

SN

中华人民共和国出入境检验检疫行业标准

SN/T 1116—2002

进出口饲料中克伦特罗、沙丁胺醇 残留量的检验方法 液相色谱法

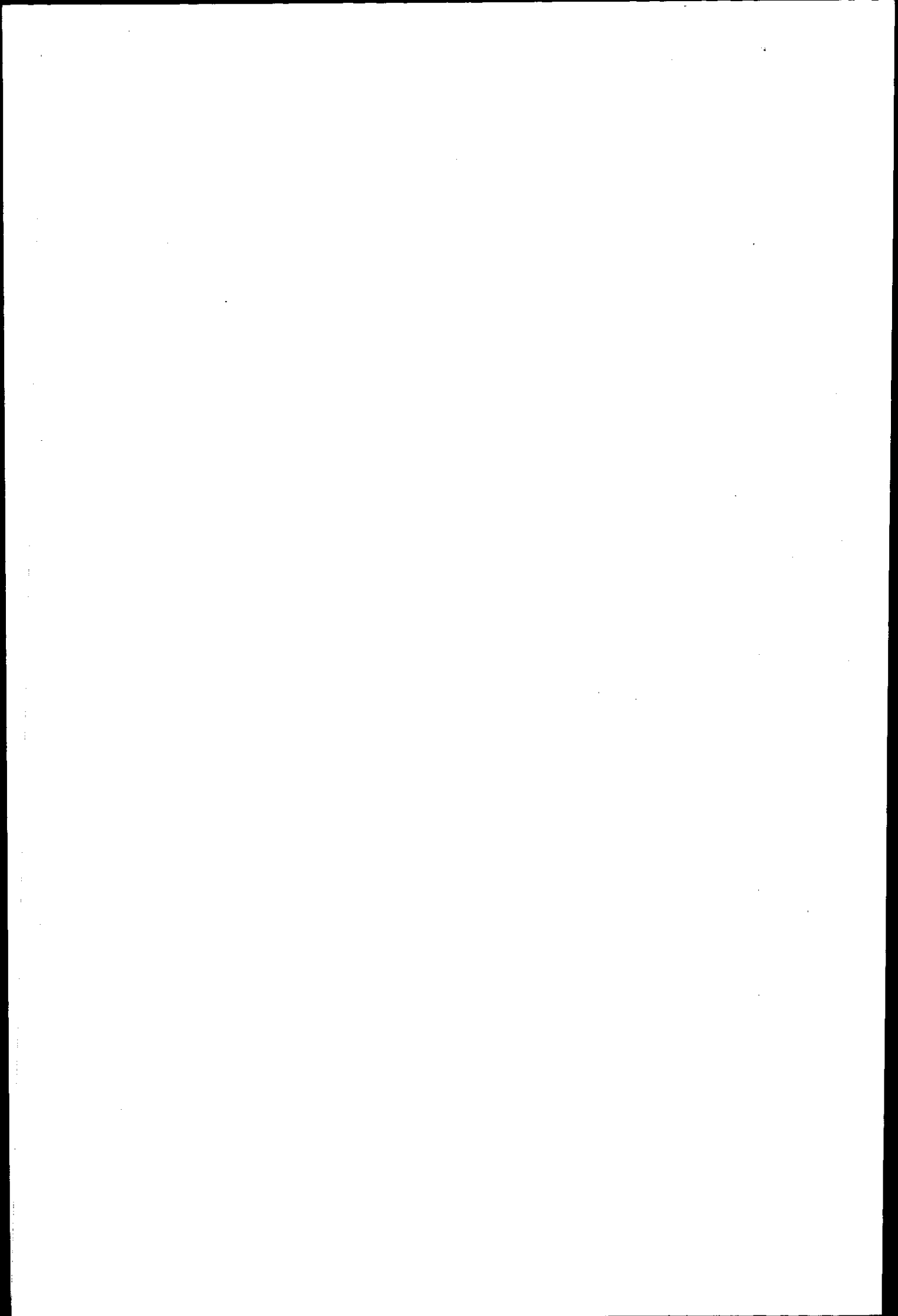
Determination of clenbuterol and salbutamol residues in
animal feed for import and export
—Liquid chromatographic method

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前 言

本标准是按照 GB/T 1.1—2000《标准化工作导则 第1部分：标准的结构和编写规则》及 SN/T 0001—1995《出口商品中农药、兽药残留量及生物毒素检验方法标准编写的基本规定》的要求进行编写的。其中测定方法是参考了国内外有关文献，经研究、改进和验证后制定的。本标准同时制定了抽样和制样方法。

测定低限是根据液相色谱测定饲料中克伦特罗、沙丁胺醇残留量方法的灵敏度而制定的。

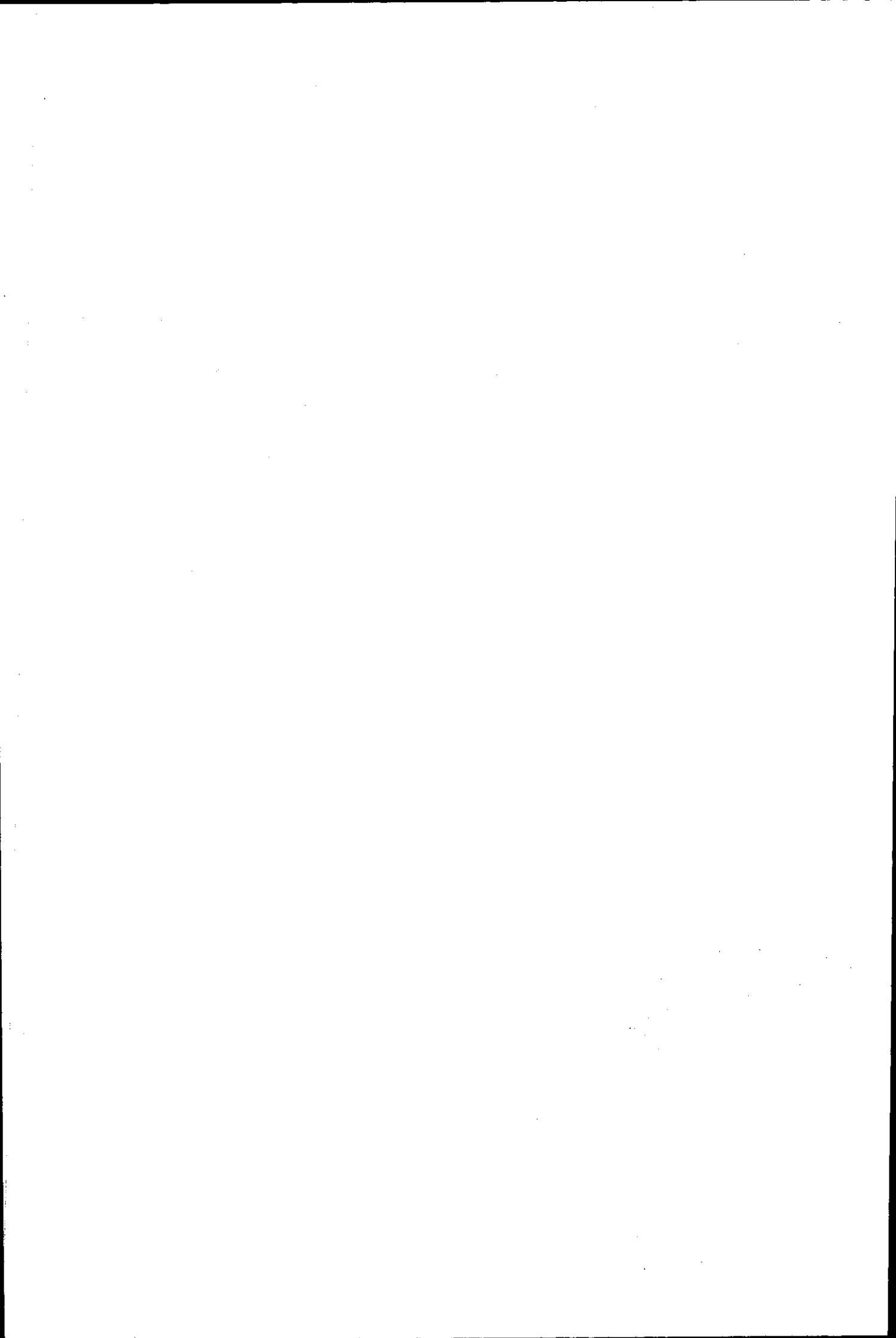
本标准的附录 A 和附录 B 为资料性附录。

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湖南出入境检验检疫局负责起草。

本标准主要起草人：戴华、袁智能、黄志强、陈新焕。

本标准系首次发布的出入境检验检疫行业标准。



进出口饲料中克伦特罗、沙丁胺醇 残留量的检验方法 液相色谱法

1 范围

本标准规定了进出口饲料中克伦特罗、沙丁胺醇残留量检验的抽样、制样和液相色谱测定方法。
本标准适用于进出口全价饲料和预混饲料中克伦特罗、沙丁胺醇残留量的检验。

2 抽样和制样

2.1 检验批

以不超过 2 000 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(见表 1)

批 量	最低抽样数
500 以下	15
501~1 000	25
1 001~2 000	30

2.3 抽样工具

2.3.1 取样勺。

2.3.2 分样板。

2.3.3 分样布。

2.3.4 盛样器:筒或袋,可密封。

2.4 抽样方法

按 2.2 规定的应抽样袋数从堆垛的各部位随机抽取样袋,逐件开启。从每袋中用取样勺抽取代表性样品约 500 g。将抽取样品立即倒入盛样器内。每袋所取样品的量应基本一致,每批所抽取的样品总量应不少于 4 kg。

将所取样品全部倒于分样布上,用分样板按四分法缩分样品至不少于 2 kg。倒入盛样器内,密封并标明标记,及时送实验室。

2.5 试样制备

2.5.1 制样工具

a) 粉碎机。

b) 筛子:2 mm 圆孔筛、20 目筛。

c) 分样板。

d) 盛样器:具塞广口瓶。

2.5.2 制样方法

将取回样品全部粉碎使通过 2 mm 圆孔筛。混匀,用四分法缩分至约 500 g。继续粉碎使全部通过

20 目筛。混匀,用四分法均分成两份,盛于盛样器内作为试样。密封并标明标记。

2.6 试样保存

将试样于-5℃以下避光保存。

注:在抽样和制样的操作过程中,必须防止样品受到污染或发生残留物含量的变化。

3 测定方法

3.1 方法提要

试样中残留的克伦特罗、沙丁胺醇经含甲醇的盐酸溶液超声提取,液-液萃取后,用固相萃取小柱净化,净化液中的克伦特罗、沙丁胺醇用配有二极管阵列检测器或紫外检测器的高效液相色谱仪测定,外标法定量。

3.2 试剂和材料

除特殊规定外,所有试剂均为分析纯,水为重蒸馏水。

3.2.1 甲醇。

3.2.2 乙腈:HPLC 级。

3.2.3 正己烷。

3.2.4 85%磷酸。

3.2.5 0.10 mol/L 盐酸溶液:取 8.33 mL 浓盐酸溶液用水稀释至 1 000 mL。

3.2.6 0.030 mol/L 盐酸溶液:取 300 mL 0.1 mol/L 盐酸溶液用水稀释至 1 000 mL。

3.2.7 0.10 mol/L 盐酸-甲醇溶液:盐酸+甲醇(9+1)。

3.2.8 2.0 mol/L 氢氧化钠溶液:称取 40.0 g 氢氧化钠用水溶解并稀释至 500 mL。

3.2.9 乙酸乙酯+正丁醇溶液(6+4),用水饱和。

3.2.10 0.007 5 mol/L 磷酸二氢钾溶液:pH 值 3.0。溶解 1.02 g 磷酸二氢钾于 800 mL 水中,用磷酸(3.2.4)调 pH 值至 3.0,然后用水定容至 1 000 mL。

3.2.11 4%(V/V)氨化甲醇溶液:取 4.0 mL 氨水(密度 0.88 g/mL)用甲醇稀释至 100 mL。

3.2.12 阳离子交换小柱:SUPELLEAN LC-SCX Sep-Pak 小柱,500 mg,3 mL 或相当者。

3.2.13 盐酸克伦特罗标准品:纯度≥99%。

3.2.14 沙丁胺醇标准品:纯度≥99%。

3.2.15 标准储备液(1 000 mg/L):称取 0.057 0 g 盐酸克伦特罗(相当于 0.050 0 g 克伦特罗)于 50 mL 容量瓶中,用甲醇溶解并定容至刻度;称取 0.050 0 g 沙丁胺醇于 50 mL 容量瓶中,用甲醇溶解并定容至刻度,作为标准储备液。根据需要再用甲醇将标准储备液稀释成适当浓度的混合标准工作液。

3.3 仪器和设备

3.3.1 高效液相色谱仪,配二极管阵列检测器或紫外检测器。

3.3.2 超声波水浴。

3.3.3 Sep-Pak 真空抽滤装置,带真空泵。

3.3.4 离心机:4 000 r/min。

3.3.5 混匀器。

3.3.6 pH 计。

3.4 测定步骤

3.4.1 提取

准确称取 5.00 g 试样于 50 mL 离心管中,加入 20 mL 盐酸-甲醇溶液(3.2.7),于混匀器上混匀后,置超声波水浴中超声 30 min(期间每 10 min 取出混匀一次),超声提取后冷却至室温,于 2 500 r/min 离心 5 min,将上清液过滤到 50 mL 容量瓶中,残渣用 2×10 mL 盐酸-甲醇溶液(3.2.7)混匀提取两次,合并提取液,用盐酸-甲醇溶液(3.2.7)洗涤滤纸并定容至刻度。

3.4.2 净化

定量取 25.0 mL 提取液于 50 mL 离心管中,加入 5 mL 正己烷,于混匀器上混匀 1 min,1 500 r/min 离心 5 min 后,用尖嘴吸管将正己烷相去掉,共处理二次。再用 2.0 mol/L NaOH 溶液(3.2.8)调节提取液 pH 值至 12,用 3×5 mL 乙酸乙酯-正丁醇溶液(3.2.9)提取样液,每次混匀 2 min,于 2 500 r/min 离心 5 min 后,用尖嘴吸管将上层有机相合并取到另一离心管中,在(70±5)℃下,用氮气流吹至近干,用 1 mL 盐酸-甲醇溶液(3.2.7)溶解残渣。

将两根 SCX 小柱(3.2.12)串联接好安装在真空抽滤装置(3.3.3)上,保持洗脱流速为 1 mL/min,依次用 5 mL 甲醇、5 mL 水和 5 mL 0.030 mol/L 盐酸溶液(3.2.6)处理小柱;然后将 1 mL 样品提取液加到小柱上;再用 1 mL 盐酸-甲醇溶液(3.2.7)洗涤试管并一起转移至小柱中;依次用 5 mL 水、5 mL 甲醇淋洗小柱。在溶剂流过固相萃取柱后,保持真空抽气状态至少保持 5 min。

于真空装置下放好刻度收集管,用 5 mL 4% 氯化甲醇溶液(3.2.11)洗脱,并收集洗脱液。将洗脱液于(70±5)℃下,用氮气流吹至近干,用甲醇溶解残渣并定容至 2.0 mL,过 0.45 μm 滤膜后,供液相色谱测定。

3.4.3 测定

3.4.3.1 色谱条件

- 色谱柱:CN 柱,250 mm×4.6 mm(内径),粒径 10 μm,或相当者;
- 流动相:乙腈+0.007 5 mol/L 磷酸二氢钾溶液(85+15),混匀后用磷酸调节 pH 值至 3.0,过 0.45 μm 微孔滤膜;
- 流速:1.0 mL/min;
- 检测波长:215 nm;
- 进样量:20 μL。

3.4.3.2 色谱测定

根据样液中克伦特罗、沙丁胺醇的含量情况,选定峰面积相近的标准工作溶液。标准工作溶液和样液中的克伦特罗、沙丁胺醇的响应值均应在仪器的检测线性范围内。对标准工作溶液和样液等体积参插进样测定。在上述色谱条件下,克伦特罗、沙丁胺醇的保留时间分别约为 13.5 min、17.4 min。标准品色谱图见附录 A 中图 A.1。标准品紫外吸收光谱图见附录 B 中图 B.1 和图 B.2。

3.4.4 空白实验

除不加试样外,均按上述测定步骤进行。

3.5 结果计算和表述

用色谱数据处理机或按下式(1)计算试样中克伦特罗或沙丁胺醇的含量:

$$X = \frac{A \times c_s \times V}{A_s \times m} \quad \dots\dots\dots (1)$$

式中:

- X ——试样中克伦特罗或沙丁胺醇的含量,mg/kg;
 A ——样液色谱图中克伦特罗或沙丁胺醇的峰面积,mm²;
 c_s ——标准工作液中克伦特罗或沙丁胺醇的浓度,μg/mL;
 A_s ——标准工作液色谱图中克伦特罗或沙丁胺醇的峰面积,mm²;
 V ——样液最终定容体积,mL;
 m ——最终样液所代表的试样量,g。

注:计算结果须扣除空白值。

4 测定低限、回收率

4.1 测定低限

本方法克伦特罗、沙丁胺醇的测定低限分别为 0.10 mg/kg、0.36 mg/kg。

4.2 回收率

4.2.1 饲料中克伦特罗添加浓度及其回收率的实验数据：

在 0.10 mg/kg 时，回收率为 89.4%；

在 1.00 mg/kg 时，回收率为 93.6%；

在 8.00 mg/kg 时，回收率为 96.3%。

4.2.2 饲料中沙丁胺醇添加浓度及其回收率的实验数据：

在 0.36 mg/kg 时，回收率为 75.1%；

在 3.60 mg/kg 时，回收率为 74.9%；

在 28.8 mg/kg 时，回收率为 83.6%。

附录 A
(资料性附录)
标准品色谱图

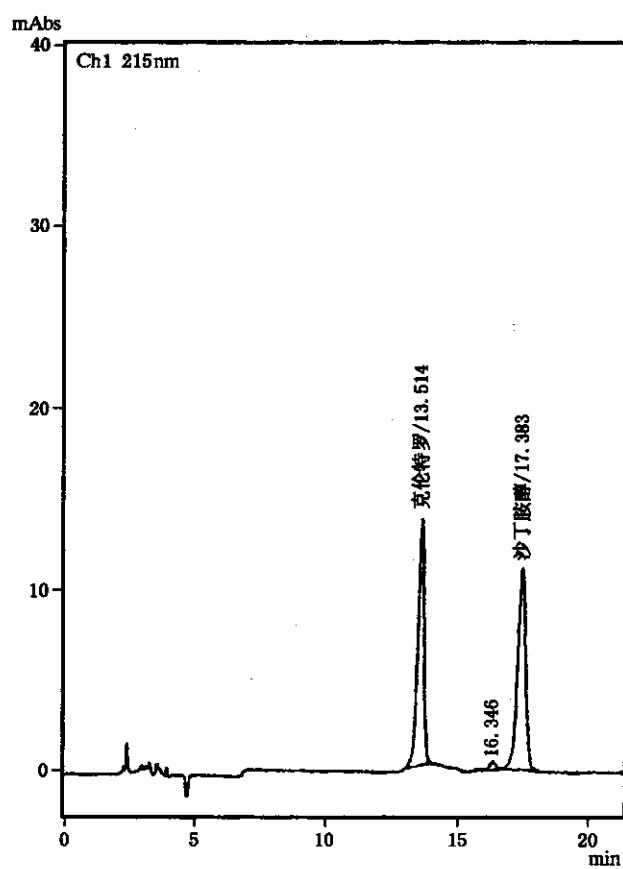


图 A.1 克伦特罗、沙丁胺醇标准品色谱图

附录 B
(资料性附录)
标准品紫外光谱图

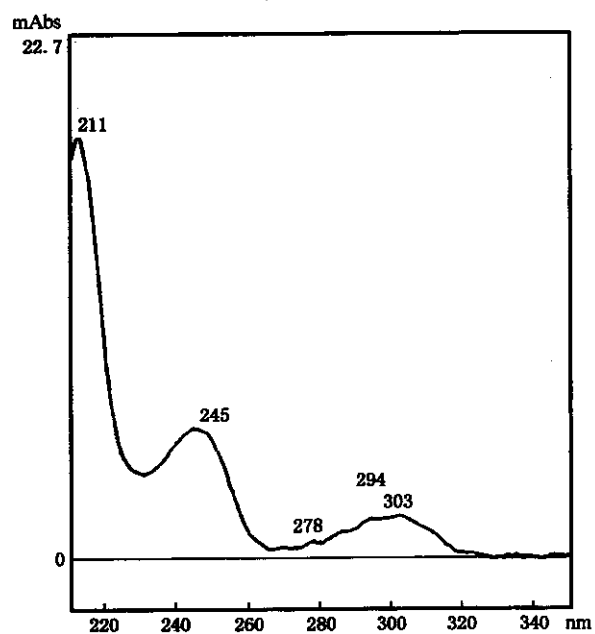


图 B.1 克伦特罗紫外吸收光谱图

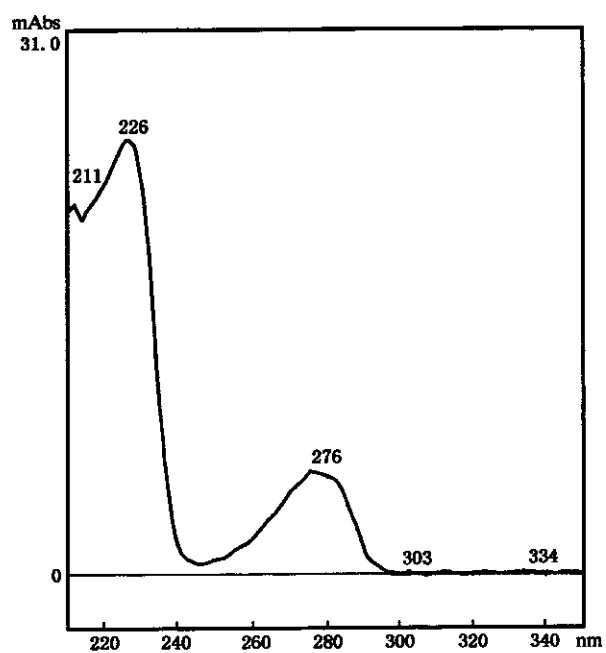


图 B.2 沙丁胺醇紫外吸收光谱图

Foreword

This standard was drafted in accordance with the requirement of GB/T 1.1—2000 “Directives for of standardization—Part 1: Rules for the structures and drafting of standards” and SN/T 0001—1995 “General rules for drafting the standard methods for the determination of pesticide, veterinary drug residues and biotoxins in commodities for export”. The method of determination of this standard was drafted by referring to relevant domestic and foreign literatures through research, modification and verification. In addition, method of sampling and sample preparation are also specified in this standard.

The limit of determination is defined in this standard on the basis of the sensitivity of the method by liquid chromatography.

Annex A and annex B of this standard are informative annexes.

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

This standard was drafted by Hunan Entry – Exit Inspection and Quarantine Bureau of the People's Republic of China.

The main drafters of this standard are Dai Hua, Yuan Zhineng, Huang Zhiqiang and Chen Xinhuan.

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

Determination of clenbuterol and salbutamol residues in animal feed for import and export —Liquid chromatographic method

1 Scope

This standard specifies the methods of sampling, sample preparation and determination of clenbuterol and salbutamol residues in animal feed for import and export by liquid chromatography. This standard is applicable to the determination of clenbuterol and salbutamol residues in pre-mixed feed and fodder feed for import and export.

2 Sampling and sample preparation

2.1 Inspection lot

Each inspection lot should not exceed 2 000 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 (see Table 1)

Table 1

Number of packages in an inspection lot	Minimum number of packages to be taken
below 500	15
501 ~ 1 000	25
1 001 ~ 2 000	30

2.3 Sampling tools

2.3.1 Sampling ladle.

2.3.2 Plate for quartering.

2.3.3 Sheet: For sample dividing (quartering).

2.3.4 Sample container: Can or bag, which can be sealed.

2.4 Sampling procedure

Draw the number of bags specified in 2.2 at any parts of the pile at random, unseal and open the bag one by one. From each bag, ca 500 g of representative sample shall be taken. Promptly place the samples in a sample container. The quantity of the sample drawn from each bag should be basically the same, and the total weight of the sample drawn from each lot should be not less than 4 kg.

Pour all the sample onto a clean sheet and reduce to not less than 2 kg by quartering with a plate. Place in a sample container, seal, label and send to the laboratory in time.

2.5 Preparation of test sample

2.5.1 Tools for sample preparation

- a) Pulverizer.
- b) Sieve: 2 mm round – hole sieve, 20 mesh sieve.
- c) Plate for quartering.
- d) Sample container: Wide – mouth bottle, with ground stopper.

2.5.2 Procedure

Pulverize all the sample taken back to let pass through a 2 mm round – hole sieve, mix and reduce by quartering to ca 500 g. Pulverize again to let all pass thorough a 20 – mesh sieve. Mix thoroughly and divide into two equal portions, place in clean sample containers as the test samples, seal and label.

2.6 Storage of test sample

The test samples should be stored below – 5°C and kept away from light.

Note : In the course of sampling and sample preparation, precautions must be taken to avoid contamination or any factors which may cause the change of residue content.

3 Method of determination

3.1 Principle

The clenbuterol and salbutamol residues are extracted from the test sample by hydrochloric acid solution containing methanol, and the extract is clean – up by liquid – liquid extraction and solid phase extraction before determination by HPLC with PAD or UV detector, using external standard method.

3.2 Reagents and materials

Unless otherwise specified, all reagents should be analytical pure, “water” is redistilled water.

3.2.1 Methanol.

3.2.2 Acetonitrile: HPLC grade.

3.2.3 n-Hexane.

3.2.4 85% Phosphoric acid.

3.2.5 0.10 mol/L Hydrochloric acid solution: Dilute 8.33 mL hydrochloric acid (HCl) to 1 000 mL with water.

3.2.6 0.030 mol/L Hydrochloric acid solution: Dilute 300 mL 0.1 mol/L HCl to 1 000 mL with water.

3.2.7 Hydrochloric acid - methanol solution: Dilute 100 mL methanol to 1 000 mL with 0.10 mol/L HCl (3.2.5).

3.2.8 2.0 mol/L Sodium hydroxide (NaOH) solution: Dissolve 40.0 g of NaOH in 500 mL water.

3.2.9 Ethyl - acetate + n - butanol solution (6 + 4), Saturated with water.

3.2.10 Potassium dihydrogen phosphate (KH_2PO_4) solution: 0.007 5 mol/L at pH value 3.0, Dissolve potassium dihydrogen phosphate in 800 mL water, adjust the pH value to 3.0 with concentrated phosphoric acid (3.2.4) and dilute to 1 000 mL with water.

3.2.11 4% Ammoniacal methanol: Dilute 4 mL ammonia solution (sp. gr. 0.88) to 100 mL with methanol.

3.2.12 Anion - exchange cartridge: SUPELLEAN LC - SCX Sep - pak cartridge, 500 mg, 3 mL or equivalent.

3.2.13 Clenbuterol hydrochloride: purity $\geq 99\%$.

3.2.14 Salbutamol: purity $\geq 99\%$.

3.2.15 Standard solution (1 000 mg/L): Weigh accurately about 0.057 0 g clenbuterol hydrochloride (equivalent to 0.050 0 g of clenbuterol) standard into a 50 mL volumetric flask. Dissolve and make up to mark with methanol. Weigh accurately about 0.050 0 g salbutamol standard into a 50 mL volumetric flask. Dissolve and make up to mark with methanol as the standard stock solution. Dilute the standard stock solution with methanol to the required concentration as the mix standards working solution.

3.3 Apparatus and equipment

3.3.1 HPLC equipped with PAD (photodiode array detector) or UV detector.

3.3.2 Ultrasonic bath.

3.3.3 Sep – Pak vacuum manifold with mechanical vacuum pump.

3.3.4 Centrifuge: 4 000 r/min.

3.3.5 Mixer.

3.3.6 pH Meter.

3.4 procedure

3.4.1 Extraction

Weigh accurately 5.00 g sample into 50 mL centrifuge tube, add 20 mL hydrochloric acid – methanol solution (3.2.7). Sonicate for 30 minutes in an ultrasonic bath (shaking at least every 10 min), let cool to room temperature. Decant solution through filter paper into 50 mL volumetric flask. Repeat extraction of solid with 20 × 10 mL Hydrochloric acid – methanol solution (3.2.7) shake vigorously, centrifuge and filter supernatant through filter paper in the same volumetric flask. Wash the tube and filter cake with small amount of hydrochloric acid – methanol solution (3.2.7), and combine the filterates and make up to 50 mL.

3.4.2 Cleanup

Pipet 25.0 mL of the extract into 50 mL centrifuge tube. Add 5 mL n – hexane into the centrifuge tube, mix on a mixer for 1 min, centrifugalize at 1 500 r/min for 5 min, draw out the n – hexane layer with a capillarytipped pipet and discard, repeat the step two times. The pH value was adjusted to 12 by 2.0 mol/L Sodium hydroxide solution (3.2.8). A volume of 3 × 5 mL of ethylacetate – n – butanol solution (3.2.9) was added to the extract and the solution was mixed on a mixer for 2 min. The sample was centrifuged (2 500 r/min, 5 min) and the organic phase was collected by capillarytipped pipet. The organic phase was evaporated to near dryness under a stream of nitrogen at $(70 \pm 5)^\circ\text{C}$. Dissolve the residue in 1 mL hydrochloric acid – methanol solution (3.2.7).

Set up the vacuum manifold and mechanical pump (3.3.3). Condition two SCX cartridges (in series) (3.2.12) by washing with 5 mL methanol, 5 mL water and then 5 mL 0.030 mol/L HCl (3.2.6), then transfer the sample extract 1 mL into the cartridges. Wash the test tube with 1 mL of hydrochloric acid and make up to 2.0 mL with methanol, and filter through 0.45 μm membrane filter. The solution is acid – methanol solution (3.2.7) and add to the cartridge. Wash the cartridges with 5 mL water and then 5 mL methanol. Drain off excessive liquid in the cartridges by continuous suction for at least 5 minutes.

Elute with 5 mL 4% ammoniacal methanol (3.2.11) and collect the eluant in a glass tube with stopper, evaporate the eluate to near dryness under nitrogen at $(70 \pm 5)^\circ\text{C}$. Dissolve the residues used for HPLC determination.

3.4.3 Determination

3.4.3.1 HPLC operating condition

- a) Column: CN column (25 cm × 4.6 mm i. d., 10 μm particle size) or equivalent;
- b) Mobile phase: Acetonitrile + 0.007 5 mol/L K₂PO₄ solution (85 + 15), the pH value was adjusted to 3.0 by phosphoric acid (3.2.4), filter through 0.45 μm membrane filter;
- c) Flow rate: 1.0 mL/min;
- d) Detector wavelength: 215 nm;
- e) Injection volume: 20 μL.

3.4.3.2 HPLC determination

According to the approximate concentration of clenbuterol and salbutamol in the sample solution, select the standard working solution with similar peak area to that of the sample solution. The responses of clenbuterol and salbutamol in the mix standard working solution and sample solution should be within the linear range of the instrumental detection. The mix standard working solution should be randomly injected in – between the injections of the sample solution of equal volume. Under the above operating condition, the retention time of clenbuterol is about 13.5 min and salbutamol about 17.4 min. For chromatogram of the standard, see Figure A.1 in annex A. For ultraviolet spectrum of clenbuterol and salbutamol, see Figure B.1 and Figure B.2 in annex B.

3.4.4 Blank test

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

3.5 Calculation and expression of the result

The calculation of the residue content of clenbuterol or salbutamol in the test sample is carried out by HPLC data processor or according to the formula (1):

$$X = \frac{A \times c_s \times V}{A_s \times m} \quad \dots\dots\dots (1)$$

Where:

X —the residue content of clenbuterol or salbutamol in the test sample, mg/kg;

A —the peak area of clenbuterol or salbutamol in the sample solution, mm²;

c_s —the concentration of clenbuterol or salbutamol in the standard working solution, μg/mL;

A_s —the peak area of clenbuterol or salbutamol in the standard working solution mm²;

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

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

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of clenbuterol is 0.10 mg/kg and salbutamol is 0.36 mg/kg.

4.2 Recovery

4.2.1 According to the experiment data, the fortifying concentrations of clenbuterol in fodders, and their corresponding recoveries are:

0.10 mg/kg, the recovery 89.4% ;

1.00 mg/kg, the recovery 93.6% ;

8.00 mg/kg, the recovery 96.3% .

4.2.2 According to the experiment data, the fortifying concentrations of salbutamol in fodders, and their corresponding recoveries are:

0.36 mg/kg, the recovery 75.1% ;

3.60 mg/kg, the recovery 74.9% ;

28.8 mg/kg, the recovery 83.6% .

Annex A
(informative)
Chromatogram of standards

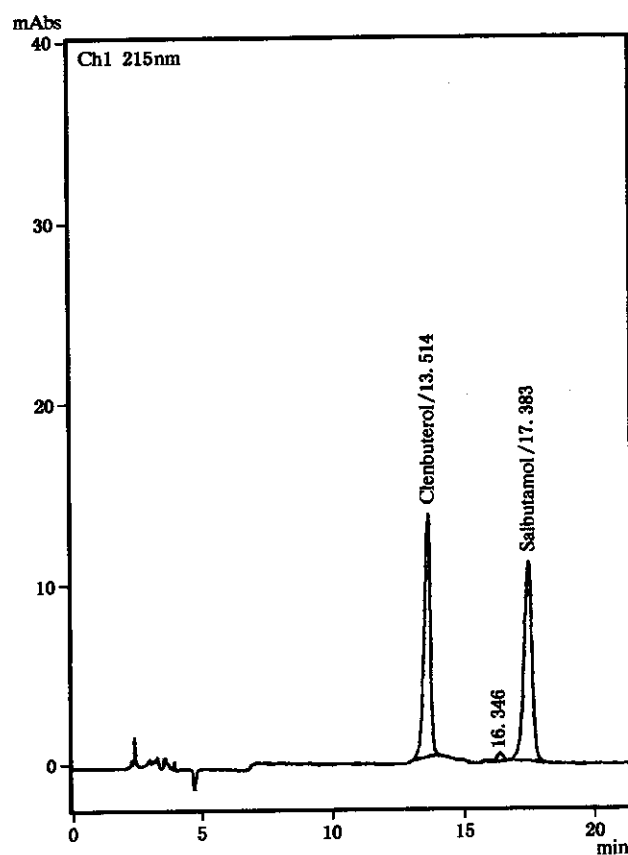


Figure A.1—Chromatogram of clenbuterol and salbutamol standards

Annex B
(informative)
Ultraviolet spectrum of the standards

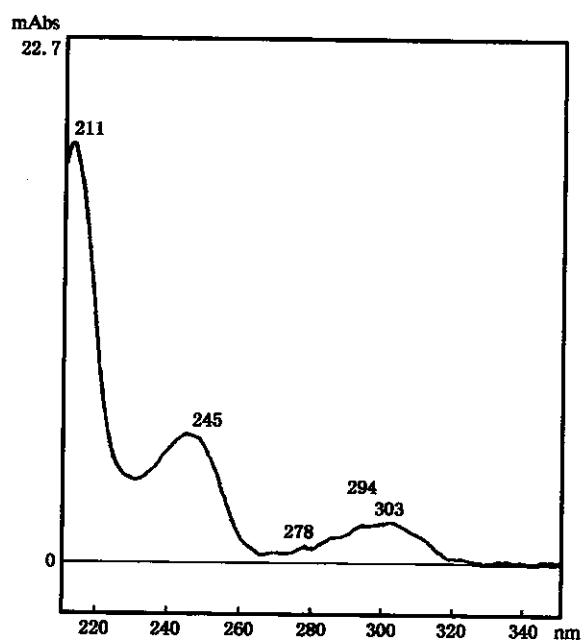


Figure B.1—Ultraviolet spectrum of Clenbuterol standard

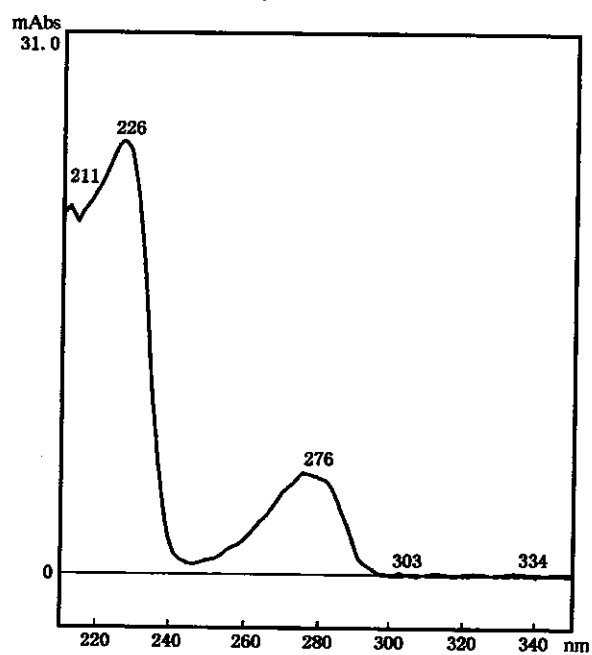
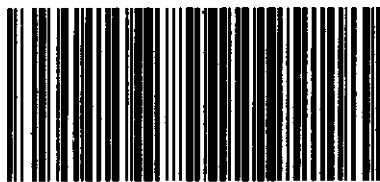


Figure B.2—Ultraviolet spectrum of salbutamol standard

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