SN

中华人民共和国进出口商品检验行业标准

SN 0530－1996

出口肉品中呋喃唑酮残留量的检验方法 液相色谱法

Method for the determination of furazolidone residues in meat for export —Liquid chromatographic method

1996-04-29发布　　1996-10-01实施

中华人民共和国国家进出口商品检验局　发布

前　言

本标准是根据我国 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》中对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。

测定低限是根据国际通常规定的肉品中呋喃唑酮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 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。

本标准起草单位：中华人民共和国浙江进出口商品检验局。

本标准主要起草人：鲍晓霞、陈笑梅。

本标准系首次发布的行业标准。

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1 范围

本标准规定了出口肉品中呋喃唑酮残留量检验的抽样、制样和液相色谱测定方法。

本标准适用于出口冻猪分割肉中呋喃唑酮残留量的测定。

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。从每件中取一袋作为原始样品，其总重量不少于 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(用酒精灭菌过)在抽出的包件中，每件割取不少于 500 g 试样，混合后置清洁容器内，作为混合原始样。混合原始样的总量不少于 2 kg。加封后，标明标记，及时送交实验室。

2.4 试样制备

从所取原始样品中取出部分有代表性样品，经高速组织捣碎机捣碎均匀，充分混匀。用四分法缩分出不少于 500 g 试样，混匀，均分成两份。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：在抽样及制样操作过程中必须防止样品受到污染或发生残留含量的变化。

中华人民共和国国家进出口商品检验局 1996-04-29 批准　　1996-10-01 实施

3 测定方法

3.1 方法提要

试样中的呋喃唑酮残留用二氯甲烷提取，浓缩提取液，用正己烷液-液萃取除去脂肪及其他杂质，离心，取下层清液供高效液相色谱仪测定，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 乙腈。

3.2.2 二氯甲烷。

3.2.3 正己烷。

3.2.4 甲醇。

3.2.5 无水硫酸钠：经 650℃灼烧 4 h 后，贮于密闭容器中备用。

3.2.6 乙腈水溶液：乙腈-水(80+20，V/V)。

3.2.7 无水硫酸钠柱：6 cm×1.8 cm(id)，内装 5 cm 高的无水硫酸钠。

3.2.8 呋喃唑酮标准品：纯度≥99%。

3.2.9 呋喃唑酮标准储备液：称取 10.0 mg 呋喃唑酮，用乙腈水溶液溶解并稀释定容至 50 mL 棕色容量瓶中，保存于冰箱中。该液 1 mL 相当于 200 μg 呋喃唑酮。

3.2.10 呋喃唑酮标准工作液：准确吸取呋喃唑酮标准储备液 0.5 mL，用水稀释定容至 100 mL 棕色容量瓶中。该液 1 mL 相当于 1.0 μg 呋喃唑酮。保存于冰箱中。

3.3 仪器和设备

3.3.1 高效液相色谱仪：配有紫外检测器。

3.3.2 旋涡混匀器或超声波提取仪。

3.3.3 离心机。

3.3.4 K-D 真空浓缩器。

3.3.5 蒸发烧瓶：100 mL。

3.3.6 锥形瓶：100 mL，具塞。

3.3.7 离心管：5 mL，具塞带刻度。

3.3.8 微量进样器，25 μL。

3.4 测定步骤

3.4.1 提取

称取试样约 10 g(精确至 0.01 g)，于 100 mL 锥形瓶中，加 25 mL 二氯甲烷，在旋涡混匀器或超声波提取器上提取 5 min，提取液通过无水硫酸钠柱滤入 100 mL 蒸发烧瓶中，继用 25 mL 二氯甲烷重复提取二次，均通过无水硫酸钠柱滤入同一蒸发烧瓶中，用二氯甲烷淋洗无水硫酸钠柱，滤液经 K-D 浓缩器(50℃水浴)浓缩至干。

3.4.2 净化

准确加入 1.0 mL 乙腈水溶液溶解残渣，同时加入 1 mL 正己烷混匀 2 min。溶液移入 5 mL 离心管中，3 000 r/min 离心 2 min 后，用尖嘴吸管移去上层正己烷层，再向离心管中加入 1 mL 正己烷，混匀 2 min，离心，用吸管移去上层正己烷层，将下层清液用乙腈水溶液定容至 1.0 mL，供高效液相色谱测定。

3.4.3 测定

3.4.3.1 液相色谱条件

a) 色谱柱：ODS-C_{18}柱，250 mm×4.0 mm(id)，粒度 10 μm；

b) 流动相：乙腈-水(40+60)，500 mL 溶液中加 0.5 mL 磷酸(85%)；

c) 流速：1.0 mL/min；

d) 检测波长:365 nm;

e) 柱温:室温;

f) 进样量:20 μL。

3.4.3.2 液相色谱测定

根据样液中呋喃唑酮残留量情况,选定峰高相近的标准工作溶液。标准工作溶液和样液中呋喃唑酮响应值均应在仪器检测线性范围内。对标准工作溶液和样液等体积参插进样进行测定。在上述色谱条件下,呋喃唑酮保留时间约为 6.0 min。

3.4.4 空白试验

除不加试样外,均按上述测定步骤进行。

3.4.5 结果计算和表述

用色谱数据处理机或按式(1)计算试样中呋喃唑酮残留含量:

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

式中:X——试样中呋喃唑酮残留量,mg/kg;

h——样液中呋喃唑酮的峰高,mm;

h_s——标准工作溶液中呋喃唑酮的峰高,mm;

c——标准工作溶液中呋喃唑酮的浓度,μg/mL;

V——样液最终定容体积,mL;

m——称取的试样量,g。

注:计算结果需要扣除空白值。

4 测定低限、回收率

4.1 测定低限

本方法的测定低限为 0.01 mg/kg。

4.2 回收率

回收率的实验数据:

呋喃唑酮的添加浓度在 0.01 mg/kg 时,回收率为 90.5%;

呋喃唑酮的添加浓度在 0.03 mg/kg 时,回收率为 92.2%;

呋喃唑酮的添加浓度在 0.10 mg/kg 时,回收率为 87.7%;

呋喃唑酮的添加浓度在 0.30 mg/kg 时,回收率为 96.1%;

呋喃唑酮的添加浓度在 3.00 mg/kg 时,回收率为 98.3%。

附 录 A
（提示的附录）
标准品色谱图

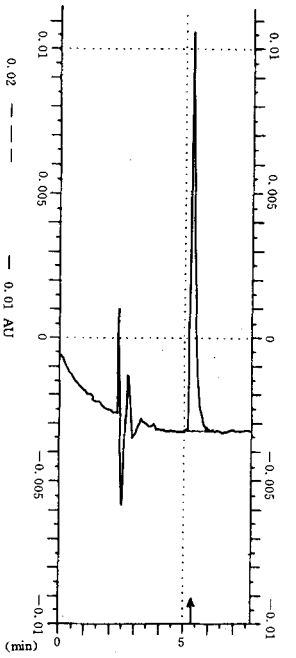


图 A1 呋喃唑酮标准品色谱图

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" on general rules for drafting and presentation 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 in the standard was drafted by referring to relevant domestic and foreign literatures through research, verification and modification. In addition, methods of sampling and sample preparation are also specified in the standard.

The limit of determination is defined in the standard on the basis of the current international maximum limit for furazolidone residues in meat and sensitivity of this method.

Annex A is the annex for information purpose.

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 Zhejiang Import and Export Commodity Inspection Bureau of the People's Republic of China.

The main drafters of this standard are Bao Xiaoxia, Chen Xiaomei.

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

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

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

Method for the determination of furazolidone residues in meat for export —Liquid chromatographic method

SN 0530—1996

1 Scope

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

This standard is applicable to the determination of furazolidone residues in frozen pork for export.

2 Sampling and sample preparation

2.1 Inspection lot

Each inspection lot should not exceed 2 500 packages.

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 the samples taken

Number of packages in each inspection lot	Minimum 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

According to 2.2, a number of packages are taken at random and opened one by one. From each package, one bag shall be taken as a primary sample. The total weight of all the primary samples should not be less than 2 kg, which shall be placed in a clean container, sealed, labeled and sent to laboratory in time.

In case there are no small bags inside each package, or if there are small bags inside the package, but the content of a bag exceeds 2 kg, cut out a part from the meat in each package of no less than 500 g with a sharp knife(disinfected with alcohol). 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 laboratory in

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Implemented from Oct. 1, 1996

time.

2.4 Preparation of test samples

Part of the representative sample is taken from the primary sample, and homogenized by grinding in a blender. The homogenized sample is thoroughly mixed and reduced to at least 500 g by quartering . Mix and divide into two equal portions. Each portion is placed in a clean container, which is then sealed and labeled.

2.5 Storage of test sample

The test sample should be stored below −18℃.

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.

3 Method of determination

3.1 Principle

The furazolidone residues in the test sample are extracted with dichloromethane. Concentrate, clean up by liquid-liquid partition with n-Haxane and centrifuge. The furazolidone is determined by high performance liquid chromatography with UV detector, using external standard method.

3.2 Reagents and materials

Unless otherwise specified, all reagents are analytical pure; "water" is distilled water.

3.2.1 Acetonitrile.

3.2.2 Dichloromethane.

3.2.3 n-Hexane.

3.2.4 Methanol.

3.2.5 Anhydrous sodium sulfate: Ignite for 4 h at 650℃, and keep in a tightly closed container.

3.2.6 Acetonitrile aqueous solution: acetonitrile-water (80+20, v/v).

3.2.7 Anhydrous sodium sulfate column: 6 cm×1.8 cm(id), packed with 5 cm height of anhydrous sodium sulfate.

3.2.8 Furazolidone standard: Purity≥99%.

3.2.9 Furazolidone standard stock solution: Accurately weigh 10.0 mg of furazolidone, dissolve in acetonitrile aqueous solution. Transfer the solution into a 50 mL brown volumetric flask and make up to volume with acetonitrile solution. Store the solution in a refrigerator, 1 mL of this solution corresponds to 200 μg of frazolidone.

3.2.10 Furazolidone standard working solution: Accurately pipette 0.5 mL of furazolidone standard stock solution into a 100 mL brown volumetric flask and make up to volume with water. Store the solution in a refrigerator, 1 mL of the solution corresponds to 1.0 μg of frazolidone.

3.3 Apparatus and equipment

3.3.1 High performance liquid chromatograph: Equipped with UV detector.

3.3.2 Whirl blender or supersonic extractor.

3.3.3 Centrifuger.

3.3.4 K-D vacuum rotary concentrator.

3.3.5 Evaporating flask: 100 mL.

3.3.6 Conical flask: 100 mL, with ground stopper.

3.3.7 Centrifugal tube: 5 mL, graduated, with ground stopper.

3.3.8 Micro-syringe: 25 μL.

3.4 Procedure

3.4.1 Extraction

Weigh ca 10 g of sample (accurate to 0.01 g) into a 100 mL conical flask. Add 25 mL of dichloromethane (3.2.2), shake for 5 min in a whirl blender or supersonic extractor. Filtrate the extracted solution through an anhydrous sodium sulfate column to a 100 mL evaporating flask. Repeat the extraction twice with additional 25 mL dichloromethane portions, also let pass the column, and finally rinse the column with a small amount of dichloromethane. Combine the filtrates in the same evaporating flask, where the filtrated solution is evaporated to dryness at the 50℃ in a water-bath by a K-D vacuum rotary concentrator.

3.4.2 Cleanup

Dissolve the residue with exactly 1.0 mL of acetonitrile aqueous solution (3.2.6), add 1.0 mL of n-Hexane and shake for 2 min. Transfer the mixture into a 5 mL centrifugal tube and centrifuge for 2 min at a speed of 3 000 r/min. Discard the upper n-Hexane phase with a pipette. Add 1.0 mL of n—Hexane into the centrifugal tube again, shake for 2 min and centrifuge. Discard the upper n-Hexane phase. The lower clear phase is diluted to exactly 1.0 mL with acetonitrile aqueous solution (3.2.6) for liquid chromatographic determination.

3.4.3 Determination

3.4.3.1 HPLC operating condition

a) Column: ODS-C_{18}, 250 mm×4.0 mm(id), particle size 10μm;

b) Mobile phase: Add 0.5 mL of 85% phosphoric acid into 500 mL of acetonitrile-water (40+60) solution;

c) Flow rate: 1.0 mL/min;

d) Detection wavelength: 365 nm;

e) Column temperature: Room temperture;

f) Injection volume: 20 μL.

3.4.3.2 HPLC determination

According to the estimated approximate concentration of furazolidone in the sample solution, select the standard working solution of similar peak height to that of the sample solution. The response values of furazolidone in the srandard solution and the sample solution should be within the linear detecting range of the instrument. The standard working solution and the sample solution of the same volume should be injected alternately into the instrument each time.

Under the above chromatographic condition, the retenton time of furazolidone is about 6.0 min.

3.4.4 Blank test

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

3.5 Calculation and expression of result

The content of furazolidone in the test sample is calculated by HPLC data processor or according to formula(1).

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

where

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

h—the peak hight of furazolidone in the sample solution, mm;

h_s—the peak hight of furazolidone in standard working solution, mm;

c—the concentration of furazolidone in the standard working solution, μg/mL;

V—the final volume of test 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 0.01 mg/kg.

4.2 Recovery

According to the experimental data: respective the fortifying concentrations of forazolidone and their respective recoveries are:

0.01 mg/kg, the recovery is 90.5%;

0.03 mg/kg, the recovery is 92.2%;

0.10 mg/kg, the recovery is 87.7%;

0.30 mg/kg, the recovery is 96.1%;

3.0 mg/kg, the recovery is 98.3%.

Annex A

(informative)

Chromatogram of the standard

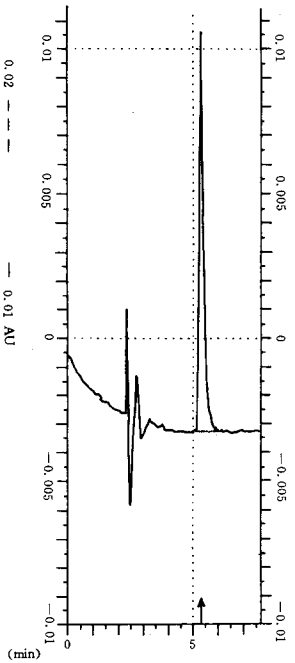


Fig. A1 Chromatogram of the forazolidone standard