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中华人民共和国出入境检验检疫行业标准

SN/T 3148—2012

出口食品中过氧化苯甲酰含量的测定 高效液相色谱法

Determination of benzoyl peroxide in food for export—HPLC

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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：中华人民共和国浙江出入境检验检疫局、中华人民共和国连云港出入境检验检疫局。

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出口食品中过氧化苯甲酰含量的测定 高效液相色谱法

1 范围

本标准规定了食品中过氧化苯甲酰含量检测的高效液相色谱测定方法。

本标准适用于小麦粉、米粉和马铃薯淀粉等食品中过氧化苯甲酰含量的检测。

第一法 还 原 法

2 方法提要

用甲醇-水(2+8,体积比)提取样品中的过氧化苯甲酰,在提取液中加入亚硫酸钠溶液,将过氧化苯甲酰还原为苯甲酸,过 0.45 μm 滤膜后,用高效液相色谱仪测定,外标法定量。

3 试剂和材料

除另有规定外,试剂均为分析纯,水为重蒸水或去离子水。

3.1 乙腈:液相色谱级。

3.2 甲醇:液相色谱级。

3.3 亚硫酸钠。

3.4 乙酸铵。

3.5 甲醇-水(2+8,体积比):取 100 mL 甲醇(3.2),与 400 mL 水混匀。

3.6 4%亚硫酸钠溶液:称取 8.0 g 亚硫酸钠(3.3),用水溶解并定容至 200 mL。

3.7 0.005 mol/L 乙酸铵溶液:称取 0.39 g 乙酸铵(3.4),用水溶解并定容至 1 000 mL。

3.8 过氧化苯甲酰(分子式:$C_{14}H_{10}O_4$,CAS 号:94-36-0)标准品:纯度大于等于 98.0%。

3.9 过氧化苯甲酰标准储备溶液:准确称取适量过氧化苯甲酰标准品(3.8),用乙腈配制成 200 μg/mL 标准储备液,0 ℃～4 ℃储存。

3.10 过氧化苯甲酰标准工作溶液:根据需要,用乙腈将标准储备液(3.9)稀释成适当浓度的标准工作溶液。

3.11 0.45 μm 有机系滤膜。

4 仪器和设备

4.1 高效液相色谱仪,配有紫外检测器或二极管阵列检测器。

4.2 分析天平:感量 0.1 mg 和 0.01 g。

4.3 涡旋混匀器。

4.4 离心机:转速不低于 4 000 r/min。

5 试样制备与保存

5.1 试样制备

取代表性样品约 500 g，混匀后均分成两份，装入洁净容器内，作为试样，密封，并标明标记。

5.2 试样保存

将试样置于 0 ℃～4 ℃保存。在制样操作过程中，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 1 g 试样(精确至 0.01 g)，置于 50 mL 离心管中。加入 10 mL 甲醇-水(3.5)，涡旋混匀5 min 后离心(4 000 r/min)5 min，过滤。

6.2 还原

取 5.0 mL 滤液，转移至另一支离心管中。加入 5.0 mL 4%亚硫酸钠溶液(3.6)，涡旋混匀 5 min 后静置 10 min。过 0.45 μm 滤膜(3.11)后供高效液相色谱分析。

6.3 标准工作曲线

在 5 个离心管中分别加入适量过氧化苯甲酰标准工作溶液，用甲醇-水(3.5)补至 5.0 mL，再加入 5.0 mL 4%亚硫酸钠溶液(3.6)，使混匀后溶液中过氧化苯甲酰的浓度分别为 0.25 μg/mL、0.5 μg/mL、1 μg/mL、5 μg/mL、10 μg/mL。涡旋混匀 5 min 后静置 10 min。过 0.45 μm 滤膜后供高效液相色谱分析。

6.4 测定

6.4.1 色谱条件

色谱条件如下：

a) 色谱柱：C_{18}，5 μm，250 mm×4.6 mm(内径)，或相当的色谱柱；
b) 流动相：梯度洗脱条件见表 1；
c) 流速：1.0 mL/min；
d) 检测波长：230 nm；
e) 进样量：50 μL。

表 1 梯度洗脱条件

时间/min	甲醇/%	0.005 mol/L 乙酸铵/%
0.00	10	90
4.00	10	90
10.00	40	60
11.00	10	90
16.00	10	90

6.4.2 色谱测定

根据样液中苯甲酸含量，选择浓度相近的标准工作溶液。还原所得标准溶液和样液中苯甲酸的响应值均应在仪器检测的线性范围内。对还原所得标准溶液和样液等体积穿插进样测定。在上述色谱条件下，苯甲酸的保留时间为 7.1 min。还原所得苯甲酸标准溶液的色谱图参见图 A.1，苯甲酸的紫外光谱图参见图 A.2。

6.5 空白试验

除不加试样外，均按上述步骤进行。

7 结果计算和表述

用色谱数据处理机或按式(1)计算试样中过氧化苯甲酰含量，计算结果应扣除空白值。

$$X=\frac{c\times V}{m} \qquad \cdots\cdots(1)$$

式中：

X ——试样中过氧化苯甲酰的含量，单位为毫克每千克(mg/kg)；

c ——由标准曲线得到的样液中过氧化苯甲酰的浓度，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)；

m ——最终样液所代表的试样量，单位为克(g)。

8 测定低限、回收率

8.1 测定低限

本方法对食品中过氧化苯甲酰的测定低限为 5 mg/kg。

8.2 回收率

在小麦粉、米粉和马铃薯淀粉样品中添加过氧化苯甲酰的浓度在 5 mg/kg、60 mg/kg、150 mg/kg 时，还原法测定的回收率范围见表 2。

表 2 还原法测定小麦粉、米粉和马铃薯淀粉样品中过氧化苯甲酰的回收率范围

%

添加浓度/(mg/kg)	小麦粉	米粉	马铃薯淀粉
5	83.6～92.9	86.0～93.7	83.6～91.3
60	88.6～92.3	88.7～92.4	89.4～93.5
150	98.3～102.4	90.2～94.1	92.2～96.0

第二法 直接提取法

9 方法提要

用乙腈提取试样中的过氧化苯甲酰，过 0.45 μm 滤膜后，用高效液相色谱仪测定，外标法定量。

10 试剂和材料

除另有规定外，试剂均为分析纯，水为重蒸水或去离子水。

10.1 乙腈：液相色谱级。

10.2 甲醇：液相色谱级。

10.3 过氧化苯甲酰(分子式：$C_{14}H_{10}O_4$，CAS号：94-36-0)标准品：纯度大于等于98.0%。

10.4 过氧化苯甲酰标准储备溶液：准确称取适量过氧化苯甲酰标准品(10.3)，用乙腈配制成200 μg/mL标准储备液，0 ℃～4 ℃储存。

10.5 过氧化苯甲酰标准工作溶液：根据需要，用乙腈将标准储备液(10.4)稀释成适当浓度的标准工作溶液。

10.6 0.45 μm有机系滤膜。

11 仪器和设备

11.1 高效液相色谱仪，配有紫外检测器或二极管阵列检测器。

11.2 分析天平：感量0.1 mg和0.01 g。

11.3 涡旋混匀器。

11.4 离心机(4 000 r/min)。

12 试样制备与保存

同第5章。

13 测定步骤

13.1 提取

称取1 g试样(精确至0.01 g)，置于50 mL离心管中。加入10 mL乙腈，涡旋混匀5 min后离心(4 000 r/min)5 min。取上清液过0.45 μm滤膜(10.6)后供高效液相色谱分析。

13.2 测定

13.2.1 色谱条件

色谱条件如下：

a) 色谱柱：C_{18}，3.5 μm，150 mm×4.6 mm(内径)，或相当的色谱柱；

b) 流动相：梯度洗脱条件见表3。

c) 流速：1.0 mL/min；

d) 检测波长：235 nm；

e) 进样量：25 μL。

表 3 梯度洗脱条件

时间/min	水/%	甲醇/%
0.00	50	50
3.00	50	50
8.00	10	90
10.00	10	90
10.10	50	50
15.00	50	50

13.2.2 色谱测定

根据样液中过氧化苯甲酰含量，选择浓度相近的标准工作溶液。标准工作溶液和样液中过氧化苯甲酰的响应值均应在仪器检测的线性范围内。对标准工作溶液和样液等体积穿插进样测定。在上述色谱条件下，过氧化苯甲酰的保留时间为 10.1 min。标准品的色谱图参见图 A.3，标准品的紫外光谱图参见图 A.4。

13.3 空白试验

除不加试样外，均按上述步骤进行。

14 结果计算和表述

用色谱数据处理机或按式(2)计算试样中过氧化苯甲酰含量，计算结果应扣除空白值。

$$X=\frac{A\times c\times V}{A_s\times m} \qquad \cdots\cdots(2)$$

式中：

X ——试样中过氧化苯甲酰的含量，单位为毫克每千克(mg/kg)；

A ——样液中过氧化苯甲酰的峰面积；

c ——标准工作液中过氧化苯甲酰的浓度，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——标准工作液中过氧化苯甲酰的峰面积；

m ——最终样液所代表的试样量，单位为克(g)。

15 测定低限、回收率

15.1 测定低限

本方法对食品中过氧化苯甲酰的测定低限为 5 mg/kg。

15.2 回收率

在小麦粉、米粉和马铃薯淀粉样品中添加过氧化苯甲酰的浓度在 5 mg/kg、60 mg/kg、150 mg/kg 时，直接提取法测定的回收率范围见表 4。

表 4　直接提取法测定小麦粉、米粉和马铃薯淀粉样品中过氧化苯甲酰的回收率范围

%

添加浓度/(mg/kg)	小麦粉	米粉	马铃薯淀粉
5	85.8～96.8	89.6～101.3	88.7～97.6
60	88.3～91.6	86.4～89.7	88.4～92.1
150	96.5～99.2	98.4～102.1	98.1～101.5

附 录 A
（资料性附录）
标准品的液相色谱图和紫外光谱图

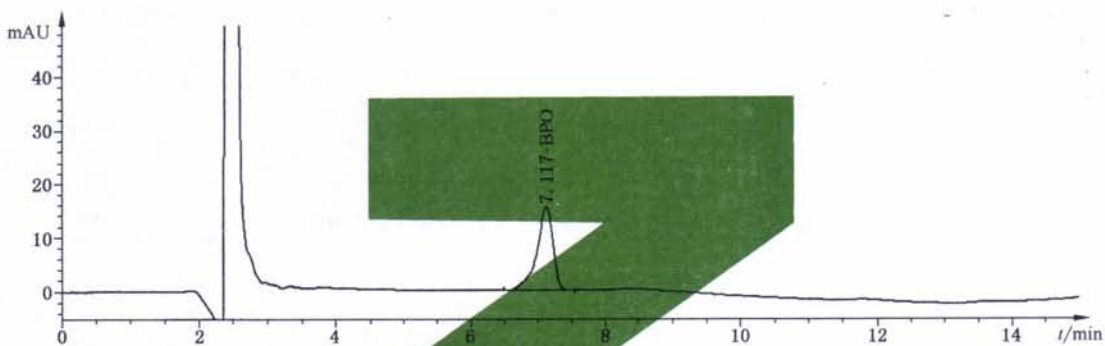

图 A.1 苯甲酸的液相色谱图

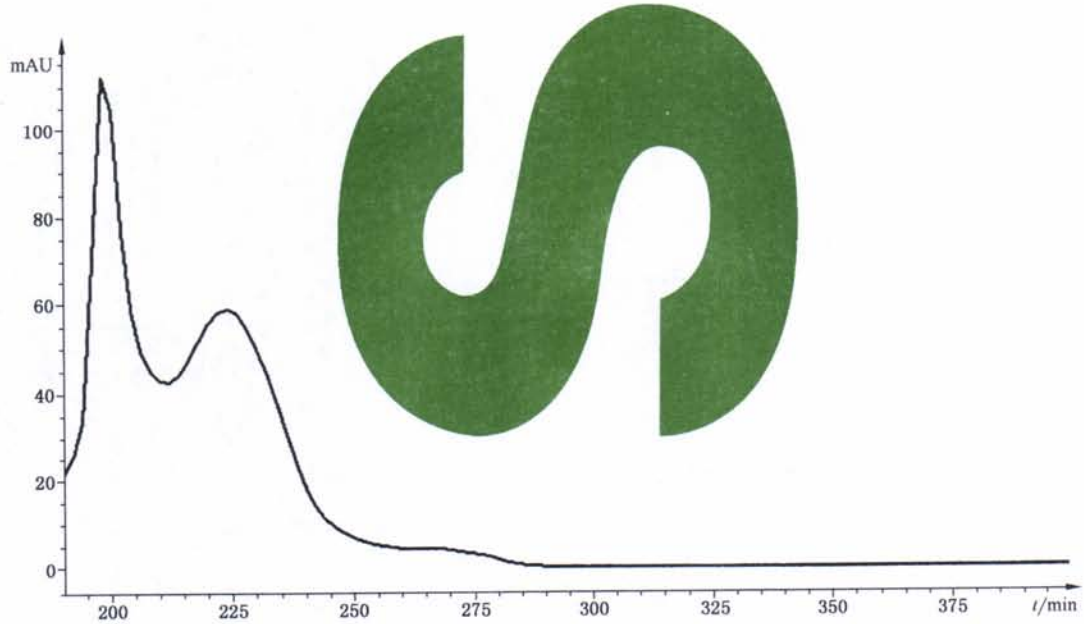

图 A.2 苯甲酸的紫外光谱图

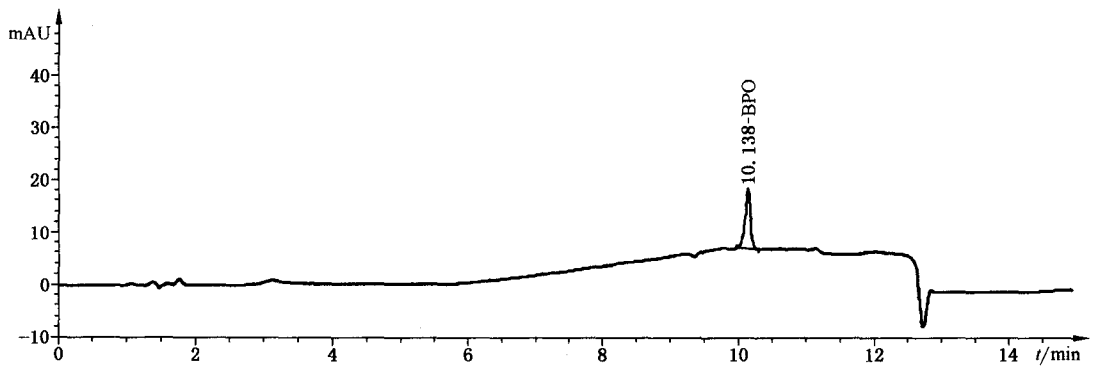

图 A.3 过氧化苯甲酰标准品的液相色谱图

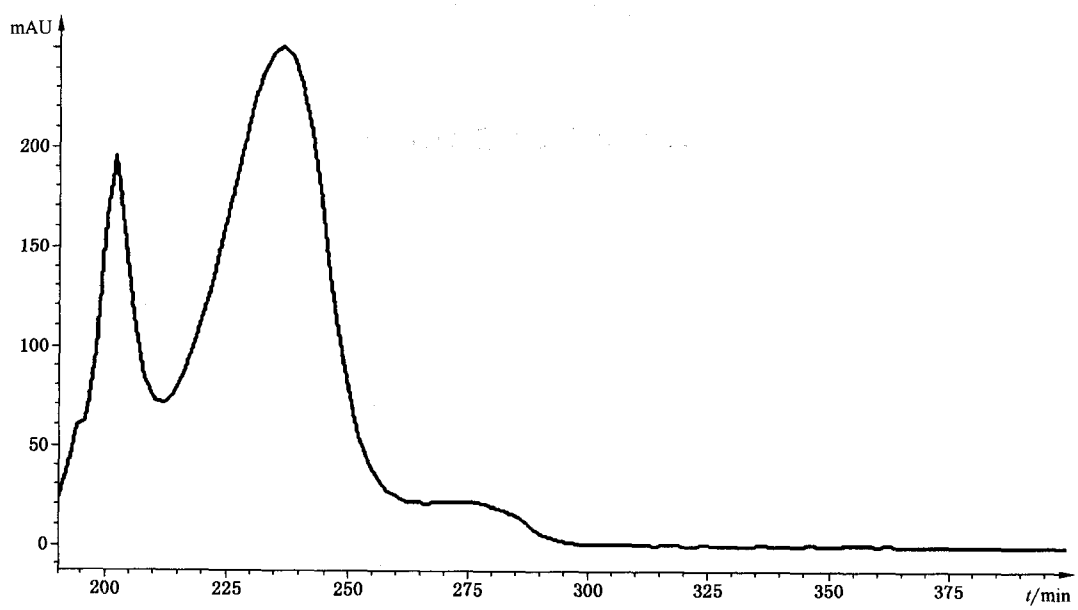

图 A.4 过氧化苯甲酰标准品的紫外光谱图

Foreword

This standard was drafted in accordance with the rule of GB/T 1. 1—2009.

Please pay attention that some contents of this document may be involved in patent. The promulgator will not take the responsibility of identifying the patents.

This standard was proposed by and is under the charge of Certification and Accreditation administration of the People's Republic of China.

This standard was drafted by Zhejiang Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Lianyungang Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Liu Haishan, Chen Xiaomei, Huang Chaoqun, Shi Lei, Lou Chengjie, Tang Hangyan, Wang Haitao.

Determination of benzoyl peroxide in food for export—HPLC

1 Scope

This standard specifies the method of sample preparation and determination of benzoyl peroxide in foods by high performance liquid chromatography.

This standard is applicable to the determination of benzoyl peroxide in wheat flour, rice flour and potato starch.

Method I Reduction method

2 Principle

Benzoyl peroxide is extracted by methanol-water (2 + 8, *V* + *V*) from the sample. Reduce benzoyl peroxide to benzoic acid by adding sodium sulfite solution into the extraction.

Determine benzoyl peroxide by high performance liquid chromatograph, quantitate with external standard method.

3 Reagents and materials

Unless otherwise specified, all reagents used should be analytically pure, "water" is redistilled or deionized.

3.1 Acetonitrile: HPLC grade.

3.2 Methanol: HPLC grade.

3.3 Sodium sulfite.

3.4 Ammonium acetate.

3.5 Methanol-water (2 + 8, *V* + *V*): Mix 100 mL methanol (3.2) with 400 mL water.

3.6 4% sodium sulfite solution: Dissolve 8.0 g sodium sulfite (3.3) in 200 mL water.

3.7 0.005 mol/L ammonium acetate solution: Dissolve 0.39 g ammonium acetate (3.4) in 1 000 mL water.

3.8 Benzoyl peroxide (molecular formula: $C_{14}H_{10}O_4$, CAS number: 94-36-0) standard: Purity is not lower than 98.0%.

3.9 Benzoyl peroxide standard stock solution: Accurately weigh an appropriate amount of benzoyl peroxide standard (3.8) and dissolve with acetonitrile to prepare a standard stock solution of 200 μg/mL. This standard stock solution should be stored at 0 ℃～4 ℃.

3.10 Benzoyl peroxide standard woking solution: According to the requirement, pipette adequate amount of standard stock solution (3.9) and dilute with acetonitrile to prepare standard working solution of suitable concentration.

3.11 Filter: 0.45 μm.

4 Apparatus and equipment

4.1 High performance liquid chromatograph, equipped with UV-detector or diode array detector.

4.2 Analytical balance: readabilities are 0.1 mg and 0.01 g respectively.

4.3 Vortex mixer.

4.4 Centrifuge: Max speed is not lower than 4 000 r/min.

5 Preparation and storage of test sample

5.1 Sample preparation

Take approximately 500 g representative sample. Mix the sample and divided it into two equal portions. Each portion is placed in a clear container as the test sample, which is sealed and labeled.

5.2 Sample storage

The test sample shall be stored at 0 ℃～4 ℃. In the course of sampling and sample preparation, precaution shall be taken to avoid the contamination or any factors which may cause the change of residue content.

6 Determination procedure

6.1 Extraction

Weigh ca 1 g test sample(accurate to 0.01 g) in 50 mL centrifuge tube. Add 10 mL methanol-water (3.5), shake the centrifuge tube with vortex mixer for 5 min. Then centrifuge at 4 000 r/min for 5 min and filtrate.

6.2 Reduction

Transfer 5.0 mL filtrate to another centrifuge tube, add 5.00 mL 4% sodium sulfite solution(3.6), shake the centrifuge tube with vortex mixer for 5 min and then leave it standing for 5 min. Filtrate the solution by 0.45 μm filter for high-performance liquid chromatographic determination.

6.3 Standard solution

Transfer, adequate amount of benzoyl peroxide standard woking solution to 50 mL centrifuge tube. Add methanol-water (3.5) to make the total volume equal 5.00 mL, add 5.0 mL 4% sodium sulfite solution(3.6). The final concentrations of benzoyl peroxide in the above solutions are 0.25 μg/mL, 0.5 μg/mL, 1 μg/mL, 5 μg/mL and 10 μg/mL respectively. Shake the centrifuge tube with vortex mixer for 5 min and then leave it standing for 5 min. Filtrate the solution by 0.45 μm filter for high-performance liquid chromatographic determination.

6.4 Determination

6.4.1 HPLC conditions

The HPLC conditions are as follows:

a) Chromatographic column: C_{18}, 5 μm, 250 mm × 4.6 mm(i.d.), or equivalent;

b) Mobile phase: for gradient elute condition, see table 1;

c) Mobile phase flow rate: 1.0 mL/min;

d) Detection wavelength: 230 nm;

e) Injection volume: 50 μL.

Table 1—Gradient elute condition

Time/min	Methanol/%	0.005 mol/L ammonium acetate/%
0.00	10	90
4.00	10	90
10.00	40	60
11.00	10	90
16.00	10	90

6.4.2 HPLC determination

According to the approximate concentration of benzoic acid in the sample solution, select the standard working solution with similar concentration to that of sample solution. The responses of benzoic acid in the standard working solution and the sample solution should be in the linear range of the instrumental detection. The standard solution should be randomly injected between the injections of sample solution of equal volume. Under the above operating conditions, the retention time of benzoic acid is about 7.1 min. For the chromatogram of the standard, see figure A.1 in annex A, and for the UV spectrum of the standard, see figure A.2 in annex A.

6.5 Blank test

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

7 Calculation and expression of result

The calculation of result is carried out by data processor or according to formula (1), and the blank value shall be subtracted from the result of calculation.

$$X = \frac{c \times V}{m} \qquad \cdots\cdots(1)$$

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

c ——the concentration of benzoyl peroxide in the sample solution accquired from the calibration curve, μg/mL;

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

m ——the corresponding mass of the test sample in the final sample solution, g.

8 Limit of quantification (LOQ) and recovery

8.1 Limit of quantification

The limit of determination of this method for benzoyl peroxide in food is 5 mg/kg.

8.2 Recovery

The fortifying concentrations of benzoyl peroxide in wheat flour, rice flour, potato starch and corresponding recovery ranges according to reduction method are shown in table 2.

Table 2—Recovery ranges of benzoyl peroxide in wheat flour, rice flour, potato starch accoding to reduction method %

Spiked level/(mg/kg)	Wheat flour	Rice flour	Potato starch
5	83.6~92.9	86.0~93.7	83.6~91.3
60	88.6~92.3	88.7~92.4	89.4~93.5
150	98.3~102.4	90.2~94.1	92.2~96.0

Method Ⅱ Direct Extraction method

9 Principle

Benzoyl peroxide is extracted by acetonitrile from the sample. Determine benzoyl peroxide by high performance liquid chromatograph, quantitate with external standard method.

10 Reagents and materials

Unless otherwise specified, all reagents used should be analytically pure, "water" is redistilled or deionized.

10.1 Acetonitrile: HPLC grade.

10.2 Methanol: HPLC grade.

10.3 Benzoyl peroxide (molecular formula: $C_{14}H_{10}O_4$, CAS number: 94-36-0) standard: Purity is not lower than 98.0%.

10.4 Benzoyl peroxide standard stock solution: Accurately weigh an appropriate amount of benzoyl

peroxide standard (10.3) and dissolve with acetonitrile to prepare a standard stock solution of 200 μg/mL. This standard stock solution should be stored at 0 ℃～4 ℃.

10.5 Benzoyl peroxide standard woking solution: According to the requirement, pipette adequate amount of standard stock solution (10.4) and dilute with acetonitrile to prepare standard working solution of suitable concentration.

10.6 Filter: 0.45 μm.

11 Apparatus and equipment

11.1 High performance liquid chromatograph, equipped with UV-detector or diode array detector.

11.2 Analytical balance: readabilities are 0.1 mg and 0.01 g respectively.

11.3 Vortex mixer.

11.4 Centrifuge: Max speed is not lower than 4 000 r/min.

12 Preparation and storage of test sample

The section is the same as section 5.

13 Determination procedure

13.1 Extraction

Weigh 1 g test sample(accurate to 0.01 g) in 50 mL centrifuge tube. Add 10 mL acetonitrile, shake the centrifuge tube with vortex mixer for 5 min. Then centrifuge at 4 000 r/min for 5 min. Filtrate the supernatant by 0.45 μm filter for high-performance liquid chromatographic determination.

13.2 Determination

13.2.1 HPLC conditions

The HPLC conditions are as follows:

a) Chromatographic column: C_{18}, 3.5 μm, 150 mm × 4.6 mm(i.d.), or equivalent;

b) Mobile phase: for gradient elute condition, see table 3;

c) Mobile phase flow rate: 1.0 mL/min;

d) Detection wavelength: 235 nm;

e) Injection volume: 25 μL.

Table 3—gradient elute condition

Time/min	Water/%	Methanol/%
0.00	50	50
3.00	50	50
8.00	10	90
10.00	10	90
10.10	50	50
15.00	50	50

13.2.2 HPLC determination

According to the approximate concentration of benzoyl peroxide in the sample solution, select the standard working solution with similar concentration to that of sample solution. The responses of benzoyl peroxide in the standard working solution and the sample solution should be in the linear range of the instrumental detection. The standard solution should be randomly injected between the injections of sample solution of equal volume. Under the above operating conditions, the retention time of benzoyl peroxide is about 10.1 min. For the chromatogram of the standard, see figure A.3, and for the UV spectrum of the standard, see figure A.4.

13.3 Blank test

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

14 Calculation and expression of result

The calculation of result is carried out by data processor or according to formula(2), and the balnk value shall be subtracted from the result of calculation.

$$X = \frac{A \times c \times V}{A_s \times m} \qquad \cdots\cdots(2)$$

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

A ——the peak area of benzoyl peroxide in sample solution;

c ——the concentration of benzoyl peroxide in the standard workig solution, μg/mL;

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

A_s——the peak area of benzoyl peroxide in the standard working solution;

m ——the corresponding mass of the test sample in the final sample solution, g.

15 Limit of quantification (LOQ) and recovery

15.1 Limit of quantification

The limit of determination of this method for benzoyl peroxide in food is 5 mg/kg.

15.2 Recovery

The fortifying concentrations of benzoyl peroxide in wheat flour, rice flour, potato starch and corresponding recovery ranges according to direct extration method are shown in table 4.

Table 4—Recovery ranges of benzoyl peroxide in wheat flour, rice flour, potato starch accoding to direct extraction method

%

Spiked level/(mg/kg)	Wheat flour	Rice flour	Potato starch
5	85.8～96.8	89.6～101.3	88.7～97.6
60	88.3～91.6	86.4～89.7	88.4～92.1
150	96.5～99.2	98.4～102.1	98.1～101.5

Annex A
(informative annex)
Chromatogram and UV spectrum of standard

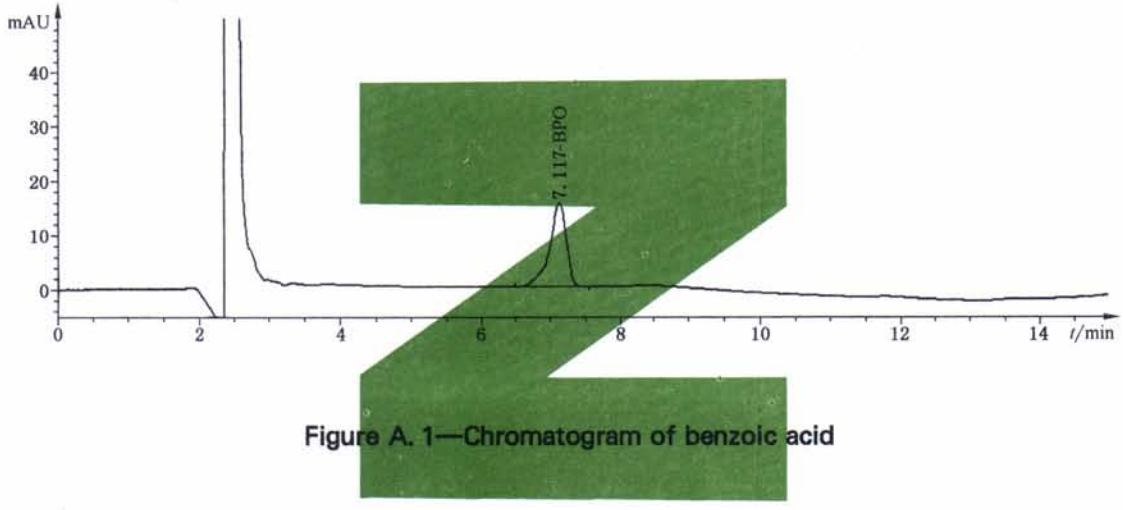

Figure A. 1—Chromatogram of benzoic acid

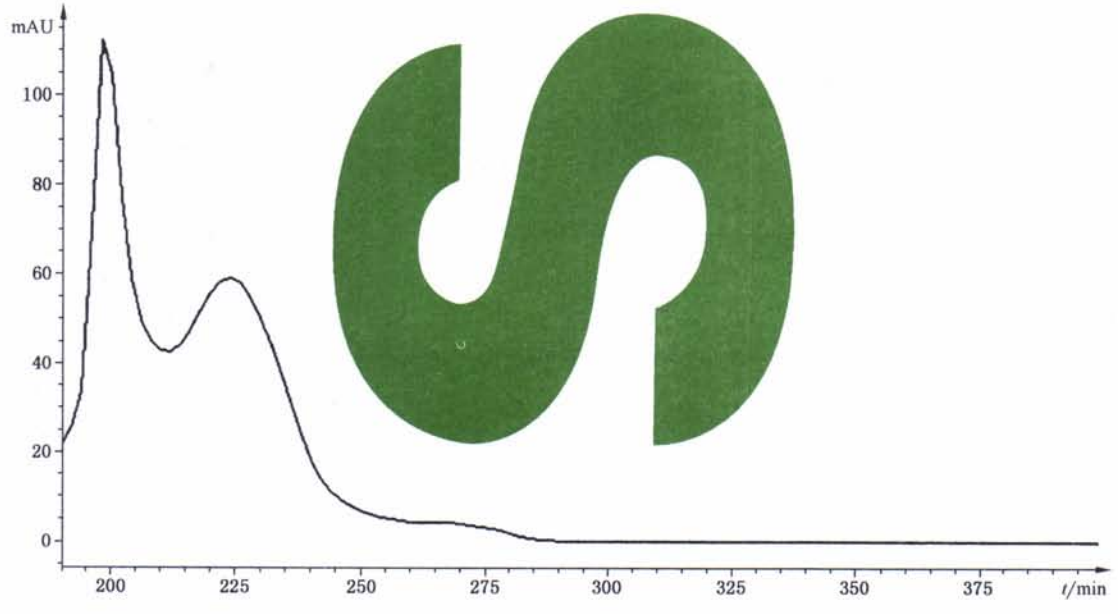

Figure A. 2—UV spectrum of benzoic acid

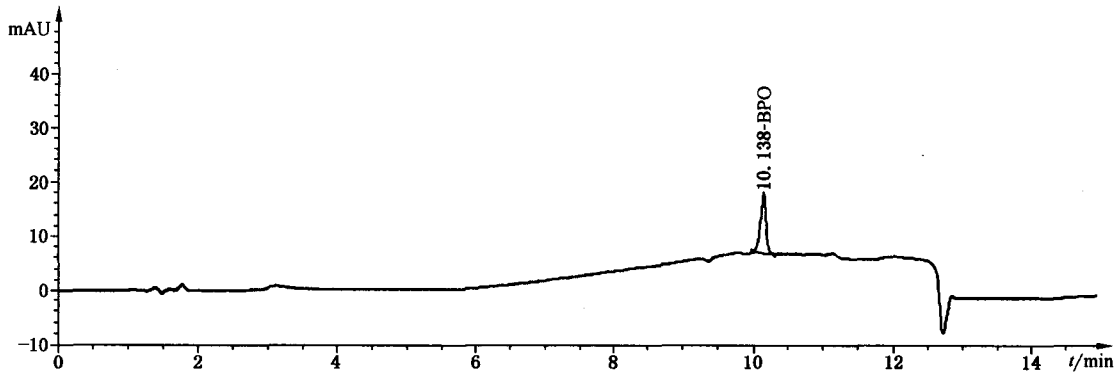

Figure A. 3—Chromatogram of benzoyl peroxide

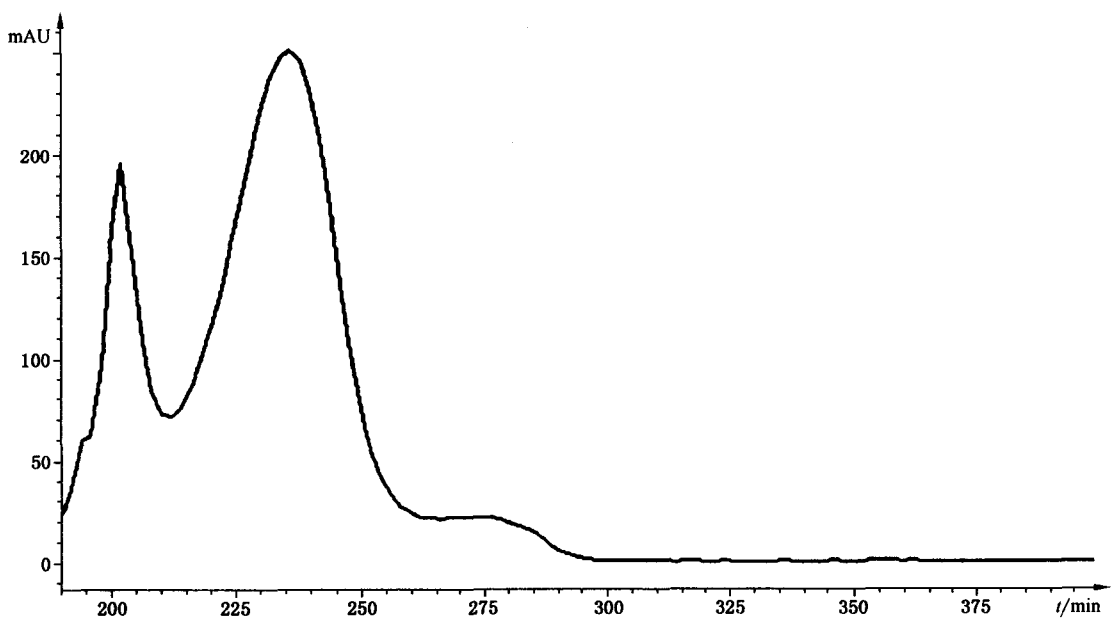

Figure A. 4—UV spectrum of benzoyl peroxide