

4.3 振荡器。

4.4 旋转蒸发器。

4.5 无水硫酸钠柱：7.5 cm×1.5 cm(内径)，内装 5 cm 高无水硫酸钠。

4.6 具塞锥形瓶：250 mL。

4.7 分液漏斗：250 mL。

4.8 浓缩瓶：50 mL、250 mL。

4.9 滤膜：0.45 μm。

5 试样制备与保存

5.1 试样制备

5.1.1 水果或蔬菜类

抽取水果或蔬菜样品500 g，或去壳、去籽、去皮、去茎、去根、去冠(不可用水洗涤)，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，均分成两份作为试样，分装入洁净的盛样袋内，密闭，标明标记。

5.1.2 肉及肉制品类

从所取全部样品中取出有代表性样品约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

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

称取试样约20 g(精确至0.1 g)于250 mL具塞锥形瓶中，加入100 mL水+丙酮(1+4)，振荡提取30 min，将提取液抽滤于250 mL浓缩瓶中。残渣再用50 mL丙酮重复提取一次，合并滤液，于40℃水浴中旋转浓缩至约20 mL。

将浓缩提取液转移至250 mL分液漏斗中，加入150 mL硫酸钠水溶液和50 mL二氯甲烷，振摇3 min，静置分层，收集二氯甲烷相。水相再用2×50 mL二氯甲烷重复提取两次，合并二氯甲烷相。经无水硫酸钠柱脱水，收集于250 mL浓缩瓶中，于40℃水浴中旋转浓缩至近干，加入5 mL环己烷+乙酸乙酯(1+1)溶解残渣，并用0.45 μm滤膜过滤，待净化。

6.2 净化

6.2.1 凝胶色谱净化(GPC)

6.2.1.1 凝胶色谱条件

a) 凝胶净化柱：700 mm×25 mm，Bio Beads S-X3，或相当者；
b) 流动相：环己烷+乙酸乙酯(1+1)；
c) 流速：5.0 mL/min；
d) 样品定量环：5.0 mL；
e) 预淋洗体积：50 mL；
f) 洗脱体积：150 mL；
g) 收集体积：95 mL～135 mL。

6.2.1.2 凝胶色谱净化步骤

将5 mL待净化液按6.2.1.1规定的条件进行净化，合并馏分收集器中的收集液于50 mL浓缩瓶中，于40℃水浴中旋转浓缩至近干，加入2 mL正己烷+乙酸乙酯(4+1)以溶解残渣，待净化。

6.2.2 固相萃取净化(SPE)

使用前用5 mL正己烷预淋洗氟罗里硅土固相萃取柱，将样液倾入柱中，然后用8 mL正己烷+乙酸乙酯(4+1)进行洗脱。收集全部洗脱液于50 mL浓缩瓶中，于40℃水浴中旋转浓缩至干。用丙酮溶解并定容至2 mL，供气相色谱-质谱测定和确证。

6.3 测定

6.3.1 气相色谱-质谱条件

a) 色谱柱：30 m×0.25 mm(内径)，膜厚0.25 μm，DB-5 MS石英毛细管柱，或相当者；
b) 色谱柱温度：50℃(2 min)$\xrightarrow{30℃/min}$180℃(1 min)$\xrightarrow{10℃/min}$270℃(10 min)；
c) 进样口温度：280℃；

d) 色谱-质谱接口温度：270℃；

e) 载气：氦气，纯度≥99.999%，流速 1.2 mL/min；

f) 进样量：1 μL；

g) 进样方式：无分流进样，1.5 min 后开阀；

h) 电离方式：EI；

i) 电离能量：70 eV；

j) 测定方式：选择离子监测方式；

k) 选择监测离子(m/z)：定量 304，定性 102，138，304，306；

l) 溶剂延迟：5 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四螨嗪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，则根据选择离子 m/z102，138，304，306(其丰度比 82：100：98：60)对其进行确证；根据选择离子 m/z304 对其进行外标法定量。在上述气相色谱-质谱条件下，四螨嗪标准物的参考保留时间约为 16.1 min，四螨嗪标准物的气相色谱-质谱选择离子色谱图和质谱图见附录 A 中图 A.1 和附录 B 中图 B.1。

6.4 结果计算和表述

用色谱数据处理机或按式(1)计算试样中四螨嗪残留量：

$$X = \frac{A \cdot c \cdot V}{A_S \cdot m} \qquad \cdots\cdots(1)$$

式中：

X——试样中四螨嗪残留量，单位为毫克每千克(mg/kg)；

A——样液中四螨嗪的色谱峰高，单位为毫米(mm)；

A_S——标准工作液中四螨嗪的色谱峰高，单位为毫米(mm)；

c——标准工作液中四螨嗪的浓度，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 0.05 mg/kg。

7.2 回收率

7.2.1 柑桔中四螨嗪的添加浓度及其回收率实验数据：

在 0.010 mg/kg～0.200 mg/kg 时，回收率为 83.8%～91.2%。

7.2.2 苹果中四螨嗪的添加浓度及其回收率实验数据：

在 0.010 mg/kg～0.200 mg/kg 时，回收率为 87.0%～91.6%。

7.2.3 菠菜中四螨嗪的添加浓度及其回收率实验数据：

在 0.010 mg/kg～0.200 mg/kg 时，回收率为 80.8%～94.8%。

7.2.4 西兰花中四螨嗪的添加浓度及其回收率实验数据：

在 0.010 mg/kg～0.200 mg/kg 时，回收率为 90.5%～91.2%。

7.2.5 牛肝中四螨嗪的添加浓度及其回收率实验数据：

在 0.010 mg/kg～0.200 mg/kg 时，回收率为 83.8%～95.0%。

7.2.6 鸡肾中四螨嗪的添加浓度及其回收率实验数据：

在 0.010 mg/kg～0.200 mg/kg 时，回收率为 87.5%～97.2%。

附 录 A
（资料性附录）
四螨嗪标准物的气相色谱-质谱选择离子色谱图

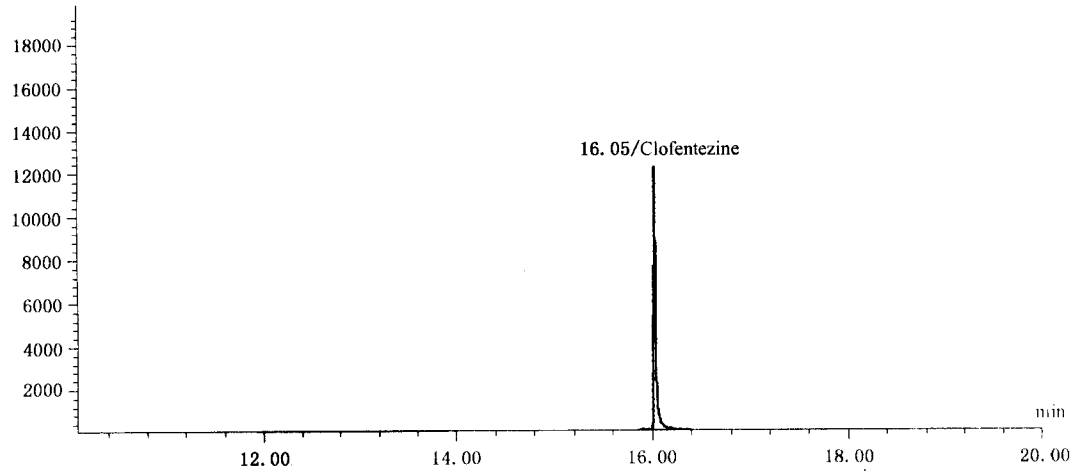

图 A.1 四螨嗪标准物的气相色谱-质谱选择离子色谱图

附 录 B
（资料性附录）
四螨嗪标准物的气相色谱-质谱图

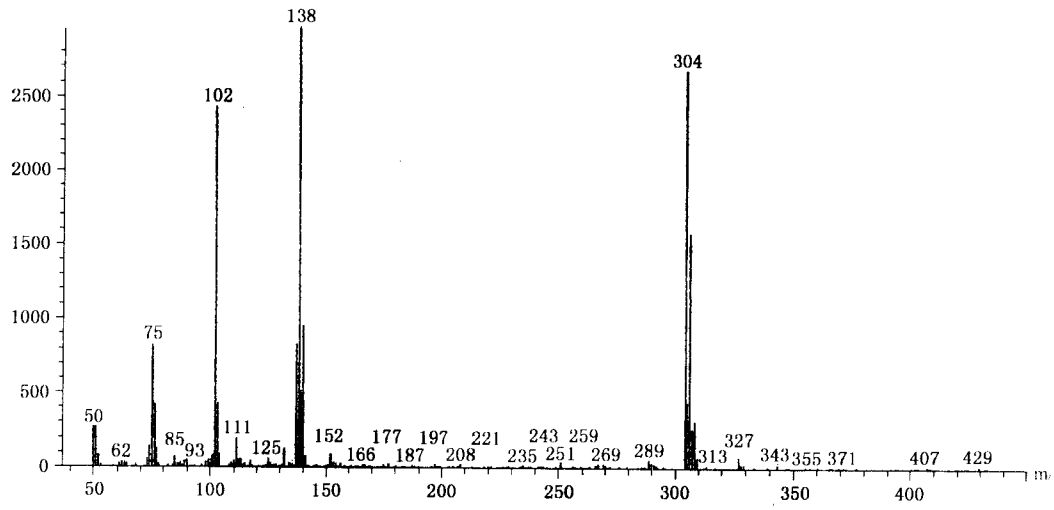

图 B.1 四螨嗪标准物的气相色谱-质谱图

Foreword

Annex A and Annex B of this standard is an informative annex.

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

This main drafter of this standard is Wang Mingtai, Mu Jun, Liu Zhiyang, Song Liguo. Li Aijun, Meng Jie.

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

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

Determination of clofentezine residues in foods for import and export— Gas chromatography mass spectrometry method

1 Scope

This standard specifies the determination and confirmation of clofentezine residues by gas chromatography-mass spectrometry in foods for import and export.

This standard is applicable to the determination and confirmation of residue content of clofentezine in orange, apple, spinach, kidney beans, cattle liver and chicken kidney for import and export.

2 Principle

The test sample are extracted with water-acetone (1 + 4), the extract is partitioned with dichloromethane. Cleaned up by passing through a on GPC and SPE of Florisil column. The elutes solution is evaporated and made up to a definite volume. Determination and confirmation is made by GC-MS, using external standard method.

3 Reagents and materials

Unless otherwise specified, all the reagents used should be analytically pure, "water" is distilled water.

3.1 Acetone.

3.2 Dichloromethane.

3.3 Cyclohexane.

3.4 Ethyl acetate.

3.5 *n*-Hexane.

3.6 Anhydrous sodium sulfate: Ignite at 650℃ for 4 h, and keep in a tightly closed container.

3.7 Sodium sulfate aqueous solution: 20 g/L.

3.8 Florisil SPE column: 1.0 g, or equivalent.

3.9 Clofentezine standard ($C_{14}H_8Cl_2N_4$, 74115-24-5): Purity≥99%.

3.10 Standard stock solution: Accurately weigh an adequate amount of clofentezine standard and dissolve in a small volume of acetone. Dilute with acetone to form a standard stock solution of 100 μg/mL in concentration.

3.11 Standard working solution: Then dilute the standard stock solution with acetone to the required concentration as the standard working solution.

4 Apparatus and equipment

4.1 Gas chromatograph equipped with mass selective detector (MSD).

4.2 Gel permeation chromatograph equipped with isocratic pump and fraction collector.

4.3 Shaker.

4.4 Rotary vacuum evaporator.

4.5 Column of anhydrous sodium sulfate: 7.5 cm × 1.5 cm (i.d.), packed with 5 cm height of anhydrous sodium sulfate.

4.6 Conical flask: 250 mL, with stopper.

4.7 Separator funnel: 250 mL.

4.8 Concentrate bottle: 50 mL, 250 mL.

4.9 Membrane filter: 0.45 μm.

5 Preparation and storage of test sample

5.1 Preparation of test sample

5.1.1 Fruits and vegetables

The combined primary sample is reduced to ca 500 g, which has been removed shell, seed, peel, stem, root, coronal (do not wash by water), then cut up the edible portions are blended and homoge-

nized thoroughly in a high speed blender (4.1.3), and then divided into two equal portions. Each portion is placed in a clean container as the test sample, which is then sealed and labeled.

5.1.2 Meats and meat products

The mixed primary sample is reduced to 1 kg. The eatable potions are thoroughly ground and homogenized in a meat grinder. Then divide into two equal portions, each portion is placed in a clean container as the test sample, which is sealed, labeled.

5.2 Storage of test sample

The test samples should be stored below −18℃. 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.

6 Procedure

6.1 Extraction

Weigh ca 20 g (accurate to 0.1 g) of the test sample into a 250 mL conical flask with stopper, add 100 mL of water-acetone(1+4), extract for 30 min on a high shaker. Filter the extract into a 250 mL concentrate bottle. Extract the residue with 50 mL of acetone once more, filter and combine the washings in the same concentrate bottle, evaporate to 20 mL in a rotary evaporator with a bath temperature below 40℃.

Transfer the concentrated solution into a 250 mL separator funnel, add 150 mL of sodium sulfate aqueous solution and 50 mL of dichloromethane, shake for 3 min and set aside for separating. Collect the dichloromethane phase. The water phase is again extracted with 2×50 mL of dichloromethane. Combined the dichloromethane phases, and let pass through a column of anhydrous sodium sulfate to remove the water. Collect the effluent in a 250 mL concentrate bottle and evaporate to near dryness in a rotary evaporator with a bath temperature below 40℃. Dissolve the residue with 5 mL of cyclohexane-ethylacetate(1+1).

6.2 Clean up

6.2.1 GPC Clean up

6.2.1.1 GPC operating condition

a) GPC column: 700 mm×25 mm(i.d.), Bio Beads S-X3 or equivalent;
b) Mobile phase: Cyclohexane-ethyl acetate (1+1);
c) Flow rate: 5 mL/min;

d) Injection volume:5 mL;
e) Rinse the column volume:50 mL;
f) Elute the column volume:150 mL;
g) Collect the eluate volume:95 mL～135 mL.

6.2.1.2 GPC clean up operating

Transfer the above solution into an GPC column, proceed as section 6.2.1.1. Combined the eluates in the 50-mL pear-shaped bottle, evaporate to dryness in a rotary evaporator with a bath temperature below 40℃. Dissolve the residue with 2 mL of *n*-hexane-ethyl acetate (4+1).

6.2.2 SPE Clean up

Rinse the Florisil column with 5 mL of *n*-hexane before use, transfer the above solution into an Florisil column. Then elute with 8 mL of *n*-hexane-ethyl acetate (4+1), collect all the eluates in a 50 mL concentrate bottle and evaporate to dryness in a rotary evaporator with a bath temperature below 40℃. Dissolve the residue and dilute exactly to 2 mL with acetone for GC-MS determination and confirmation.

6.3 Determination

6.3.1 GC-MS operating condition

a) Chromatographic column:30 m×0.25 mm(i.d.),0.25 μm film thickness, DB-5 MS, silica capillary column or equivalent;
b) Column temperature:50℃(2 min) $\xrightarrow{30℃/min}$ 180℃(1 min) $\xrightarrow{10℃/min}$ 270℃(10 min);
c) Injection port temperature:280℃;
d) Interface temperature:270℃;
e) Carrier gas:Helium, purity≥99.999%, flow rate 1.2 mL/min;
f) Injection volume:1 μL;
g) Injection mode:Splitless, purge on after 1.5 min;
h) Electron ionization mode:EI;
i) Ionization energy:70 eV;
j) Determination mode:SIM mode;
k) Selected monitoring ion(m/z):Determined by 304, confirmed by 102, 138, 304, 306;
l) Solvent protection delay:5 min.

6.3.2 GC-MS determination and confirmation

According to the approximate concentration of the pesticide in the sample solution, select the standard working solution with similar peak height to that of the sample solution. The standard working solution should be randomly injected in-between the injections of the sample solution of equal vol-

ume. The responses of clofentezine in the standard working solution and sample solution should be within the linear range of the instrumental detection.

If there is any peak of sample solution appeared at the same retention time as such peak of the standard solution, it must be confirmed by selected monitoring ions(m/z)102, 138, 304, 306 (abundance ratio is ca 82 : 100 : 98 : 60), using external standard method with(m/z)304. Under the above chromatographic condition, the retention time of clofentezine is ca 16. 1 min. For GC-MS chromatogram (TIC) of the standard and GC-MS spectrum, see Figure A. 1 in annex A and Figure B. 1 in annex B.

6.4 Calculation and expression of the result

Calculate the content of clofentezine residues in the test sample by GC-MS data processor or according to the formula(1).

$$X = \frac{A \cdot c \cdot V}{A_S \cdot m} \qquad \cdots\cdots(1)$$

where

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

A the peak height of clofentezine in the sample solution, mm;

A_S the peak height of clofentezine in the standard working solution, mm;

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

V the final volume of the sample solution, mL;

m the corresponding mass of the test sample in 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. 010 mg/kg.

7.2 Recovery

7.2.1 According to the experimental data, the fortifying concentrations of clofentezine in orange and its corresponding recoveries are:
0. 010 mg/kg～0. 200 mg/kg, the recovery 83. 8%～91. 2%.

7.2.2 According to the experimental data, the fortifying concentrations of clofentezine in apple and its corresponding recoveries are:
0. 010 mg/kg～0. 200 mg/kg, the recovery 87. 8%～91. 6%.

7.2.3 According to the experimental data, the fortifying concentration of clofentezine in spinach and its corresponding recoveries are:
0. 010 mg/kg～0. 200 mg/kg, the recovery 80. 8%～94. 8%.

7.2.4 According to the experimental data, the fortifying concentrations of clofentezine in kidney beans and its corresponding recoveries are:
0.010 mg/kg～0.200 mg/kg, the recovery 90.5%～91.2%.

7.2.5 According to the experimental data, the fortifying concentration of clofentezine in cattle liver and its corresponding recoveries are:
0.010 mg/kg～0.200 mg/kg, the recovery 83.8%～95.0%.

7.2.6 According to the experimental data, the fortifying concentrations of clofentezine in chicken kidney and its corresponding recoveries are:
0.010 mg/kg～0.200 mg/kg, the recovery 87.5% ～97.2%.

Annex A
(informative)
GC-MS chromatogram (TIC) of the clofentezine standard

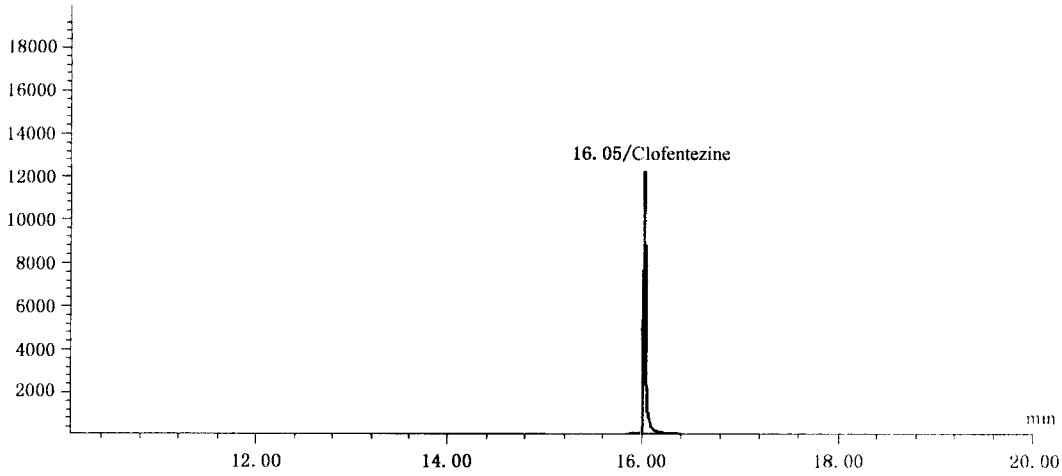

Figure A.1 GC-MS chromatogram (TIC) of the clofentezine standard

Annex B

(informative)

Gas chromatogram and mass spectrum of clofentezine standard

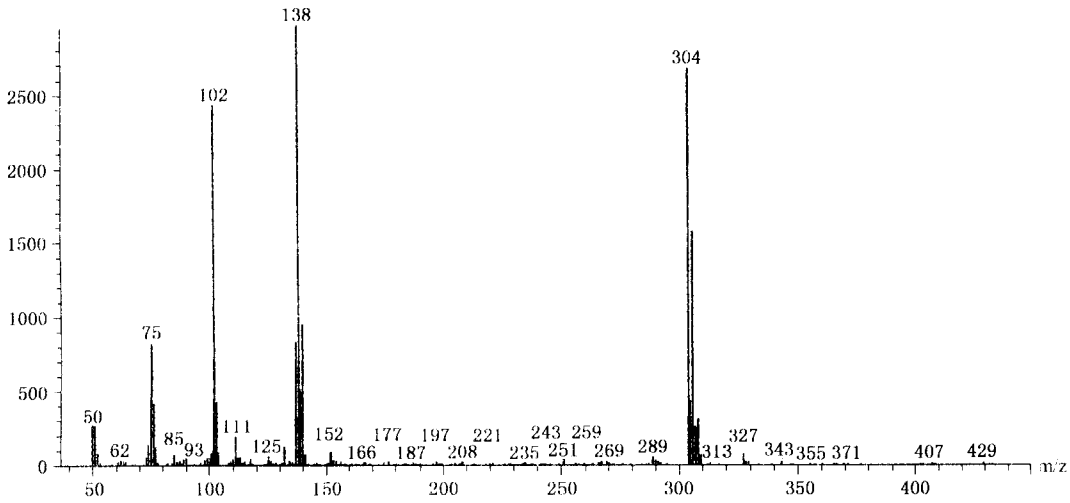

Figure B. 1 Gas chromatogram and mass spectrum of clofentezine standard

中华人民共和国出入境检验检疫
行 业 标 准
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气相色谱串联质谱法
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