SN

中华人民共和国进出口商品检验行业标准

SN 0498—95

出口肉类中磺胺间二甲氧嘧啶残留量检验方法

Method for the determination of sulfadimethoxine residues in meat for export

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中华人民共和国国家进出口商品检验局　发布

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1 主题内容与适用范围

本标准规定了出口肉类中磺胺间二甲氧嘧啶残留量的抽样、制样和气相色谱测定方法。

本标准适用于出口冻鸡、分割猪肉、分割牛肉、填鸭中磺胺间二甲氧嘧啶残留量的检验。

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 500 g(或一袋)作为原始样品，原始样品总重量不少于 2 kg。放入清洁容器内，加封后，标明标记，及时送交实验室。

2.4 试样制备

从每袋原始样品中取出部分有代表性样品，将可食部分放入高速组织捣碎机中捣碎均匀。充分混匀，用四分法缩分出不少于 500 g 试样。装入清洁的容器内，加封后，标明标记。

2.5 样品保存

将试样于－18℃以下冷冻保存。

注：在抽样及制样过程中，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

肉类中残留的磺胺间二甲氧嘧啶采用乙腈提取，提取液加盐酸溶液后蒸去乙腈，再用正己烷分配净化。用氢氧化钠溶液调节 pH 至 5.5～6.0，加三氯甲烷反提取，以重氮甲烷甲酯化，用配有电子俘获检测

中华人民共和国国家进出口商品检验局 1995-11-28 批准　　1996-01-01 实施

器的气相色谱仪测定，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，水为蒸馏水。

3.2.1 乙腈：用全玻璃器皿重蒸馏，收集 81～82℃馏分。

3.2.2 正己烷：用全玻璃器皿重蒸馏，收集 67～69℃馏分。

3.2.3 三氯甲烷：用全玻璃器皿重蒸馏，收集 61～62℃馏分。

3.2.4 乙酸乙酯：用全玻璃器皿重蒸馏，收集 76～78℃馏分。

3.2.5 丙酮。

3.2.6 乙醚：用全玻璃器皿重蒸馏，收集 33～34℃馏分。

3.2.7 柠檬酸三钠溶液：取柠檬酸三钠，用水制备成饱和溶液。

3.2.8 无水硫酸钠：650℃灼烧 4 h。冷却后贮于密封容器中备用。

3.2.9 重氮甲烷-乙醚溶液：在 125 mL 的蒸馏瓶中，加入 35 mL 乙醇、10 mL 乙醚和 10 mL 氢氧化钾水溶液(6 g/10 mL)。放入磁棒，并固定在磁力搅拌器加热板上的水浴中。连接漏斗和冷凝器并串联两个 125 mL 锥形瓶，锥形瓶中各置有 10 mL 乙醚。冷凝收集管插入乙醚液面下，锥形接收瓶置于冰浴中。滴液漏斗内盛有 21.5 g 的 N-甲基-N-亚硝基-p-甲苯磺酰胺溶于 140 mL 乙醚的溶液(重氮试剂)。加热水浴至 70℃，进行蒸馏，蒸馏瓶内溶液边用磁力搅拌，边滴加重氮试剂，滴完全部溶液的时间控制在 20 min以上。当馏出液近于无色时，停止蒸馏。将两个锥形接收瓶中的溶液合并，并在 70℃水浴上重蒸馏。将馏出液(重氮甲烷-乙醚溶液)密封，并于－18℃保存。保存期一个月。

当心：重氮甲烷有毒，有特异性过敏，而且易引起爆炸。配制重氮甲烷-乙醚溶液、甲酯化或蒸发均应在通风橱中进行。重氮甲烷-乙醚溶液应贮于冷冻器内，不要暴露于直射阳光或强烈的人工光。

3.2.10 磺胺间二甲氧嘧啶标准品：纯度≥99%。

3.2.11 磺胺间二甲氧嘧啶标准溶液(0.100 mg/mL)：准确称取 10.0 mg 的磺胺间二甲氧嘧啶标准品，用少量丙酮溶解并准确定容至 100 mL，摇匀，为标准储备液。根据需要再配制成适用浓度的标准工作溶液。

3.3 仪器和设备

3.3.1 气相色谱仪：配备电子俘获检测器和毛细管柱上进样器。

3.3.2 高速组织捣碎机。

3.3.3 振荡器。

3.3.4 旋转蒸发器。

3.3.5 恒温水浴锅。

3.3.6 全玻璃系统蒸馏装置。

3.3.7 pH 计。

3.4 测定步骤

3.4.1 提取

取经绞碎混匀的试样约 20 g(精确至 0.1 g)于 250 mL 具塞锥形瓶中，加入 50 mL 乙腈，于振荡器上提取 30 min。用中速定量滤纸将乙腈提取液过滤于蒸发瓶中，并用 30 mL 乙腈分三次洗涤残渣。加入 10 mL 盐酸溶液(1 mol/L)，然后在 35℃水浴中旋转蒸发浓缩以除去有机溶剂。

3.4.2 净化

将酸相移入至 125 mL 分液漏斗中，再用 10 mL 盐酸溶液(1 mol/L)分两次洗涤蒸发瓶，并将洗液并入 125 mL 分液漏斗中。加入 20 mL 正己烷，振摇 1 min，静置 10 min，弃去有机层。再用 2×20 mL 正己烷重复净化两次。然后在酸相中加入 20 mL 饱和柠檬酸三钠溶液，用氢氧化钠溶液(3 mol/L)调节 pH 至 5.5～6.0。用 3×20 mL 三氯甲烷振摇提取三次。合并三氯甲烷提取液，经无水硫酸钠脱水后，收集于 100 mL 蒸发瓶中，然后在 35℃水浴上旋转蒸发至干。

3.4.3 甲酯化

加入 1 mL 丙酮以溶解残渣，再加入 2 mL 重氮甲烷-乙醚溶液，加塞。室温下避光放置 30 min，其间进行断续轻摇。反应结束后，通以氮气流吹干。准确加入 2.00 mL 乙酸乙酯-正己烷混合液(3+7)以溶解残渣，供气相色谱测定。

3.4.4 标准溶液甲酯化

取适用浓度的标准工作溶液于具塞试管中，用氮气吹干，按上述操作进行甲酯化后，供气相色谱测定。

3.4.5 测定

3.4.5.1 色谱条件

a. 色谱柱：30 m×0.53 mm(id)×1.0 μm(膜厚)，HP50+石英毛细管柱，或相当者；

b. 色谱柱温度：270℃；

c. 进样口温度：280℃；

d. 检测器温度：300℃；

e. 载气、尾吹气：氮气，纯度≥99.99%，75 cm/s；尾吹气，50 mL/min；

f. 进样方式：柱上进样；

g. 进样量：2 μL。

3.4.5.2 色谱测定

分别准确注入 2 μL 的样品溶液及适用浓度的磺胺间二甲氧嘧啶甲酯标准工作溶液于气相色谱仪中，按上述条件进行色谱分析。响应值均应在仪器检测的线性范围之内。在上述色谱条件下，磺胺间二甲氧嘧啶甲酯的保留时间约为 7.5 min。

3.4.6 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计算试样中磺胺间二甲氧嘧啶残留含量：

$$X = \frac{h \cdot c \cdot V}{h_s \cdot m}$$

式中：X——试样中磺胺间二甲氧嘧啶含量，mg/kg；

h——样液中磺胺间二甲氧嘧啶甲酯的色谱峰高，mm；

h_s——标准工作液中磺胺间二甲氧嘧啶甲酯的色谱峰高，mm；

c——标准工作液中磺胺间二甲氧嘧啶浓度，μg/mL；

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 0.01 mg/kg。

4.2 回收率

回收率的实验数据：磺胺间二甲氧嘧啶添加浓度在 0.01～0.10 mg/kg 范围内，回收率为 74.0%～107.0%。

附加说明：

本标准由中华人民共和国国家进出口商品检验局提出。

本标准由中华人民共和国吉林进出口商品检验局负责起草。

本标准主要起草人牟峻、荣会。

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

SN 0498—95

Method for the determination of sulfadimethoxine residues in meat for export

1 Scope and field of application

This standard specifies the methods of sampling, sample preparation and determination by gas chromatography of sulfadimethoxine residues in meat for export.

This standard is applicable to the determination of sulfadimethoxine residues in frozen chicken, frozen pork, frozen beef, frozen duck 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, grade etc., 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 at least 500 g (or one bag) shall be taken as a primary sample. The total weight of all 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.

2.4 Preparation of test sample

Part of representative sample is taken from each bag of the primary sample, and the edible portions are homogenized by grinding in a high speed meat grinder. The homogenized sample is thoroughly mixed, reduced to at least 500 g by quartering and placed in a clean container, which is then sealed

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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 sulfadimethoxine residues in the meat are extracted with acetonitrile. After adding hydrochloric acid, expel the acetonitrile and the solution is partitioned with n-hexane. The pH of solution is adjusted to 5.5—6.0 by adding alkali, then the analyte is reextracted with chloroform, methylated by using diazomethane and determined by gas chromatography with electron capture detector, using external standard method.

3.2 Reagents and materials

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

3.2.1 Acetonitrile: Redistill with all glass apparatus, collect the distillate of 81—82℃.

3.2.2 n-Hexane: Redistill with all glass apparatus, collect the distillate of 67—69℃.

3.2.3 Chloroform: Redistill with all glass apparatus, collect the distillate of 61—62℃.

3.2.4 Ethyl acetate: Redistill with all glass apparatus, collect the distillate of 76—78℃.

3.2.5 Acetone.

3.2.6 Ether: Redistill with all glass apparatus, collect the distillate of 33—34℃.

3.2.7 Trisodium citrate solution: Prepare a trisodium citrate saturated solution with water.

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

3.2.9 Diazomethane-ether solution: Add 35 mL of ethanol, 10 mL of ether and 10 mL of KOH solution (6 g/10 mL) into a 125 mL distilling flask. Place a magnetic bar in the flask, and immerse the flask in a water-bath with a magnetic stirring device. Attach the dropping funnel and the condenser with the flask, and connect the condenser with two 125 mL conical flasks in series, each containing 10 mL of ether and let the tips of outlet tubes dip below the surface of the ether. Cool both receiving flasks in ice-bath. Place in the dropping funnel a solution of 21.5 g of N-methyl-N-nitroso-p-toluenesulfonamide dissolved in 140 mL of ether (diazo reagent). Heat the water-bath to 70℃, stir the contents of flask while adding dropwise the diazo reagent for a period of more than 20 min. Cease the distillation when the distillate becomes nearly colorless. Combine the contents of two conical flasks, and redistill it by placing in the water-bath at 70℃. Store the distillate (diazomethane-ether solution) in a sealed container under −18℃ (not exceeding one month).

Caution: Diazomethane is toxic, can cause specific allergy, and is potentially explosive. The preparation of diazomethane-ether solution, methylation, and evaporation should be carried out in a ventilated hood. Store diazomethane-ether solution in a freezer, do not expose to direct sunlight or strong artificial light.

3.2.10 Sulfadimethoxine standard: Purity≥99%.

3.2.11 Sulfadimethoxine standard solution (0.100 mg/mL): Accurately weigh 10.0 mg of sulfadimethoxine standard, dissolve in a small volume of acetone, and dilute to mark with acetone in a 100 mL volumetric flask, mix thoroughly to form a standard stock solution. Prepare a standard working solution of appropriate concentration according to the requirements.

3.3 Apparatus and equipment

3.3.1 Gas chromatograph, equipped with electron capture detector and on-column capillary injector.

3.3.2 High speed tissue grinder.

3.3.3 Shaker.

3.3.4 Rotary evaporator.

3.3.5 Constant temperature water-bath.

3.3.6 All-glass distilling system.

3.3.7 pH meter.

3.4 Procedure

3.4.1 Extraction

Weigh ca 20 g(accurate to 0.1 g)of evenly ground and mixed sample into a 250 mL conical flask with stopper, add 50 mL of acetonitrile and extract for 30 min on a shaker. The acetonitrile extract is filtrated into an evaporating flask, using mid-speed quantitative filter paper. The residue is washed for 3 times with 30 mL (in total) of acetonitrile, add 10 mL of the HCl(1 mol/L), and then evaporate off the organic solvent in water-bath at 35℃ with rotary evaporator.

3.4.2 Cleanup

Transfer the HCl phase to a 125 mL separatory funnel. Rinse the evaporating flask twice with additional 10 mL of HCl(1 mol/L) and transfer the rinsings to the separatory funnel. Add 20 mL of n-hexane. After shaking for 1 min and setting aside for 10 min, the organic phase is slowly drawn off and discarded. The above procedure is repeated twice with 2×20 mL of n-hexane. Add 20 mL of satuarated aqueous solution of trisodium citrate to HCl phase, and adjust pH to 5.5—6.0 by adding NaOH (3 mol/L). The sample solution is then extracted with 3×20 mL of chloroform. The extracts are combined, dehydrated by anhydrous sodium sulfate, collected in a 100 mL distillation flask, and then evaporated to dryness in a water-bath at 35℃ with the rotary evaporator.

3.4.3 Methylation

Add 1 mL of acetone to dissolve the residue, then add 2 mL of diazomethane-ether solution, close with a stopper. The solution is laid aside at room temperature for 30 min (out of light), with occasional gentle shaking. After the reaction is over, the solution is evaporated to dryness by blowing with N_2. Then exactly 2.00 mL of ethyl acetate-n-hexane (3+7) are added to dissolve the residue, the solution is used for GC determination.

3.4.4 Methylation of the standard solution

An appropriate amount of standard working solution is pipetted into a centrifuge tube, the solvent is expelled with nitrogen, and the solute is methylated according to above procedure. The solution is used for GC determination.

3.4.5 Determination

3.4.5.1 GC operating condition

a. Chromatographic column: 30 m×0.53 mm(id)×1.0 μm(film thickness), HP50+fused silica capillary column, or the equivalent;

b. Column temperature: 270℃;

c. Injection port temperature: 280℃;

d. Detector temperature: 300℃;

e. Carrier gas and make-up gas: Nitrogen, purity≥99.99%; carrier gas: 75 cm/s, make-up gas: 50 mL/min;

f. Injection mode: On-column;

g. Injection volume: 2 μL.

3.4.5.2 GC determination

Inject accurately 2 μL of the methylated sulfadimethoxine standard working solution and sample solution separately into gas chromatograph, and carry out the analysis under above operating condition. The responses must be within the linear range of the instrumental detection. Under the above chromatographic condition, the retention time of methylated sulfadimethoxine is about 7.5 min.

3.4.6 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 sulfadimethoxine residues in the test sample by GC data processor or according to the following formula.

$$X = \frac{h \cdot c \cdot V}{h_s \cdot m}$$

where

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

h—the peak height of methylated sulfadimethoxine in the sample solution, mm;

h_s—the peak height of methylated sulfadimethoxine in the standard working solution, mm;

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

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

m—the corresponding mass of the test sample in the final sample 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 0.01 mg/kg.

4.2 Recovery

According to the experimental data, when the fortified concentration of sulfadimethoxine is in the range of 0.01—0.10 mg/kg, the recovery is 74.0%—107.0%.

Additional explanations:

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

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

This standard was mainly drafted by Mu Jun, Rong Hui.

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