SN

中华人民共和国出入境检验检疫行业标准

SN/T 2232—2008
代替 SN/T 0644—1997

进出口食品中三唑醇残留量的检测方法 气相色谱-质谱法

Determination of triadimenol residue in foods for import and export—GC-MS method

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前　言

本标准代替 SN/T 0644—1997《出口粮谷中三唑醇残留量检验方法》。

本标准与 SN/T 0644—1997 相比，主要变化如下：

——扩大标准检测的适用范围；

——改进了样品前处理技术；

——用气相色谱-质谱法替代气相色谱法，并改用负化学电离(GC-MS/NCI)技术确证。

本标准的附录 A 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。

本标准起草单位：中华人民共和国江苏出入境检验检疫局、中华人民共和国上海出入境检验检疫局、中华人民共和国吉林出入境检验检疫局、中华人民共和国内蒙古出入境检验检疫局。

本标准主要起草人：沈伟健、陈惠兰、赵增运、邓晓军、王明泰、李刚、杨雯筌、李丽花、余可垚、沈崇钰。

本标准所代替标准历次版本发布情况为：

——SN/T 0644—1997。

进出口食品中三唑醇残留量的检测方法 气相色谱-质谱法

1 范围

本标准规定了食品中三唑醇残留量的气相色谱-质谱检测方法。

本标准适用于荷兰豆、什锦蔬菜、橙、大豆、木薯干、乌龙茶、大米、牛肉、鲳鱼、蜂王浆、龙虾仁和蜂蜜等食品中三唑醇残留量的测定和确证。

2 方法提要

试样经正己烷饱和过的乙腈(含1%冰醋酸)提取,分散固相萃取净化,气相色谱-负化学离子源质谱法进行测定与确证,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,水为去离子水。

3.1 乙腈:色谱纯。

3.2 正己烷:色谱纯。

3.3 丙酮:色谱纯。

3.4 冰醋酸。

3.5 无水硫酸镁。

3.6 无水乙酸钠。

3.7 提取溶剂(含1%冰醋酸的经正己烷饱和过的乙腈溶液):加10 mL冰醋酸到990 mL的乙腈(事先用正己烷饱和过)。

3.8 三唑醇标准物质(Triadimenol,$C_{14}H_{18}ClN_3O_2$,CAS号:55219-65-3):纯度大于等于98.0%。

3.9 三唑醇标准储备溶液:准确称取适量的三唑醇标准品,用乙腈稀释配制成200 μg/mL的标准储备液,4 ℃下保存(有效期为6个月)。

3.10 三唑醇标准工作液:根据需要用丙酮稀释成适当浓度的标准工作溶液,4 ℃下保存(有效期为3个月)。

3.11 石墨化碳黑填料:40 μm。

3.12 PSA(N-2 氨乙基)填料:40 μm。

3.13 C_{18}填料:40 μm。

3.14 微孔滤膜:0.45 μm,有机相。

4 仪器和设备

4.1 气相色谱-质谱仪:配置负化学离子源(NCI)。

4.2 分析天平:感量为0.1 mg和0.01 g。

4.3 旋转蒸发器。

4.4 组织捣碎机。

4.5 粉碎机。

4.6 均质器。

4.7 振荡器。

5 试样制备与保存

5.1 试样制备

5.1.1 荷兰豆、什锦蔬菜和橙

取代表性样品 500 g，将其可食用部分切碎后（不可用水洗涤），依次用捣碎机将样品加工成浆状。混匀，均分成两份作为试样，分装入容器内，密闭并标明标记。

5.1.2 乌龙茶、木薯干、大米和大豆

取代表性样品 500 g，用粉碎机粉碎并通过 2.0 mm 圆孔筛。混匀，分装入容器内，密闭并标明标记。

5.1.3 牛肉、鲳鱼或龙虾仁

取代表性样品 500 g，将其切碎后，依次用绞碎机将样品绞碎，混匀，分装入容器内，密封并标明标记。

5.1.4 蜂王浆和蜂蜜

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。制备好的试样均分成两份，分别装入样品瓶中，密封，并标明标记。

5.2 试样的保存

大豆、木薯干、乌龙茶、大米、蜂蜜等试样于 0 ℃～4 ℃保存；荷兰豆、什锦蔬菜、橙、牛肉、鲳鱼、龙虾仁和蜂王浆等试样于－18 ℃以下冷冻保存。

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 称样

6.1.1 大豆、木薯干、牛肉、鲳鱼、龙虾仁和蜂王浆等试样

称取 5 g 试样（精确至 0.01 g）。

6.1.2 荷兰豆、什锦蔬菜和橙等试样

称取 10 g 试样（精确至 0.01 g）。

6.1.3 米、茶叶和蜂蜜等试样

称取 5 g 试样（精确至 0.01 g），并加入 4 mL 水，混匀，浸泡或溶解。

茶叶需浸泡 30 min。

6.2 提取

将称取的样品置于 250 mL 的具塞锥形瓶中，加入 50 mL 提取溶剂（3.7），加入 15 g 无水硫酸镁（3.5）和 6 g 无水醋酸钠（3.6），振荡提取 40 min（牛肉、鲳鱼、龙虾仁等肉制品应均质提取 5min），过滤于 150 mL 浓缩瓶中。再加入 20 mL 提取溶剂（3.7）重复提取一次，合并提取液，40 ℃下旋转浓缩至干。用 2 mL 乙腈溶解残渣，待净化。

6.3 固相分散萃取净化

6.3.1 荷兰豆、什锦蔬菜、橙、乌龙茶和木薯干提取液

将 6.1 相应样品提取液转移到事先装有 50 mg PSA 填料和 200 mg 石墨化碳黑填料的小试管中，充分涡旋 1 min，待色素完全消除后，过滤膜，供气相色谱-质谱测定和确证。

6.3.2 大豆、大米、牛肉、鲳鱼、龙虾仁、蜂王浆和蜂蜜提取液

将 6.1 相应样品提取液转移到事先装有 100 mg PSA 填料，50 mg 石墨化碳黑填料和 100 mg C_{18} 填料的小试管中，充分涡旋 1 min，过滤膜，供气相色谱-质谱测定和确证。

6.4 测定

6.4.1 气相色谱-质谱条件

a) 色谱柱：DB-17 ms 石英弹性毛细管柱，30 m×0.25 mm(内径)，膜厚 0.25 μm，或相当者；

b) 色谱柱温度：150 ℃ $\xrightarrow{15\ ℃/min}$ 300 ℃(2 min)；

c) 进样口温度：300 ℃；

d) 色谱-质谱接口温度：250 ℃；

e) 载气：氦气，纯度大于等于 99.999%；流速，1.0 mL/min；

f) 进样量：1 μL；

g) 进样方式：不分流进样，1.5 min 后开阀；

h) 电离方式：NCI；

i) 电离能量：184 eV；

j) 离子源温度：150 ℃；

k) 四极杆温度：150 ℃；

l) 反应气：甲烷，纯度大于等于 99.99%，反应气流速：2 mL/min；

m) 检测方式：选择离子监测方式(SIM)；

n) 选择监测离子(m/z)：定量离子 295；定性离子 127，238，297；

o) 溶剂延迟时间：6 min。

6.4.2 气相色谱-质谱检测及确证

根据样液中三唑醇含量的情况，选定峰面积相近的标准工作溶液，对标准工作液和样液等体积参插进样。标准工作溶液和样液中三唑醇的相应值均应在仪器的线性范围内。

如果样液与标准工作溶液的选择离子色谱图中，在相同保留时间处有色谱峰出现，并且在扣除背景后的样品质量色谱图中，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)。在上述色谱条件下，三唑醇两个异构体的保留时间分别约为 7.28 min 和 7.41 min，其监测离子(m/z)丰度比为 295∶297∶127∶238＝100∶35∶27∶6 对其进行确证；根据定量离子 m/z 295 对其进行外标法定量。三唑醇标准物的气相色谱-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 和图 A.2。

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

相对离子丰度/%	>50	>20～50	>10～20	≤10
允许的相对偏差/%	±20	±25	±30	±50

6.5 空白试验

除不加试样外，均按上述测定步骤进行。

6.6 结果计算和表述

用色谱数据处理机或按式(1)计算试样中三唑醇残留量：

$$X = \frac{A_X \times c_s \times V_X}{A_s \times m} \qquad \cdots\cdots(1)$$

式中：

X——试样中三唑醇残留量(以异构体之和计)的含量，单位为毫克每千克(mg/kg)；

A_X——样液中三唑醇两个异构体的定量离子峰面积之和；

c_s——标准工作液中三唑醇的浓度，单位为微克每毫升(μg/mL)；

V_X——样液最后定容体积，单位为毫升(mL)；

A_s——标准工作液中三唑醇两个异构体的定量离子峰面积之和；

m——最终样液所代表的试样质量，单位为克(g)。

注：计算结果需将空白值扣除。

7 测定低限和回收率

7.1 测定低限

本方法各种基质的测定低限均为 0.005 mg/kg。

7.2 回收率

不同基质中添加浓度水平下的回收率范围见表 2。

表 2 三个添加水平下食品中三唑醇的回收率数据

样品名称	0.005 mg/kg	0.010 mg/kg	0.020 mg/kg
	回收率/%	回收率/%	回收率/%
荷兰豆	85.2～105.6	78.8～95.7	77.3～108.0
什锦蔬菜	63.4～89.4	66.5～75.9	68.2～75.0
橙	67.4～78.8	75.6～93.7	81.1～94.1
大豆	71.6～83.0	75.6～99.6	89.1～115.2
木薯干	76.2～94.4	67.2～84.2	62.8～74.9
乌龙茶	94.6～109.2	87.6～114.8	89.5～115.6
大米	65.4～81.8	68.1～88.4	84.4～99.1
牛肉	90.2～102.6	72.4～94.3	72.6～84.3
鲷鱼	89.8～104.8	74.5～91.4	73.6～81.4
蜂王浆	60.6～84.2	62.6～81.6	69.8～89.4
龙虾仁	89.0～108.2	79.0～91.4	66.4～76.1
蜂蜜	86.6～99.0	78.5～91.5	82.8～91.2

附 录 A
（资料性附录）
三唑醇标准溶液 GC-MSD/NCI 选择离子流色谱图和全扫描质谱图

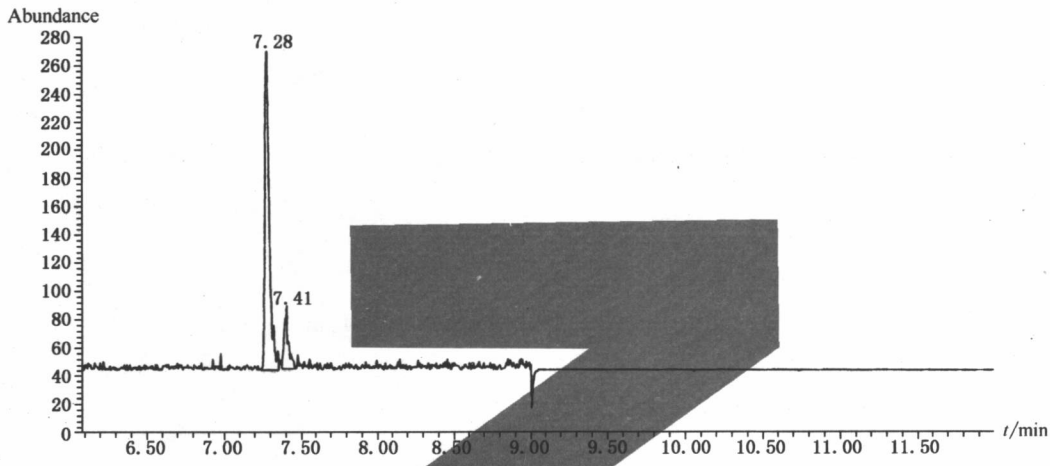

图 A.1 三唑醇标准溶液 GC-MSD/NCI 选择离子流色谱图

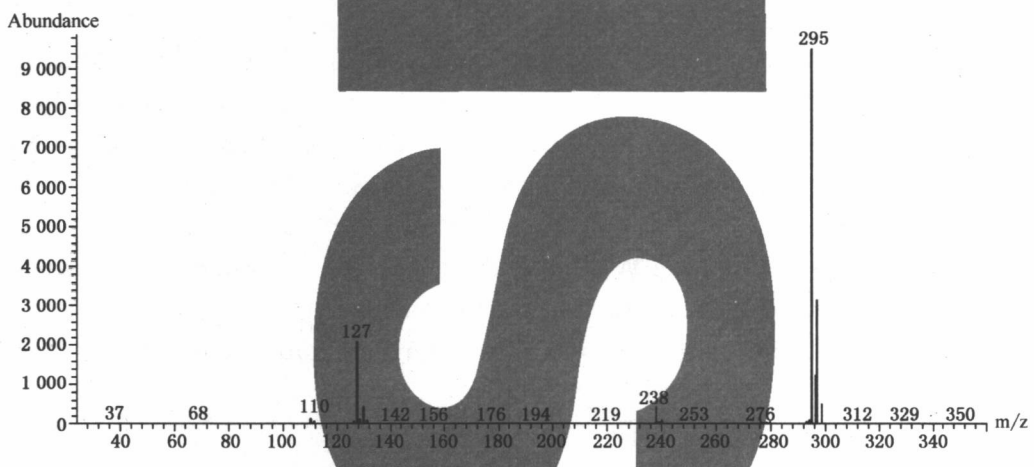

图 A.2 三唑醇标准溶液 GC-MSD/NCI 全扫描质谱图

Foreword

This standard replace SN/T 0644—1997

The mainly differences between this standard and SN/T 0644—1997 are:

—expand the applicable scope of the standard

—develop the technique of sample preparation

—GC-MS with the technique of negative ionization replace GC method.

Annex A of this standard are annexes.

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

This standard was drafted by Jiangsu Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Shanghai Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Jilin Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Neimeng-gu Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

This standard is mainly drafted by Shen Weijian, Chen Huilan, Zhao Zengyun, Deng Xiaojun, Wang Mingtai, Li Gang, Yang Wenquan, Li Lihua, Yu Keyao, Shen Chongyu.
This standard is promulgated for the first time in 1997, and modified for the first time.

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

Determination of triadmenol residue in foods for import and export— GC-MS method

1 Scope

This standard specifies preparation of test sample, determination and confirmation of triadimenol residue by gas chromatography-negative chemical ionization mass spectrometry (GC-MS/NCI) in foods for import and export.

This standard is applicable to the determination and confirmation of triadimenol residue in foods, such as snow pea, assorted seasonal vegetables, orange, bean, dry potato, oolong tea, rice, beef, long-snout catfish, royel jelly, red swamp crayfish and bee honey, etc.

2 Principle

Triadimenol residue is extracted with acetonitrile containing 1% acetic acid and simultaneous liquid-liquid partitioning formed by adding anhydrous magnesium sulfate plus sodium acetate following by a simple cleanup step known by dispersive solid-phase extraction. The aliquot is determined and confirmed by gas chromatography-negative chemical ionization mass spectrometry (GC-MS/NCI) using external standard method.

3 Reagents and materials

Unless otherwise specified, all the reagents used should be analytical grade. "Water" is redistilled water.

3.1 Acetonitrile: HPLC Grade.

3.2 Hexane: HPLC Grade.

3.3 Acetone: HPLC Grade.

3.4 Acetic acid.

3.5 Magnesium sulfate anhydrous.

3.6 Sodium acetate anhydrous.

3.7 Acetonitrile(satured by *n*-hexane) containing 1% acetic acid.

3.8 Triadimenol ($C_{14}H_{18}ClN_3O_2$, CAS No:55219-65-3), purity≥98.0%.

3.9 Triadimenol standard stock solution: Accurately weight an adequate amount of triadimenol standard, dissolve in a small volume of acetonitrile. Dilute with acetonitrile to form a standard stock solution of 200 μg/mL in concentration (Be stored below 4 ℃ for 6 months).

3.10 Triadimenol standard working solution: Then dilute the standard stock solution with acetonitrile to the required concentration as the standard working solution (Be stored below 4 ℃ for 3 months).

3.11 Graphitized Carbon Black: 40 μm.

3.12 Bondesil-PSA (Primary secondary amine): 40 μm.

3.13 AccuBOND SPE ODS(C_{18}): 40 μm.

3.14 0.45 μm organic phase fiber.

4 Apparatus and equipment

4.1 Gas chromotograph/msss spectrometry(MSD) equipped with negative chemical ionization.

4.2 Balances (0.1 mg, 0.01 g).

4.3 Rotatory evaporator.

4.4 Tissue triturator.

4.5 Grinding machine.

4.6 Homogenizer.

4.7 Vortex mixer.

5 Sample preparation and storage

5.1 Preparation of test sample

5.1.1 Vegetables or fruits, such as snow pea, assorted seasonal vegetables and orange, etc.

About 500 g representative samples should be taken from all samples, the edible parts are selected, cut into mince and homogenized thoroughly into pulp by a high speed tissue triturator. Then divide the pulp into two equal portions. Each portion is put in a clean container which is sealed and labled.

5.1.2 Tea or cereals, such as bean, dry potato, oolong tea and rice, etc.

About 500 g representative samples should be taken from all samples, and grounded into powder and then passed through a mesh with 2.0 mm round holes. The passed powder is mixed and divided into two portions. Each portion is put into one clean sample bottle which is sealed and labled.

5.1.3 Meats and meat products, such as beef, longsnout catfish and red swamp crayfish, etc.

About 500 g representative samples should be taken from all samples, the edible parts are cut into mince and homogenized by a high speed tissue triturator. The mixed primary sample is divided into two equal portion. Each portion is put into one clean sample bottle which is sealed and labled.

5.1.4 Bee products, such as royel jelly and bee honey, etc.

About 500 g representative samples should be taken from all samples, and the sample that is not crystallized shall be stirred well to make homogeneous. If the sample is crystallized, it must be warmed in a water-bath below 60 ℃ with the sample bottle covered tightly, mix thoroughly when all sample has melted, then cool immediately to room temperature. In the course of melting the sample, precautions must be taken to avoid evaporation of water from the sample. Then divide the pulp into two equal portions. Each portion is put in a clean container which is sealed and labled.

5.2 Storage of test sample

The test samples of tea, bee products, grains or cereals should be stored below 4 ℃. The test samples of fresh fruits, vegetables, meat and meat products should be stored below −18 ℃.

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

6 Procedure

6.1 Weight

6.1.1 bean, dry potato, beef, longsnout catfish, red swamp crayfish and royal jelly

Weigh ca 5 g of the test sample (accurate to 0.01 g) into 250 mL stoppered conical flask.

6.1.2 snow pea, assorted seasonal vegetables, and orange

Weigh ca 10 g of the test sample (accurate to 0.01 g) into 250 mL stoppered conical flask.

6.1.3 rice, oolong tea, and bee honey

Weigh ca 5 g of the test sample (accurate to 0.01 g) into 250 mL stoppered conical flask, and add 4 mL water homogenized.

6.2 Extraction

Add 50 mL acetonitrile with 1% acetic acid (3.7) and 15 g anhydrous magnesium sulfate (3.5) and 6 g sodium acetate anhydrous (3.6), vibrating for 40 min in the oscillator. Filter the extract into a 150 mL condensor. The residue is extracted with 20 mL acetonitrile (3.7) again, filter and combine the extracts into the same condensor. Evaporate the extract to dry by a rotary evaporator with a water bathing temperature of 40 ℃. Add 2 mL acetonitrile to dissolve the residue and waiting for cleanup operation.

6.3 Dispersive SPE Cleanup

6.3.1 Extraction solution from high pigments content sample, such as snow pea, assorted seasonal vegetables, orange and oolong tea, etc. or dry potato.

An aliquat of the extract is transferred into the 10 mL tube which contains 50 mg PSA and 200 mg graphitized carbon, and shake vigorously by hand for 1 minute, and filter 0.45 μm organic phase fiber.

6.3.2 Extraction solution from high oil content sample, such as bean, rice, beef, longsnout catfish and red swamp crayfish, etc. or bee products such as bee honey and royel jelly, etc.

Transfer the sample extract of high oil content sample into the 10 mL tube which contains 100 mg PSA, 50 mg graphitized carbon and 100 mg C_{18} and shake vigorously by hand for 1 minute, and same as 6.2.1 section.

6.4 Determination

6.4.1 GC-MSD operation conditions

a) Column: DB-17 ms fused quartz capillary column, 30 m × 0.25 mm(i.d.), film thickness 0.25 μm, or the equivalent;

b) Column temperature: 150 ℃ $\xrightarrow{15\ ℃/min}$ 300 ℃ (2 min);

c) Inlet temperature: 300 ℃;

d) Interface temperature:250 ℃;

e) Carrier gas:Helium,purity≥99.999%,flow rate=1.0 mL/min;

f) Injection volumn:1 μL;

g) Injection mode:splitless,purge after 1.5 min;

h) Ionization mode:NCI;

i) Ionization energe:184 eV;

j) Ionization source temperature:150 ℃;

k) Quadropole temperature:150 ℃;

l) Reagent gas:methane,purity≥99.99%, flow rate:1.0 mL/min;

m) Acquisition mode:Selected Ion Monitoring (SIM) mode;

n) Selected monitoring ions(m/z):Quantified by 295; Confirmed by127,238 and 297;

o) Solvent delay time:6 min.

6.4.2 *GC-MS determination and confirmation*

According to the approximate concentration of the pesticide in the sample solution,select the standard working solution with similar concentration of the sample solution. The standard working solution should be injected in-between the injections of the sample solution with one common volume. The response of triadimenol in the standard working solution and sample solution should be within the linear range of the instrument detection.

If there is a peak appeared at the same retention time for both of sample solution and standard working solution,and the qualification ions for every compound must be found,and for the same analysis batch and the same compound,the variation range of the ion ratio between the two daughter ions for the unknown sample and the standard working solution at the similar concentration cannot be out of range of table 1.

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

Under the above GC-MSD operating conditions, the retention time of two triadimenol peaks are 7.28 min and 7.41 min respectively, and the ratio of monitoring ions(m/z) is 295 : 297 : 127 : 238 = 100 : 35 : 27 : 6. The GC-MS total ion chromatogram and its mass spectrum of triadimenol standard solution are shown respectively in annex A.

6.5 Blank test

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

6.6 Calculation and expression of the result

Calculate the content of triadimenol residue in the test sample by GC-MSD data processor or using the formula (1):

$$X = \frac{A_X \times c_s \times V_X}{A_s \times m} \quad \cdots\cdots (1)$$

Where:

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

A_X—the total area of quantitative ion for two triadimenol peaks in the sample solution;

c_s—the concentration of triadimenol in the standard working solution, μg/mL;

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

A_s—the total area of quantitative ion for two triadimenol peaks in the standard working solution;

m—the corresponding weight 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 0.005 mg/kg.

7.2 Recovery

Recovery and RSD data are listed in table 2.

Table 2—Recovery and RSD data of triadimenol at three spiked levels in foods

Food type	0.005 mg/kg	0.010 mg/kg	0.020 mg/kg
	Recovery/%	Recovery/%	Recovery/%
Snow pea	85.2～105.6	78.8～95.7	77.3～108.0
Assorted seasonal vegetables	63.4～89.4	66.5～75.9	68.2～75.0
Orange	67.4～78.8	75.6～93.7	81.1～94.1
Bean	71.6～83.0	75.6～99.6	89.1～115.2
Dry potato	76.2～94.4	67.2～84.2	62.8～74.9
Oolong Tea	94.6～109.2	87.6～114.8	89.5～115.6
Rice	65.4～81.8	68.1～88.4	84.4～99.1
Pork	90.2～102.6	72.4～94.3	72.6～84.3
Longsnout catfish	89.8～104.8	74.5～91.4	73.6～81.4
Royal Jelly	60.6～84.2	62.6～81.6	69.8～89.4
Red swamp crayfish	89.0～108.2	79.0～91.4	66.4～76.1
Bee Honey	86.6～99.0	78.5～91.5	82.8～91.2

Annex A
(Informative)
GC-MSD/NCI selected ion chromatogram and full scan mass spectrum of the triadimenol standard solution

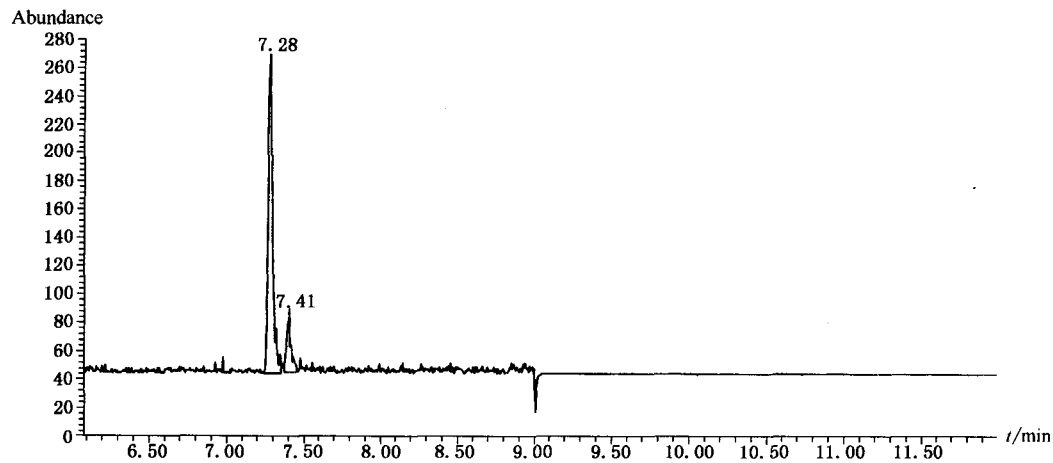

Figure A. 1—GC-MSD/NCI selected ion chromatogram of the triadimenol standard solution

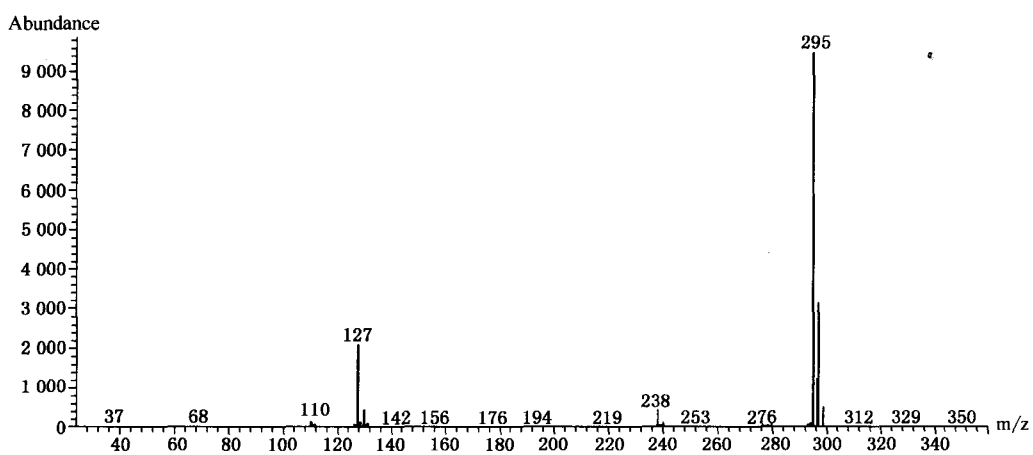

Figure A. 2—Mass spectrum of triadimenol gained from GC-MSD/NCI