



中华人民共和国出入境检验检疫行业标准

SN/T 3623—2013

出口食品中富马酸二甲酯的测定方法

Determination of dimethy fumarate in foods for export

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前 言

本标准按照 GB/T 1.1—2009 给出的规则起草。
请注意本文件的某些内容可能涉及专利。本文件的发布机构不承担识别这些专利的责任。
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、中国检验检疫科学研究院。
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出口食品中富马酸二甲酯的测定方法

1 范围

本标准规定了食品中富马酸二甲酯测定的气相色谱-质谱法(GC/MS)。
本标准适用于豆奶、葡萄、午餐肉、月饼、调料粉、奶粉等食品中富马酸二甲酯的测定。

2 规范性引用文件

下列文件对于本文件的应用是必不可少的。凡是注日期的引用文件,仅注日期的版本适用于本文件。凡是不注日期的引用文件,其最新版本(包括所有的修改单)适用于本文件。

GB/T 6682 分析实验室用水规格和试验方法

3 方法提要

采用乙酸乙酯做溶剂提取食品中的富马酸二甲酯,浓缩后过中性氧化铝与活性炭串联的固相萃取小柱净化,洗脱液浓缩并溶解定容后,用 GC/MS 仪测定,外标法定量。

4 试剂和材料

除另有说明,所用试剂均为分析纯,水 GB/T 6682 规定的一级水。

- 4.1 甲醇,HPLC 级。
- 4.2 乙酸乙酯,色谱纯。
- 4.3 无水硫酸钠:650 °C 灼烧 4 h,在干燥器内冷却至室温,贮于密封瓶中备用。
- 4.4 活性炭固相萃取小柱:500 mg,3 mL 或相当者。
- 4.5 中性氧化铝固相萃取小柱:1 g,3 mL 或相当者。
- 4.6 富马酸二甲酯标准品(Dimethyl fumarate, $C_6H_8O_4$,CAS 号 624-49-7):纯度大于或等于 98%。
- 4.7 富马酸二甲酯标准储备液:称取适量的富马酸二甲酯标准品,用甲醇配制成浓度为 1 mg/mL 的标准储备液,置-18 °C 冰箱保存,有效期为 12 个月。
- 4.8 富马酸二甲酯标准工作液:取适量富马酸二甲酯标准储备液用甲醇稀释为 10.0 μ g/mL 的工作液,置-18 °C 冰箱保存,有效期为 6 个月。

5 仪器和设备

- 5.1 气相色谱-质谱联用仪:配有电子轰击源(EI)。
- 5.2 组织捣碎机。
- 5.3 电子天平:感量分别为 0.01 g,0.000 1 g。
- 5.4 涡旋振荡器。
- 5.5 旋转蒸发器。
- 5.6 具塞塑料离心管:50 mL。

5.7 离心机,不低于 8 000 r/min。

5.8 固相萃取装置。

6 测定步骤

6.1 试样的制备与保存

6.1.1 午餐肉、月饼和葡萄等试样

取代表性样品 500 g,将其切碎后,依次用捣碎机将样品加工后混匀,分装入洁净的盛样袋内,密封并标明标记。

6.1.2 奶粉、豆奶和调料粉等试样

取代表性样品 500 g,混匀,装入洁净的容器内,密封并标明标记。

6.2 试样的保存

午餐肉、月饼、葡萄等试样于-18℃冰箱保存;豆奶试样于 0℃~4℃保存;奶粉和调料粉等可常温下避光保存。在制样的操作过程中,应防止样品受到污染或发生残留物含量的变化。

7 测定步骤

7.1 提取

称取 5 g 试样(精确到 0.01 g)置于 50 mL 具塞离心管中,再加入 15 mL 乙酸乙酯,涡旋振荡 5 min,以 8 000 r/min 离心 5 min,将上清液导入 150 mL 梨形瓶;再用 15 mL 乙酸乙酯重复上述提取步骤,合并收集液于梨形瓶。在旋转蒸发仪上浓缩至大约 2 mL,待净化。

7.2 净化

按中性氧化铝小柱在下,活性炭小柱在上的顺序将二者串联,活性炭柱上加 1 cm 高的无水硫酸钠,5 mL 乙酸乙酯活化,上样并收集,再用 3 mL 乙酸乙酯将梨形瓶清洗后上样洗脱并收集,合并收集液定容到 5 mL,待测。

7.3 测定

7.3.1 色谱条件

色谱条件如下:

- a) 色谱柱:HP-5MS 石英毛细管柱,30 m×0.25 mm×0.25 μm,或相当者;
- b) 柱温:初始温度 50℃,保持 1 min,以 8℃/min 速率,升温至 300℃,保持 5 min;
- c) 载气:高纯氮气 99.999%,柱流速 1.0 mL/min;
- d) 进样口温度:250℃;
- e) 进样模式:不分流,0.75 min 打开分流阀;
- f) 进样量:1 μL。

7.3.2 质谱条件

质谱条件如下:

- a) 接口温度:280 ℃;
- b) 离子源:电子轰击源(EI);
- c) 电子能量:70 eV;
- d) 离子源温度:230 ℃;
- e) 溶剂延迟:4 min;
- f) 检测方式:SIM,目标物的选择离子均为 m/z 113、59、114、85,定量离子为 m/z 113。

7.3.3 气相色谱-质谱测定

根据样液中被测物含量情况,选定浓度相近的标准工作溶液与样液等体积参插进样测定,标准工作溶液和待测样液中的富马酸二甲酯的响应值均应在仪器检测的线性范围内。如果样液与标准工作溶液的选择离子色谱图中,在相同保留时间有色谱峰出现,并且在扣除背景后的样品质量色谱中,所选离子均出现,并且所选择离子的丰度比与标准品对应离子的丰度比,其值在允许范围内(见表1)。在上述色谱条件下富马酸二甲酯的保留时间分别为 7.69 min,标准溶液全扫描质谱图及选择离子色谱图参见附录 A。

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

7.3.4 空白实验

除不加试样外,按上述 7.1~7.3 测定步骤进行。

8 结果计算和表述

试样中富马酸二甲酯的测定结果可有数据处理系统按外标法自动计算,也可按式(1)计算,计算结果应扣除空白值。

$$C = \frac{A \times C_s \times V}{A_s \times m}$$

.....(1)

式中:

- C ——每克试样中富马酸二甲酯的含量,单位为毫克每千克(mg/kg);
- A ——试样溶液中富马酸二甲酯的峰高或峰面积;
- C_s ——标准工作溶液中富马酸二甲酯浓度,单位为微克每毫升(μg/mL);
- V ——试样溶液最终定容体积,单位为毫升(mL);
- A_s ——标准工作溶液中富马酸二甲酯峰高或峰面积;
- m ——称样量,单位为克(g)。

9 测定低限、回收率

本方法富马酸二甲酯的测定低限为 0.050 mg/kg,不同基质的回收率范围参见表 2。

表 2 不同基质的回收率

基质	添加水平/(mg/kg)	回收率/%
豆奶	0.05	82.6~91.0
	0.1	81.2~96.8
	0.5	86.2~102
葡萄	0.05	81.0~90.4
	0.1	95.8~102
	0.5	91.7~103
月饼	0.05	78.2~96.2
	0.1	84.6~98.8
	0.5	82.4~98.4
午餐肉	0.05	83.0~95.4
	0.1	82.5~94.1
	0.5	87.8~98.4
调料粉	0.05	83.0~97.6
	0.1	86.1~101
	0.5	85.8~99.2
奶粉	0.05	83.0~95.4
	0.1	82.5~94.1
	0.5	91.2~100

附录 A
(资料性附录)

标准品全扫描质谱图 and 选择离子色谱图

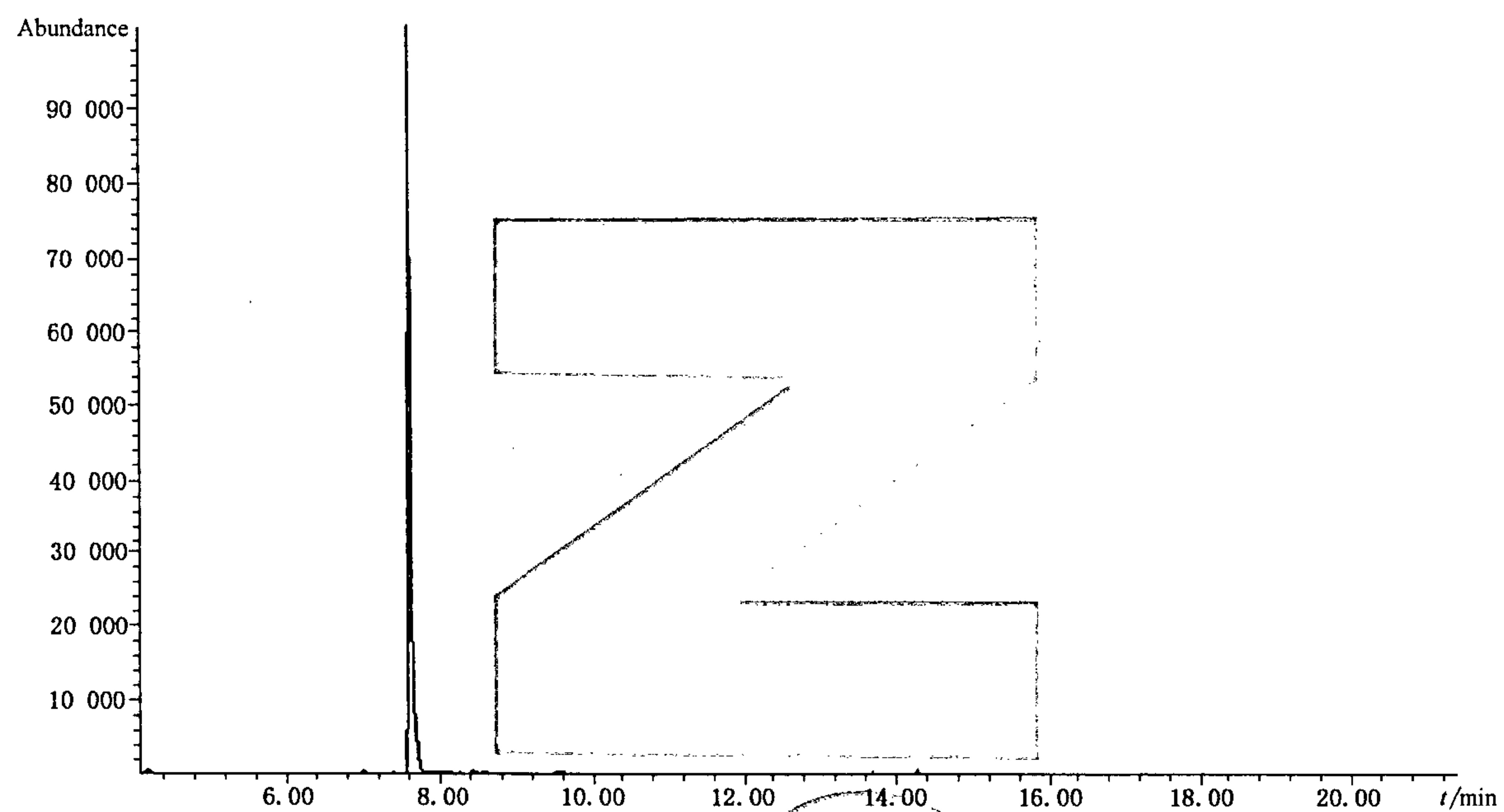


图 A.1 富马酸二甲酯选择离子色谱图

Foreword

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

Some parts of the standard may have relationship with some patents. The release department have no responsibility to recognize these patents.

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, Chinese Academy of Inspection and Quarantine.

The main drafters of this standard are HanLi, DengXiaojun, YiXionghai, PengTao, ShengYonggang, Fanxiang, ZhaoShanzhen.

Determination of dimethy fumarate in foods for export

1 Scope

This standard specifies determination of dimethyl fumarate (DMF) in food for export by GC/MS.

This standard is applicable to the determination of dimethyl fumarate (DMF) in food such as pork tin, moon cake, milk powder, soy milk, spices, grape and spices powder so on.

2 Normative reference

The following normative documents contain provisions which, through reference in this text, constitute provisions of this standard. For dated references, subsequent amendments to, or revisions of any of these publications do not apply. However parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies.

GB/T 6682 Water for analytical laboratory use—Specification and test methods

3 principle

Dimethyl fumarate residue in food is extracted with ethyl acetate. The concentrated solution was cleaned by SPE column of N-Alumina and Carb. The elution is determined by GC/MS using external standard method.

4 Reagents and materials

All the reagents used should be analytically pure unless otherwise specified. “Water” is first class water.

4.1 Methanol; HPLC grade.

4.2 Ethyl Acetate; Chromatographic purity.

4.3 Anhydrous sodium sulfate; Dried at 650 °C for 4 h, cool to room temperature in a desiccator and store in sealed container.

4.4 Envi-carb column:500 mg,3 mL or equivalent.

4.5 N-Alumina column:1 g,3 mL or equivalent.

4.6 Dimethyl fumarate standard ($C_6H_8O_4$,CAS 号 624-49-7):Purity $\geq$ 98%.

4.7 DMF standard working solution: Accurately weight DMF, dissolved with methanol to form a standard stock solution of 1.0 mg/mL in concentration, be stored at $-18\text{ }^{\circ}\text{C}$, assign a shelflife of 12 months.

4.8 DMF working standard solutions: Then dilute the standard stock solution with methanol to form the standard working solution of 10.0 $\mu\text{g/mL}$ in concentration, be stored at $-18\text{ }^{\circ}\text{C}$.assign a shelflife of 6 months.

5 Apparatus and equipment

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

5.2 Tissue triturator.

5.3 Balance,sensitivity is 0.01 g and 0.000 1 g.

5.4 Vortex mixer.

5.5 Rotary evaporator.

5.6 Plastic centrifuge tubes with cap:50 mL.

5.7 Centrifuger. Not below 8 000 r/min.

5.8 Equipment for SPE clean.

6 Procedure

6.1 Sample preparation and storage

6.1.1 Pork tin,moon cake and grape

About 500 g prerepresentative samples should be taken from all samples, and cut into mince and homogenized. The mixed to produce homogenous samples, and put in a clean container which is sealed and labeled.

6.1.2 milk powder, soy milk and spices powder

About 500 g representative samples should be taken from all samples, homogenized and put in a clean container which is sealed and labeled.

6.2 Storage of test sample

Pork tin, moon cake and grape were stored at $-18\text{ }^{\circ}\text{C}$. Soy milk was stored at $0\text{ }^{\circ}\text{C} \sim 4\text{ }^{\circ}\text{C}$. Milk powder and spices powder should be stored in room temperature. In the course of sampling and sample preparation, precaution should be taken to avoid contamination or any factors which may cause the change of residue content.

7 Procedure

7.1 Extraction

Weigh about 5 g (accurate to 0.01 g) of the test sample into 50 mL plastic centrifuge tubes, add 15 mL of ethyl acetate and vortex, centrifuge for 5 min in 8 000 r/min and transfer the supernatant to a 150 mL glass the add another 15 mL of ethyl acetate into the tubes with residues, centrifuge and Combine the extracted solution. Then evaporation the solution to 2 mL by rotary evaporator and wait for cleaning.

7.2 SPE cleanup

N-Alumin SPE column is connected with Carb SPE column that is contain Anhydrous sodium sulfate with 1 cm high, then condition with 5 mL ethyl acetate, sample and collection, and elute with 3 mL ethyl acetate, Constant volume to 5 mL and wait for determination by instrument.

7.3 Determination

7.3.1 GC operation conditions

GC operation conditions is as following:

- Column: HP-5MS capillary column, $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$, or the equivalent;
- Temperature program: $50\text{ }^{\circ}\text{C}$ for 1 min, $8\text{ }^{\circ}\text{C}/\text{min}$ to $300\text{ }^{\circ}\text{C}$ for 5min;
- Carrier gas: Helium, purity $\geq 99.999\%$, flow rate: $1.0\text{ mL}/\text{min}$;
- Inlet temperature: $250\text{ }^{\circ}\text{C}$;

- e) Injection mode: Splitless, open split valve at 0.75 min;
- f) Injection volume: 1 μ L;

7.3.2 Mass operation conditions

MS operation conditions is as following:

- a) Interface temperature: 280 $^{\circ}$ C ;
- b) Ionization source: EI;
- c) Electron impact: 70 eV;
- d) Ionization source temperature: 230 $^{\circ}$ C ;
- e) Solvent delay: 4 min;
- f) Selected monitoring ions(m/z) 113, 59, 114, 85; the quantified ion is m/z 113.

7.3.3 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 DMF 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 same conditions of experiment, the retention time of the unknown sample is the same as the standard working solution; 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.

Under the above GC-MS operating conditions, the retention time of DMF is about 7.69 min, and its mass spectrum are shown by figure A. 1 in annex A.

Table 1—Maximum permitted tolerances relative ion intensities while confirmation %

Relative intensity	>50	>20~50	>10~20	≤10
Permitted tolerances	± 20	± 25	± 30	± 50

7.3.4 Blank experiment

In addition to not sample, according to the determination of the above steps 7.1~7.3.

8 Calculation and expression of the result

Calculate the content of DMF in the test sample by GC－MS data processor or using the followed formula (1).The result will subtract the value of blank sample.

$$C = \frac{A \cdot C_s \cdot V}{A_s \cdot m}$$

.....(1)

Where;

C —the residue content of DMF in the test sample (mg/kg) ;

A —the height or area of DMF in the test sample;

C_s—the concentration of DMF in the standard working solution (μg/mL) ;

V —the final volume of the sample solution (mL) ;

A_s—the height or area of standard DMF;

m—the sample weight (g).

9 Limit of determination and recovery

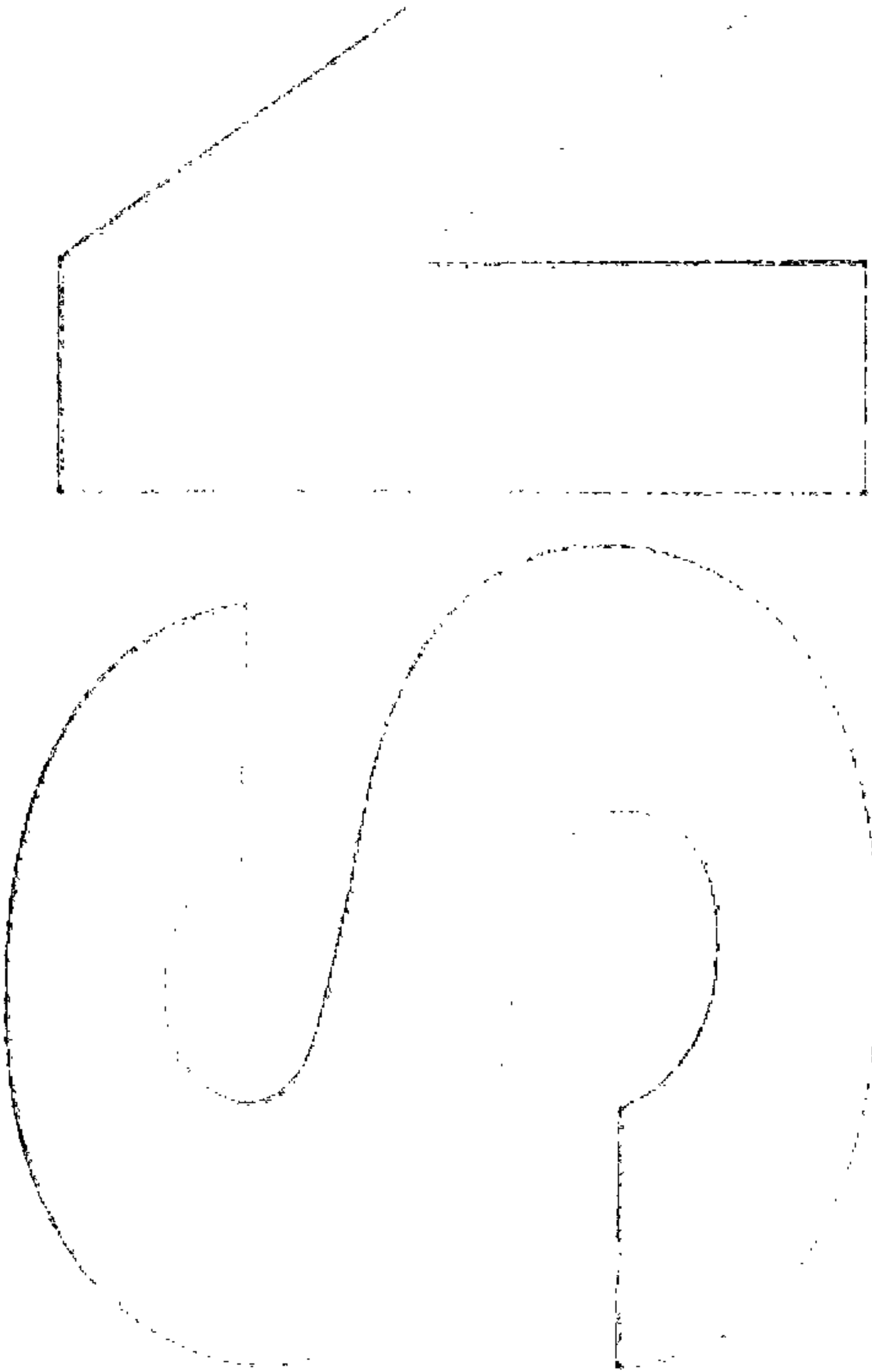
The limit of determination of DMF of this method is 0.050 mg/kg,the recovery are listed in table 2.

Table 2—Different media recovery

Material matrix	Spiked level/(mg/kg)	Recovery/%
soy milk	0.05	82.6~91.0
	0.1	81.2~96.8
	0.5	86.2~102
grape	0.05	81.0~90.4
	0.1	95.8~102
	0.5	91.7~103
moon cake	0.05	78.2~96.2
	0.1	84.6~98.8
	0.5	82.4~98.4

Table 2 (continued)

Material matrix	Spiked level/(mg/kg)	Recovery/%
pork tin	0.05	83.0~95.4
	0.1	82.5~94.1
	0.5	87.8~98.4
spices powder	0.05	83.0~97.6
	0.1	86.1~101
	0.5	85.8~99.2
milk powder	0.05	83.0~95.4
	0.1	82.5~94.1
	0.5	91.2~100



Annex A
(Informative)
GC-MS chromatogram and mass spectrum of the DMF Standard

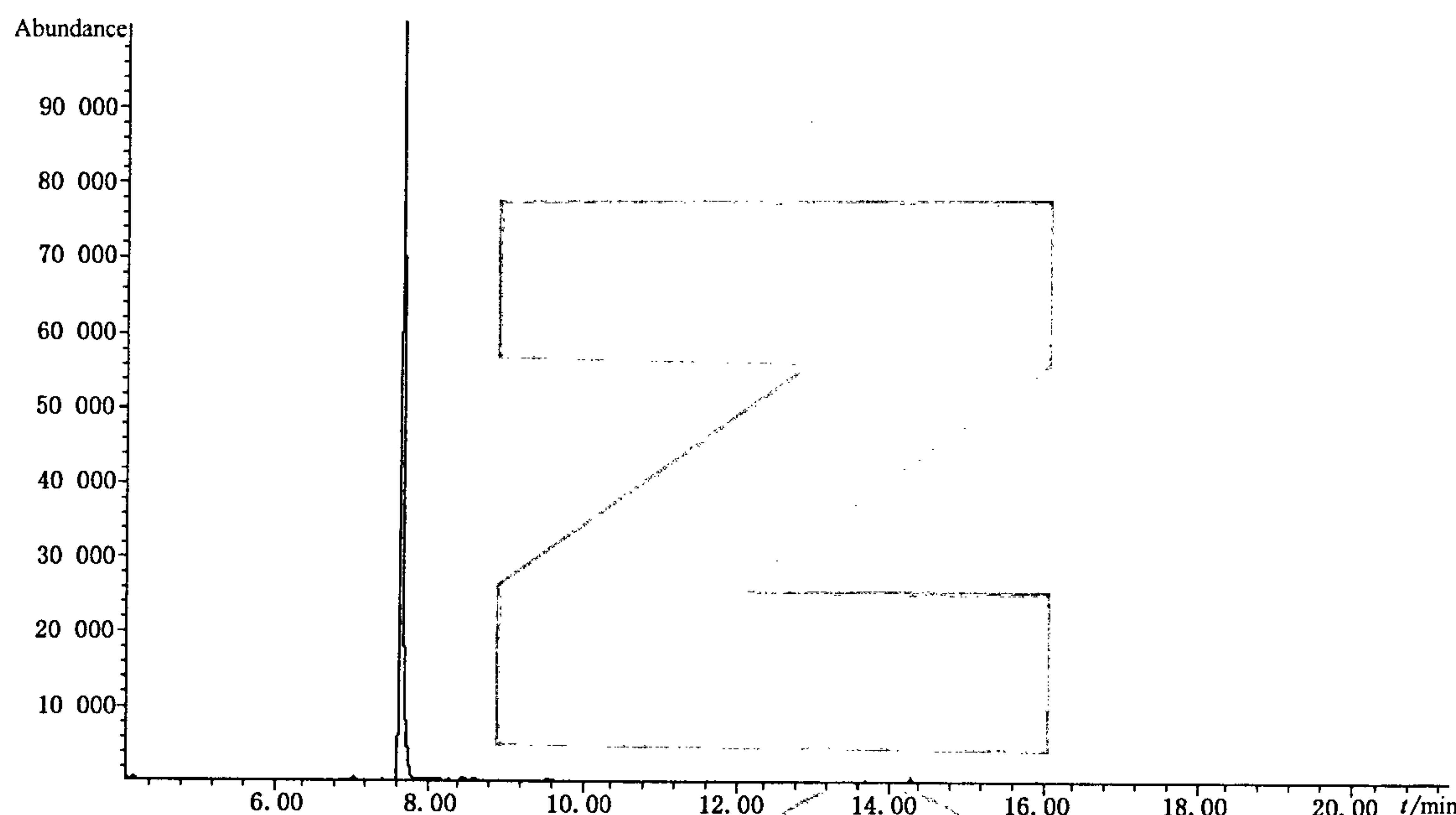


Figure A.1—SIM chromatogram of the DMF standard