SN

中华人民共和国进出口商品检验行业标准

SN 0646—1997

出口肉及肉制品中新霉素残留量检验方法 液相色谱法

Method for the determination of neomycin residues in meats and meat products for export —Liquid chromatography

1997-08-15发布　　1998-01-01实施

中华人民共和国国家进出口商品检验局　发布

前　言

本标准是根据GB/T 1.1—1993《标准化工作导则　第1单元：标准的起草与表述规则　第1部分：标准编写的基本规定》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》的要求而编写的。其中测定方法是参考国内外有关文献，经研究、改进和验证后而制定的。本标准同时制定了抽样和制样方法。

测定低限是根据国际上对肉及肉制品中新霉素残留量的最高限量和测定方法的灵敏度而制定的。

本标准的附录A是提示的附录。

本标准由中华人民共和国国家进出口商品检验局提出并归口。

本标准由中华人民共和国宁波进出口商品检验局负责起草。

本标准主要起草人：李佐卿、康继韬、吕建成、刘晓剑。

本标准系首次发布的行业标准。

中华人民共和国进出口商品检验行业标准

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1 范围

本标准规定了出口肉及肉制品中新霉素残留量检验的抽样、制样和液相色谱测定方法。

本标准适用于出口猪肉中新霉素残留量的检验。

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

批量，件	最低抽样数，件
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。每件至少取一袋作为原始样品，原始样品总量不得少于 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(经灭菌)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

将所取样品全部放入高速捣碎机中捣碎均匀，混匀后用四分法缩分出不少于 1 kg，均分成两份，作为试样，装入清洁容器内，加封后标明标记。

2.5 试样保存

将试样于－18℃以下冷冻保存。

注：在抽样及制样的操作过程中，必须防止样品受到污染或发生残留物含量的变化。

中华人民共和国国家进出口商品检验局 1997-08-15 批准　　1998-01-01 实施

3 测定方法

3.1 方法提要

用磷酸盐缓冲液-乙腈提取试样中的新霉素，提取液经加热、冷却和过滤以除去蛋白质和脂肪。在阳离子交换柱上与邻苯二甲醛进行衍生化，用四硼酸钾缓冲液-甲醇洗脱，洗脱液用带有荧光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，所用试剂均为分析纯，水为蒸馏水。

3.2.1 乙腈。

3.2.2 甲醇：紫外光谱纯。

3.2.3 三乙胺。

3.2.4 磷酸(85%)。

3.2.5 氯化钠。

3.2.6 磷酸盐缓冲液：将 33.46 g 磷酸氢二钾和 1.046 g 磷酸二氢钾溶解在水中，并定容至 1 L。

3.2.7 混合提取液：磷酸盐缓冲液-乙腈(1+1)。

3.2.8 硼酸钾缓冲液：将 2.5 g 硼酸溶于 80 mL 水中，用 50%(m/V)氢氧化钾溶液调 pH 至 10.5，用水定容至 100 mL。

3.2.9 邻苯二甲醛试剂：将 100 mg 邻苯二甲醛溶于 1 mL 甲醇中，加入 200 μL 2-巯基乙醇和 10 mL 硼酸钾缓冲液，用棕色瓶(有盖)贮存于冰箱中，一周内有效。

3.2.10 碱性缓冲液：将 76 g 四硼酸钾溶于 400 mL 水中，用 50%(m/V)氢氧化钾溶液调 pH 至 11.0，用水定容至 500 mL。

3.2.11 洗脱剂：碱性缓冲液-甲醇(1+4)。

3.2.12 阳离子交换树脂：D152。

3.2.13 硫酸新霉素标准品：生化试剂，已知纯度，700 IU/mg 以上(硫酸新霉素 1 mg=1 000 IU)。

3.2.14 硫酸新霉素标准溶液：称取适量的硫酸新霉素标准品(精确至 0.1 mg)，用水配成浓度为 0.100 mg/mL的标准储备溶液，根据需要再用水配成适当浓度的标准工作溶液。

3.2.15 新霉素衍生物标准溶液：将适量的标准工作溶液注入到阳离子交换柱(3.3.6)中，按 3.4.2 步骤进行衍生化反应，制成新霉素衍生物标准溶液。

3.3 仪器和设备

3.3.1 液相色谱仪：配有荧光检测器。

3.3.2 高速捣碎机。

3.3.3 振荡器。

3.3.4 离心机。

3.3.5 过滤漏斗：滤孔为 80～120 μm、40～80 μm。

3.3.6 阳离子交换柱：120 mm×5 mm(内径)玻璃柱。用碱性缓冲液(3.2.10)将阳离子交换树脂制成悬浮液，平衡 2 h，装入玻璃柱中，高度约为 6 cm，用 5 mL 洗脱剂淋洗，流速为 0.8 mL/min，然后用水洗至流出液呈中性，备用。

3.4 测定步骤

3.4.1 提取

称取试样约 10 g(准确至 0.1 g)于一 100 mL 锥形瓶中，加入 60 mL 混合提取液(3.2.7)和 2 g 氯化钠，振荡 30 min，经滤孔为 80～120 μm 的过滤漏斗抽滤，残渣用 3×10 mL 混合提取液洗涤。合并滤液和洗液于一 250 mL 烧杯中。在沸水中加热 30 min，取下，于 3 000 r/min 离心 20 min，置 4℃冰箱中冷却 20 min，然后经滤孔为 40～80 μm 的过滤漏斗抽滤，收集滤液。

3.4.2 衍生化

将滤液注入阳离子交换柱(3.3.6)中，用 10 mL 水洗柱，弃去流出液。将 1 mL 邻苯二甲醛试剂注入柱中，反应 5 min。然后加 2 mL 洗脱剂洗脱柱上衍生物。弃去前面 1 mL 洗脱液，收集后面 1 mL 洗脱液，立即放置在−18℃冰箱中，15 min 后进行液相色谱测定。

3.4.3 测定

3.4.3.1 色谱条件

a) 色谱柱：ODS Hypersil，3 μm，60 mm×4.6 mm(内径)，或相当者；

b) 柱温：35℃；

c) 流动相：77.5%甲醇水溶液中含 0.09 mol/L 三乙胺和 0.45 mol/L 磷酸；

d) 流速：1.0 mL/min；

e) 激发波长：345 nm；发射波长：445 nm；

f) 进样量：10 μL。

3.4.3.2 色谱测定

根据样液中新霉素含量情况，选定峰面积相近的新霉素衍生物标准溶液。标准液和样液中新霉素衍生物响应值均应在仪器检测线性范围内。对标准液和样液等体积参插进样测定。在上述色谱条件下，新霉素衍生物的保留时间约为 6 min。标准品衍生物液相色谱图见附录 A 中图 A1。

3.4.4 空白试验

除不加试样外，均按上述操作步骤进行。

3.4.5 结果计算和表述

用色谱数据处理机或按式(1)进行计算：

$$X = \frac{A \cdot c \cdot V \times 0.855}{A_s \cdot m} \quad \cdots\cdots(1)$$

式中：X——试样中新霉素含量，mg/kg；

A——样液中新霉素衍生物的峰面积，mm^2；

A_s——新霉素衍生物标准溶液中新霉素衍生物峰面积，mm^2；

c——新霉素衍生物标准溶液中相当的硫酸新霉素浓度，μg/mL；

V——最终样液的体积，mL；

m——最终样液相当的试样量，g；

0.855——由硫酸新霉素换算成新霉素的换算系数。

注：计算结果需扣除空白值。

4 测定低限和回收率

4.1 测定低限

本方法的测定低限为 0.05 mg/kg。

4.2 回收率

猪肉中新霉素添加浓度及其回收率实验数据：

在 0.05 mg/kg 时，回收率为 90.4%；

在 0.10 mg/kg 时，回收率为 91.6%；

在 0.50 mg/kg 时，回收率为 96.3%。

附 录 A
（提示的附录）
标准品色谱图

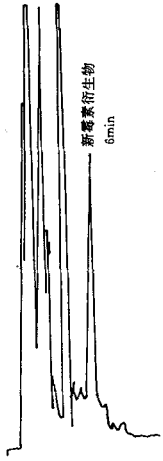


图 A1 新霉素标准品衍生物的液相色谱图

Foreword

This standard was drafted in accordance with the requirements of GB/T 1.1—1993"Directives for the work of standardization—Unit 1:Drafting and presentation of standards—Part 1:General rules for drafting 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, methods 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 current international maximum limits for neomycin residues in meats and meat products and the sensitivity of the method.

Annex A of this standard is an informative annex.

This standard was proposed by and is under the charge of the State Administration of Import and Export Commodity Inspection of the People's Republic of China.

This standard was drafted by Ningbo Import and Export Commodity Inspection Bureau of the People's Republic of China.

The main drafters of this standard are Li Zuoqing, Kang Jitao, Lu Jiancheng and Liu Xiaojian.

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

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

Professional Standard of the People's Republic of China for Import and Export Commodity Inspection

Method for the determination of neomycin residues in meats and meat products for export —Liquid chromatography

SN 0646—1997

1 Scope

This standard specifies the methods of sampling, sample preparation and determination by high performance liquid chromatography of neomycin residues in meats and meat products for export.

This standard is applicable to the determination of neomycin residues in pork for 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

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. From each package, at least one bag shall be taken as a primary sample. The total weight of all primary samples should not be less than 2.0 kg, which shall be placed in a clean container, sealed, labeled and sent to the laboratory in time.

In case the meat-pieces are not contained in small bags inside each package, or if there are small bags inside but the content of 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). Mix the parts of the meat as the mixed primary sample, which shall not be less than 2 kg. Place in a clean container, seal, label, and send to the laboratory in time.

Approved by the State Administration of Import and Export Commodity Inspection of the People's Republic of China on Aug. 15, 1997

Implemented from Jan. 1, 1998

2.4 Preparation of test sample

Blend the sample at high speed with a high-speed blender and mixed thoroughly, then reduced to not less than 1 kg by quartering. Divide into two equal portions, each is placed in a clean container as test sample, which is then sealed and labeled.

2.5 Storage of test sample

The test samples should be stored below −18℃.

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 neomycin residues are extracted from the test sample with phosphate buffer-acetonitrile. After heating, cooling and filtering, the extract is deproteinized and defatted, then derivatizated with phthalaldehyde reagent on cation exchange resin column. The derivative on column is then eluted with potassium tetraborate buffer-methanol. Determination is made by HPLC with fluorescence detector, using external standard method.

3.2 Reagents and materials

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

3.2.1 Acetonitrile.

3.2.2 Methanol: Ultraviolet spectrographic grade.

3.2.3 Triethylamine.

3.2.4 Phosphoric acid (85%).

3.2.5 Sodium chloride.

3.2.6 Phosphate buffer solution: Dissolve 33.46 g of dibasic potassium phosphate and 1.046 g of monobasic potassium phosphate and make up to 1 000 mL with water.

3.2.7 Mixed extraction solution: Phosphate buffer-acetonitrile (1+1).

3.2.8 Potassium borate buffer solution: Dissolve 2.5 g of boric acid in 80 mL of water, adjust to pH 10.5 with 50% (m/V) potassium hydroxide solution and dilute to 100 mL with water.

3.2.9 *o*-phthalaldehyde reagent: Dissolve 100 mg of *o*-phthalaldehyde in 1 mL of methanol. Add 200 μL 2-mercaptoethanol and 10 mL of potassium borate buffer. Store in a capped amber vial in the refrigerator. This reagent is effective within a week.

3.2.10 Alkaline buffer solution: Dissolve 76 g of potassium tetraborate in 400 mL of water, adjust to pH 11.0 with 50% (m/V) potassium hydroxide solution and dilute to 500 mL with water.

3.2.11 Eluting agent: Alkaline buffer solution-methanol (1+4).

3.2.12 Cation exchange resin: D152.

3.2.13 Neomycin sulfate standard: Biochemical reagent, more than 700 IU/mg. 1 mg pure neomycin sulfate =1 000 IU)

3.2.14 Neomycin sulfate standard solution: Accurately weigh an adequate amount of neomycin sulfate standard (accurate to 0.1 mg), dissolve in water to prepare a solution of 0.100 mg/mL in concentration as the standard stock solution. According to the requirement, prepare a standard working solution of appropriate concentration by diluting the stock solution with water.

3.2.15 Neomycin derivative standard solution: Pour an appropriate amount of standard working so-

lution into the cation exchange resin column (3.3.6) and proceed as described in 3.4.2 to prepare a neomycin derivative standard solution.

3.3 Apparatus and equipment

3.3.1 High performance liquid chromatograph: Equipped with fluorescence detector.

3.3.2 High-speed blender.

3.3.3 Shaker.

3.3.4 Centrifuge.

3.3.5 Filter funnel: Hole size, 80—120 μm, 40—80 μm.

3.3.6 Cation exchange resin column: 120 mm×5 mm(id) glass column. A suspension is made by shaking D152 resin with the alkaline buffer solution (3.2.10) and equilibrated for 2 h. Then fill into a glass column to about 6.0 cm height and rinse the column with 5 mL of eluting agent. The flow rate is 0.8 mL/min. Rinse with water until the effluent is neutral.

3.4 Procedure

3.4.1 Extraction

Weigh ca 10.0 g of the test sample (accurate to 0.1 g)into a 100 mL conical flask, add 60 mL of mixed extraction solution (3.2.7) and 2 g of sodium chloride. Shake for 30 min on a shaker. Filter through a filter funnel with 80—120 μm hole size and wash the residues with 3×10 mL of portions of mixed extraction solution. Combine the filtrates and the washings in a 250 mL beaker and heat for 30 min in a boiling water-bath. Then centrifugalize at 3 000 r/min for 20 min and cool for 20 min in a refrigerator at 4℃. Filter through a filter funnel with 40—80 μm hole size and collect the filtrate.

3.4.2 Derivatization

Pour the filtrate into the cation exchange resin column (3.3.6), wash the column with 10 mL of water and discard the effluent. Then add 1 mL of *o*-phthalaldehyde reagent into the column and let react for 5 min. Elute the derivative with 2 mL of eluting agent. Discard the first 1 mL of the eluate and collect the following 1 mL of eluate and place the eluate in a refrigerator at −18℃ immediately. The solution is ready for HPLC determination after 15 min.

3.4.3 Determination

3.4.3.1 HPLC operating condition

a) Column: ODS Hypersil, 3 μm, 60 mm×4.6 mm(id), or the equivalent;

b) Column temperature: 35℃;

c) Mobile phase: 77.5% methanol aqueous solution containing 0.09 mol/L triethylamine and 0.45 mol/L phosphoric acid;

d) Flow rate: 1.0 mL/min;

e) Excitation wavelength: 345 nm, emission wavelength: 445 nm;

f) Injection volume 10 μL.

3.4.3.2 HPLC determination

According to the approximate concentration of neomycin in the sample solution, select the neomycin derivative standard solution with similar peak area to that of sample solution. The responses of neomycin derivative in the standard solution and sample solution should be within the linear range of the instrumental detection. The standard solution should be randomly injected in-between the injections of the sample solution of equal volume. Under above operating conditions, the retention time of neomycin derivative is about 6 min. For HPLC chromatogram of the neomycin derivative standard, see fig. A1 in annex A.

3.4.4 Blank test

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

3.4.5 Calculation and expression of result

Calculation of result is carried out by HPLC data processor or according to the formula(1):

$$X = \frac{A \cdot c \cdot V \times 0.855}{A_s \cdot m} \quad \cdots\cdots(1)$$

where

X—Residue content of neomycin in the test sample, mg/kg;

A—Peak area of neomycin derivative in the sample solution, mm^2;

A_s—Peak area of neomycin derivative in neomycin derivative standard solution, mm^2;

c—Corresponding concentration of neomycin sulfate in neomycin derivative standard solution, μg/mL;

V—Final volume of the sample solution, mL;

m—Corresponding mass of the test sample in the final sample solution, g;

0.855—Conversion factor of neomycin sulfate to neomycin.

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 0.05 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentrations of neomycin in pork and its corresponding recoveries are:

0.05 mg/kg, the recovery 90.4%;

0.10 mg/kg, the recovery 91.6%;

0.50 mg/kg, the recovery 96.3%.

Annex A

(informative)

Chromatogram of the standard

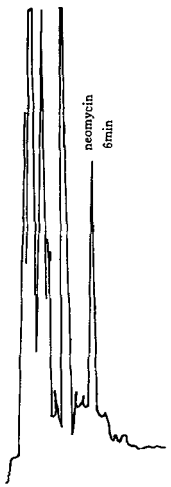


Fig. A1 Liquid chromatogram of neomycin derivative standard