SN

中华人民共和国出入境检验检疫行业标准

SN/T 1392—2004

进出口肉及肉制品中2甲4氯及2甲4氯丁酸残留量检验方法

Determination of MCPA and MCPB residues in meat and meat products for import and export

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前　言

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。

本标准由中华人民共和国天津出入境检验检疫局、天津中医学院第一附属医院起草。

本标准主要起草人：林安清、许泓、唐丹舟、垢静。

本标准系首次发布的出入境检验检疫行业标准。

进出口肉及肉制品中2甲4氯及2甲4氯丁酸残留量检验方法

1 范围

本标准规定了进出口肉及肉制品中2甲4氯及2甲4氯丁酸残留量检验的抽样、制样和气相色谱-质谱测定方法。

本标准适用于进出口冻分割牛肉中2甲4氯及2甲4氯丁酸残留量的检验。

2 抽样和制样

2.1 检验批

以不超过2 500件为一检验批。

同一检验批的商品应具有相同的特征，如包装、标记、产地、规格和等级等。

2.2 抽样数量

抽样数量见表1。

表1

单位为件

批量	最少抽样数
1～25	1
26～100	5
101～250	10
251～500	15
501～1 000	17
1 001～2 500	20

2.3 抽样方法

按2.2规定的抽样件数随机抽取，逐件开启。

肉及肉制品：从每件中取一袋作为原始样品，其总量不少于2 kg，放入清洁容器内，加封后，标明标记，及时送交实验室。

如每件中无小包装或有小包装但每袋重量超过2 kg者，则可用锋利刀(用酒精灭菌过)在抽出的包件中，每件割取不少于100 g，混合后置于清洁容器内，作为混合原始样。混合原始样的重量不少于2 kg。加封后，标明标记，及时送交实验室。

2.4 试样制备

肉及肉制品：从所取全部样品中取出有代表性样品约1 kg，充分搅碎，混匀，均分成两份，分别装入洁净容器内。密封作为试样，标明标记。在抽样和制样的操作过程中，应防止样品受到污染或发生残留物含量的变化。

2.5 试样保存

将试样于－18℃冷冻保存。

3 测定方法

3.1 方法提要

在酸性条件下，用三氯甲烷提取组织中残留的2甲4氯、2甲4氯丁酸及其钠盐，并转移至碱液中，

用有机溶剂洗涤后再将其酸化，2 甲 4 氯、2 甲 4 氯丁酸再用三氯甲烷提取。蒸除溶剂将其甲酯化，用气相色谱-质谱仪检测，外标法定量。

3.2 试剂和材料

除另有规定外，试剂均为分析纯，水为蒸馏水。

3.2.1 三氯甲烷：重蒸馏。

3.2.2 乙醇。

3.2.3 正己烷：重蒸馏。

3.2.4 甲醇。

3.2.5 乙醚：重蒸馏。

3.2.6 硫酸-水(1+9)：用优级纯浓硫酸配制。

3.2.7 氢氧化钠：优级纯。

3.2.8 氢氧化钠溶液(30 g/L)：称取 30 g 氢氧化钠(3.2.7)溶于 1 000 mL 蒸馏水。

3.2.9 氯化钠：优级纯，于 600℃灼烧 4 h，贮于具塞瓶中。

3.2.10 饱和氯化钠溶液：用足量氯化钠(3.2.9)溶解于蒸馏水中直至不溶解。

3.2.11 无水硫酸钠：于 650℃灼烧 4 h，储于密封容器中备用。

3.2.12 硫酸钠溶液(40 g/L)：称取 4 g 无水硫酸钠(3.2.11)溶解于 100 mL 蒸馏水中。

3.2.13 三氟化硼-乙醚溶液：市售，避光保存。

3.2.14 甲酯化溶液：将三氟化硼-乙醚(3.2.13)和甲醇，于－15℃下预冷后，将 30 mL 冷三氟化硼-乙醚试剂和 120 mL 冷甲醇混合，于 0～4℃储存备用。

3.2.15 2 甲 4 氯、2 甲 4 氯丁酸标准品：纯度≥99%。

3.2.15.1 2 甲 4 氯、2 甲 4 氯丁酸标准溶液：准确称取适量的 2 甲 4 氯、2 甲 4 氯丁酸标准品，用甲醇配制成浓度为 0.1 mg/mL 标准储备溶液。再以甲醇稀释成适用浓度的标准工作溶液。

3.3 仪器和设备

3.3.1 气相色谱-质谱联用仪。

3.3.2 高速均质器。

3.3.3 心形瓶：250 mL。

3.3.4 密封试管：15 mL，带螺旋盖(配聚四氟乙烯内衬密封垫)。

3.3.5 恒温水浴箱。

3.4 测定步骤

3.4.1 提取及净化

称取搅碎混匀的试样约 20 g(精确到 0.01 g)于锥形瓶中，加 15 mL 乙醇、5 mL 硫酸-水(3.2.6)、10 g氯化钠(3.2.9)和 100 mL 三氯甲烷(3.2.1)。在高速均质器上均质提取 5 min。通过快速滤纸过滤，滤液收集于 250 mL 分液漏斗中。用约 50 mL 三氯甲烷分三次洗涤锥形瓶及滤渣，合并洗液于分液漏斗中，于上述分液漏斗中加 25 mL 氢氧化钠溶液(3.2.8)和 50 mL 蒸馏水，再加入 10 mL 饱和氯化钠(3.2.10)。此时水相 pH 应大于 12，否则应补加适量氢氧化钠溶液(3.2.8)对于极易乳化的样品可再补加约 3 g 氯化钠(3.2.9)，振摇提取 2 min。静置分层，弃去三氯甲烷层。用 25 mL 三氯甲烷洗涤水层两次，弃去三氯甲烷层。再用 25 mL 乙醚洗涤水层，静置分层，将下层水相转移至另一 250 mL 分液漏斗中，弃去乙醚层。于上述水相中，加入 25 mL 硫酸-水(3.2.6)进行酸化，摇匀。水相 pH 应小于 2，否则应适量补加硫酸-水(3.2.6)，然后分别用 50 mL、25 mL、25 mL 三氯甲烷提取水相。将三氯甲烷提取液收集于 250 mL 心形瓶中。于 60℃水浴中通以氮气流将三氯甲烷挥发至约 3～5 mL。完全转移至密封试管(3.3.4)中。再用氮气流于 50℃水浴中吹干。

3.4.2 甲酯化

于上述密封试管中，加 1 mL 甲酯化溶液(3.2.14)，用螺旋盖密封。充分振动混匀，于 70℃水浴酯

化 1 h，放冷至室温。以约 10 mL 硫酸钠溶液(3.2.12)将甲酯液转移至 25 mL 具塞试管中，分别以 4 mL正己烷提取两次。用滴管将正己烷层转移至另一 25 mL 具塞试管中，用 2 mL 硫酸钠溶液洗涤正己烷层两次，用滴管吸除水层，将正己烷层转移至 10 mL 容量瓶并以正己烷定容。吸取上述正己烷溶液 3～5 mL 于 10 mL 具塞试管中，加约 1 g 无水硫酸钠，振摇脱水。供气相色谱-质谱分析。

3.4.3 2 甲 4 氯、2 甲 4 氯丁酸标准工作溶液的制备

准确吸取适用浓度的 2 甲 4 氯、2 甲 4 氯丁酸标准溶液 1 mL(3.2.16)于带螺旋帽盖的试管(3.3.4)中，用氮气流于 50℃水浴中吹干，按 3.4.2 操作进行甲酯化。

3.4.4 测定

3.4.4.1 色谱条件

a) 色谱柱：弹性石英毛细柱 DB5MS 30 m×0.32 mm(内径)×0.25 μm 或相当者；

b) 柱温程序：50℃ $\xrightarrow{20℃/min}$ 240℃(10 min)；

c) 汽化室温度：250℃；

d) 载气：氦气，纯度 99.999%，流速 1.2 mL/min；

e) 进样方式：无分流；

f) 进样量：1 μL。

3.4.4.2 质谱条件

a) 接口温度：250℃；

b) 离子源：电子轰击源(EI)；

c) 电子能量：70 eV；

d) 离子源温度：200℃；

e) 检测方式：SIR；

f) 选择离子(m/z)及相对丰度(%)：见表 2。

表 2 选择离子及相对丰度

被测组分	2 甲 4 氯甲酯				2 甲 4 氯丁酸甲酯			
选择离子/(m/z)	141	157	214	216	101	107	142	211
相对丰度/(%)	85	20	100	34	100	13	9	8

3.4.4.3 气相色谱-质谱测定

对样液(3.4.2)及标准工作溶液(3.4.3)等体积参插进样测定。实际应用的标准工作溶液及待测样液中，2 甲 4 氯甲酯、2 甲 4 氯丁酸甲酯的响应值均应在仪器线性范围内。在上述条件下 2 甲 4 氯甲酯、2 甲 4 氯丁酸甲酯保留时间分别约为 7.8 min 和 9.1 min。标准品气相色谱-质谱图参见附录 A。

3.4.4.4 空白试验

除不加样品外，按上述相同条件和步骤进行。

3.5 结果计算和表述

用色谱数据处理机或按式(1)计算，计算结果需扣除空白值。

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

式中：

X——试样中 2 甲 4 氯或 2 甲 4 氯丁酸含量，单位为毫克每千克(mg/kg)；

A——样液中 2 甲 4 氯甲酯或 2 甲 4 氯丁酸甲酯的峰面积，单位为毫米(mm)；

A_s——标准溶液中 2 甲 4 氯甲酯或 2 甲 4 氯丁酸甲酯的峰面积，单位为毫米(mm)；

c——标准工作溶液中 2 甲 4 氯或 2 甲 4 氯丁酸浓度，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量，单位为克(g)。

4 方法的测定低限、回收率

4.1 测定低限

本方法测定低限为：0.02 mg/kg。

4.2 回收率

牛肉中 2 甲 4 氯添加浓度及回收率的实验数据：

在 0.02 mg/kg 时，回收率为 85.5%；

在 0.10 mg/kg 时，回收率为 95.0%；

在 0.20 mg/kg 时，回收率为 92.0%。

牛肉中 2 甲 4 氯丁酸添加浓度及回收率的实验数据：

在 0.02 mg/kg 时，回收率为 90.0%；

在 0.10 mg/kg 时，回收率为 90.0%；

在 0.20 mg/kg 时，回收率为 93.0%。

附 录 A
（资料性附录）
标准物气相色谱-质谱图

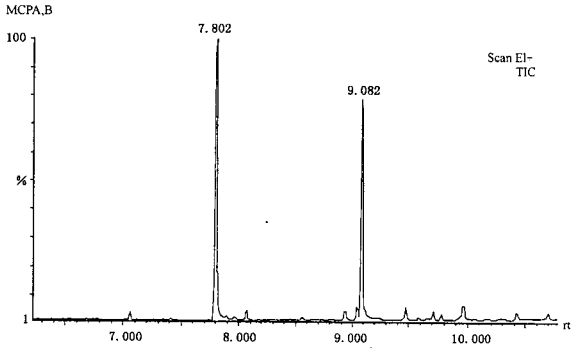

图 A.1 2甲4氯及2甲4氯丁酸标准衍生物气相色谱-质谱图(TIC)

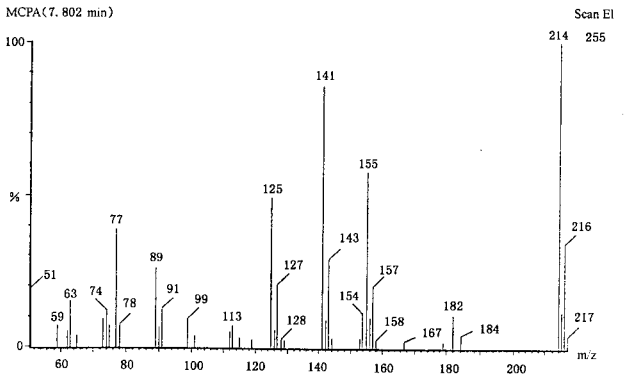

图 A.2 2甲4氯标准衍生物质谱图

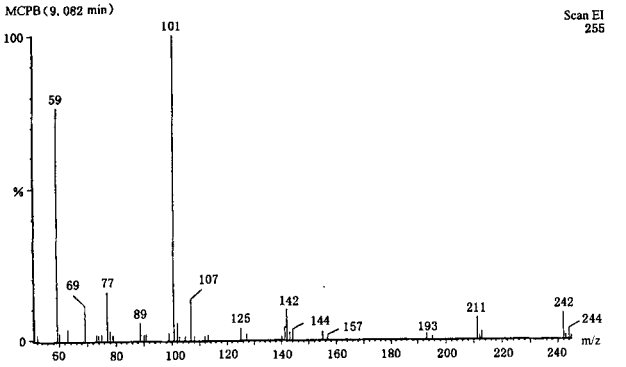

图 A.3　2 甲 4 氯丁酸标准衍生物质谱图

Foreword

Annex A of this standard is on informative annex.

This standard was proposed by and is under the charge of National Regulation Commission for Certification and Accreditation.

This standard was drafted by Tianjin Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, No. 1 Preaching Hospital Tianjin University of accessorial Chinese medicine.

This standard was mainly drafted by Lin Anqing, Xu Hong, Tang Danzhou, Goujing.

This standard is a professional standard promulgated for the first time.

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

Determination of MCPA and MCPB residues in meat and meat products for import and export

1 Scope

This standard specifies the methods of sampling, sample preparation and determination of residues by GC-MS of (4-cholro-2-methyphenoxy) acetic acid (MCPA) and 4-(4-chloro-o-toloxy) butyric acid (MCPB) residues in meat and meat products for import and export.

This standard is applicable to the determination of MCPA and MCPB residues in frozen cuts of beef for import and export.

2 Sampling and sample preparation

2.1 Inspection lot

The quantity of an inspection lot should not be more than 2 500 packages.
The characteristics of the cargo within the same inspection lot, such as packing, mark, origin, specification and grade, should be the same.

2.2 Quantity of sample taken

Quantity of sample taken see table 1.

Table 1

Number of packages in each inspection lot	Minimum number of packages to be taken
1～25	1
26～100	5
101～250	10
251～500	15
501～1 000	17
1 001～2 500	20

2.3 Sampling procedure

A number of packages specified in 2.2 are taken at random and opened one by one.
Meat and meat products: From each, at least one bag shall be taken as a primary sample. The total weight of all the primary samples should not be less than 2 kg, which shall be placed in a clean container, sealed, labeled and sent to the laboratory in time.
In case the frozen meat-pieces are not contained in small bags inside each package, or if there are

small bags inside the package but the content of the bag exceeds 2 kg, cut out a part from the meat in each package of not less than 100 g with a sharp knife (disinfected with alcohol). Mix the parts of the mixed primary samples, which shall not be less than 2 kg. Sealed, labeled and sent to the laboratory in time.

2.4 Preparation of test sample

Meat and meat products: The combined primary sample is reduced to 1 kg which is blended mixed and divided into two equal portions, each portion is placed in a clean vessel as a test sample, which is then sealed and labeled. In the course of sampling and sample preparation, precaution should be taken to avoid contamination or any factor which may causes the change of residue content.

2.5 Storage of sample

The test sample should be stored at −18℃.

3 Method of determination

3.1 Principle

MCPA and MCPB residues and its sodium salts are extracted from the tissue with chloroform under acidic condition, then transfer into alkaline solution. The alkaline solution is washed with organic solvents and acidified, MCPA and MCPB are then extracted with chloroform and derivatisation after the chloroform has been evaporated. Determination is made by GC-MS and quantified by using the external standard.

3.2 Reagents and materials

Unless otherwise specified, all reagents used should be analytically pure, "water" is distilled water.

3.2.1 Chloroform: redistilled.

3.2.2 Ethanol.

3.2.3 n-Hexane: redistilled.

3.2.4 Methanol.

3.2.5 Ether: redistilled.

3.2.6 Sulfuric acid-water (1+9): Prepare the solution with GR grade of sulfuric acid.

3.2.7 Sodium hydroxide: GR grade.

3.2.8 Sodium hydroxide solution (30 g/L): Weigh 30 g Sodium hydroxide (3.2.7) into 1 000 mL distilled H_2O.

3.2.9 Sodium chloride: GR grade, ignite at 600℃ for 4 h, store in a glass jar with stopper.

3.2.10 Saturated sodium chloride solution: Sodium chloride dissolved in distilled H_2O to saturation.

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

3.2.12 Sodium sulfate solution(40 g/L): Weigh 4 g of anhydrous sodium sulfate (3.2.11)and dissolve in 100 mL water.

3.2.13 Boron trifluoride-ether solution: commercially, kept away from light.

3.2.14 Derivatization reagent: Pre-cool the boron trifluoride-ether solution (3.2.13)and methanol at −15℃, add 30 mL cold boron trifluoride-ether into 120 mL cold methanol and mix thoroughly, store at 0 −4 ℃.

3.2.15 MCPA and MCPB standard: Purity≥99%.

3.2.16 MCPA and MCPB standard solution: Prepare 0.1 mg/mL standard stock solution with methanol, then dilute to suitable concentration with methanol as standard working solution.

3.3 Apparatus and equipments

3.3.1 Gas chromatograph equipped with mass spectrograph (GC-MS).

3.3.2 High-speed homogenizer.

3.3.3 Heart shape flask: 250 mL.

3.3.4 Sealed test tube: 15 mL, with teflon-lined screw cap.

3.3.5 Thermostatic water bath.

3.4 Procedure

3.4.1 Extraction and clean up

Weigh ca 20 g (accurate to 0.01 g)of test sample in a conical flask, add 15 mL ethanol, 5 mL sulfuric

acid-water(3.2.6), 10 g sodium chloride (3.2.9) and 100 mL chloroform (3.2.1). Extract 5 min with high-speed homogenizer. Then filter the extract through rapid filter paper into a 250 mL separating funnel, rinse the conical flask and filter residue thrice with ca 50 mL chloroform. Combine all rinsing solutions into the separating funnel. Add 25 mL sodium hydroxide solution (3.2.8) and 50 mL distilled water. Add 10 mL saturated sodium chloride solution(3.2.10). pH of the aqueous phase should be more than 12, otherwise, additional sodium hydroxide solution(3.2.8) is needed. Additional 3 g sodium chloride (3.2.9) should be added to the easily emulsified sample, shake for 2 min. Stand to separate, discard the chloroform layer. Wash the aqueous phase twice with 25 mL chloroform, discard the chloroform layer. And then wash the aqueous phase with 25 mL ether, stand to separate, transfer the below layer to a 250 mL separating funnel, discard the ether layer. The above aqueous phase is acidified with 25 mL sulfuric acid-water (3.2.6) and mix thoroughly, pH of the aqueous phase should be less than 2, otherwise, additional sulfuric acid-water(3.2.6) is needed. Then extract the aqueous phase with 50, 25 and 25 mL chloroform respectively. Collect the extract of chloroform into 250 mL heart shape flask. Evaporate the chloroform to ca 3-5 mL in a water bath at 50℃ under nitrogen flow. Then transfer entirely to the sealed test tube (3.3.4), make the chloroform to dryness under nitrogen flow in a water bath at 50℃.

3.4.2 Derivatisation

Add 1 mL derivatization reagent (3.2.14)into the above tube and seal tightly, mix thoroughly, place the tube in water bath for 1 h at 70℃. Cool to room temperature, transfer the resulting mixture with ca 10 mL sodium sulfate solution (3.2.12) to a 25 mL tube with stopper. Extract with n-hexane twice each 4 mL. Transfer the n-hexane layer to another 25 mL tube with stopper. Extract the n-hexane layer with each 2 mL of sodium sulfate solution twice and discard the aqueous layer. Using a dropper transfer the n-hexane layer to 10 mL volumetric flask, dilute to volume with n-hexane. Pipette 3-5 mL n-hexane layer into a 10 mL tube with stopper, add ca 1 g of anhydrous sodium sulfate (3.2.11), mix well. The solution is used for GC-MS determination.

3.4.3 Preparation of MCPA and MCPB standard working solution

Accurately pipette 1 mL MCPA and MCPB standard solution of suitable concentration into tube with screw cap (3.3.4), remove the solvent under nitrogen flow in a water bath at 50℃, proceed as section 3.4.2.

3.4.4 Determination

3.4.4.1 GC operating conditions

a. Column: DB5MS 30 m × 0.32(id) mm × 0.25 μm(film thickness) or equivalent;

b. Column temperature program: 50℃ $\xrightarrow{20℃/min}$ 240℃ (10 min);

c. Injection port temperature: 250℃;

e. Carrier Gas: He, Purity≥99.999%; flow rate: 1.2 mL/min;

f. Injection mode: Splitless;
g. Injection volume: 1 μL.

3.4.4.2 MS operating conditions

a. Interface temperature: 250℃;
b. Ion Source: Electron Impact Ion Source (EI);
c. Electron Energy: 70 eV;
d. Source temperature: 200℃;
e. Detection mode: SIR;
f. Selected ions (m/z) and relative intensity (%): see Table 2.

Table 2 Selected ions and relative intensity

analyte	MCPA methyl ester				MCPB methyl ester			
Selected ions (m/z)	141	157	214	216	101	107	142	242
Relative intensity(%)	85	20	100	34	100	13	9	8

3.4.4.3 GC-MS determination

The mix standard working solution should be randomly injected in-between the injections of the sample solution of equal volume. The responses of the MCPA methyl ester and MCPB methyl ester in the standard working solution and sample solution should be within the linear range of the detector. The retention time of MCPA and MCPB methyl ester is ca 7.8 min and 9.0 min under the above conditions. For the chromatogram of the standard, see annex A.

3.4.4.4 Blank test

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

3.5 Calculation and expression of result

Calculate the content of MCPA and MCPB by GC data processor or according to formula(1) the blank value shall be subtracted from the result of calculation.

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

Where

X——the residue content of MCPA or MCPB in test sample, mg/kg;
A——the peak area of MCPA methyl ester or MCPB methyl ester in sample solution, mm;
A_s——the peak area of MCPA methyl ester or MCPB methyl ester in standard solution, mm;
c——the concentration of MCPA or MCPB in standard solution, μg/mL;
V——the final volume of the sample solution, mL;
m——the corresponding mass of test sample in the final sample solution ,g.

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 0.02 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentration of MCPA in beef and its corresponding recoveries are:
0.02 mg/kg, recovery 85.5%;
0.10 mg/kg, recovery 95.0%;
0.20 mg/kg, recovery 92.0%.

According to the experimental data, the fortifying concentration of MCPB in beef and its corresponding recoveries are:

0.02 mg/kg, recovery 90.0%;
0.10 mg/kg, recovery 90.0%;
0.20 mg/kg, recovery 93.0%.

Annex A
(informative)
GC-MS Chromatogram of the standard

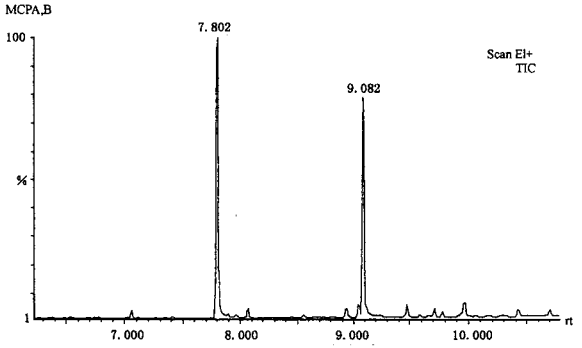

Fig A.1 GC-MS chromatogram(TIC)of the MCPA methyl ester and MCPB methyl ester standard

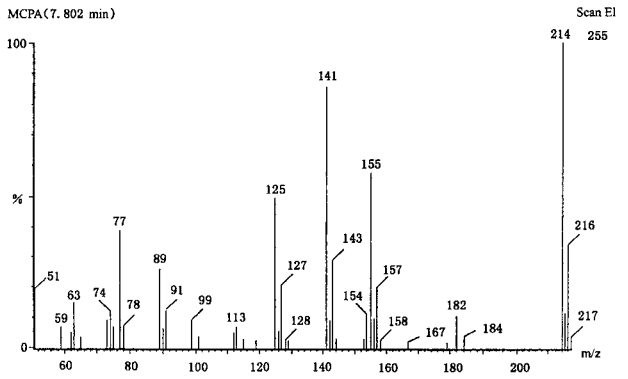

Fig A.2 Mass spectrogram of the MCPA methyl ester standard

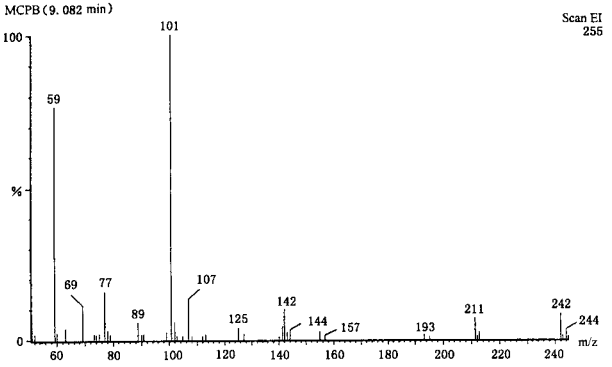

Fig A. 3 Mass spectrogram of the MCPB methyl ester standard