SN

中华人民共和国出入境检验检疫行业标准

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进出口食品中莠去津残留量的检测方法 气相色谱-质谱法

Determination of atrazine residue in foods for import and export—Gas chromatography-mass spectrometry（GC-MS）method

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前　言

本标准附录A、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。

本标准起草单位：中华人民共和国吉林出入境检验检疫局、中华人民共和国天津出入境检验检疫局、中华人民共和国深圳出入境检验检疫局、中华人民共和国辽宁出入境检验检疫局。

本标准主要起草人：王明泰、牟峻、林安清、周晓、卫锋、蓝芳、芦春梅、李爱军。

本标准系首次发布的出入境检验检疫行业标准。

进出口食品中莠去津残留量的检测方法 气相色谱-质谱法

1 范围

本标准规定了食品中莠去津残留量的气相色谱-质谱检测方法。

本标准适用于柑橘、苹果、菠菜、大葱、胡萝卜、松子仁、核桃仁、茶叶、蜂蜜、鱼、牛肝、鸡肾、玉米、糙米、甘草、辣椒酱中莠去津残留量的测定和确证。

2 方法提要

试样用水-丙酮均质提取，经二氯甲烷液-液分配，以凝胶色谱净化，再经活性炭和弗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：残留级。

3.2 二氯甲烷：残留级。

3.3 环已烷：残留级。

3.4 乙酸乙酯：残留级。

3.5 正已烷：残留级。

3.6 氯化钠。

3.7 无水硫酸钠：650℃灼烧 4 h，贮于密封容器中备用。

3.8 氯化钠水溶液：20 g/L，称取氯化钠 20 g，用水溶解并稀释至 1 000 mL。

3.9 莠去津标准品(Atrazine，$C_8H_{14}ClN_5$，CAS No. 1912-24-9)：纯度大于等于 98%。

3.10 莠去津标准储备溶液：准确称取适量的莠去津标准品，用丙酮配制成浓度为 100 μg/mL 的标准储备溶液。该溶液在 0℃～4℃冰箱中保存。

3.11 莠去津标准工作溶液：根据需要再用丙酮稀释成适用浓度的标准工作溶液。该溶液在 0℃～4℃冰箱中保存。

3.12 弗罗里硅土固相萃取柱：Florisil，500 mg，6 mL，或相当者。

3.13 石墨化炭固相萃取柱：ENVI-Carb，250 mg，6 mL，或相当者。

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

3.15 滤膜：0.45 μm。

4 仪器与设备

4.1 气相色谱-质谱仪：配有电子轰击源(EI)。

4.2 凝胶色谱仪：配有单元泵、馏分收集器。

4.3 均质器。

4.4 旋转蒸发器。

4.5 具塞锥型瓶：250 mL。

4.6 分液漏斗：250 mL。

4.7 浓缩瓶：50 mL、250 mL。

5 试样制备与保存

5.1 试样制备

5.1.1 玉米、糙米、茶叶、松子仁、核桃仁、甘草

取代表性样品约 500 g，用粉碎机粉碎，混匀，装入洁净容器，密封，标明标记。

5.1.2 柑桔、苹果、菠菜、大葱、胡萝卜

取代表性样品约 500 g，将其可食用部分(不可用水洗)切碎后，用捣碎机将样品加工成浆状，混匀，装入洁净容器，密封，标明标记。

5.1.3 鱼、牛肝、鸡肾

取代表性样品约 1 kg，取可食部分，经捣碎机充分捣碎均匀，装入洁净容器，密封，标明标记。

5.1.4 蜂蜜

取代表性样品约 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。装入洁净容器，密封，标明标记。

5.1.5 辣椒酱

取代表性样品约 500 g，搅拌均匀，装入洁净容器，密封，标明标记。

5.2 试样保存

粮谷类、坚果类、茶叶、蜂蜜、辣椒酱试样于 0℃～4℃保存；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

称取试样约 20.0 g(松子仁、核桃仁及辣椒酱称取 10.0 g，甘草、茶叶称取 5.0 g)于 250 mL 具塞锥形瓶中，加入 20 mL 水，然后加入 100 mL 丙酮，均质提取 3 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℃水浴中浓缩至约 20 mL。

将浓缩提取液转移至 250 mL 分液漏斗中，加入 100 mL 氯化钠水溶液和 100 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℃水浴中浓缩至近干。加入 10 mL 环己烷＋乙酸乙酯(1＋1)溶解残渣(茶叶样品浓缩至近干后加 3 mL 正己烷溶解残渣)，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(内径)，Bio Beads S-X3，或相当者；

b) 流动相：环己烷＋乙酸乙酯(1＋1)；

c) 流速：4.7 mL/min；

d) 样品定量环：10 mL；

e) 预淋洗时间：10 min；

f) 凝胶色谱平衡时间：5 min；

g) 收集时间：21 min～28 min。

6.2.1.2 凝胶色谱净化步骤

将 10 mL 待净化液按 6.2.1.1 规定的条件进行净化，将收集组分于 40℃下浓缩至近干，并用 3 mL 正己烷溶解残渣，待固相萃取净化(松子仁及核桃仁样品液须按 6.2.1.1 规定的条件重复进行一次凝胶

净化)。

茶叶样品不用经过凝胶色谱净化,按照6.2.2直接进行固相萃取净化。

6.2.2 固相萃取(SPE)净化

自上而下将活性炭固相萃取柱(3.12)与氟罗里硅土固相萃取柱(3.13)串联连接,使用前用8 mL正己烷+乙酸乙酯(3+2)预淋洗,弃去流出液。将样品提取液倾入柱中(柑橘、菠菜、胡萝卜、玉米、糙米、蜂蜜、甘草样品只进行活性炭固相萃取即可),用8 mL正己烷+乙酸乙酯(3+2)进行洗脱。收集全部洗脱液于50 mL浓缩瓶中,于40℃水浴中浓缩至近干。用丙酮溶解并定容至1.0 mL,供气相色谱-质谱仪测定和确证。

6.3 测定

6.3.1 气相色谱-质谱条件

a) 色谱柱:HP-1701石英毛细管柱,30 m×0.25 mm(内径)×0.25 μm,或相当者;

b) 色谱柱温度:50℃(2 min)$\xrightarrow{20℃/min}$200℃(1 min)$\xrightarrow{5℃/min}$270℃(18 min);

c) 进样口温度:280℃;

d) 色谱-质谱接口温度:280℃;

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:无分流进样,1.2 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):定量离子200,定性离子200、173、202、215;

l) 溶剂延迟:9.0 min。

6.3.2 气相色谱-质谱检测及确证

根据样液中莠去津含量情况,选定浓度相近的标准工作溶液,标准工作溶液和待测样液中莠去津的响应值均应在仪器检测的线性范围内。标准工作溶液与样液等体积参插进样测定。

标准溶液及样液均按6.3.1规定的条件进行测定,如果样液中与标准溶液相同的保留时间有峰出现,则对其进行确证。经确证分析被测物质量色谱蜂保留时间与标准物质相一致,并且在扣除背景后的样品谱图中,所选择的离子均出现;同时所选择离子的丰度比与标准样物质相关离子的相对丰度一致,相似度在允差之内(见表1),被确证的样品可判定为莠去津阳性检出。莠去津标准物质的气相色谱-质谱选择离子色谱图和质谱图参见附录A中图A.1和附录B中图B.1。

表1 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

除不称取试样外,均按上述步骤进行。

6.5 结果计算和表述

用色谱数据处理机或按式(1)计算试样中莠去津残留量,计算结果应扣除空白值。

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

式中:

X——试样中莠去津残留量,单位为毫克每千克(mg/kg);

h——样液中莠去津的色谱峰高;

h_S——标准工作液中莠去津的色谱峰高；

c——标准工作液中莠去津的浓度，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 测定低限和回收率

7.1 测定低限和确证低限

本方法的测定低限和确证低限：柑橘、苹果、菠菜、大葱、胡萝卜、蜂蜜、鱼、牛肝、鸡肾、玉米、糙米为0.005 mg/kg；茶叶、松子仁、核桃仁、甘草、辣椒酱为0.010 mg/kg。

7.2 添加浓度范围及回收率

本方法添加浓度范围及回收率见表2。

表2 本方法添加浓度及回收率范围

样品名称	添加浓度范围/(mg/kg)	回收率范围/%
柑橘	0.005～0.200	89.9～104.0
苹果	0.005～0.200	84.8～107.0
菠菜	0.005～0.200	89.6～102.0
大葱	0.005～0.200	87.0～98.1
胡萝卜	0.005～0.200	87.8～92.5
松子仁	0.010～0.200	92.8～105.0
核桃仁	0.010～0.200	89.3～105.0
茶叶	0.010～0.200	93.0～99.0
蜂蜜	0.005～0.200	93.0～99.0
鱼肉	0.005～0.200	91.0～104.0
牛肝	0.005～0.200	81.8～94.6
鸡肾	0.005～0.200	93.5～110.0
玉米	0.005～0.200	80.7～91.0
糙米	0.005～0.200	92.5～98.0
甘草	0.010～0.200	87.0～102.0
辣椒酱	0.005～0.200	81.0～105.0

附 录 A
（资料性附录）
莠去津气相色谱-质谱选择离子色谱图

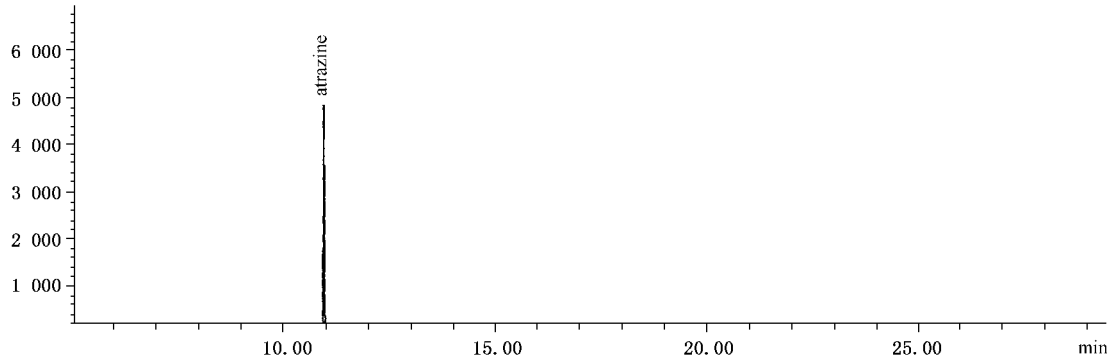

图 A.1 莠去津(1.0 μg/mL)气相色谱-质谱选择离子色谱图

附 录 B
（资料性附录）
莠去津气相色谱-质谱图

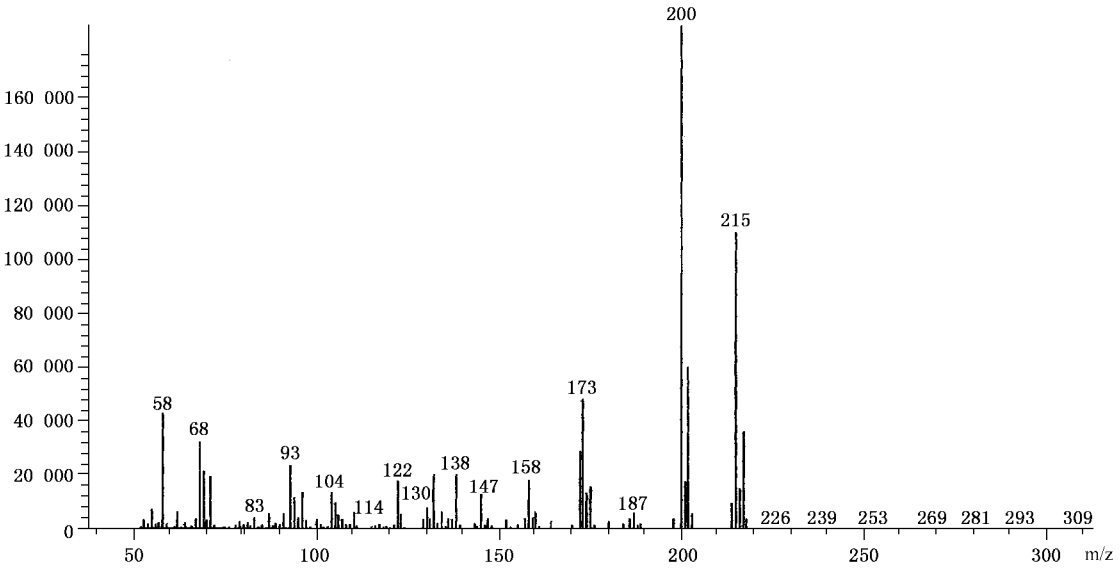

图 B.1 莠去津气相色谱-质谱图

Foreword

Annex A and Annex B of this standard are informative.

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 P. R. C, Tianjin Entry-Exit Inspection and Quarantine Bureau of the P. R. C, Shenzhen Entry-Exit Inspection and Quarantine Bureau of the P. R. C and Liaoning Entry-Exit Inspection and Quarantine Bureau of the P. R. C.

The main drafters of this standard are Wang Mintai, Mu Jun, Lin Anqing, Zhou Xiao, Wei Feng, Lan Fang, Lu Chunmei, and Li Aijun.

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 atrazine residue in foods for import and export—Gas chromatography-mass spectrometry (GC-MS) method

1 Scope

This standard specifies the method for determination of atrazine residue in foods for import and export by gas chromatography-mass spectrometry (GC-MS).

This standard is applicable to determination and confirmation of atrazine residue in orange, apple, spinach, scallion, carrot, pine-nut kernel, walnut kernel, tea, honey, fish, bovine liver, chicken kidney, corn, brown-rice, licorice and chili paste for import and export.

2 Principle

The test sample is extracted with water-acetone followed by partition with dichloromethane. Primary clean-up procedure is based on the gel permeation chromatography (GPC), then an active carbon ENVI-Carb column coupled with one florisil solid phase extraction (SPE) column is used for the secondary procedure. The elute is condensed and dissolved for determination and confirmation by GC-MS using 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: Grade for residue analysis.

3.2 Dichloromethane: Grade for residual analysis.

3.3 Cyclohexane: Grade for residual analysis.

3.4 Ethyl acetate: Grade for residual analysis.

3.5 *n*-Hexane: Grade for residual analysis.

3.6 Sodium chloride.

3.7 Anhydrous sodium sulfate: Dried at 650℃ for 4 hours, then stored in a sealed container.

3.8 Sodium chloride aqueous solution: 20 g/L. Weigh 20 g of sodium chloride. Dissolve with water and dilute to final volume of 1 000 mL.

3.9 Atrazine standard (atrazine, $C_8H_{14}ClN_5$, CAS No. 1912-24-9): Purity≥98%.

3.10 Standard stock solution: Accurately weigh appropriate amount of atrazine standard and dissolve with a little volume of acetone. Dilute with acetone to make final concentration of 100 μg/mL. The solution is stored in a refrigerator at range of 0℃～4℃.

3.11 Standard working solution: dilute the standard stock solution with acetone to make required concentration. The solution is stored in a refrigerator at 0℃～4℃.

3.12 Florisil SPE tube: florisil, 500 mg, 6 mL, or equivalent.

3.13 Active carbon SPE tube: ENVI-Carb, 250 mg, 6 mL, or equivalent.

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

3.15 Film: 0.45 μm.

4 Apparatus and equipment

4.1 GC-MS: equipped with electro-impact source (EI).

4.2 GPC: equipped with isocratic pump and fraction collector.

4.3 Homogenizer.

4.4 Rotary vacuum evaporator.

4.5 Stoppered Erlenmeyer flask: 250 mL.

4.6 Separatory funnel: 250 mL.

4.7 Concentrate bottle: 50 mL and 250 mL.

5 Preparation and storage of test sample

5.1 Preparation of test sample

5.1.1 Corn, brown-rice, tea, pine-nut kernel, walnut kernel and licorice

Take approximately 500 g of representative sample. Smash thoroughly by a pulverizer. Mix thoroughly. Put into clean containers. Seal and label them.

5.1.2 Orange, apple, spinach, scallion, and carrot

Take approximately 500 g of representative sample. Collect the edible parts (do not wash with water) and cut into minces. Crush with a crusher into pulp. Mix thoroughly. Put into a clean containers. Seal and label them.

5.1.3 Fish, bovine liver, chicken kidney

Take approximately 1 kg of representative sample. Collect the edible parts. Crush with a crusher. Mix thoroughly. Put into clean containers. Seal and label them.

5.1.4 Honey

Take about 500 g of representative sample. The non-crystallized sample should be stirred well to make homogeneous while the crystallized sample must be warmed under a water-bath at no more than 60℃ with the sample bottle covered tightly for prevention of loss of water, mix thoroughly when all sample has melted, then cool immediately to room temperature. Take the prepared sample into two sample bottles, seal and label them.

5.1.5 Chilli paste

Take approximately 500 g of representative sample. Mix thoroughly. Put into clean containers. Seal and label them.

5.2 Storage of test sample

The test samples of cereals, nuts, tea, honey and chilli paste should be stored at the temperature ranged from 0℃ to 4℃. Other samples should be frozen and stored at the temperature below −18℃. In course of sampling and sample preparation, it must be taken to avoid contamination or any factors which may cause the change of residue content.

6 Procedure

6.1 Extraction

Weigh approximately 20.0 g (weigh 10.0 g for pine-nut kernel, walnut kernel or chilli paste; 5.0 g for tea or licorice) of the test sample into a 250 mL stoppered Erlenmeyer flask. Add 20 mL of water, then 100 mL of acetone into the flask. Homogenize for 3 min by a high speed homogenizer. Filter the extract into a 250 mL concentrate bottle under vacuum. Extract the residue with 50 mL of acetone again. Combine the filtrates into the same concentrate bottle. Condense to 20 mL by a rotary evaporator under water bath at 40℃.

Transfer the concentrated extract into a 250 mL separatory funnel. Add 100 mL of sodium chloride aqueous solution and 100 mL of dichloromethane. Shake for 3 min and stand still for layering. Collect the dichloromethane phase. The water phase is re-extracted with 2 × 50 mL of dichloromethane twice. Combine the dichloromethane phases, and pass through a column of anhydrous sodium sulfate to remove water. Collect the effluent into a 250 mL concentrate bottle and condense to nearly dryness under water bath at 40℃. Add 10 mL of cyclohexane-ethyl acetate (1+1) to dissolve the residue (for tea, add 3 mL of *n*-hexane to dissolve the residue after the sample is condensed to nearly dryness). Filter through 0.45 μm film for further clean-up procedure.

6.2 Cleaning up

6.2.1 GPC Cleaning 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: 4.7 mL/min;

d) Injection volume at sample loop: 10 mL;

e) Time of pre-rinsing: 10 min;

f) GPC balance time: 5 min;

g) Time of collecting the eluate: 21 min~28 min.

6.2.1.2 GPC cleaning up procedure

Take 10 mL of the solution above for purity according to the condition described as the section 6.2.1.1. Condense the solution collected to nearly dryness. Dissolve the residue with 3 mL of *n*-hexane for SPE cleaning up (sample solutions of pine-net kernel and walnut kernel should employ GPC cleaning up procedure described as the section 6.2.1.1 again).

The sample of tea can employ the SPE cleaning up directly without GPC procedure.

6.2.2 SPE Cleaning up

Couple the active carbon SPE tube (3.12) and flofisil SPE tube (3.13) up to down. Rinse the two columns with 8 mL of *n*-hexane-ethyl acetate in advance. Discard the washing. Transfer the sample solution into the upper column (sample of orange, spinach, carrot, corn, brown rice, honey or licorice need only undergo active carbon SPE cleaning-up). Then elute with 8 mL of *n*-hexane-ethyl acetate (3+2). Collect all eluates into a 50 mL concentrate bottle. Evaporate to nearly dry in a 40℃ water bath. Dissolve the residue and dilute exactly to 1.0 mL with acetone for the GC-MS determination and confirmation.

6.3 Determination

6.3.1 GC-MS operating condition

a) Chromatographic column: HP-1701 silica capillary column, 30 m×0.25 mm (i.d.)×0.25 μm, or equivalent;

b) Column temperature: 50℃ (2 min) $\xrightarrow{20℃/min}$ 200℃ (1 min) $\xrightarrow{5℃/min}$ 270℃ (18 min);

c) Injector temperature: 280℃;

d) Interface temperature: 280℃;

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

f) Injection volume: 1 μL;

g) Injection mode: Splitless. Open the valve after 1.2 min;

h) Ionization mode: EI;

i) Ionization energy: 70 eV;

j) Acquired mode: SIM;

k) Selected monitoring ions (m/z): ion for quantification is 200 and ions for qualification are 200, 173,202, 215;

l) Solvent protection delay: 9.0 min.

6.3.2 GC-MS determination and confirmation

Based on proposed content of atrazine, select the working standard solution with similar concentration to that of the sample solution. The responses of atrazine in the working standard solution and in the sample solution should both be within the linear range of the instrumental detection. The working standard solution is injected in-between the injections of the sample solutions with identical volume.

The working standard solution and the sample solution are tested according to the condition described in section 6.3.1. A respected peak of sample solution at the same retention time with that of the working standard solution will be conformed if all selected ions appear in the subtracted chromatogram, furthermore, the relative ionic abundance tolerance can meets the prescription listed as table 1, then a positive result for atrazine residue can be provided. The chromatogram and mass spectrum of the atrazine standard are shown as the figure A.1 in annex A and as the figure B.1 in annex B.

Table 1—The maximum tolerance of relative ionic abundance for the qualification and quantification

Relative ionic abundance/%	>50	>20~50	>10~20	≤10
Allowed relative deviation/%	±20	±25	±30	±50

6.4 Blank test

Blank test will be conducted according to the procedures above without sample addition.

6.5 Calculation and expression of the result

Calculate the content of atrazine residue in the test sample by GC-MS data processor or according to the formula (1). The result of calculation should be deducted with blank value.

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

where

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

h—the peak height of atrazine in the sample solution;

h_S—the peak height of atrazine in the standard working solution;

c—the concentration of atrazine 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 Detection limit and recovery

7.1 Limit of quantification

The limit of quantification of the method: orange, apple, spinach, scallion, carrot, honey, fish, bovine live, chicken kidney, corn, brown-rice, 0.005 mg/kg; tea, pine-nut kernel, walnut kernel, licorice and chili paste, 0.010 mg/kg.

7.2 Range of fortification and recovery

7.3 The ranges of fortification and recovery of this method are shown in table 2.

Table 2—The range of fortification and recovery of this method

Sample	Fortified range/(mg/kg)	Recovery range/%
orange	0.005～0.200	89.9～104.0
apple	0.005～0.200	84.8～107.0
spinach	0.005～0.200	89.6～102.0
scallion	0.005～0.200	87.0～98.1
carrot	0.005～0.200	87.8～92.5
pine-nut kernel	0.010～0.200	92.8～105.0
walnut kernel	0.010～0.200	89.3～105.0
tea	0.010～0.200	93.0～99.0
honey	0.005～0.200	93.0～99.0
fish	0.005～0.200	91.0～104.0
bovine liver	0.005～0.200	81.8～94.6
chicken kidney	0.005～0.200	93.5～110.0
corn	0.005～0.200	80.7～91.0
brown-rice	0.005～0.200	92.5～98.0
licorice	0.010～0.200	87.0～102.0
chili paste	0.005～0.200	81.0～105.0

Annex A
(informative)
GC-MS selected ion chromatogram of the atrazine standard

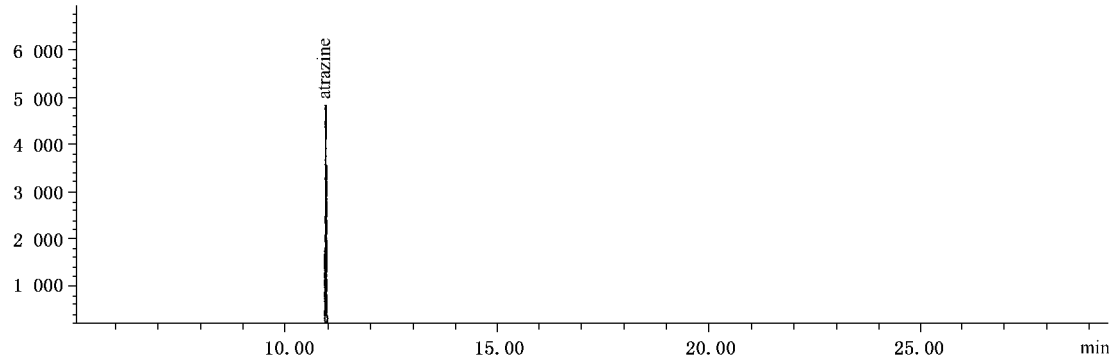

Figure A. 1—GC-MS selected ion chromatogram of the atrazine standard (1.0 μg/mL)

Annex B
(informative)
GC-MS spectrum of atrazine standard

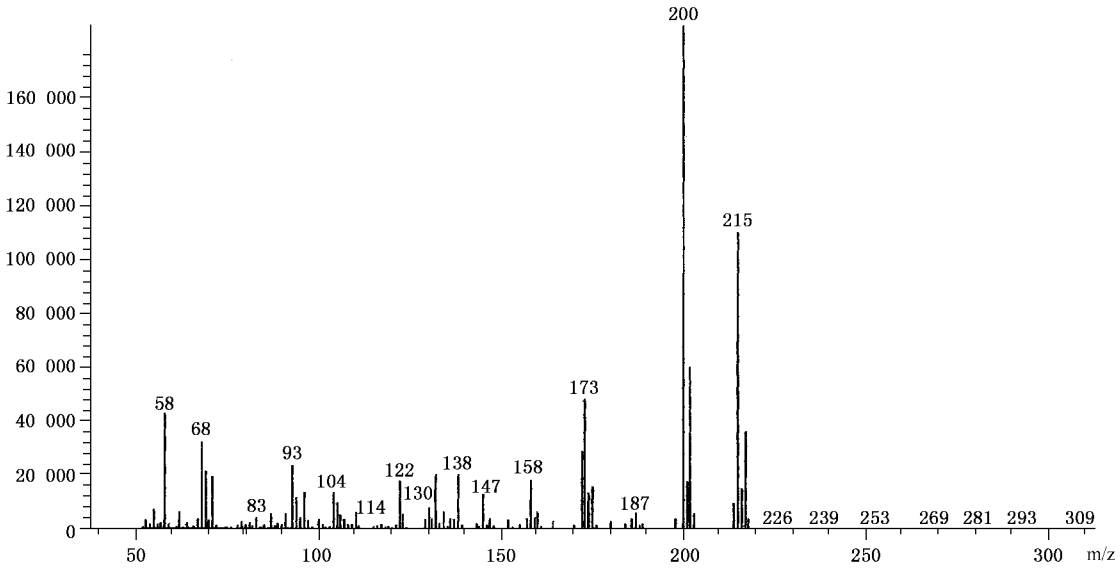

Figure B. 1—GC-MS spectrum of atrazine standard

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