SN

中华人民共和国进出口商品检验行业标准

SN/T 0212.2--93

出口禽肉中二氯二甲吡啶酚残留量检验方法 甲基化-气相色谱法

Method for determination of clopidol residues in poultry meat for export —Methylation-gas chromatography

1993-06-04发布　　1993-08-01实施

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

本标准规定了出口禽肉中二氯二甲吡啶酚残留量检验的抽样、制样和气相色谱测定方法。

本标准适用于出口鸡肉中二氯二甲吡啶酚残留量的检验。

2 抽样和制样

2.1 检验批

以不超过2 500箱为一个检验批。

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(箱)	最低抽样数(箱)
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规定的抽样箱数随机抽取，逐箱开启。每箱至少取一袋作为原始样品；原始样品总量不少于2 kg，加封后，标明标记，及时送实验室。

2.4 试样制备

从每袋原始样品中取部分有代表性鸡肉样，用绞肉机绞碎，将全部绞碎的样品混匀，用四分法缩分出试样量不少于500 g，分装入清洁容器内，加封后，标明标记。

2.5 试样保存

试样于－18℃下冷冻保存。

注：在抽样和制样过程中，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

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用甲醇提取鸡肉组织内的二氯二甲吡啶酚，离心后的上清液经中性氧化铝和阴离子交换柱净化。洗脱液经重氮甲烷试剂甲基化，生成的二氯二甲吡啶甲醚(3,5-二氯-4-甲氧基-2,6-二甲基吡啶)用配有电子俘获检测器的气相色谱仪测定，以2,4-二硝基氯苯为内标进行定量。

3.2 试剂和材料

除特殊规定外，试剂均为分析纯，水为蒸馏水或相应的去离子水。

3.2.1 甲醇。

3.2.2 中性氧化铝：层析用，100～200目；300℃灼烧4 h，置于干燥器备用。

3.2.3 阴离子交换树脂：Dowex 1-X8，100～200目，氯型。或等效品。

3.2.4 乙酸甲醇溶液：0.6% (*V*/*V*)。3 mL冰乙酸溶于497 mL甲醇中。

3.2.5 正己烷：加碱重蒸馏。

3.2.6 内标：2,4-二硝基氯苯，纯度>99%。

3.2.7 提取用的硝基氯苯正己烷溶液：0.1 μg2,4-二硝基氯苯/mL正己烷溶液。

3.2.8 重氮甲烷试剂：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。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-重氮甲烷试剂密封，并于－18℃保存。保存期一个月。

3.2.9 二氯二甲吡啶酚标准品：纯度>99%。

3.2.9.1 二氯二甲吡啶酚标准贮备液：100 μg/mL。准确称取100.0 mg二氯二甲吡啶酚标准品，用甲醇移入1 000 mL容量瓶至总体积约600 mL，振荡溶解(约需2～3 h)。用甲醇定容到刻度，混匀。

3.2.9.2 中间标准溶液(Ⅰ)：10 μg/mL。用移液管移取上述标准贮备液10 mL于100 mL容量瓶内，用甲醇稀释至刻度。

3.2.9.3 中间标准溶液(Ⅱ)：1 μg/mL。用移液管移取中间标准溶液(Ⅰ)10 mL于100 mL容量瓶内，用甲醇稀释至刻度。

3.2.9.4 标准工作溶液：0.1 μg/mL。用移液管移取中间标准溶液(Ⅱ)10 mL于100 mL容量瓶内，用甲醇稀释至刻度。

3.3 仪器和设备

3.3.1 搅肉机：电动。

3.3.2 均质机：3 000 r/min。

3.3.3 离心机：日立20 PR-520 4 000 r/min或相当的设备。

3.3.4 中性氧化铝柱：称取2 g中性氧化铝(3.2.2)，填入100 mm×10 mm(内径)玻璃层析柱内。

3.3.5 阴离子交换层析柱：将Dowex 1-X8阴离子交换树脂伴以去离子水移入250 mm×10 mm(内径)层析柱内，沉降后柱床高度为1.5 cm。以100 mL 1 mol/L的NaOH水溶液淋洗树脂，继以去离子水淋洗至中性；再以100 mL 0.5 mol/L的乙酸钠水溶液淋洗，继以去离子水淋洗至中性。流速均为2 mL/min。注入甲醇水溶液(8∶2)50 mL备用。使用前放出甲醇水溶液，使液面略高于树脂层。

3.3.6 试管：带旋盖(耐压并气密性的)，100 mm×13 mm(内径)。

3.3.7 气相色谱仪并配有电子俘获检测器。

3.3.8 微量注射器：10 μL。

3.4 测定步骤

3.4.1 提取

称取约20 g试样(精确至0.1 g)于均质瓶内，加入100 mL甲醇，3 000 r/min均质4 min后，将均

质瓶移入离心机，4 000 r/min 离心 10 min。

3.4.2 净化

将阴离子交换柱置于中性氧化铝柱下，用移液管移 20 mL 提取的上清液于氧化铝柱中，使提取液经中性氧化铝和阴离子交换柱流入烧杯。用 10 mL 甲醇清洗中性氧化铝柱的柱壁。移去氧化铝柱和烧杯，将 25 mL 的比色管置于阴离子交换柱的下方，用两份 10 mL 的 0.6%乙酸甲醇溶液洗脱二氯二甲吡啶酚，洗提液流速：1 mL/min。用甲醇定容并混匀。

3.4.3 甲基化

用移液管移取定容的洗提液 5 mL 于试管(3.3.6)内，试管置于 70℃水浴内，通以氮气流吹干。取出试管，加入 0.2 mL 甲醇水溶液(8∶2)，微温使残渣溶解，冷却至室温。加 1 mL 重氮甲烷试剂，拧上旋盖，将试管置于 70℃水浴中保温反应 2 min。反应时，试管内的液面和水浴面持平。取出试管，冷却至室温。拧去旋盖，仅使管底部接触水面，直至乙醚蒸除。于除去乙醚后的试管内加 5 mL 去离子水和 1.00 mL提取用的硝基氯苯正己烷溶液(3.2.7)，加盖，在混和器上混和 1 min。取出试管，4 000 r/min 离心3 min。所制成的上层正己烷样液供气相色谱测定。

3.4.4 测定

3.4.4.1 色谱条件

a. 色谱柱：玻璃柱，2 m×2 mm(内径)，填充物为 10%(*m*/*m*)DC-200 硅油涂于 Chromosorb W HP 载体(80～100 目)；

b. 氮气：纯度≥99.99%，60 mL/min；

c. 柱温：170℃；

d. 进样口温度：200℃；

e. 检测器温度：300℃。

3.4.4.2 色谱测定

将样液(如有必要，可多加提取用的硝基氯苯正己烷溶液，作进一步稀释)注入气相色谱柱，进样量 2 μL。在上述色谱条件下二氯二甲吡啶甲醚出峰保留时间约为 2 min，2,4-二硝基氯苯出峰保留时间约为 4 min。

3.4.5 标准曲线的绘制

分别取二氯二甲吡啶酚标准工作溶液 0.0.1.0.2,0.4,0.8,1.6 mL 于各个试管(3.3.6)内，按上述步骤(3.4.3～3.4.4)进行，但略去“定容的洗提液 5 mL”。以所得各浓度二氯二甲吡啶甲醚色谱峰高和内标峰高之比.对应实际标准溶液的浓度(μg/mL)绘制标准曲线。

3.4.6 回收系数 R

称取三份 19.9～20.1 g(精确至 0.1 g)不含二氯二甲吡啶酚的试样，分别加入相当于 0.0,0.01 和 0.05 μg/g 的二氯二甲吡啶酚中间标准溶液(Ⅱ)(分别为 0.0,0.2 和 1 mL)。按 3.4.1～3.4.4 条操作，并计算出平均回收系数 R。

3.4.7 空白试验：除不称取试样外，均按上述测定步骤(3.4.1～3.4.4)进行。

3.4.8 结果计算和表述

按下式计算试样中二氯二甲吡啶酚的含量：

$$X = \frac{c \cdot V}{R \cdot m}$$

式中：X——鸡肉中二氯二甲吡啶酚含量，mg/kg；

R——平均回收率；

c——由标准曲线查得最终样液中二氯二甲吡啶酚的含量，μg/mL；

V——最终样液的体积，mL；

m——最终样液所代表的样品量，g；

二氯二甲吡啶酚含量亦可由色谱数据处理机直接求得。

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

本方法测定低限为 0.005 mg/kg。

4.2 回收率

回收率实验数据：

二氯二甲吡啶酚添加浓度在 0.005～0.025 mg/kg 范围内，回收率为 65.4%～86.2%。

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上海、河北、北京、山东进出口商品检验局负责起草。

本标准主要起草人朱治平、郭德华、朱坚、沈礼兵、葛修丽、王凤池、张军、刘心同。

参考文献：

AOAC-Official Methods of Analysis，13th ed. 41.013-41.018，1980.

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

Method for determination of clopidol residues in poultry meat for export —Methylation-gas chromatography

SN/T 0212.2—93

1 Scope and field of application

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

This standard is applicable to the determination of clopidol residue content in broilers for export.

2 Sampling and sample preparation

2.1 Inspection lot

The quantity of an inspection lot should not be more than 2 500 cartons.

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 sample taken

Number of cartons in each inspection lot	Minimum number of cartons 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 cartons specified in 2.2 are taken at random and opened one by one. From each carton at least 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 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 homogenized by grinding in a meat grinder. The homogenized sample is thoroughly mixed and reduced to at least 500 g by quartering. The test sample is placed in clean container which is labeled and sealed.

2.5 Storage of the sample

The test samples should be stored under −18℃.

Note: In the course of sampling and sample preparation, precaution must be taken to avoid contamination or any

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factors which may cause the change of the residue content.

3 Method of determination

3.1 Principle

The clopidol in the broiler tissue is extracted with methanol, the extract is centrifugalized, and the supernatant liquid is cleaned up through neutral alumina and anion exchange columns. The eluate is methylated with diazomethane, producing methyl ether of clopidol(3,5-dichloro -4 -methoxy -2,6-lutidine),which is detected by GC with electron capture detector. Dinitrochlorobenzene is used as an internal standard for quantitative measurement.

3.2 Reagents and materials.

Unless otherwise specified, the reagents should be of analytical grade, "water" is distilled water or de-ionized water.

3.2.1 Methanol.

3.2.2 Neutral alumina: For chromatography, 100—200 mesh, heated at 300 C for 4 h and cooled in a desiccator, ready for use.

3.2.3 Anion exchange resin: Dowex 1-X8,100—200 mesh, chlorinate form, or equivalent.

3.2.4 Methanolic acetic acid solution:0.6% (*V*/*V*), add 3 mL of glacial acetic acid to 497 mL of methanol.

3.2.5 n-Hexane: Redistilled with alkali.

3.2.6 Internal standard: 2,4-dinitrochlorobenzene, purity>99%.

3.2.7 n-Hexane extractive solution: 0.1 μg dinitrochlorobenzene/mL n-hexane.

3.2.8 Diazomethane reagent: 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 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 reagent) in a sealed container under −18℃ (not exceeding one month).

3.2.9 Clopidol standard: Purity>99%.

3.2.9.1 Clopidol standard stock solution:100 μg/mL. Accurately weigh 100.0 mg of clopidol standard, transfer it into a 1 L volumetric flask with methanol to a total volume of ca 600 mL, shake to dissolve clopidol (about 2—3 h). Dilute to volume with methanol and mix well.

3.2.9.2 Intermediate standard solution(Ⅰ):10 μg/mL. Pipet 10 mL of the stock standard solution into a 100 mL volumetric flask and dilute to volume with methanol.

3.2.9.3 Intermediate standard solution(Ⅱ): 1 μg/mL. Pipet 10 mL of the intermediate standard solution (Ⅰ) into a 100 mL volumetric flask and dilute to volume with methanol.

3.2.9.4 Standard working solution: 0.1 g/mL. Pipet 10 mL of the intermediate standard solution (Ⅱ) into a 100 mL volumetric flask and dilute to volume with methanol.

3.3 Apparatus and equipment

3.3.1 Meat grinder: Electric.

3.3.2 Homogenizer: 3 000 r/min.

3.3.3 Centrifuge: Model 20 PR-520 Hitachi 4 000 r/min, or equivalent.

3.3.4 Neutral alumina column: Add 2 g alumina(3.2.2) into a 100 mm×10 mm(id) glass column.

3.3.5 Anion exchange resin column: Fill a 250 mm×10 mm (id) column with Dowex1-X8 resin, using de-ionized water to transfer the resin to a height of 1.5 cm(after setting down). Elute the column with 100 mL of 1 mol/L NaOH solution, rinse with de-ionized water until neutralized; then elute the column with 100 mL of 0.5 mol/L CH_3COONa solution, rinse with de-ionized water until neutralized. Keep the flow rate always at 2 mL/min. Add 50 mL of methanol aqueous solution(8 + 2) into the column, ready for use. Prior to use, drain off the methanol aqueous solution(8 + 2), keep the liquid surface sligtly above the resin surface.

3.3.6 Test tube: With screw cap, pressure-resistant and air-tight, 100 mm×13 mm(id).

3.3.7 Gas chromatograph: With electron capture detector.

3.3.8 Micro-syringe: 10 μL.

3.4 Procedure

3.4.1 Extraction

Weigh ca 20 g of the test sample (accurate to 0.1 g) into a homogenizing bottle. Add 100 mL of methanol, homogenize for 4 min at 3 000 r/min. Remove the bottle to the centrifuge and centrifugalize for 10 min at 4 000 r/min.

3.4.2 Clean up

Place the anion exchange column under the alumina column, pipet 20 mL of supernatant extract solution into the alumina column, and let the solution pass through both columns into a beaker. Wash the column and its wall with 10 mL of methanol by adding it to the alumina column. Remove the alumina column and the beaker. Place a 25 mL colorimetric tube under the anion exchange column and elute the clopidol with two portions of 10 mL of 0.6% methanolic acetic acid solution with the elution rate of 1 mL/min. Dilute the eluate to volume with methanol, and mix well.

3.4.3 Methylation

Pipet 5 mL of the eluate into a test tube (3.3.6) and evaporate to dryness by placing the tube in a 70℃ water-bath with a gentle stream of nitrogen. Remove the tube, add 0.2 mL of methanol aqueous solution (8 + 2), and warm gently to redisscIve the residue. Cool to room temperature. Add 1 mL of diazomethane reagent, close with the screw cap, and heat for 2 min by placing the tube in a 70℃ water-bath with the water level at the same height as the solution in the tube. Remove the tube and let cool to room temperature. Take off the cap and evaporate gently by placing the tube with its lower end touching the water surface of the bath (70℃) until all the ether is expelled. Add 5 mL of de-ionized water and 1.00 mL of n-hexane extractive solution(3.2.7)to the tube. Cap the tube and mix for 1 min by a mixer. Remove the tube and centrifugalize for 3 min at 4 000 r/min. The upper n-hexane layer of the sample solution is used for GC determination.

3.4.4 Determination

3.4.4.1 GC operating conditions:

a. GC column: Glass, 2 m×2 mm(id), packed with 10%(*m*/*m*) DC-200 silicone oil on Chromosorb W HP(80—100 mesh);

b. Nitrogen: Purity≥99.99%, 60 mL/min;

c. Column temperature: 170℃;

d. Injection port temperature: 200℃;

e. Detector temperature: 300℃.

3.4.4.2 Gas chromatographic determination: Inject the test solution (if necessary, dilute further with more n-hexane extractive solution) onto the column of gas chromatograph. Injection volumn: 2 μL. Retention time under the above conditions: ca 2 min for methyl ether of clopidol ,ca 4 min for 2,4-dinitrochlorobenzene.

3.4.5 Preparation of the standard curve: Pipet respectively 0, 0.1, 0.2, 0.4, 0.8, 1.6 mL of the standard working solution into a series of test tubes (3.3.6) ,proceed by following the above procedures (3.4.3—3.4.4) with the omission of the sample eluate. Prepare the standard curve by plotting the ratio of peak heights of the standard and internal standard against the concentration (μm/mL) of the standards.

3.4.6 Recovery factor R: Weigh three samples of 19.9—20.1 g (accurate to 0.1 g) each of the homogenized clopidol-free tissue and add separately 0.0, 0.2 and 1 mL (equivalent to 0.0, 0.01 and 0.05 μg/g clopidol) of intermediate standard solution (Ⅱ). Proceed as in 3.4.1—3.4.4 and calculate the average recovery factor R.

3.4.7 Blank test: Proceed by following the above procedures (3.4.1—3.4.4) with the omission of sampie weighing.

3.4.8 Calculation and expression of the result

Calculate the content of the clopidol according to the following formula:

$$X = \frac{c \cdot V}{R \cdot m}$$

where

X—Content of clopidol in test sample, mg/kg;

R—Average recovery factor;

c—Concentration of clopidol in the final sample solution, obtained from the standard curve, μg/mL;

V—Volume of the final sample solution, mL;

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

The content of clopidol in the sample can also be calculated by GC data processor.

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

4 Limit of determination and recovery

4.1 Limit of determination

The limit of determination of this method is 0.005 mg/kg;

4.2 Recovery

According to the experimental data, when the fortifying concentration of clopidol is in the range of 0.005—0.025 mg/kg, the recovery is 65.4%—86.2%.

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 Shanghai, Hebei, Beijing, Shandong Import and Export Commodity Inspection Bureau of the People's Republic of China.

Principal drafters: Zhu Zhiping, Zhujian, Shen Libing, Ge Xiuli, Zhang Jun, Wang Fenchi, Liu Xintong.

Reference:

AOAC-Official Methods of Analysis, 13 th ed. 41.013-41.018 1980.

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