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中华人民共和国出入境检验检疫行业标准

SN/T 1873—2007

进出口食品中硫丹残留量的检测方法 气相色谱-质谱法

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

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前　言

本标准附录A为资料性附录。

本标准由中华人民共和国国家认证认可监督管理委员会提出并归口。

本标准由中华人民共和国天津出入境检验检疫局、中华人民共和国广东出入境检验检疫局、中华人民共和国上海出入境检验检疫局负责起草。

本标准主要起草人：葛宝坤、王云凤、常春艳、陈捷、陈其勇、刘培、郭德华、焦红、韩丽。

本标准系首次发布的检验检疫行业标准。

进出口食品中硫丹残留量的检测方法 气相色谱-质谱法

1 范围

本标准规定了食品中α-硫丹、β-硫丹、硫丹硫酸盐残留量的气相色谱-质谱检测方法。

本标准适用于鳗鱼、泥鳅、鲶鱼、黄鳝、牛肉、大豆、蘑菇、毛豆、菠菜、蒜薹、西红柿、甘蓝、苹果、柑橘、茶叶中硫丹残留量的测定。

2 测定方法

2.1 方法提要

样品经溶剂提取、凝胶层析柱或硅镁吸附剂净化，采用气相色谱-质谱测定，外标法定量。

2.2 试剂和材料

除另有规定外，所有的有机试剂为色谱纯，水为二次蒸馏水。

2.2.1 丙酮。

2.2.2 乙酸乙酯。

2.2.3 环己烷。

2.2.4 石油醚：沸程 30℃～60℃。

2.2.5 无水硫酸钠：分析纯，650℃灼烧 4 h，在干燥器内冷却至室温，贮于密封瓶中备用。

2.2.6 氯化钠：分析纯。

2.2.7 洗脱液：乙酸乙酯-环己烷(1∶1，体积比)混合溶液。

2.2.8 凝胶及溶胀：Bio-Beads® S-X3 200 目～400 目或相当者；凝胶的溶胀按每克凝胶加 4.6 mL 乙酸乙酯-环己烷(1∶1，体积比)的混合溶液浸泡，溶胀 6 h 以上备用。

2.2.9 凝胶层析柱：柱长 20 cm、内径 2.0 cm 具活塞玻璃层析柱，柱底垫少许玻璃棉。用洗脱剂乙酸乙酯-环己烷(1∶1，体积比)溶胀的凝胶以湿法转入装柱，柱床高约 20 cm，胶床始终保持在洗脱剂中；洗脱流速约 1 mL/min，上样前用洗脱液以两分之一的洗脱流速淋洗一个柱体积。

2.2.10 固相萃取柱：硅镁吸附剂，1 g，或相当者。用前分别用 5 mL 丙酮-正己烷(1∶9，体积比)、5 mL正己烷淋洗活化小柱。

2.2.11 α-硫丹：CAS 编码 959-98-8，纯度大于 99.5%。

2.2.12 β-硫丹：CAS 编码 33213-65-9，纯度大于 99.5%。

2.2.13 α-硫丹＋β-硫丹混合物：CAS 编码 115-29-7，纯度(66.1%＋33.4%)。

2.2.14 硫丹硫酸盐：CAS 编码 1031-07-8，纯度大于 99.5%。

2.2.15 硫丹标准储备液：准确称取适量的硫丹标准品，用少量甲苯溶解后，以正己烷稀释成一定浓度的储备液，4℃冰箱保存。

2.2.16 硫丹标准工作液：取一定量的标准储备液，用正己烷稀释成适当浓度的标准工作液。

2.3 仪器和设备

2.3.1 气相色谱-质谱仪，配备负化学源(NCI)。

2.3.2 旋转蒸发装置。

2.3.3 氮吹仪。

2.3.4 组织匀浆机。

2.3.5 植物粉碎机。

2.3.6 振荡器。

2.4 测定步骤

2.4.1 样品的制备

a) 动物产品：取鳗鱼、泥鳅、鲶鱼、黄鳝、牛肉产品取可食部分 500 g，用组织捣碎机充分捣碎均匀，均分成两份，分别装入洁净容器中，密封、标记、－18℃冷冻保存。

b) 植物产品：取大豆、蘑菇、毛豆、菠菜、蒜苔、西红柿、甘蓝、苹果、柑橘、茶叶 500 g，组织粉碎机粉碎，分别装入洁净容器中，密封、标记、4℃冷藏保存。

2.4.2 样品的提取

a) 动物产品：称取试样 20 g(精确到 0.01 g)，于 100 mL 具塞三角瓶中，加水 6 mL(视样品水分含量加水使总水量约 20 g，水产品的肉通常在 70%左右，加水 6 mL 即可)，加 40 mL 丙酮，匀浆 1 min，加氯化钠 6 g，充分摇匀，再加 30 mL 石油醚，振摇 30 min。取 35 mL 有机层上清液，经无水硫酸钠滤于旋转蒸发瓶中，浓缩至约 1 mL，加 2 mL 乙酸乙酯-环己烷溶液再浓缩，如此重复 3 次，浓缩至约 2 mL。

b) 水果蔬菜：称取试样 25 g(精确到 0.01 g)，于 200 mL 烧杯中，加 50 mL 乙腈，匀浆 1 min，转移至预先加入 6 g 氯化钠的 100 mL 的具塞量筒中，剧烈振荡 2 min，静置 20 min，取 10 mL 上清液于10 mL比色管中，在 40℃水浴中氮吹仪吹干，2 mL 正己烷溶解。

c) 粮谷、茶叶等：称取粉碎试样 10 g(精确到 0.01 g)，于 100 mL 三角瓶中，添加 20 mL 正己烷，振荡 30 min。过滤，吸取 2 mL 上清液于 10 mL 比色管中。

2.4.3 样品的净化

a) 对 2.4.2.1 的提取样品上凝胶层析柱，并以乙酸乙酯-环己烷溶液洗脱，弃去 0 mL～35 mL 流分，收集 35 mL～70 mL 流分。将其旋转蒸发浓缩至约 2 mL，再经凝胶层析柱重复净化一次，收集 35 mL～70 mL 流分，蒸发浓缩，用氮吹仪吹除溶剂(40℃)，以正己烷定容至 5 mL，留待 GC/MS 分析。

b) 对 2.4.2.2 和 2.4.2.3 的浓缩液全部上固相萃取柱，再用 10 mL 丙酮-正己烷洗脱，将盛有淋洗液的离心管置于氮吹仪上，40℃水浴氮吹至近干，蔬菜、水果类用正己烷准确定容至 5 mL，粮谷类用正己烷准确定容至 1 mL，在旋涡混合器上混匀，移入样品瓶中待测。

2.4.4 测定

2.4.4.1 气相色谱-质谱条件

a) 色谱柱：DB-1701 柱，30 m×0.25 mm×0.25 μm，或相当者；

b) 载气：氦气，纯度不低于 99.999%，流速：1.3 mL/min；

c) 柱温：40℃保持 1 min，以 30℃/min 速度升至 130℃，再以 5℃/min 升至 260℃，保持 1 min；

d) 进样口温度：250℃；

e) 进样方式：不分流；

f) 开阀时间：0.75 min；

g) 进样量：2 μL；

h) 电离方式：NCI，30 eV；

i) 接口温度：280℃；

j) 反应气：甲烷气，CH_4；

k) 离子源温度：150℃；

l) 溶剂延迟：10 min；

m) 离子检测模式：选择离子监测(SIM)，监测离子及其相对丰度比见表 1。

表 1 选择离子和相对丰度

被测组分	α-硫丹、β-硫丹	硫丹硫酸盐
选择离子 m/z	374，404，406(定量)，408，410	384，386(定量)，388，390
相对丰度/(%)	28∶47∶100∶84∶32	62∶100∶72∶30

2.4.4.2 定量测定

根据样液中被测硫丹的含量情况，选定浓度相近的标准工作液，其响应值应在方法检测的线性范围内。在上述气相色谱-质谱条件下，保留时间分别为 α-硫丹 23.7 min、β-硫丹 27.7 min、硫丹硫酸盐 30.4 min，选择离子色谱图和质谱图见附录 A 中的图 A.1～图 A.5。

2.4.4.3 定性测定

按照色谱-质谱条件测定样品和标准工作溶液，如果检测的质量色谱峰的保留时间与标准品一致，相对丰度允许偏差小于 20%，则可判断样品中存在对应的被测物。

2.4.4.4 空白试验

除不加试样外，均按上述步骤进行。

2.4.5 结果计算与表述

按式(1)计算试样中硫丹的含量(以 α-硫丹、β-硫丹、硫丹硫酸盐的总量计)：

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

式中：

X——试样中硫丹的含量，单位为毫克每千克(mg/kg)；

A——试样中硫丹的色谱峰面积；

c_s——标准工作溶液中硫丹的浓度，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——标准工作溶液中硫丹的色谱峰面积；

m——最终样液所代表的量，单位为克(g)。

2.4.6 测定低限、回收率

2.4.6.1 测定低限

本方法的测定低限：动物产品 0.004 mg/kg、植物产品 0.01 mg/kg。

2.4.6.2 回收率

动物产品、植物产品样品添加回收率见表 2。

表 2 动物产品、植物产品样品添加回收率

样品	添加水平/(mg/kg)	回收率范围/(%)	样品	添加水平/(mg/kg)	回收率范围/(%)
鳗鱼	0.004	67.5～77.5	鲶鱼	0.004	70.0～82.5
	0.01	71.0～79.0		0.01	77.0～87.5
	0.05	70.0～88.0		0.05	78.0～94.0
泥鳅	0.004	70.0～85.0	牛肉	0.004	75.0～87.5
	0.01	79.0～87.0		0.01	81.0～88.0
	0.05	78.0～94.0		0.05	80.0～96.0
大豆	0.01	70.0～80.0	苹果	0.01	71.0～88.0
	0.04	72.5～85.0		0.04	72.5～90.0
	0.10	80.0～90.0		0.10	80.0～96.0
甘蓝	0.01	76.0～87.0	蒜苔	0.01	70.0～78.0
	0.04	75.0～92.5		0.04	70.0～82.5
	0.10	86.0～97.0		0.10	79.0～89.0
茶叶	0.01	70.0～76.0	菠菜	0.01	74.0～88.0
	0.04	70.0～80.0		0.04	72.5～90.0
	0.10	77.0～85.0		0.10	83.0～97.0

附 录 A
（资料性附录）
标准品质谱图

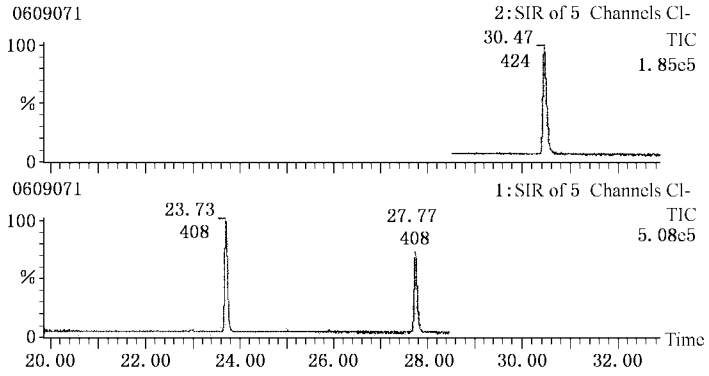

图 A.1 α-硫丹、β-硫丹、硫丹硫酸盐选择离子色谱图

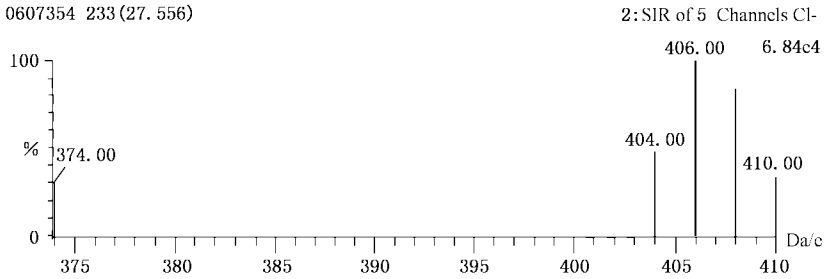

图 A.2 α-硫丹、β-硫丹选择离子质谱图

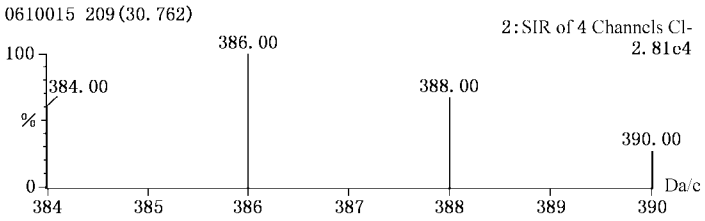

图 A.3 硫丹硫酸盐的选择离子质谱图

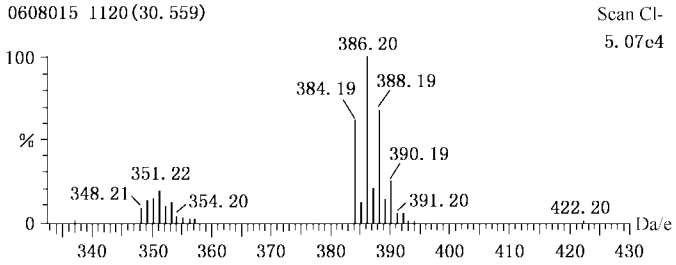

图 A.4 硫丹硫酸盐全扫描质谱图

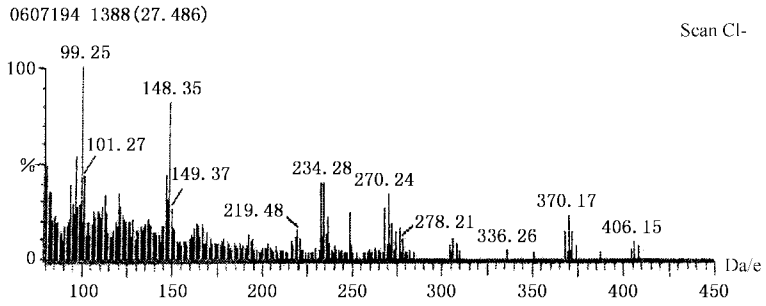

图 A.5 α-硫丹、β-硫丹全扫描质谱图

Foreword

Annex A of this standard is an information annex.

This standard was proposed by and is under the charged of certification and accreditation administation of the People's Republic of China.

This standard was drafted by Tianjin Entry-Exit Inspection and Quarantine Bureau, and Guangdong Entry-Exit Inspection and Quarantine Bureau and Shanghai Entry-Exit Inspection and Quarantine Bureau.

The standard was mainly drafted by Ge-bao Kun, Wang-yun Feng, Chang－chun Yan, Chen-jie,Chen-qi Yong, Liu Pei, Guo-de Hua, Jiao-hong, Han-li.

This standard is professional standard for entry-exit inspection and quarantine promulgated for the first time.

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

1 Scope

This standard specifies the determination and confirmation of α-endosulfan, β-endosulfan and endosulfan sulfate in food by gas chromatography－mass spectrum.

This standard is applicable for the detemination and confirmation of endosulfan in eel, loach, catfish, rice field eel, beef, soybean, mushroom, green soy bean, spinage, garlic, tomato, cabbage, apple, orange and tea samples.

2 Method of determination

2.1 Principle

Endosulfan in the test sample are extracted with the solvent, then cleaned up with gelatin chromatographic column. Determination by gas chromatography－mass spectrum and quanyified by using the external standard.

2.2 Reagents and materials

Unless otherwise specified, all the reagent used should be chromatograph pure, and "water" is deionized water.

2.2.1 Acetone.

2.2.2 Ethyl acetate.

2.2.3 Cyclhexane.

2.2.4 Petroleum ether: boils the regulation.

2.2.5 Anhydrous sodium sulfate: ignite at 650℃ for 4 h, and store in air-tight container.

2.2.6 Sodium chloride (analytical pure).

2.2.7 Elute solvet: ethyl acetate-cyclohexane(1 ∶ 1).

2.2.8 Gel and swelling: Bio－Beads® S-X3 200 mesh～400 mesh or equivalent; The swelling of gel through every 4 g gel adding 4.6 mL Ethyl acetate－cyclohexane(1∶1) immersion. The gel to be used when swelling 6 h.

2.2.9 Gelatin chromatographic column: the length of the column is 20 cm, Inside diameter 2.0 cm with a piston, and the material quality is glass. Put some glass wool in the base of the column. Put the gelatin soaks with Ethyl acetate-cyclohexane(1∶1) in the column by aqueous method. The length of the Column bed is nearly 20 cm, gelatin bed throughout in the Ethyl acetate-cyclohexane (1∶1); The elute flow rate is about 1 mL/min. Elute the column use 1 column volume elute solution at a flow rate of 0.5 mL/min before pour extract solution.

2.2.10 SPE cartridge: florisil, 1 g, or equivalent. It was conditioned with 5 mL acetone-*n*-hexane (10+90) followed by 5 mL *n*-hexan.

2.2.11 α-endosulfan: CAS code 959-98-8, purity＞99.5%.

2.2.12 β-endosulfan: CAS code 33213-65-9, purity＞99.5%.

2.2.13 The mixture of α-endosulfan and β-endosulfan:CAS code 115-29-7.

2.2.14 Endosulfan sulfate: CAS code 1031-07-8, purity＞99.5%.

2.2.15 Stock standard solution of endosulfan: accurately weigh adequate amounts of endosulfan standard, dissolution by a little toluene, then dissolved in N－hexane to make up standard stock solution of the density, should be stored at 4℃ in refrigeratory.

2.2.16 Working standard solution of endosulfan: dilute the stock standard solution with *n*-hexane to 1.0 μg/mL as working standard solution.

2.3 Apparatus and equipments

2.3.1 Gas chromatograph combined with negative chemical ionization mass spectrogram (GC-MS).

2.3.2 Rotary vacuum evaporator.

2.3.3 Nitrogen evaperator.

2.3.4 High-speed homogenizer.

2.3.5 Plant disintegrator.

2.3.6 Oscillators.

2.4 Procedure

2.4.1 Sample preparation

a) Creatural sample: Take the edible part of original sample is taken and homogenized, then it is divided in two angd sealed, labeled for lab test. The test sample is stored in −18℃ refrigeratory.
b) Botanic sample: Take original sample is taken and homogenized, then it is divided in two angd sealed, labeled for lab test. The test sample is stored in 4℃ refrigeratory.

2.4.2 Sample Extraction

a) Creatural sample: Weight 20 g(accurate to 0.01 g) of the test sample into 100 mL conical flask, accurately adding 6 mL water, adding 40 mL acetone, homogenize 1 min and add 6 g of sodium chloride, mix well. Adding 30 mL petroleum ether, shake 30 min, Taking the upper liquid 35 mL flask, combine extracted solution and evaporate to nearly 1 mL, adding 2 mL of ethyl acetate-cyclohexane (1 ∶ 1), combine thrice according to above process, combine the extract solution nearly 2 mL.
b) Fruit and vegetable: Weight 25 g(accurate to 0.01 g) of the test sample into 200 mL beaker, accurately adding 50 mL acetonitrile, homogenize 1 min and transfer into the graduated cylinders with stopper, then add 6 g of sodium chloride into the graduated cylinders. Shake vigorously 2 min, taking the upper liquid into 10 mL centrifuge tube, below it to dryness with nitrogen flow at 40℃ water-bath, dissolve with 2 mL petroleum ether.
c) Grain and tea: Weight 10 g(accurate to 0.01 g) of the test sample into 100 mL conical flask, adding 20 mL *n*-hexane, shake 30 min, then filter and taking the 2 mL upper liquid into 10 mL centrifuge tube.

2.4.3 Sample clean up

a) Wash the extract sample solution of 2.4.2.1 with Ethyl acetate-cyclohexane(1 ∶ 1) by gelatin chromatographic column, discarding 0～35 mL effluent, collect 35 mL～70 mL elution, combine the elution and evaporate to nearly 2 mL, clean up twice according to above process. Combine all the elution and evaporate to dryness under nitrogen flow, make up to 5 mL with petroleum ether, The solution is used for GC-MS determination.
b) Pour the sample solution of 2.4.2.2 and 2.4.2.3 into the cleanup column, elute with 5 mL acetone-*n*-hexane (10+90). Repeat again according to above process. Evaporate to dryness under nitrogen flow at 40℃, then make up to 5 mL with *n*-hexane in fruit and vegetable and make up to 1 mL with *n*-hexane in grain; mix well. The solution is used for GC-MS determination.

2.4.4 Determination

2.4.4.1 GC-MS operating conditions

a) Column: DB-1701, 30 m × 0.25 mm × 0.25 μm or equivalent;
b) Carrier gas: He(purity>99.999%), flow rate of carrier gas: 1.3 mL/min;
c) Temperature program: 40℃(keep 1 min) 30℃/min to 130℃ 5℃/min to 260℃(keep 1 min);
d) Injection temperature: 250℃;
e) Injection mode: splitless;
f) Purge time: 0.75 min;
g) Injection volume: 2 μL;
h) Electron mode:NCI, 30 eV;
i) Interface temperature: 280℃;
j) Reaction gas: CH_4;
k) Ion source temperature: 150℃;
l) Solvet delay: 10 min;
m) Detection mode:SIM: Selection ions (m/z) and relative intensity(%) see table1.

Table 1—Selected ions and relative intensity

Analyte	α-endosulfan α、β-endosulfan	endosulfan sulfate
Selected ions m/z	374,404,406(quantitative),408,410	384,386(quantitative),388,390
Relative intensity/(%)	28 : 47 : 100 : 84 : 32	62 : 100 : 72 : 30

2.4.4.2 GC-MS determination

According to the values of endosulfan in sample, select the standard working solution with similar peak area to that of sample solution. The responses of endosulfan in the standard working and the sample solution should be within the liner range of the instrumental detection. Under the above GC-MS operating condition, The retention time of for chromatogram of α-endosulfan is 23.7 min, β-endosulfan is 27.7 min and endosulfan sulfate is 30.4 min, The chromatogram and mass spectrum of the standard, see Figure A.1～Figure A.5. of annex A.

2.4.4.3 Confirmation of GC-MS

Under GC-MS conditions, the working solution and solution is injected. If the retention times of sample chromatogram peaks are consistent with the standards, and the deviation of the abundance ratio between sample and standard within 20%, according to the selected ions and relative intensity to confirmate.

2.4.4.4 Blank test

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

2.4.5 Calculation and expression of result

Calculation the content of endosulfan residue in the test sample according the formula(1),(the result

is the total of α-endosulfan, β-endosulfan and endosulfan sulfate):

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

Where:

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

A—the peak area of c in sample solution;

c_s—the concentration of endosulfan in standard working solution;

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

A_s—the peak area of endosulfan in standard working solution;

m—Mass of test sample,g.

2.4.6 Limit of determination and recovery

2.4.6.1 Limit of determination

The limit of determination of this method: the creatural sample is 0.004 mg/kg, the Botanic sample is 0.01 mg/kg.

2.4.6.2 Recovery

According to the experimental data, the corresponding recoveries of fortifying concentrations fortifying concentrations in creatural sample see table 2.

Table 2—The corresponding recoveries of fortifying concentrations in creatural sample and botanic sample

sample	fortifying level/(mg/kg)	recovery/(%)	sample	fortifying level/(mg/kg)	recovery/(%)
eel	0.004	67.5～77.5	catfish	0.004	70.0～82.5
	0.01	71.0～79.0		0.01	77.0～87.5
	0.05	70.0～88.0		0.05	78.0～94.0
rice field eel	0.004	70.0～85.0	beef	0.004	75.0～87.5
	0.01	79.0～87.0		0.01	81.0～88.0
	0.05	78.0～94.0		0.05	80.0～96.0
soybean	0.01	70.0～80.0	apple	0.01	71.0～88.0
	0.04	72.5～85.0		0.04	72.5～90.0
	0.10	80.0～90.0		0.10	80.0～96.0
cabbage	0.01	76.0～87.0	garlic	0.01	70.0～78.0
	0.04	75.0～92.5		0.04	70.0～82.5
	0.10	86.0～97.0		0.10	79.0～89.0
tea	0.01	70.0～76.0	spinage	0.01	74.0～88.0
	0.04	70.0～80.0		0.04	72.5～90.0
	0.10	77.0～85.0		0.10	83.0～97.0

Annex A
(informative annex)
Chromatogram of the standard

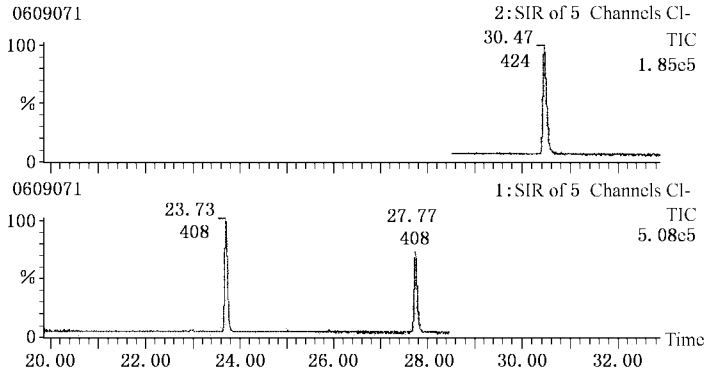

Figure A. 1—GC-MS Chromatogram of α-endosulfan, β-endosulfan and endosulfan sulfate

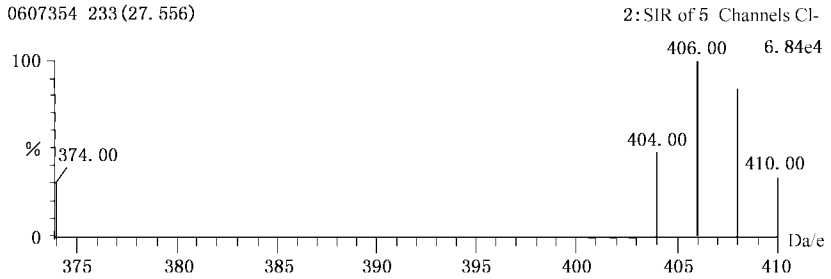

Figure A. 2—SIM mass spectrogram of the α-endosulfan and β-endosulfan

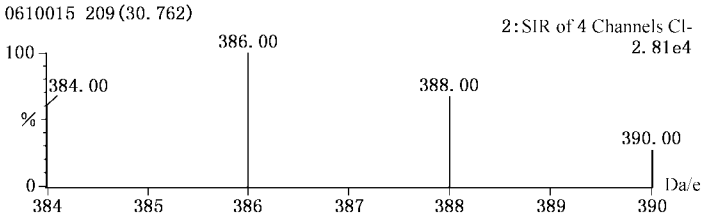

Figure A. 3—SIM mass spectrogram of the endosulfan sulfate

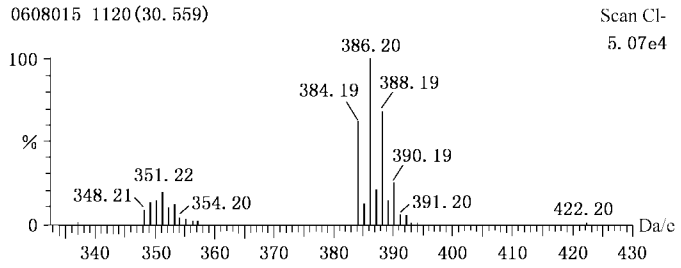

Figure A. 4—Scan mass spectrogram of the endosulfan sulfate

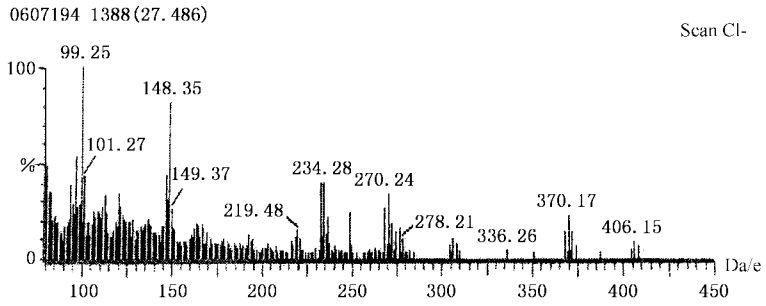

Figure A. 5—Scan mass spectrogram of the α-endosulfan and β-endosulfan

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