SN

中华人民共和国进出口商品检验行业标准

SN 0606－1996

出口乳及乳制品中噻菌灵残留量检验方法 荧光分光光度法

Method for the determination of thiabendazole residues in milk and milk products for export —Fluorescence spectrophotometry

1996-11-15发布　　1997-05-01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》的要求而进行编写的。其中测定方法是参考国内外有关文献，经研究、改进和验证后制定。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鲜乳中噻菌灵残留量的最高限量和测定方法的灵敏度而制订的。

本标准的附录A为提示的附录。

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。

本标准负责起草单位：中华人民共和国广东进出口商品检验局。

本标准主要起草人：张思群、李辉、梁伟大。

本标准系首次发布的行业标准。

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1 范围

本标准规定了出口乳及乳制品中噻菌灵残留量检验的抽样、制样和荧光分光光度测定方法。

本标准适用于出口鲜乳中噻菌灵残留量的检验。

2 抽样和制样

2.1 检验批

以不超过 50 000 瓶为一检验批。

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，瓶	最低抽样数，瓶
10 000 以下	2
10 000～20 000	3
20 001～30 000	4
30 001～40 000	5
40 001～50 000	6

2.3 抽样方法

按 2.2 规定的抽样瓶数随机抽取，在所取的样瓶上标明记号，及时送实验室。

2.4 试样制备

将所取回的样瓶，充分混匀，分取约 250 mL 作为试样。装入洁净的容器内，密封，标明标记。

2.5 试样保存

将试样于－5℃以下保存。

注：在抽样和制样的操作过程中，必须防止样品受到污染或发生残留物含量的变化。

3 测定方法

3.1 方法提要

用氢氧化钾皂化试样中的脂肪，用乙酸乙酯提取噻菌灵，再用盐酸溶液抽提乙酸乙酯提取液中噻菌灵。用荧光分光光度法测定，标准曲线法定量。

3.2 试剂和材料

除另有规定外，所用试剂均为分析纯，水为蒸馏水。

中华人民共和国国家进出口商品检验局 1996-11-15 批准　　1997-05-01 实施

3.2.1 氢氧化钾溶液：50%(*m*/*V*)水溶液。

3.2.2 氢氧化钾溶液：0.05%(*m*/*V*)水溶液。

3.2.3 盐酸溶液：0.1 mol/L。

3.2.4 乙酸乙酯。

3.2.5 噻菌灵标准品：纯度≥99%。

3.2.6 噻菌灵标准溶液：准确称取适量的噻菌灵标准品，用盐酸溶液配成浓度为 0.100 mg/mL 的标准贮备液。根据需要再用盐酸溶液稀释成适当浓度的标准工作溶液。

3.3 仪器和设备

3.3.1 荧光分光光度计。

3.3.2 冷凝管。

3.3.3 分液漏斗：125 mL。

3.3.4 锥形瓶：100 mL，具磨口。

3.3.5 电热水浴锅。

3.3.6 容量瓶：10 mL。

3.4 测定步骤

3.4.1 皂化

称取试样 10 g(精确至 0.1 g)于锥形瓶中，加入 7 mL 氢氧化钾溶液(3.2.1)，接上冷凝管，在沸腾的水浴上回流皂化 40 min，取下，充分冷却。

3.4.2 提取

将皂化液移入分液漏斗中，用 10 mL 水洗涤锥形瓶，洗液并入同一分液漏斗。加入 15 mL 乙酸乙酯，轻摇 0.5 min，静止分层。将水层转入另一分液漏斗，用 15 mL 乙酸乙酯再提取一次，剧烈振摇 1 min，静止分层。合并乙酸乙酯提取液。

3.4.3 净化

用 20 mL 氢氧化钾溶液(3.2.2)洗涤乙酸乙酯提取液，剧烈振摇 1 min，分层后，弃去水层。再加入 20 mL 氢氧化钾溶液(3.2.2)轻摇洗涤一次，弃去水层。用 2×5 mL 盐酸溶液(0.1 mol/L)提取乙酸乙酯层。合并盐酸提取液于10 mL容量瓶中，并用盐酸溶液(0.1 mol/L)定容。供荧光分光光度法测定。

3.4.4 测定

3.4.4.1 荧光分光光度法测定条件

激发波长：307 nm；发射波长：359 nm。不同型号仪器，可根据实际情况调节，以获得最佳激发波长和发射波长。

3.4.4.2 标准曲线的绘制

分别吸取 0.2，0.5，1.0，5.0 和 10.0 mL 标准工作溶液至一组 10 mL 容量瓶中，用盐酸溶液(0.1 mol/L)定容，于荧光分光光度计上测定各荧光吸光度，以荧光吸光度对噻菌灵浓度绘制标准曲线。标准品的荧光吸光度扫描图见附录 A 中图 A1。

3.4.4.3 样液测定

取 3.4.3 中定容后的样液，于荧光分光光度计上测定样液的荧光强度。从标准曲线上查得样液中噻菌灵浓度。

3.4.5 空白试验

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

按式(1)计算试样中噻菌灵的残留含量：

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

式中：X——试样中噻菌灵含量，mg/kg；

c——从标准曲线上查得的样液中噻菌灵的浓度，μg/mL；

V——定容后样液的体积，mL；

m——试样的重量，g。

注：计算结果需将空白值扣除。

4 测定低限、回收率

4.1 测定低限

本方法的测定低限为 0.02 mg/kg。

4.2 回收率

回收率的实验数据：

噻菌灵的添加浓度在 0.02 mg/kg 时，回收率为 102%；

噻菌灵的添加浓度在 0.10 mg/kg 时，回收率为 96.5%；

噻菌灵的添加浓度在 0.50 mg/kg 时，回收率为 99.6%。

附 录 A
（提示的附录）
标准品荧光吸光度扫描图

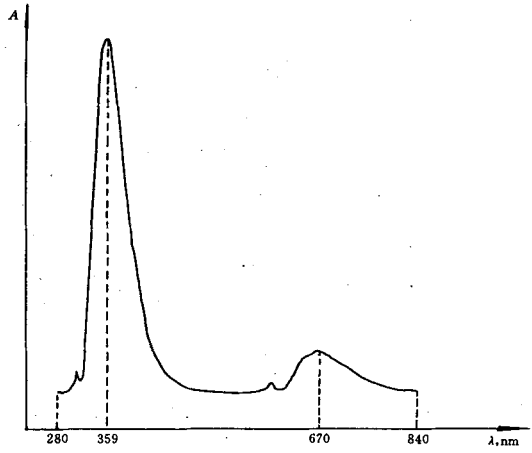

图 A1 噻菌灵标准品荧光吸光度扫描图

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 in this standard is defined on the bases of the current international maximum limits for thiabendazole residues in milk 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 Guangdong Import and Export Commodity Inspection Bureau of the People's Republic of China.

The main drafters of this standard are Zhang Siqun, Li Hui, Liang Weida.

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

SN 0606—1996

Method for the determination of thiabendazole residues in milk and milk products for export —Fluorescence spectrophotometry

1 Scope

This standard specifies the methods of sampling, sample preparation and determination of thiabendazole residues by fluorescence spectrophotometry in milk and milk products.

This standard is applicable to the determination of thiabendazole residues in fresh milk for export.

2 Sampling and sample preparation

2.1 Inspection lot

The quantity of an inspection lot should not be more than 50 000 bottles.

The characteristics of the cargo within the same inspection lot, such as packing, mark, origin, specification, grade etc., should be the same.

2.2 Quantity of sample taken

Number of bottles in an inspection lot	Minimum number of bottles to be taken
Less than 10 000	2
10 000—20 000	3
20 001—30 000	4
30 001—40 000	5
40 001—50 000	6

2.3 Sampling procedure

A number of bottles specified in 2.2 are taken at random. The sample should be marked and sent to laboratory in time.

2.4 Preparation of test sample

Mix the samples drawn in bottles evenly, and take ca 250 mL as the test sample, which is placed in a clean container, sealed and labeled.

2.5 Storage of test sample

The test sample should be stored below −5℃.

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

Approved by the State Administration of Import and Export Commodity Inspection of the People's Republic of China on Nov. 15, 1996

Implemented from May. 1, 1997

3 Method of determination

3.1 Principle

The fat in the test sample is saponified with potassium hydroxide, the thiabendazole is extracted with ethyl acetate. The thiabendazole in the ethyl acetate extract is re-extracted with hydrochloric acid solution and determined by fluorescence spectrophotometry, using standard curve method.

3.2 Reagents and materials

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

3.2.1 Potassium hydroxide solution: 50%(m/V)aqueous solution.

3.2.2 Potassium hydroxide solution: 0.05%(m/V)aqueous solution.

3.2.3 Hydrochloric acid solution: 0.1 mol/L aqueous solution.

3.2.4 Ethyl acetate.

3.2.5 Thiabendazole standard: Purity≥99%.

3.2.6 Thiabendazole standard solution: Accurately weigh an adequate amount of thiabendazole standard, dissolve in hydrochloric acid solution and prepare a standard stock solution of 0.100 mg/mL in concentration. According to the requirement, dilute the stock solution with hydrochloric acid solution to appropriate concentration as the standard working solution.

3.3 Apparatus and equipment.

3.3.1 Fluorescence spectrophotometer.

3.3.2 Condenser.

3.3.3 Separatory funnel: 125 mL.

3.3.4 Conical flask: 100 mL, with ground stopper.

3.3.5 Electric water-bath.

3.3.6 Volumetric flask: 10 mL.

3.4 Procedure

3.4.1 Saponification

Weigh ca 10 g of the test sample (accurate to 0.1 g) into a conical flask. Add 7 mL of potassium hydroxide solution (3.2.1), connect with condenser. Saponify by refluxing on a boiling water-bath for 40 min. Remove and cool down completely.

3.4.2 Extraction

Transfer the saponified solution to a 125 mL separatory funnel. Wash the conical flask with 10 mL of distilled water and combine the washings into the same separatory funnel. Add 15 mL of ethyl acetate and shake gently for 0.5 min. Let stand to separate and transfer the aqueous phase to another separatory funnel. Repeat the extraction with 15 mL of ethyl acetate by shaking vigorously for 1 min. Let stand to separate and combine the ethyl acetate extracts.

3.4.3 Cleanup

Wash the ethyl acetate layer with 20 mL of potassium hydroxide solution (3.2.2). Shake vigorously for 1 min. Let stand to separate and discard the aqueous layer. Add again 20 mL of potassium hydroxide solution (3.2.2), shake gently and discard the aqueous layer. Extract the ethyl acetate layer with 2×5 mL of 0.1 mol/L hydrochloric acid solution, combine the extracts of hydrochloric acid solution in a 10 mL volumetric flask and dilute to mark with hydrochloric acid solution. The solution is ready for fluorescence spectrophotometric determination.

3.4.4 Determination

3.4.4.1 Fluorescence spectrophotometric operating condition

Excitation wavelength: 307 nm; emission wavelength: 359 nm.

For different types of instrument, adjustment should be made according to different conditions of the instruments to obtain the optimum excitation and emission wavelengths.

3.4.4.2 Preparation of standard curve

Add 0.2, 0.5, 1.0, 5.0 and 10.0 mL standard working solution to a series of 10 mL volumetric flask, and dilute to mark with 0.1 mol/L hydrochloric acid solution. Measure the absorbance of each by the fluorescence spectrophotometer and plot the absorbances against concentrations of thiabendazole to prepare the standard curve. For the scanning curve of fluorescence absorbance of the standard, see figure A1 in Annex A.

3.4.4.3 Determination of sample solution

Measure the absorbance of the sample solution obtained from 3.4.3 by fluorescence spectrophotometer. Obtain the concentration of thiabendazole in the sample solution from the standard curve.

3.4.5 Blank test

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

3.5 Calculation and expression of result

Calculate the content of thiabendazole residues in the test sample according to formula(1):

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

where

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

c—the concentration of thiabendazole in the sample solution, obtained from standard curve, μg/mL;

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

m—the mass of the test sample, 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 0.02 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentrations of thiabendazole in fresh milk and its corresponding recoveries are:

0.02 mg/kg, the recovery 102%;

0.10 mg/kg, the recovery 96.5%;

0.50 mg/kg, the recovery 99.6%.

Annex A

(informative)

Scanning curve of fluorescence absorbance of the standard

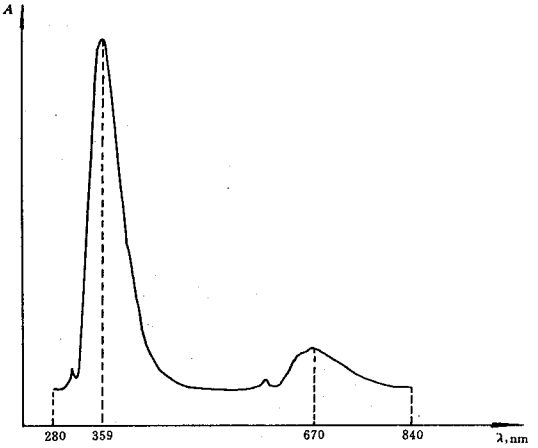

Fig. A1 Scanning curve of fluorescenec absorbance of the thiabendazole standard