SN

SN/T 0190—2012
代替 SN 0190—1993

中华人民共和国出入境检验检疫行业标准

出口水果和蔬菜中乙撑硫脲残留量测定方法　气相色谱质谱法

Determination of ethylenethiourea residues in fruit and vegetable for export—GC-MS method

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中华人民共和国
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前　言

本标准按照 GB/T 1.1—2009 给出的规则起草。

本标准代替 SN 0190—1993《出口水果中乙撑硫脲残留量检验方法》。

本标准与 SN 0190—1993 相比，主要技术变化如下：

——对标准的名称进行了修改；

——对原标准的检测范围进行了扩展，除水果外增加了蔬菜；

——对衍生化方法进行改进，并由气相色谱法改为 GC-MS 法；

——略去了抽样步骤。

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、中华人民共和国广州出入境检验检疫局新沙办事处。

本标准主要起草人：韩丽、高军、樊祥、王敏、伊雄海、邓晓军、杨慧琴。

本标准所代替标准的历次版本发布情况为：

——SN 0190—1993。

出口水果和蔬菜中乙撑硫脲残留量测定方法　气相色谱质谱法

1　范围

本标准规定了出口水果和蔬菜中乙撑硫脲残留量的测定方法。

本标准适用于鲜桔、马蹄、苹果、西兰花、菠菜等水果和蔬菜中乙撑硫脲残留量的测定。

2　方法提要

甲醇提取试样中残留的乙撑硫脲，提取液与1%苄基氯-甲醇溶液于沸水浴中回流反应生成乙撑苄硫脲，通过调节酸碱度和液液萃取净化，再用三氟乙酸酐衍生，于气相色谱-质谱仪上测定，外标法定量。

3　试剂和材料

除另有规定外，试剂均为分析纯，水为蒸馏水。

3.1　甲醇：液相色谱纯。

3.2　二氯甲烷：残留级。

3.3　正己烷：残留级。

3.4　甲苯：残留级。

3.5　乙撑硫脲标准品(Ethylenethiourea，分子式 $C_3H_6N_2S$，CAS No 96-45-7)：纯度＞99%。

3.6　苄基氯。

3.7　无水硫酸钠：650 ℃灼烧 4 h，贮于干燥器中，冷却后备用。

3.8　5 mol/L 氢氧化钠溶液：称取 20 g 氢氧化钠溶于适量水，并用水稀释至 100 mL。

3.9　6 mol/L 盐酸溶液：吸取 54 mL 浓盐酸，加水稀释至 100 mL。

3.10　三氟乙酸酐/甲苯溶液：1＋9，体积比。

3.11　1%苄基氯-甲醇溶液：1＋99，体积比。

3.12　乙撑硫脲标准溶液：准确称取乙撑硫脲标准品，用甲醇溶解，并配制成浓度为 1.0 mg/mL 标准储备溶液。根据需要再用甲醇将标准溶液稀释成适用浓度的标准工作液。

4　仪器和设备

4.1　气相色谱-质谱仪：配有电子轰击电离源。

4.2　组织搅拌机。

4.3　振荡器。

4.4　离心机。

4.5　电热恒温水浴锅。

4.6　涡旋混合器。

4.7　旋转蒸发仪。

4.8　氮吹仪。

4.9　塑料离心管，50 mL。

4.10　2 mL 带螺旋帽盖的衍生化小瓶(配聚四氟乙烯内衬密封垫)。

5 试样的制备与保存

5.1 试样制备

分取出部分有代表性样品，取可食部分切碎，用四分法缩分出 500 g，经组织搅拌机搅碎后再混匀，均分成两份，装入洁净容器内，密封，标明标记。

5.2 试样保存

将试样于—18 ℃冷冻保存。在抽样和制样过程中，应防止样品受到污染和发生残留物含量的变化。

6 测定步骤

6.1 提取

称取约 20 g 试样(精确到 0.1 g)于 50 mL 塑料离心管中，加入甲醇 35 mL，振荡 30 min，以 3 000 r/min 离心 5 min，在漏斗中放一小块脱脂棉后过滤。在盛有残渣的离心管内再加入甲醇35 mL，振荡 30 min，再离心过滤，用甲醇洗涤残渣。合并提取液并用甲醇定容于 100 mL 容量瓶中，混匀。

6.2 苄基化

吸取提取液 10 mL，加水 10 mL、1%苄基氯-甲醇溶液 1 mL，接上冷凝管，并在沸水浴上加热 30 min。移去冷凝管，冷却后加入 6 mol/L 盐酸 1 mL，于 40 ℃水浴中旋转蒸发，除去甲醇。

6.3 净化

将上述剩余水溶液移入 50 mL 塑料离心管中，并用 10 mL 水清洗后合并，用 20 mL 二氯甲烷振荡洗涤，以 3 000 r/min 离心 5 min，弃去下层的二氯甲烷层，向水层加入 5 mol/L 氢氧化钠 5 mL，用 40 mL 二氯甲烷分二次萃取，合并两次的二氯甲烷层，经无水硫酸钠脱水后浓缩至近干，加正己烷溶解残留物，并转移到 2 mL 的自动进样小瓶中。

6.4 三氟乙酰化

将上述小瓶中的溶剂用氮气吹干，加三氟乙酸酐/甲苯溶液 0.5 mL，室温下反应 15 min，再次用氮气吹干，加甲苯 1 mL 溶解残渣，供气相色谱测定。

6.5 标准衍生物的制备

取适量标准工作液，加甲醇 10 mL、水 10 mL，1%苄基氯-甲醇溶液 1 mL，然后按 6.2～6.4 步骤操作。

6.6 测定

6.6.1 色谱-质谱条件

6.6.1.1 色谱柱：HP-5MS 毛细管柱，30 m×0.25 mm(内径)×0.25 μm，或相当者。

6.6.1.2 升温程序：初始温度 100 ℃，保持 1 min，以 30 ℃/min 升高到 150 ℃，保持 2 min，再以 3 ℃/min 升高到 205 ℃，以 10 ℃/min 升高到 260 ℃，保持 20 min。

6.6.1.3 进样口温度：220 ℃。

6.6.1.4　流速：1.0 mL/min。

6.6.1.5　载气：氦气，纯度≥99.999%。

6.6.1.6　进样模式：不分流进样。

6.6.1.7　进样量：1 μL。

6.6.1.8　电子轰击电离源：70 eV。

6.6.1.9　离子源温度：230 ℃。

6.6.1.10　四极杆温度：150 ℃。

6.6.1.11　接口温度：280 ℃。

6.6.1.12　溶剂延迟：8 min。

6.6.1.13　监测离子(m/z)：288(定量离子)、289、255、219。

6.6.2　色谱测定

根据样液中乙撑硫脲含量的情况，选定与样液中乙撑硫脲浓度相近的标准工作溶液。标准工作溶液和样液中乙撑硫脲衍生物的响应值应在仪器检测的线性范围内。标准溶液和样液等体积穿插进样测定。在上述色谱条件下，乙撑硫脲衍生物的保留时间约为 18.6 min，标准物质的总离子流图参见附录A中图 A.1。乙撑硫脲的两步衍生物的定性离子(m/z)为 288、289、255、219，定量离子为 m/z 288，在扣除背景后的样品质谱图中，所选择的离子必须出现，离子丰度比变化范围见表 1。

表 1　乙撑硫脲衍生物离子与相对丰度比

化合物	定性离子 m/z	标准丰度比/%	允许相对偏离范围/%
乙撑硫脲衍生物	219	100	—
	288	85～95	10
	289	25～35	15
	255	15～25	15

6.7　空白实验

除不加试样外，按上述测定步骤进行。

7　结果计算

用色谱数据处理机或按式(1)计算试样中乙撑硫脲的残留量：

$$X=\frac{A\times c_s\times V\times 10}{A_s\times m} \qquad \cdots\cdots(1)$$

式中：

X ——试样中乙撑硫脲残留含量，单位为毫克每千克(mg/kg)；

A ——试样中乙撑硫脲衍生物的峰高或峰面积；

c_s ——衍生后标准工作液的浓度，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——标准乙撑硫脲衍生物的峰高或峰面积；

m ——称样量，单位为克(g)；

10——稀释倍数。

8 测定低限、回收率

8.1 测定低限

本方法中乙撑硫脲的测定低限为 0.05 mg/kg。

8.2 添加浓度和回收率

回收率数据见表 2。

表 2 不同基质中乙撑硫脲添加水平及回收率范围

样品名称	添加水平 mg/kg	回收率范围 %
鲜桔	0.05	82.6～91.0
	0.1	81.2～96.8
	0.3	84.7～97.7
马蹄	0.05	81.0～90.4
	0.1	84.6～97.7
	0.3	91.7～103
苹果	0.05	78.2～96.2
	0.1	84.6～98.8
	0.3	89.3～99.7
西兰花	0.05	83.0～97.6
	0.1	86.1～101
	0.3	86.0～101
菠菜	0.05	83.0～95.4
	0.1	82.5～94.1
	0.3	90.3～102

附 录 A
（资料性附录）
乙撑硫脲标准品衍生物全扫描色谱图和质谱图

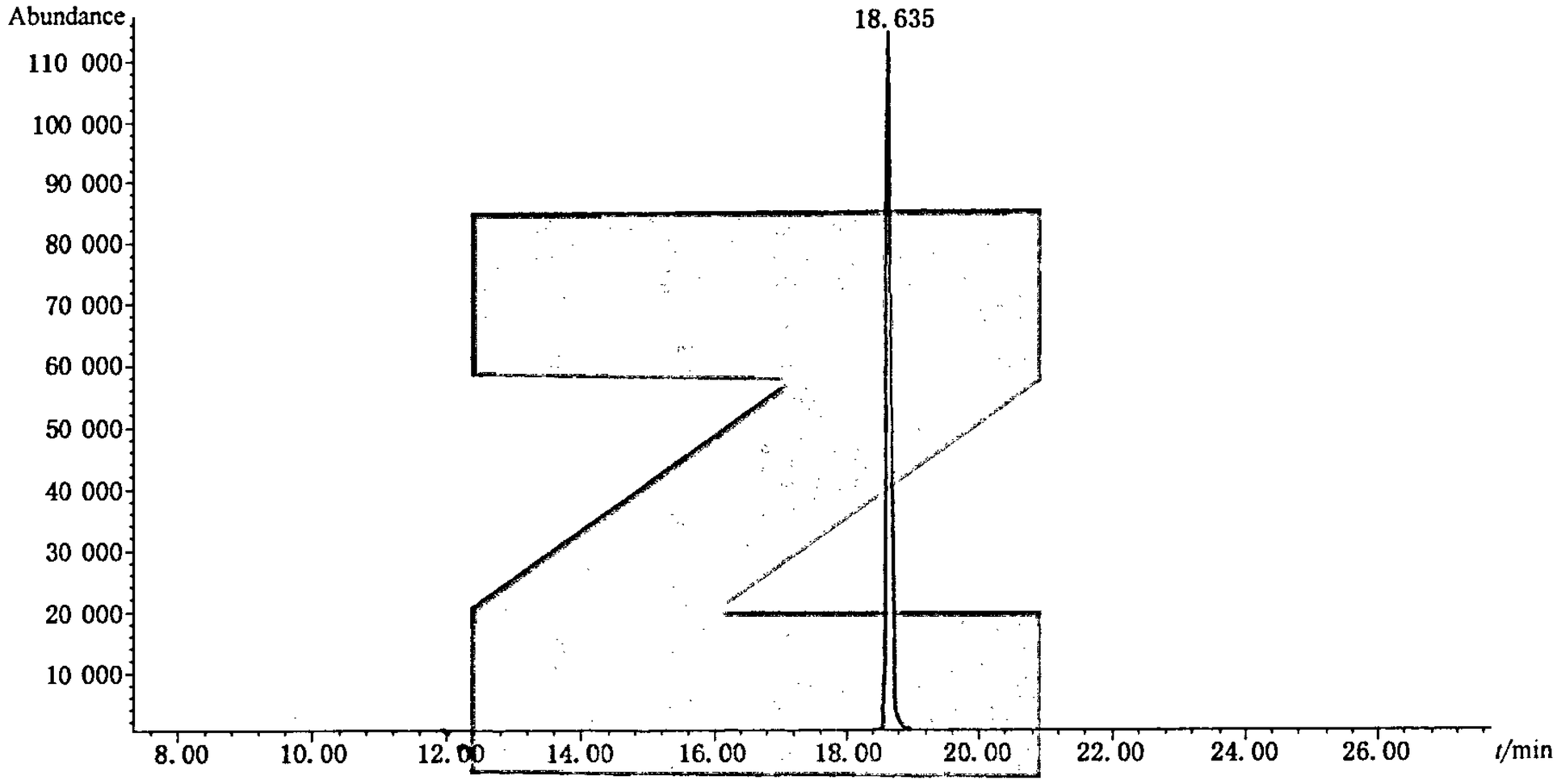

图 A.1 乙撑硫脲标准品衍生物全扫描色谱图

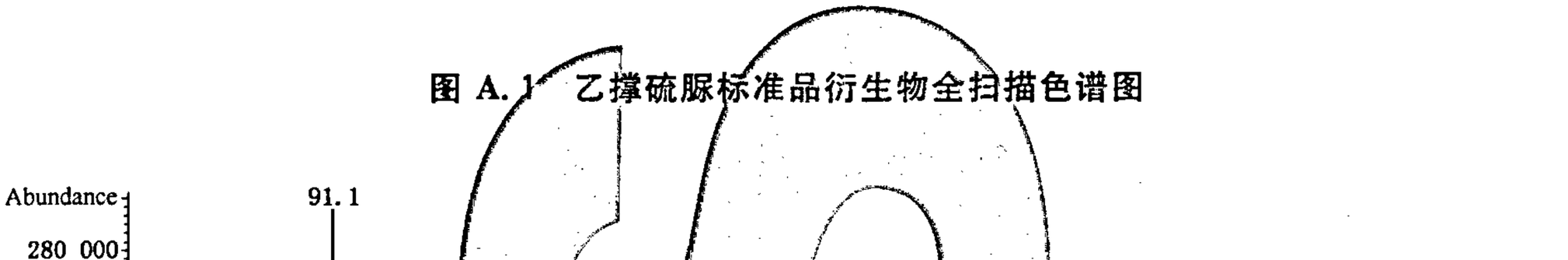

图 A.2 乙撑硫脲标准品衍生物全扫描质谱图

Foreword

The standard is drafted according to GB/T 1.1—2009.

This standard is replace of SN 0190—1993《Method for determination of ethylenethiourea residues in fruits for export》.

The main modification is:

—the topic of standard is modified;

—the scope is extended and amend the matrix of vegetable;

—improvement the method of deriviation and the method of GC/MS is replace of GC;

—cancellation the procedure of sample.

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

The main drafters of this standard are HanLi, WangMin, FanXiang, ShengYonggang, DengXiaojun, MaHongqing.

The origin standard was published in 1993, this standard is revised firstly.

Determination of ethylenethiourea residues in fruits and vegetables for export—GC-MS method

1 Scope

This standard specifies determination of ethylenethiourea(ETU) residue in fruits and vegetables for import and export.

This standard is applicable to the determination of ethylenethiourea residue in fruits and vegetables.

2 principle

ethylenethiourea residue in fruits is extracted with methanol, 1% Benzyl chloride-methanol is added and contents are refluxed for 30 min. The value of pH is adjusted and cleaned up by liquid-liquid extraction, dericatived by 10% trifluoroacetic anhydride in toluene. The aliquot is determined by GC-MS 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 Methanol: HPLC grade.

3.2 Dichloromethane: Residue grade.

3.3 Hexane: Residue grade.

3.4 Toluene: Residue grade.

3.5 Ethylenethiourea ($C_3H_6N_2S$, CAS No:96-45-7), purity>99%.

3.6 Benzyl chloride.

3.7 Anhydrous sodium sulfate: Roasted at 650 ℃ for 4 h, and stored in a tightly closed container.

3.8 Sodium hydroxide solution: 5 mol/L.

3.9 Hydrochloric acid: 6 mol/L.

3.10 Trifluoroacetic anhydride + toluene: 1 + 9, *V*/*V*.

3.11 1% benzyl chloride-methanol solution: 1 + 99, *V*/*V*.

3.12 Ethylenethiourea standard working solution: Accurately weight ethylenethiourea, dissolved with methanol to form a standard stock solution of 1.0 mg/mL in concentration. Then dilute the standard stock solution with methanol to the required concentration as the standard working solution.

4 Apparatus and equipment

4.1 Gas chromatography-mass spectrometry(MSD) equipped with electron impact ionization.

4.2 Tissue triturator.

4.3 Oscillator.

4.4 Centrifuger.

4.5 Electric-heated thermostatic water bath.

4.6 Vortex mixer.

4.7 Rotary evaporator.

4.8 Nitrogen blowing instrument.

4.9 Plastic centrifuge tubes, 50 mL.

4.10 2 mL Derivative vial with a screw cap cover (equipped with Teflon-lined gasket).

5 Sample preparation and storage

5.1 Preparation of test sample

Representative samples shall be taken from all samples, the edible parts are selected, cut into mince, about 500 g is selected by Criss and cross method, put into a tissue triturator and homogenized. 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

Samples shall be stored below −18 ℃. In the course of sampling and sample preparation, precaution shall be taken to avoid contamination or any factors which may cause the change of residue content.

6 Procedure

6.1 Extraction

Weigh about 20 g (accurate to 0.1 g) of the test sample into 50 mL plastic centrifuge tubes, add 35 mL of methanol vibrating for 30 min, centrifuge for 5 min in 3 000 r/min and filte. add another 35 mL of methanol into the tubes with residues, vibrating for 30 min, centrifuge and filte, clean up the residues with methanol. Combine the extracts into the same 100 mL of condenser and blending.

6.2 Benzylation

Get 10 mL of extract solution, add 10 mL water, 1 mL 1% benzyl chloride-methanol solution, and the contents were refluxed for 30 min. Condenser was removed and 1 mL 6 mol/L hydrochloric acid was added after the contents was cooled, methanol was removed with a rotary evaporator (waterbath at 40 ℃).

6.3 Cleanup

The samples were then transferred to a 50 mL plastic centrifuge tube, washed with 10 mL water and Combine the extracts, extracted with 20 mL dichloromethane, which was discarded after shaked and centrifuged at 3 000 r/min for 5 min. After the addition of 5 mol/L KOH (5 mL) to the aqueous phase, and extracted twice with 20 mL of dichloromethane, the dichloromethane extract was dried by passage through sodium sulphate and the solvent was removed with a rotary evaporator. The residue was dissolved with *n*-hexane and moved to 2 mL automatic injection vials.

6.4 Trifluoroacetyl

Blow the solvents dry with nitrogen, a solution containing 10% trifluoroacetic anhydride in toluene (0.5 mL) was added to the dry residue and the sample was allowed to react at room temperature for 15 min. The solvent was evaporated to dryness under a stream of nitrogen and dissolved in 1 mL of toluene for GC analysis.

6.5 Preparation of standard derivatives

Take appropriate standard working solution, add 10 mL of methanol, 10 mL water, 1 mL 1% benzyl chloride-methanol solution, and the same as 6.2 to 6.4 sections.

6.6 Determination

6.6.1 GC-MS operation conditions

6.6.1.1 Column: HP-5MS capillary column, 30 m × 0.25 mm (i.d.) × 0.25 μm, or the equivalent.

6.6.1.2 Temperature program: 100 ℃ for 1 min, 30 ℃ /min to 150 ℃ for 2 min; 3 ℃/min to 205 ℃, 10 ℃/min to 260 ℃ and final time 20 min.

6.6.1.3 Inlet temperature: 220 ℃.

6.6.1.4 Flow rate: 1.0 mL/min.

6.6.1.5 Carrier gas: Helium, purity ≥99.999%.

6.6.1.6 Injection mode: Splitless.

6.6.1.7 Injection volume: 1 μL.

6.6.1.8 Electron impact: 70 eV.

6.6.1.9 Ionization source temperature: 230 ℃.

6.6.1.10 Quadrupole temperature: 150 ℃.

6.6.1.11 Interface temperature: 280 ℃.

6.6.1.12 Solvent delay: 8 min.

6.6.1.13 Selected monitoring ions (*m/z*): 288, 289, 255, 219.

6.6.2 GC-MS determination

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 response of ETU derivatization in the standard working solution and the sample solution should be within the linear range of the instrument detection. The standard working solution should be injected in-between the injections of the sample solution with one common volume. Under the above GC-MS operating conditions, the retention time of ETU derivatization is about 18.6 min, and its mass spectrum are shown by figure A.1 in annex A. Two-step derivatization of ETU were confirmed by (*m/z*) 288, 289, 255, and 219; quantified by *m/z* 288, the qualification ions must be found in the sample mass spectrum after deducting the background, the variation range of the ion ratio are shown by table 1.

Table 1—ETU derivatization ions and relative ion intensities

Compound	Confirmed ions m/z	Standard intensity/%	Permitted tolerances/%
ETU derivatization	219	100	—
	288	85～95	10
	289	25～35	15
	255	15～25	15

6.7 Blank experiment

In addition to not sample,according to the determination of the above steps.

7 Calculation and expression of the result

Calculate the content of ETU in the test sample by GC-MS data processor or using the followed formula(1).

$$X = \frac{A \times c_s \times V \times 10}{A_s \times m} \quad \cdots\cdots(1)$$

Where :

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

A —the height or area of ETU derivatization in the test sample;

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

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

A_s —the height or area of standard ETU derivatization;

m —the sample weight,g;

10 —Dilution times.

8 Limit of determination and recovery

8.1 Limit of determination

The limit of determination of ETU of this method is 0.05 mg/kg.

8.2 Add concentration and recovery

The recovery data was shown in table 2.

Table 2—Add levels of ETU and the recoveries from different fruits and vegetables

Sample type	ann level mg/kg	average recovery %
Fresh orange	0.05	82.6～91.0
	0.1	81.2～96.8
	0.3	84.7～97.7
Zantedeschia	0.05	81.0～90.4
	0.1	84.6～97.7
	0.3	91.7～103
apple	0.05	78.2～96.2
	0.1	84.6～98.8
	0.3	89.3～99.7
broccoli	0.05	83.0～97.6
	0.1	86.1～101
	0.3	86.0～101
spinage	0.05	83.0～95.4
	0.1	82.5～94.1
	0.3	90.3～102

Annex A
(Informative)
GC-MS chromatogram and mass spectrum of the ETU standard derivatization

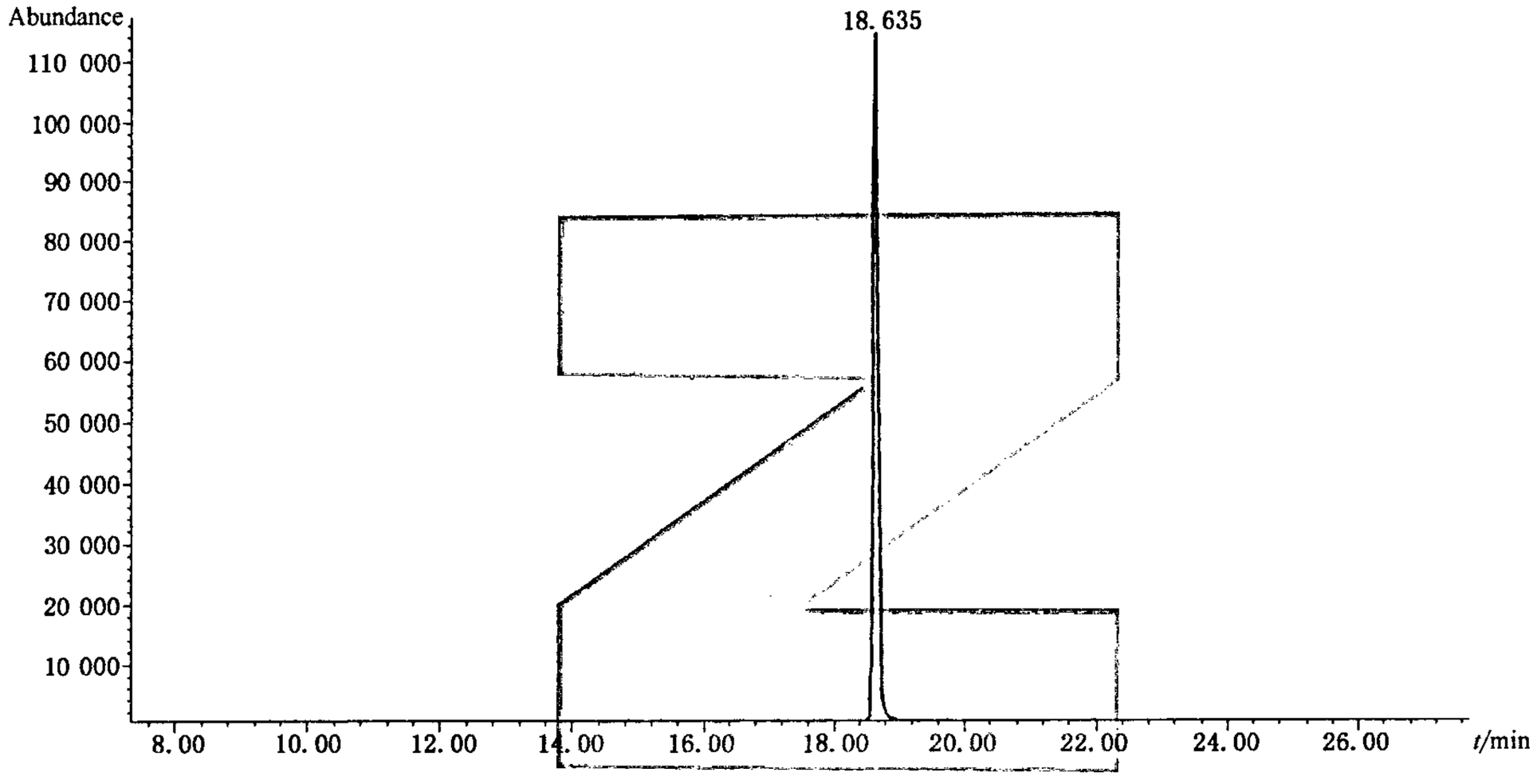

Figure A.1—GC-MS chromatogram of the ETU standard derivatization

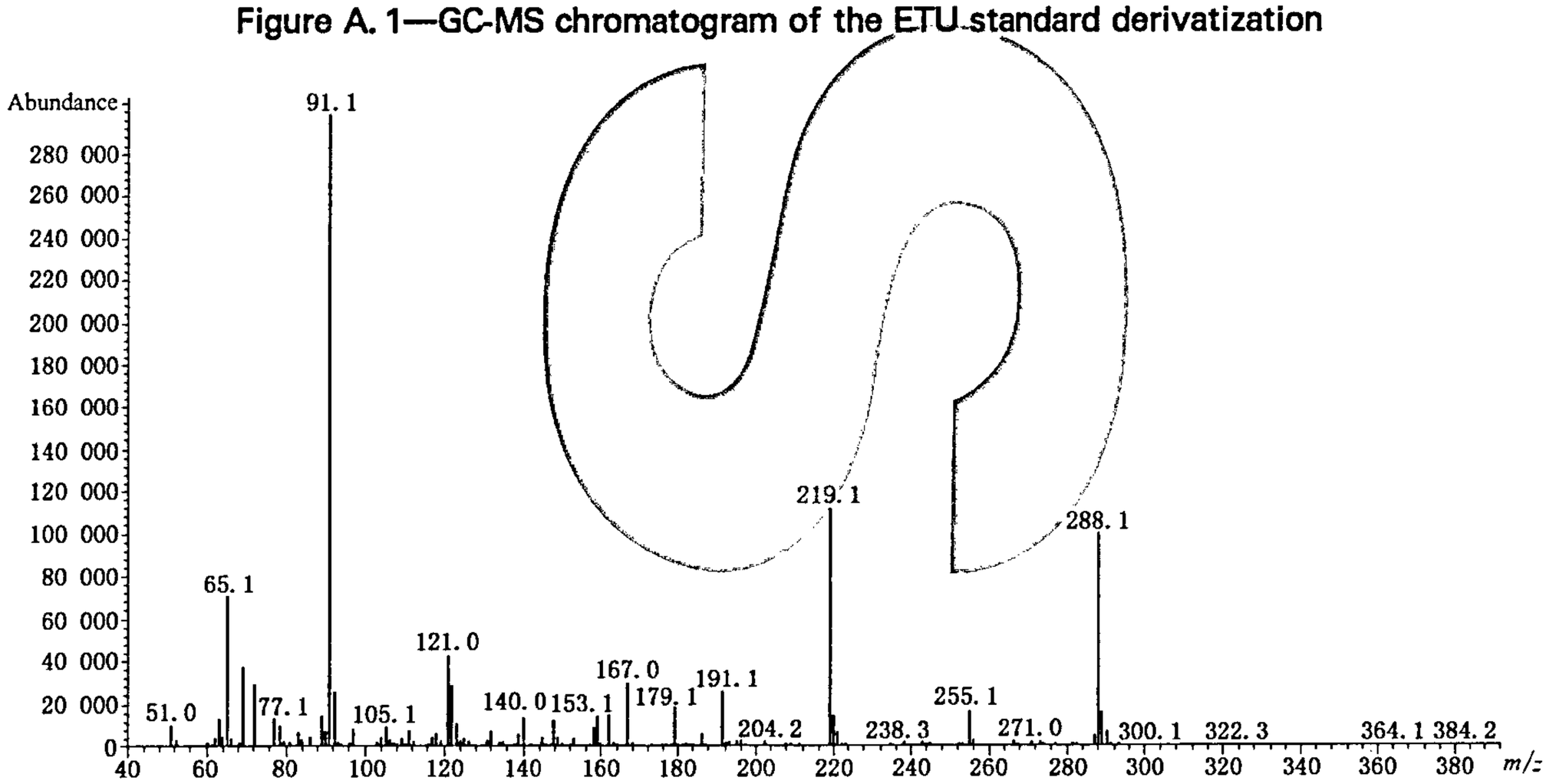

Figure A.2—Mass spectrum of the ETU standard derivatization gained from GC-MS