SN

# 中华人民共和国出入境检验检疫行业标准

SN/T 0529—2013
代替 SN 0529—1996

# 出口肉品中甲氧滴滴涕残留量检验方法 气相色谱/质谱法

**Determination of methoxychlor residues in meat for export—GC/MS method**

2013-03-01 发布　　2013-09-16 实施

中华人民共和国
国家质量监督检验检疫总局 发布

# 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 0529—1996《出口肉品中甲氧滴滴涕残留量检验方法》。

本标准与 SN 0529—1996 相比，主要技术变化如下：

——标准名称修改为《出口肉品中甲氧滴滴涕残留量检验方法　气相色谱/质谱法》；

——去掉了抽样部分；

——将填充柱更换为毛细柱；

——气相色谱-质谱同时确证和测定代替气相色谱-电子捕获检测器测定。

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：中华人民共和国潍坊出入境检验检疫局。

本标准主要起草人：孙军、吕建霞、田国宁、宫小明、刘永强、董静、张靖。

本标准于 1996 年首次发布，本次为第一次修订。

# 出口肉品中甲氧滴滴涕残留量检验方法 气相色谱/质谱法

## 1 范围

本标准规定了出口肉品中甲氧滴滴涕残留量的气相色谱/质谱测定方法。

本标准适用于出口鸡肉、鸭肉、猪肉中甲氧滴滴涕残留量的测定。

## 2 方法提要

样品中的甲氧滴滴涕经乙酸乙酯-环己烷(1+1,体积比)提取,提取液浓缩后经凝胶渗透色谱(GPC)系统净化,用乙酸乙酯-环己烷(1+1,体积比)洗脱,洗脱液浓缩至干,定容后,气相色谱-质谱仪选择离子检测,外标法定量。

## 3 试剂和材料

所有试剂除另有说明外,均为农残级,水为二次蒸馏水。

3.1 乙酸乙酯。

3.2 环己烷。

3.3 正己烷。

3.4 无水硫酸钠:分析纯,用前在 650 ℃灼烧 4 h,储存于干燥器中,冷却后备用。

3.5 甲氧滴滴涕标准品(Methoxychlor,$C_{16}H_{15}Cl_3O_2$,相对分子质量:345.66,CAS 号:72-43-5):纯度≥99%。

3.6 甲氧滴滴涕标准溶液:溶解 10.0 mg 甲氧滴滴涕标准品于少量正己烷中,用正己烷定容至 100 mL,摇匀,作为标准储备溶液,质量浓度为 100 μg/mL。根据需要再用正己烷稀释配成适当浓度的标准工作溶液。

## 4 仪器和设备

4.1 气相色谱-质谱仪:配备电子轰击源(EI)。

4.2 组织捣碎机。

4.3 均质器。

4.4 旋转蒸发仪。

4.5 天平:感量为 0.1 mg 和 0.01 g 各一台。

4.6 离心机。

4.7 凝胶渗透色谱系统(GPC):内装 Bio-Beads S-X3 填料的净化柱。

4.8 涡旋混合器。

## 5 样品制备与保存

### 5.1 试样的制备

从取回原始样品中取出部分有代表性样品,将可食部分放入高速组织捣碎机中捣碎均匀。充分混

匀,用四分法缩分出不少于 500 g 作为试样。装入清洁的容器中,加封后,标明标记。

### 5.2 试样保存

将试样于−18 ℃以下冷冻保存。

### 5.3 制备与保存要求

在试样制备与保存的操作过程中,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 10 g(精确至 0.01 g)样品于 100 mL 具塞离心管中,加入 20 g 无水硫酸钠(3.4),加入乙酸乙酯-环己烷(1+1,体积比)40 mL,均质 2 min,4 000 r/min 离心 5 min,上清液通过装有无水硫酸钠的漏斗,收集于 100 mL 旋蒸瓶中。残渣中再加入 20 mL 乙酸乙酯-环己烷(1+1,体积比),按上述步骤提取一次,合并提取液于 40 ℃下旋转蒸发浓缩至近干。

### 6.2 净化

#### 6.2.1 凝胶渗透色谱条件

凝胶渗透色谱条件如下:

a) 净化柱:200 mm×25 mm,内装 Bio-Beads S-X3 填料;
b) 流动相:乙酸乙酯-环己烷混合溶剂(1+1,体积比);
c) 流速:4.7 mL/min;
d) 进样量:5 mL;
e) 开始收集时间:9.5 min;
f) 结束收集时间:13 min。

#### 6.2.2 凝胶渗透色谱净化

用 10 mL 乙酸乙酯-环己烷(1+1,体积比)溶解残渣并洗涤,转移入凝胶渗透色谱进样瓶中,充分混匀。如有颗粒状物质存在,则离心或过滤。5 mL 样液注入经校准的 GPC 进样管,通过凝胶渗透色谱系统进行净化。用乙酸乙酯-环己烷溶液(1+1,体积比)洗脱,洗脱液收集于 100 mL 梨形烧瓶中。于 40 ℃以下旋转蒸发至近干。用正己烷(或其他合适的溶剂)将其溶解并定容至 1.0 mL。

### 6.3 测定

#### 6.3.1 气相色谱-质谱条件

气相色谱-质谱条件如下:

a) 色谱柱:DB-35MS(30 m×0.25 mm×0.25 μm)石英毛细管柱或相当者;
b) 色谱柱温度:80 ℃保持 1 min,然后以 20 ℃/min 程序升温至 180 ℃,保持 3 min,再以 20 ℃/min 程序升温至 230 ℃,保持 7 min,以 10 ℃/min 程序升温至 290 ℃,保持 10 min;
c) 进样口温度:260 ℃,不分流进样,进样量:1 μL;
d) 载气:氦气,纯度≥99.999%,流速 1.0 mL/min;
e) 电子轰击源:70 eV;
f) 离子源温度:230 ℃;

g) GC-MS 接口温度:280 ℃;

h) 选择离子监测:定量离子:227,定性离子:228,344。

### 6.3.2 定性测定

分别等体积注入标准工作溶液及样品溶液于气相色谱-质谱仪中,按上述条件进行分析,甲氧滴滴涕出峰时间为 23.5 min。标准品色谱图参见附录 A 中图 A.1、A.2、A.3。进行样品测定时,如果检出的色谱峰保留时间与标准品的保留时间相一致,并且所选择的离子的丰度与标准品对应离子的丰度相比,偏差在表 1 允许的范围内,则可判断样品中存在甲氧滴滴涕。

**表 1 使用气相色谱-质谱定性时相对离子丰度最大允许偏差**

| 相对丰度/% | ≥50 | >20～50 | >10～20 | ≤10 |
|---|---|---|---|---|
| GC/MS 定性时相对离子丰度最大允许偏差/% | ±10 | ±15 | ±20 | ±50 |

### 6.3.3 定量测定

定量采用单个离子定量,配制系列浓度的标准溶液,得到峰面积与浓度的关系,外标法定量测定。

## 6.4 空白实验

除不称取试样外,均按上述步骤进行。

# 7 结果计算和表述

按式(1)计算试样中甲氧滴滴涕残留含量:

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

式中:

$X$ ——试样中甲氧滴滴涕残留含量,单位为毫克每千克(mg/kg);

$A$ ——样液中甲氧滴滴涕的色谱峰面积;

$A_s$——标准工作溶液中甲氧滴滴涕的色谱峰面积;

$c$ ——标准工作溶液中甲氧滴滴涕的质量浓度,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);

$m$ ——最终样液所代表的样品质量,单位为克(g)。

# 8 测定低限、回收率和精密度

## 8.1 测定低限

采用本方法对鸡肉、鸭肉和猪肉中的甲氧滴滴涕残留进行测定,其测定低限为 0.005 mg/kg。

## 8.2 回收率和精密度

采用本方法对鸡肉、鸭肉和猪肉进行添加回收实验,添加水平为 0.005 mg/kg、0.01 mg/kg、0.02 mg/kg,甲氧滴滴涕的添加回收率数据和相对标准偏差数据见表 2。

表 2　鸡肉、鸭肉和猪肉中甲氧滴滴涕的添加浓度和回收率、相对标准偏差

| 样品名称 | 添加浓度/(μg/kg) | 回收率/% | 相对标准偏差/% |
|---|---|---|---|
| 鸡肉 | 5 | 82.8～89.6 | 2.89 |
| | 10 | 98.6～104.7 | 2.54 |
| | 20 | 100.9～103.2 | 0.86 |
| 鸭肉 | 5 | 97.4～102.8 | 2.08 |
| | 10 | 99.6～103.1 | 1.60 |
| | 20 | 101.9～102.7 | 1.10 |
| 猪肉 | 5 | 85.2～93.4 | 4.25 |
| | 10 | 95.8～97.5 | 0.73 |
| | 20 | 93.2～96.2 | 1.41 |

# 附 录 A
（资料性附录）
甲氧滴滴涕标准品气相色谱质谱图

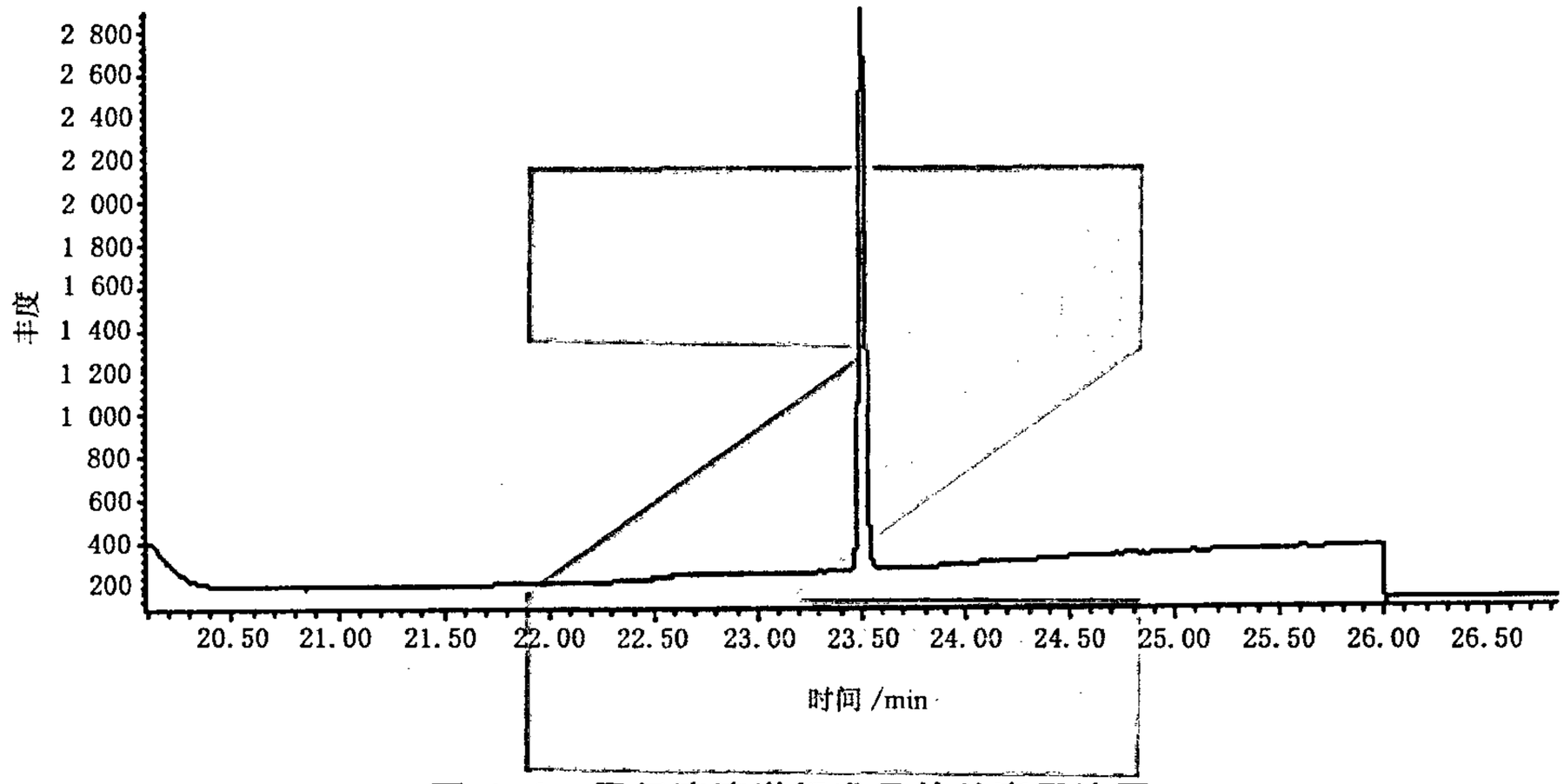


图 A.1 甲氧滴滴涕标准品的总离子流图

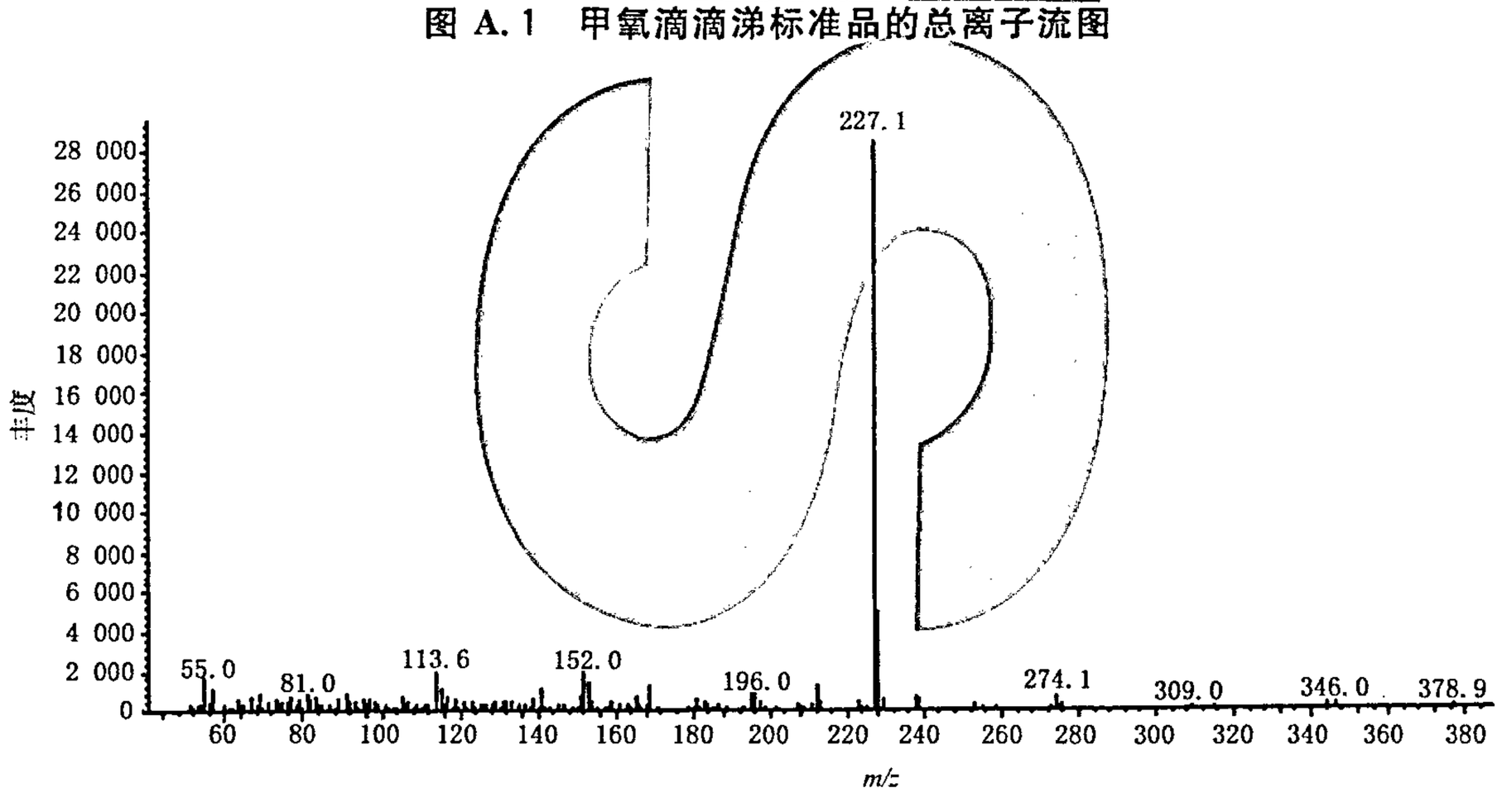


图 A.2 甲氧滴滴涕标准品的全扫描质谱图

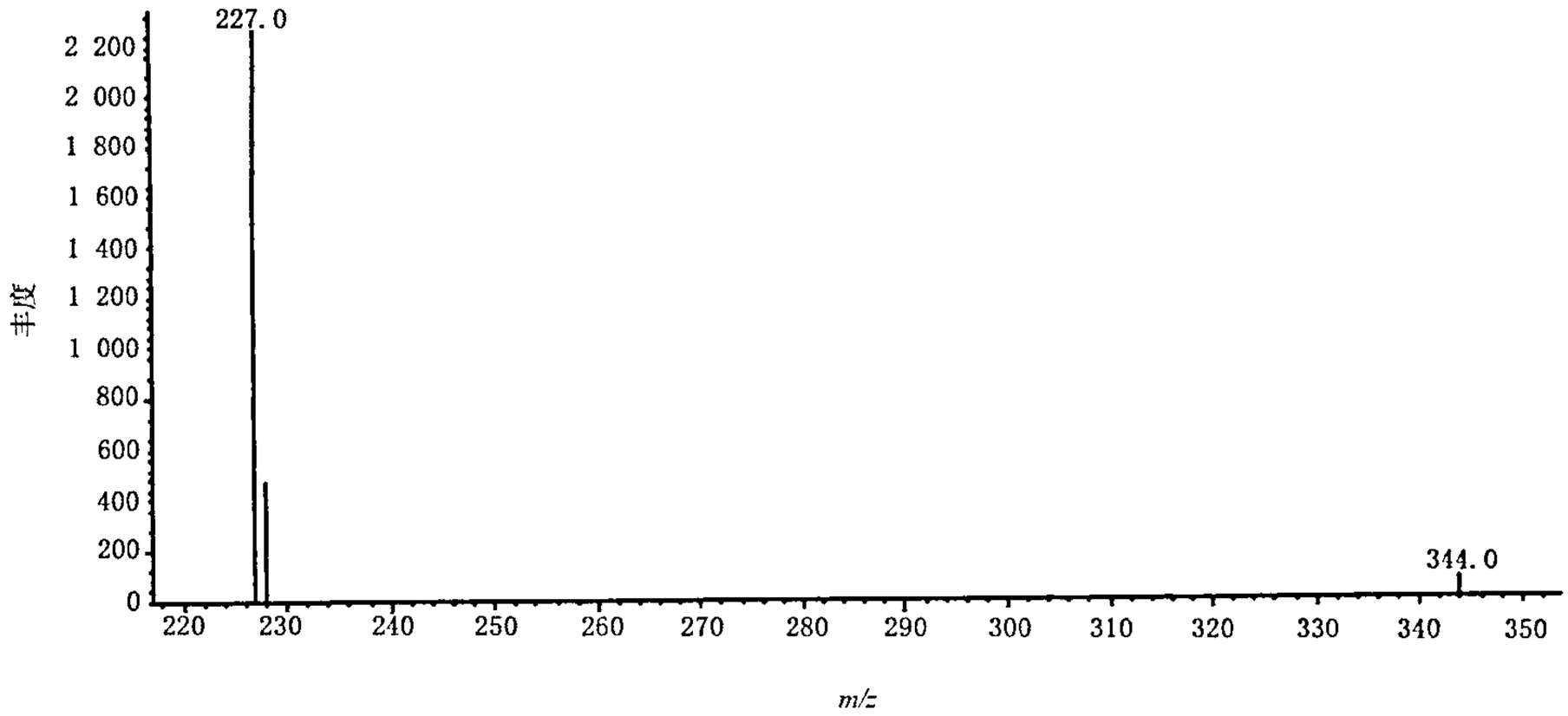


图 A.3　甲氧滴滴涕标准品的选择离子质谱图

# Foreword

This standard is drafted according to GB/T 1. 1—2009.

This standard substitute for SN 0529—1996 Method for the determination of methoxychlor residues in meat for export.

Comparing with SN 0529—1996, the main changes are as follows:

—The name of the standard is changed to "Determination of methoxychlor residues in meat for export—GC/MS method";

—The sampling section is deleted;

—Packed column is replaced by capillary column;

—Methoxychlor is determined and confirmed by GC/MS at the same time, while not by GC/ECD.

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 Shandong Weifang Entry-exit Inspection and Quarantine Bureau of the People's Republic of China.

This main drafters of this standard are Jun Sun, Jianxia Lu, Guoning Tian, Xiaoming Gong, Yongqiang Liu, Jing Dong, Jing Zhang.

This standard is published for the first time in 1996. The standard is modified for the first time.

# Determination of methoxychlor residues in meat for export—GC/MS method

## 1 Scope

This standard specifies the determination of methoxychlor residues in meat for export by GC/MS.

This standard is applicable to the determination of methoxychlor residues in chicken, duck and pork for export.

## 2 Principle

The residues of methoxychlor in the test sample are extracted with ethyl acetate-cyclohexane(1 + 1, *V*/*V*). After cleaned up by gel permeation chromatography (GPC), the residues are determined by gas chromatograph mass spectrometry (GC-MS) under SIM mode. External standard method is used for quantitation.

## 3 Reagents and materials

Unless otherwise specified, the reagents should be pesticide grade, water is redistilled water.

**3.1** Ethyl acetate.

**3.2** Cyclohexane.

**3.3** Hexane.

**3.4** Sodium sulfate: Ignite at 650 ℃ for 4 h, cool to room temperature in a desiccator and store in sealed container.

**3.5** Methoxychlor standard ($C_{16}H_{15}C_{13}O_2$, Molecular weight: 345.66, CAS Number: 72-43-5): Purity ≥99%.

**3.6** Methoxychlor standard solution: Dissolve 10.0 mg of the methoxychlor standard in hexane, and make up to volume with hexane in a 100 mL volumetric flask. Mix thoroughly as the standard stock solution of 100 μg/mL in concentration. From which standard working solutions of suitable concentration are prepared by diluting with hexane according to the requirement.

## 4 Apparatus and equipment

4.1 Gas chromatograph mass spectrometry:equipped with electron ionization (EI) .

4.2 Tissue blender.

4.3 Homogenizer.

4.4 Rotary evaporator.

4.5 Analytical balance:0.1 mg and 0.01 g.

4.6 Centrifuge.

4.7 Gel permeation chromatographic system (GPC):with cleaning column packed with Biobeads S-X3 resin.

4.8 Vortex mixer.

## 5 Sample preparation and storage

### 5.1 Preparation of test samples

Part of representative sample is taken from the mixed primary sample and the edible portions are blended in a high-speed grinder. The homogenized sample is thoroughly mixed and reduced to at least 500 g by quartering as the test sample,which is then placed in a clean container,sealed and labeled.

### 5.2 Storage of the test samples

The test sample should be stored below −18 ℃.

### 5.3 Requirment of preparation and storage

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 10 g (accurate to 0.01 g) test sample into a 100 mL plastic centrifuge tube,then add 20 g

sodium sulfate (3.4) and 40 mL ethyl acetate-cyclohexane (1+1, *V*/*V*) into the centrifuge tube. Homogenize for 2 min, then centrifuge at 4 000 r/min for 5 min. Transfer the supernatant to a 100 mL evaporated flask through a funnel with sodium sulfate. The residue was homogenized and extracted with 20 mL ethyl acetate-cyclohexane(1 + 1, *V*/*V*). After centrifuging. combine the supernatant to the evaporated flask. Evaporate the solvent to dryness with ratary evaporator below 40 ℃.

### 6.2 GPC cleanup

#### 6.2.1 Conditions

Conditions are as follows:

a) Purification column:200 mm × 25 mm, packed with Bio-Beads S-X3;

b) Mobile phase:ethyl acetate-cyclohexane (1+1, *V*/*V*);

c) Flow rate:4.7 mL/min;

d) Injection volume:5 mL;

e) Time to start collecting:9.5 min;

f) Time to end collecting:13 min.

#### 6.2.2 Clean-up

The residues are dissolved and transferred into GPC injection bottle with 10 mL ethyl acetate-cyclohexane(1+1, *V*/*V*)and mixed thoroughly. Filter or centrifuge if particulate matter is visible. Fill the calibrated GPC sample loop with 5 mL of sample solution. Cleanup is carried out by passing through GPC system and elute with ethyl acetate-cyclohexane(1+1, *V*/*V*), collect the eluate in a 100 mL long-necked flask and evaporate nearly to dryness on a rotary evaporator below 40 ℃. Quantitatively transfer the residue and make the volume up to 1.0 mL with hexane (or other solvent suitable for GC/MS detection).

### 6.3 Determination

#### 6.3.1 GC/MS operating conditions

GC/MS operating conditions are as follows:

a) Column:DB-35MS (30 m × 0.25 mm × 0.25 μm) capillary column or equivalent;

b) Oven:80 ℃ (1 min), ramp at 20 ℃/min to 180 ℃, hold for 3 min, ramp at 20 ℃/min to 230 ℃,

hold for 7 min, ramp at 10 ℃/min to 290 ℃, hold for 10 min.

c) Injection temperature: 260 ℃; Injection mode: splitless; injection volume: 1 μL;

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

e) Electron mode: EI, 70 eV;

f) Ion source temperature: 230 ℃;

g) GC-MS interface temperature: 280 ℃;

h) Selected ion monitoring: quantitative ion: 227; qualitative ions: 228, 344.

### 6.3.2 Qualitative determination

Injecting an equal volume of standard working solution and sample solution into the gas chromatography-mass spectrometry and analyzed according to the above conditions, the retention time for methoxychlor is 23.5 min. Standard chromatograms are in Appendix A. Methoxychlor is measured in the sample, if the detected chromatographic peak retention time is consistent with the standard and that the abundance ratio of the selected ions is the similar (the deviation is in the extent permitted scope in Table 1).

**Table 1—Maximum permitted tolerances for relative ion abundance while confirmation**

| Relative abundance/% | ≥50 | >20～50 | >10～20 | ≤10 |
|---|---|---|---|---|
| Permitted tolerances/% | ±10 | ±15 | ±20 | ±50 |

### 6.3.3 Quantitative determination

Quantitation is carried out with single ion. Series of concentrations of the standard solution are prepared, and then the relationship between peak area and concentration is obtained, so external standard method is used for quantitative determination.

## 6.4 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.

# 7 Calculation and expression of result

The calculation of methoxychlor residue content in sample is carried out according to formula(1):

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

Where:

$X$ —Content of methoxychlor in the test sample, mg/kg;

$A$ —Peak area of methoxychlor in sample solution;

$A_s$—Peak height of methoxychlor in standard working solution;

$c$ —Concentration of methoxychlor in standard working solution, μg/mL;

$V$ —Final volume of the sample solution, mL;

$m$ —Corresponding mass of the sample in the final sample solution, g.

## 8 Limit of determination, recovery and precision

### 8.1 Limit of determination

The limit of determination of this method used in chicken, duck and pork is 0.005 mg/kg.

### 8.2 Recovery and precision

The method is used to determine the methoxychlor content in chicken, duck and pork. When the spiked level is 0.005 mg/kg, 0.01 mg/kg, 0.02 mg/kg, the recovery and RSD is listed in Table 2.

Table 2—The recovery and RSD of methoxychlor in chicken, duck and pork

| Samples | Spiked concentration/(μg/kg) | Recoveries/% | RSD/% |
|---|---|---|---|
| chicken | 5 | 82.8～89.6 | 2.89 |
| | 10 | 98.6～104.7 | 2.54 |
| | 20 | 100.9～103.2 | 0.86 |
| duck | 5 | 97.4～102.8 | 2.08 |
| | 10 | 99.6～103.1 | 1.60 |
| | 20 | 101.9～102.7 | 1.10 |
| pork | 5 | 85.2～93.4 | 4.25 |
| | 10 | 95.8～97.5 | 0.73 |
| | 20 | 93.2～96.2 | 1.41 |

# Annex A
(informative)
Chromatogram of methoxychlor standard

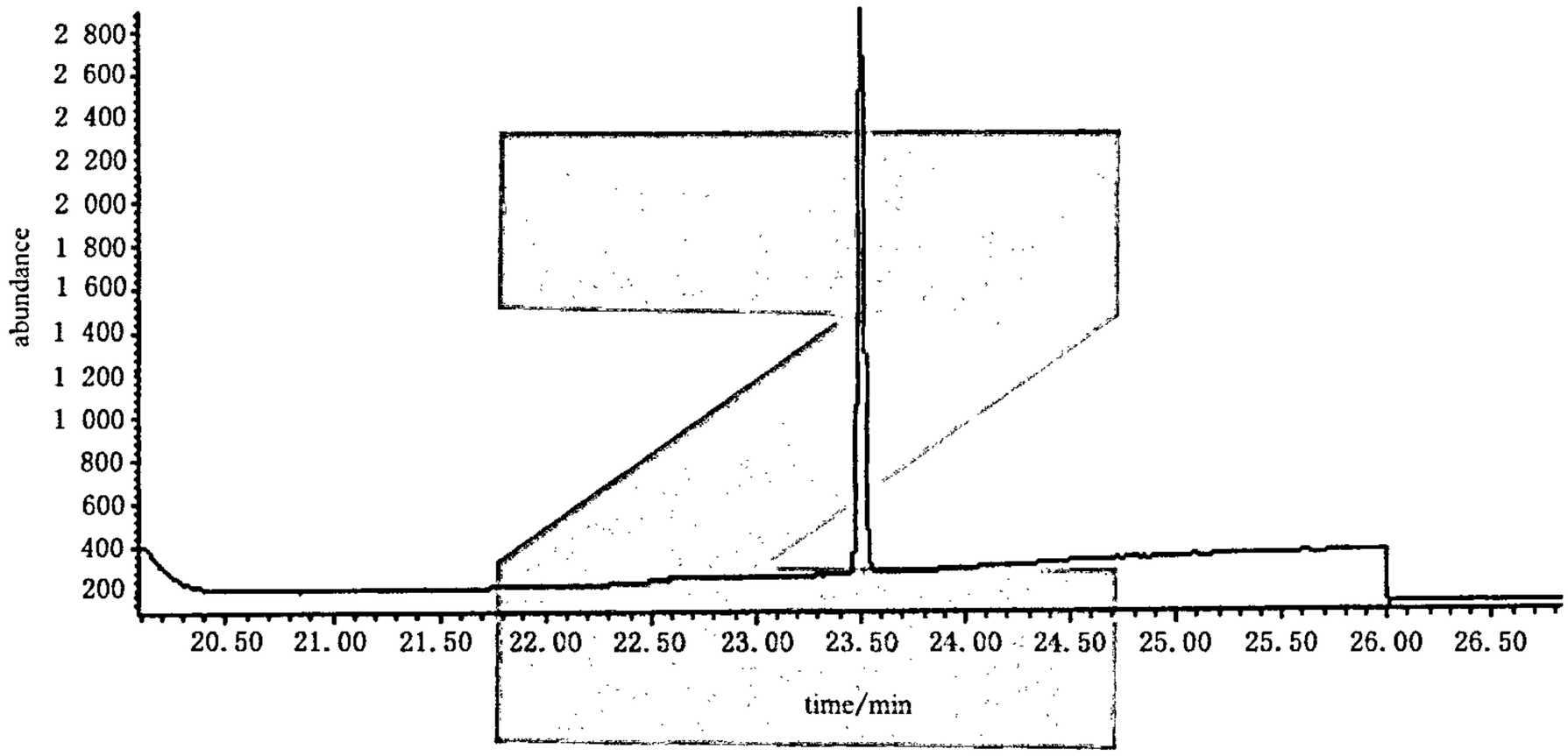


Figure A. 1—Total ion chromatogram of methoxychlor standard by GC/MS

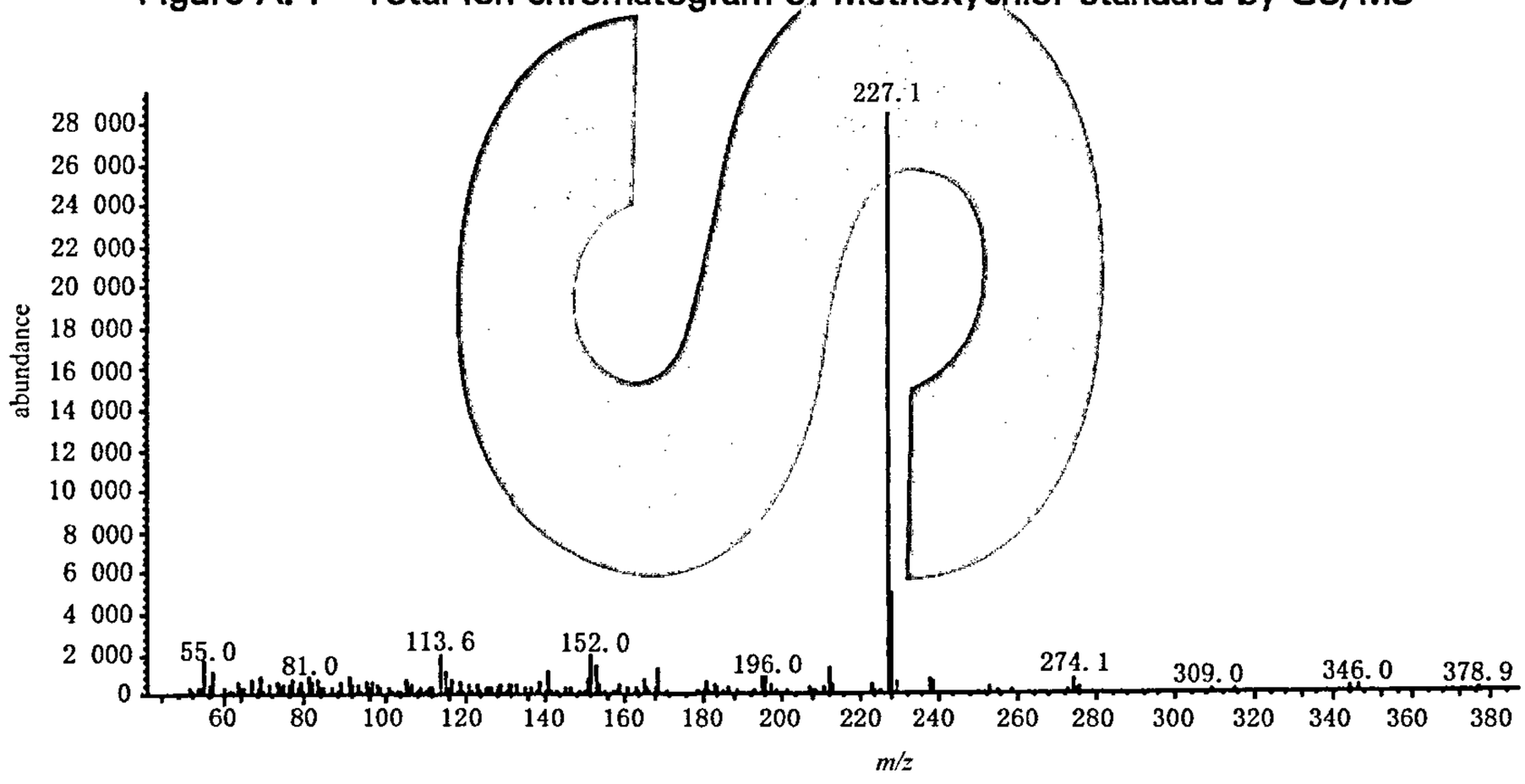


Figure A. 2—Mass spectrum of methoxychlor standard using scan mode

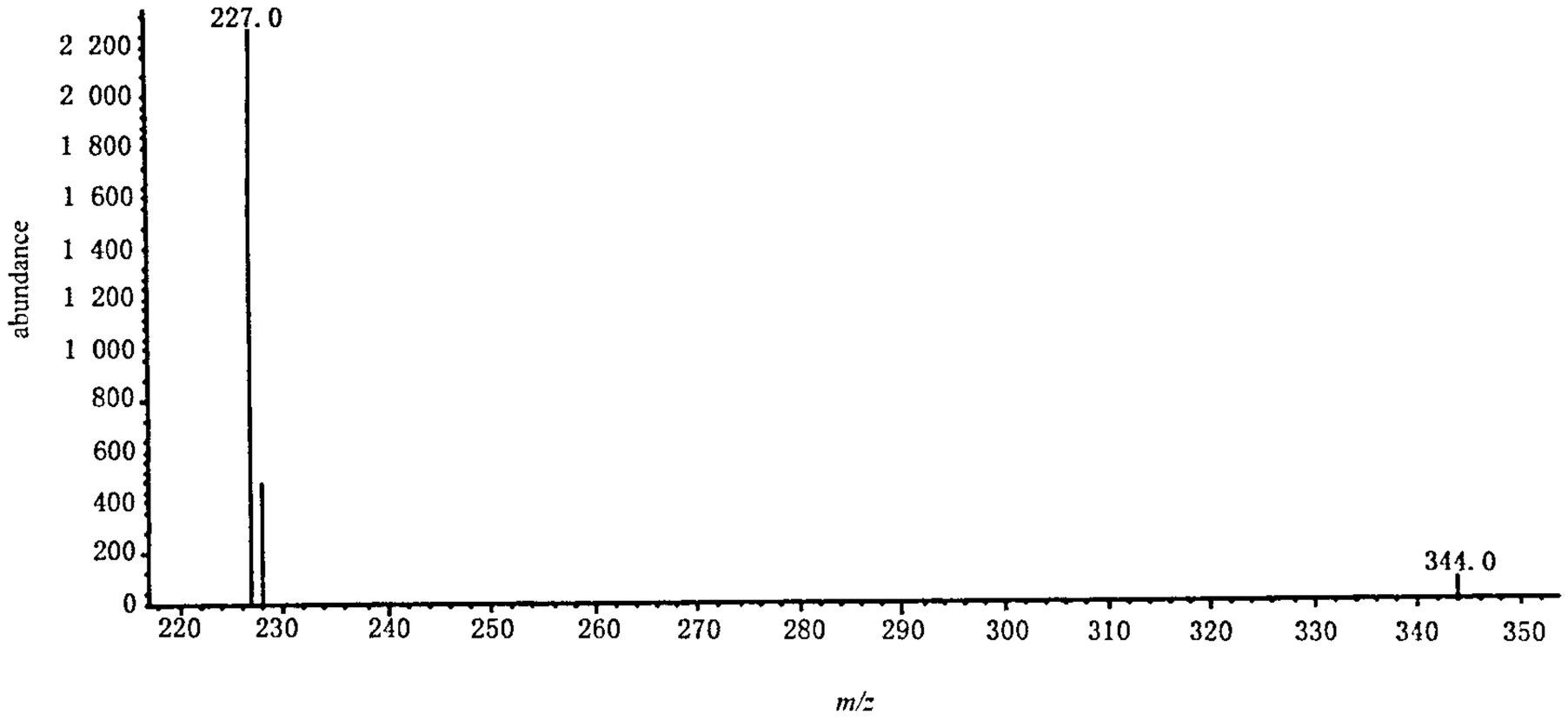


Figure A. 3—Mass spectrum of methoxychlor standard using selected ion mode