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中华人民共和国出入境检验检疫行业标准

SN/T 1121—2002

中药制剂中苯甲酸、山梨酸和对羟基苯甲酸酯类防腐剂的检验方法　液相色谱法

Determination of benzoic acid, sorbic acid and parabens in Chinese traditional medicine preparation —Liquid chromatographic method

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前　言

本标准中的测定方法是参考国内有关文献，经研究、改进和验证后制定的。

本标准附录 A 为资料性的附录。

本标准由国家认证认可监督管理委员会提出并归口。

本标准负责起草单位：中华人民共和国广州出入境检验检疫局。

本标准主要起草人：刘青、林峰、蔡纯、梁伟大。

本标准系首次发布的出入境检验检疫行业标准。

中药制剂中苯甲酸、山梨酸和对羟基苯甲酸酯类防腐剂的检验方法　液相色谱法

1　范围

本标准规定了中药合剂、糖浆剂的抽样、制样方法，以及中药合剂、糖浆剂中苯甲酸、山梨酸、对羟基苯甲酸甲酯、对羟基苯甲酸乙酯、对羟基苯甲酸丙酯和对羟基苯甲酸丁酯的液相色谱法测定方法。

本标准适用于中药合剂、糖浆剂中苯甲酸、山梨酸、对羟基苯甲酸甲酯、对羟基苯甲酸乙酯、对羟基苯甲酸丙酯和对羟基苯甲酸丁酯的检验。

2　抽样和制样

2.1　检验批

以不超过 5 000 件为一检验批。

每一检验批应以同一生产批次的产品划分。

2.2　抽样方法

2.2.1　100 件以下最低抽取 3 件；101～1 000 件最低抽取 5 件；1 000 件以上最低抽取 7 件。

2.2.2　按 2.2.1 规定的数量随机抽取。逐件开启，每件取出 1 瓶。瓶装中药合剂、糖浆剂，每批抽样不得小于 3 瓶(每瓶以 500 mL 计)，平均供试样品的量一般不少于实验室所需 3 倍数。

2.3　试样的制备

将取回的样品充分混合，分取 500 mL 作为试样，均分成两份，装入洁净的容器内，密封标明标记。

2.4　试样的保存

将试样于室温下保存。

注：在抽样和制样的操作过程中，必须防止样品受到污染。

3　测定方法

3.1　方法提要

在酸性条件下用乙醚提取试样中的防腐剂，提取液浓缩后分为苯甲酸、山梨酸和对羟基酯类两部分分别进行测试，液相色谱二极管阵列或紫外检测器外标法定量。

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　甲醇：液相色谱纯。

3.2.2　乙醚：无水乙醚。

3.2.3　乙酸铵水溶液：0.02 mol/L。

3.2.4　盐酸水溶液：6 mol/L。

3.2.5　苯甲酸、山梨酸、对羟基苯甲酸甲、乙、丙、丁酯标准品：纯度≥98%。

3.2.6　标准溶液：准确称取适量的防腐剂标准品，用甲醇配成为 1.0 mg/mL 的标准储备溶液，根据需要再用甲醇稀释，配成适当浓度的标准工作溶液。

3.3 仪器和色谱条件

3.3.1 高效液相色谱仪：配二极管阵列或紫外检测器。

3.3.2 分液漏斗：50 mL。

3.3.3 容量瓶：50 mL。

3.3.4 带刻度的离心试管：5.0 mL。

3.3.5 水浴锅：可调温。

3.4 测试步骤

3.4.1 样品处理

称取 1.0 g(准确至 0.001 g)试样于 50 mL 分液漏斗中，加 25 mL 水，混匀后加入 0.2 mL 6 mol/L 盐酸水溶液，用 2×20 mL 无水乙醚提取两次，合并提取液于容量瓶中，用乙醚定容至 50.0 mL 并混匀。静置片刻后，准确移取 5.0 mL 提取液于带刻度的离心试管中，在 40℃水浴上蒸发至近干，用甲醇定容至 2.0 mL，溶液经 0.45 μm 滤膜过滤后供液相色谱分析。

3.4.2 测定

3.4.2.1 色谱条件

a) 色谱柱：SUPELCO Discovery C_{18}柱，150 mm×4.6 mm(内径)，粒度 5 μm，或相当者；

b) 测定波长：苯甲酸、山梨酸：230 nm；

对羟基苯甲酸甲、乙、丙、丁酯：254 nm；

c) 柱温：40℃；

d) 流动相：苯甲酸、山梨酸：0.02 mol/L 乙酸铵水溶液-甲醇(体积比，98+2)；

对羟基苯甲酸甲、乙、丙、丁酯：0.02 mol/L 甲醇-乙酸铵水溶液(体积比，70+30)；

e) 流速：1.0 mL/min；

f) 进样体积：20 μL。

3.4.2.2 测定方法

根据样液中防腐剂的含量情况，选定峰面积相近的标准工作溶液。标准工作溶液和样液中防腐剂的响应值均应在仪器检测的线性范围内。在选定的分析条件下，对标准工作溶液和测定样液等体积交叉进行试验，苯甲酸、山梨酸的保留时间分别约为 5.7 min、8.1 min，对羟基苯甲酸甲、乙、丙、丁酯的保留时间分别约为 4.4 min、5.5 min、7.8 min、12.3 min。标准品色谱图参见附录 A 中图 A.1、A.2。

3.5 空白试验

除不加试样外，按上述测定步骤进行。

3.6 结果计算和表达

用色谱管理系统处理数据或按式(1)计算：

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

式中：X——试样中防腐剂的含量，单位为毫克每千克(mg/kg)；

A——试样中防腐剂的峰面积，单位为平方毫米(mm^2)；

A_s——标准工作液中防腐剂的峰面积，单位为平方毫米(mm^2)；

c——标准工作液中防腐剂浓度，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——最终样液中所代表的试样量，单位为克(g)。

注：计算结果需扣除空白值。

4 测定低限、回收率

4.1 测定低限

本方法中苯甲酸、山梨酸和对羟基苯甲酸甲、乙、丙、丁酯的测定低限均为 1.00 mg/kg。

4.2 回收率

4.2.1 苯甲酸、山梨酸回收率

在 1.00 mg/kg～3 000 mg/kg 范围内，苯甲酸的回收率为 91.0%～103%；

在 1.00 mg/kg～3 000 mg/kg 范围内，山梨酸的回收率为 91.7%～104%。

4.2.2 对羟基苯甲酸甲、乙、丙、丁酯

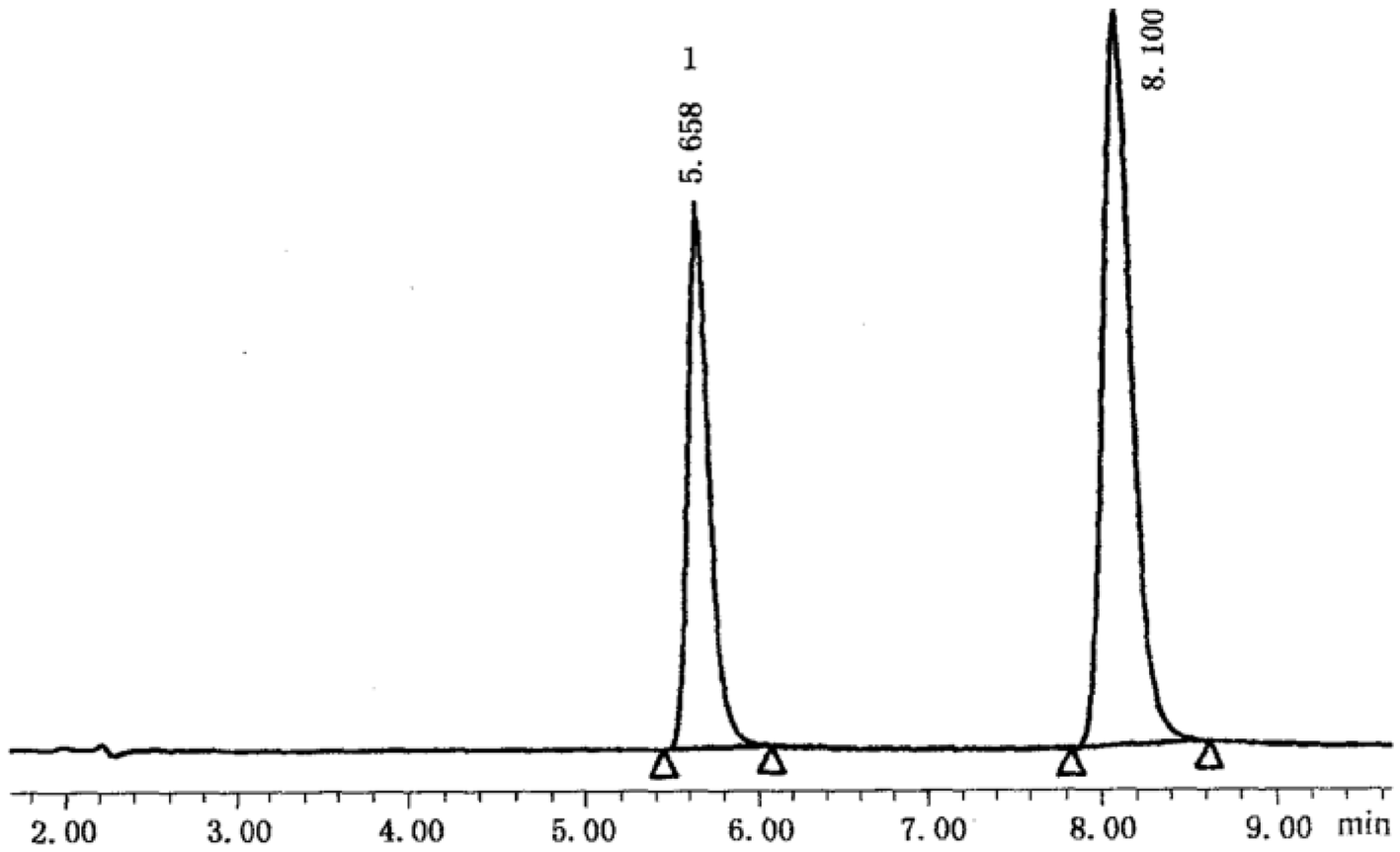
在 1.00 mg/kg～500 mg/kg 范围内，对羟基苯甲酸甲酯的回收率为 92.0%～104%；

在 1.00 mg/kg～500 mg/kg 范围内，对羟基苯甲酸乙酯的回收率为 91.0%～102%；

在 1.00 mg/kg～500 mg/kg 范围内，对羟基苯甲酸丙酯的回收率为 91.0%～98.8%；

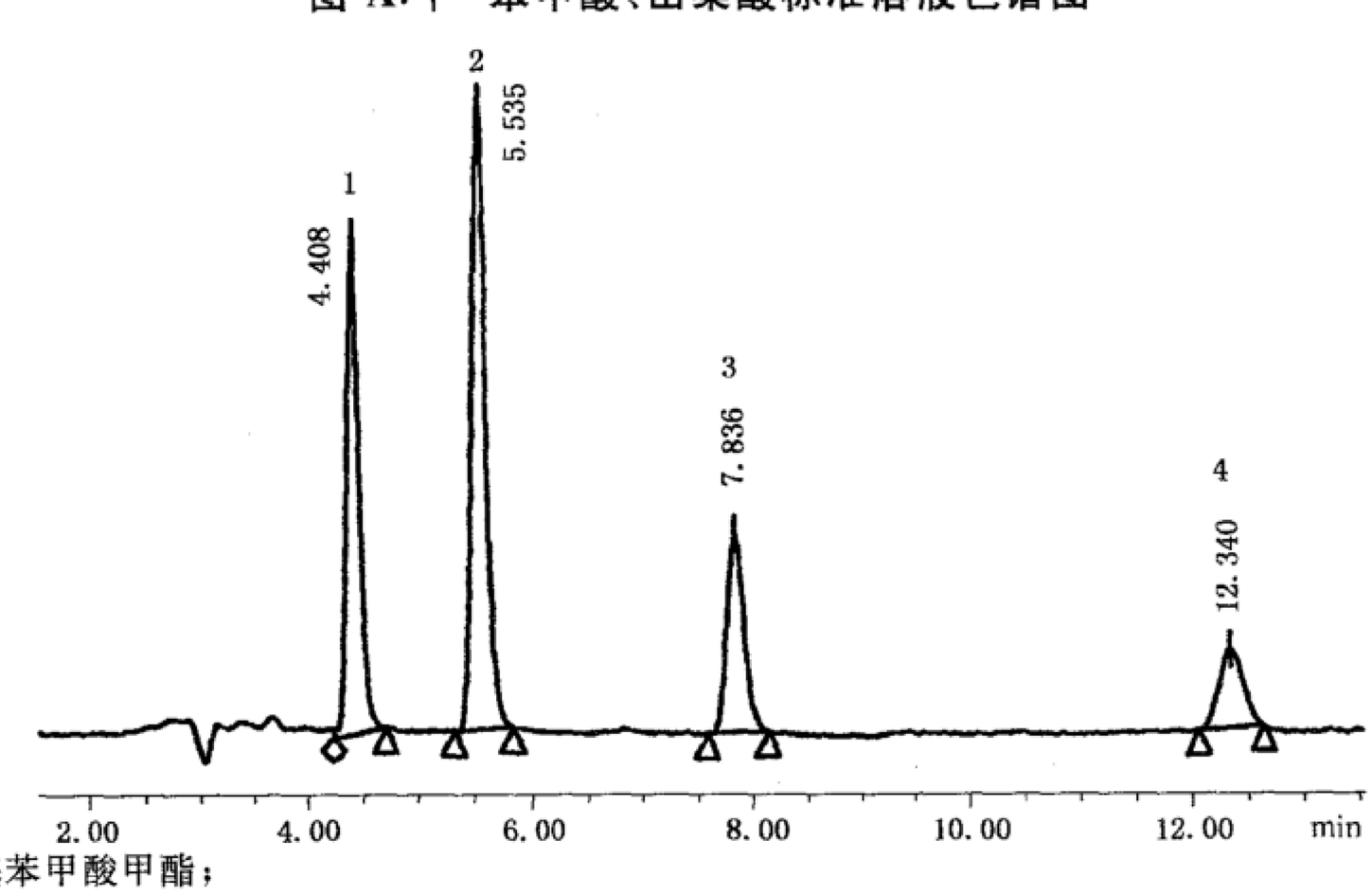
在 1.00 mg/kg～500 mg/kg 范围内，对羟基苯甲酸丁酯的回收率为 92.6%～99.6%。

附 录 A
（资料性附录）
标准品色谱图



1——苯甲酸；
2——山梨酸。

图 A.1 苯甲酸、山梨酸标准溶液色谱图



1——对羟基苯甲酸甲酯；
2——对羟基苯甲酸乙酯；
3——对羟基苯甲酸丙酯；
4——对羟基苯甲酸丁酯。

图 A.2 对羟基苯甲酸甲、乙、丙、丁酯标准溶液色谱图

Foreword

The method for determination of this standard was drafted by referring to relevant domestic and foreign literatures through research, modification and verification.

Annex A of this standard is an informative annex.

This standard was proposed and administrated by National Regulatory Commission for Certification and Accreditation.

This standards wad drafted by Guangzhou Entry-Exit Inspection and Quarantine of the Peoples Republic of China.

The main drafters of this standard are Liu qing, Lin Feng, Cai Chun, Liang Weida.

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

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

Determination of benzoic acid, sorbic acid and parabens in Chinese traditional medicine misture and julep —Liquid chromatographic method

1 Scope

This standard specifies the methods of sampling, sample preparation and determination of benzoic acid, sorbic acid and parabens in Chinese traditional medicine misture and julep by liquid chromatographic method.

The standard is applicable to the determination of benzoic acid, sorbic acid and parabens in Chinese traditional medicine misture and julep for export.

2 Sampling and sample preparation

2.1 Inspection lot

Each Inspection lot should not exceed 5 000 packages.

The product in an inspection should be of the same production batch.

2.2 Sampling method

2.2.1 For 100 packages, take 3 packages at least; between 101 ~ 1 000 packages at least; above 1 000 packages, take 10 packages at least.

2.2.2 Take the packages inside according to the number specified in 2.2.1 at random and opened one by one. From each package, take one bottle. The bottled samples taken back will not less than 3 bottles each lot(as 500 mL). The number of them should be three times of that of the test sample.

2.3 Sample preparation

Mix all the samples taken back thoroughly; take 500 mL as the test sample. Divide into two equal portions and place them in clean bottles, which is sealed and labeled.

2.4 Storage of the test sample

The test sample should be stored at room temperature.

Note: In the course of sampling and preparation of sample, precaution must be taken to avoid the contamination.

3 Method of determination

3.1 Principle

Extract the preservatives with ether in acid condition, after concentration, the test solution is used for two tests, by HPLC equipped with PDA or UV detector, one is the determination of benzoic acid and sorbic acid, another is that of parabens, using external standard method.

3.2 Reagents and materials

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

3.2.1 Methanol: HPLC grade.

3.2.2 Ether: Dehydrated ether.

3.2.3 Acetic ammonium aqueous solution: 0.02 mol/L.

3.2.4 Hydrochloric acid aqueous solution: 6 mol/L.

3.2.5 Benzoic acid, sorbic acid, methylphydroxybenzoate, ethylphydroxybenzoate, propylphydroxybenzoate and butylphydroxybenzoate standard: purity $\geqslant$ 98%.

3.2.6 Standard solution: Accurately weigh an adequate amount of antiseptic standard, dissolve in methanol and prepare a solution of 1.0 mg/mL as the standard solution. Then, as required, dilute it with water to appropriate concentration to serve as the standard working solution.

3.3 Instrument and apparatus

3.3.1 High performance liquid chromatograph: Equipped with PDA detector of UV detector.

3.3.2 Separatory funnel: 50 mL.

3.3.3 Volumetric flask: 50 mL.

3.3.4 Centrifuge tube: 5.0 mL, graduated.

3.3.5 Water bath: Temperature controlled.

3.4 Procedure

3.4.1 Preparation

Weigh 1.0 g test sample(Accurate to 0.001 g) into 50 mL separatory funnel, add 25 mL water. After mixing thoroughly, add 0.2 mL 6 mol/L hydrochloric acid aqueous solution. Extract with 2 × 20 mL ether, combine the ether extract into a volumetric flask, add to 50.0 mL. Stewing for a moment, accurately transfer 5.0 mL extract to a graduated centrifuge tube, evaporate in a 40℃ water bath to dryness, add exactly 2.0 mL of methanol.

3.4.2 Determination

3.4.2.1 LC parameters

a) Column: SUPELCO Discovery C_{18}, 150 mm × 4.6 mm(i.d.), particle size 5 μm, or equivalent;

b) Wavelength: benzoic acid and sorbic acid: 230 nm;
parabens: 254 nm;

c) Oven temperature: 40℃;

d) Mobile phase:
Benzoic acid and sorbic acid: 0.02 mol/L acetic ammonium-Methanol(*V*/*V*, 98 + 2);
Parabens: 0.02 mol/L Methanol-acetic ammonium (*V*/*V*, 70 + 30);

e) Flow rare: 1.0 mL/min;

f) Injection volume: 20 μL.

3.4.2.2 According to the approximate concentration of the preservatives in the sample solution, select the standard working solution with similar peak area to that of the sample solution. The standard working solution should be randomly injected in-between the injections of the sample solution of equal volume. The retention time of benzoic acid and sorbic acid is 5.7 min and 8.1 min. The retention time of parabens are separately 4.4 min, 5.5 min, 7.8 min and 12.3 min.

3.5 Blank test

The operation of the blank test is the same as that of described in the method of determination, but without addition of sample.

3.6 Calculation and expression of result

The calculation of result is carried out by an LC chromatography system or according to the formula(1):

$$X = \frac{A \times c \times V}{A_s \times m} \qquad \text{.....(1)}$$

where:

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

A—Peak area of preservative in the test solution, mm^2;

A_s—Peak area of preservative in the standard working solution, mm^2;

c—Concentration of preservative in the standard working solution, $\mu g/mL$;

V—Final volume of test sample solution, mL;

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

Note: The blank value should be subtracted from the above result of calculation.

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 1.00 mg/kg.

4.2 Recovery

4.2.1 Recovery of benzoic acid and sorbic acid

Fortifying concentration 1.00 mg/kg ~ 3 000 mg/kg;

The recovery of benzoic acid is 91.0% ~ 103%;

The recovery of sorbic acid is 91.7% ~ 104%.

4.2.2 Recovery of parabens

Fortifying concentration 1.00 mg/kg ~ 500 mg/kg;

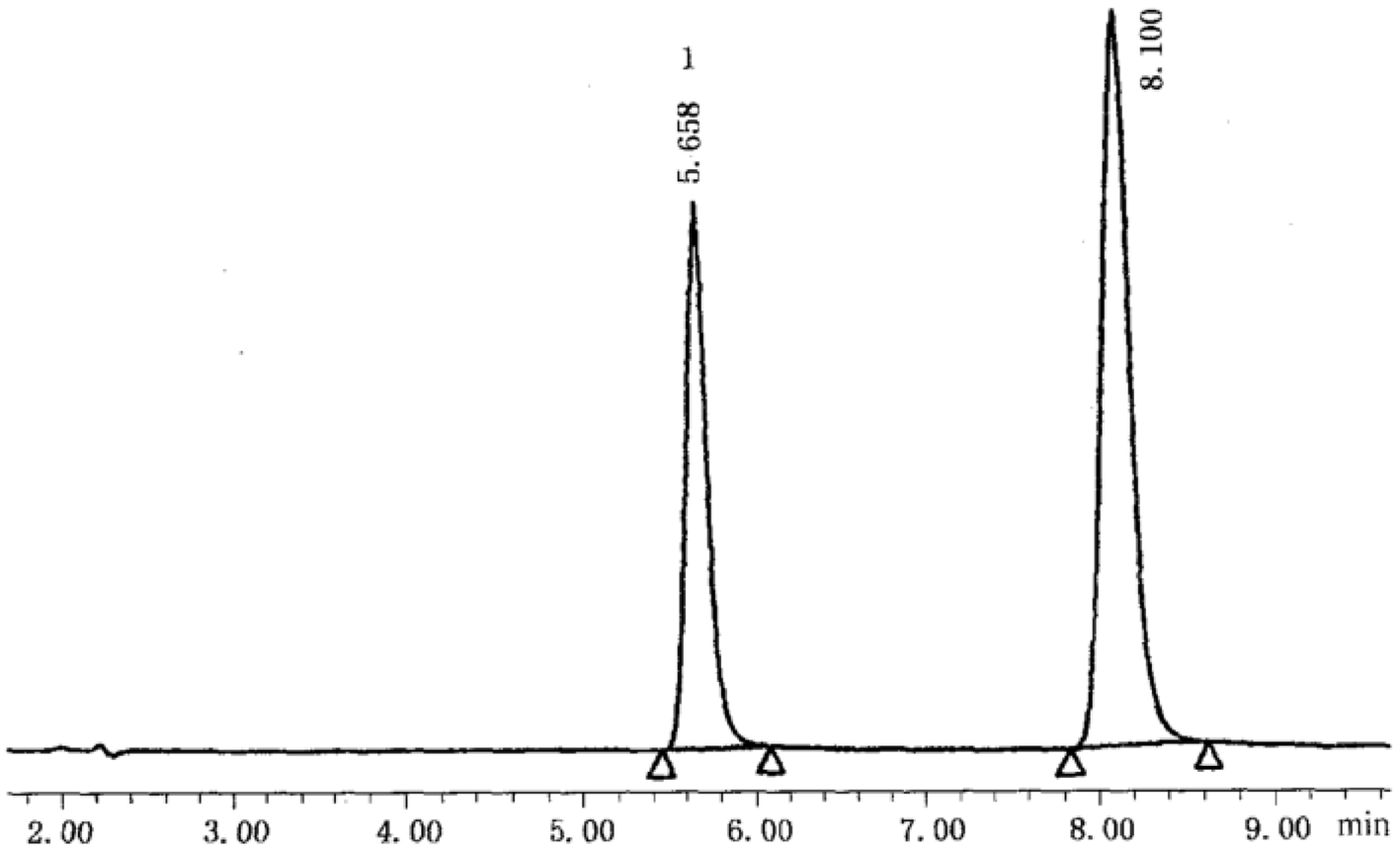
The recovery of methylphydroxybenzoate is 92.0% ~ 104%;

The recovery of ethylphydroxybenzoate is 91.0% ~ 102%;

The recovery of propylphydroxybenzoate is 91.0% ~ 98.8%;

The recovery of butylphydroxybenzoate is 92.6% ~ 99.6%.

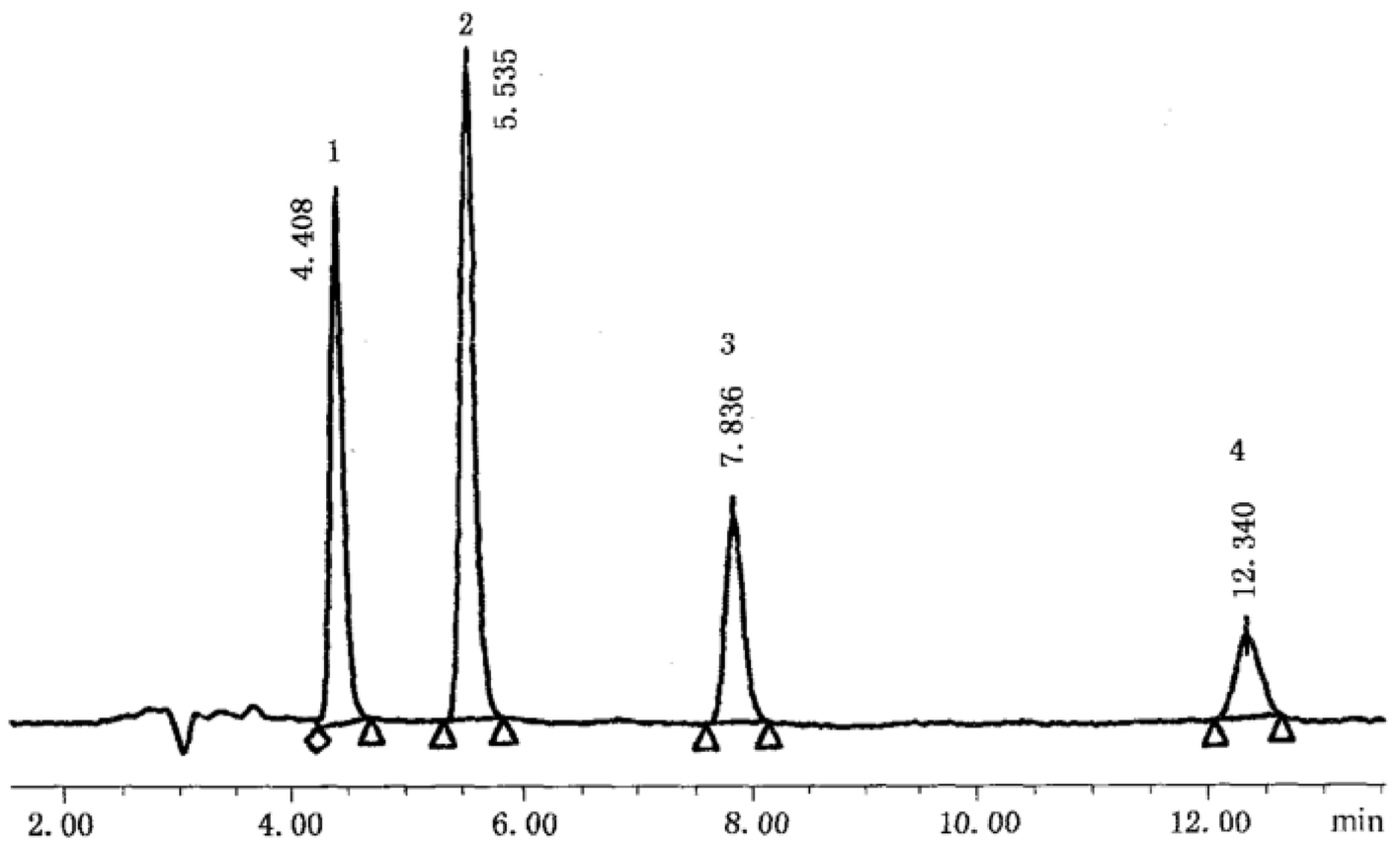
Annex A
(informative)
Chromatogram of the standard



1—benzoic acid;

2—sorbic acid.

Fig A.1 HPLC chromatogram of benzoic acid and sorbic acid standard



1—methylphydroxybenzoate;

2—ethylphydroxybenzoate;

3—propylphydroxybenzoate;

4—butylphydroxybenzoate.

Fig A.2 HPLC chromatogram of the parabens standard

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