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中华人民共和国进出口商品检验行业标准

SN 0195—93

出口肉及肉制品中2,4-滴残留量检验方法

Method for determination of 2,4-D residues in meat and meat products for export

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

本标准规定了出口肉及肉制品中2,4-滴残留量检验的抽样、制样和气相色谱测定方法。

本标准适用于出口冻分割肉、清蒸猪肉罐头和咸牛肉中2,4-滴残留量的检验。

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)	最少抽样数(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。

肉及肉制品(罐头除外)：从每件中取一袋作为原始样品，其总量不少于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。

罐头：每件随机取一罐。

所抽取的样品应标明标记，及时送交实验室。

2.4 试样制备

肉及肉制品(罐头除外)：从所取全部样品中取出有代表性样品约1 000 g，充分搅碎，混匀，均分成两份，分别装入洁净容器内。密封，作为试样，标明标记。

罐头：将所取全部样品开罐后，充分搅碎、混匀，取出约1 000 g，装入洁净容器内，密封，作为试样，标明标记。

2.5 试样保存

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将试样于－18℃冷冻保存。

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

在酸性条件下，以三氯甲烷提取组织中残留的2,4-滴及其钠盐，并转移至碱液中。用有机溶剂洗涤后再将其酸化，2,4-滴再用三氯甲烷提取。蒸除溶剂将其甲酯化，用气相色谱仪电子俘获检测器检测，外标法定量。

3.2 试剂和材料

3.2.1 三氯甲烷：分析纯，重蒸馏。

3.2.2 乙醇：分析纯，重蒸馏。

3.2.3 正己烷：分析纯，重蒸馏。

3.2.4 无水乙醚：分析纯，重蒸馏。

3.2.5 硫酸-水(1+9)：以优级纯浓硫酸配制。每500 mL硫酸-水(1+9)用50 mL三氯甲烷抽提两次后备用。

3.2.6 氢氧化钠溶液(30 g/L)：以优级纯氢氧化钠配制。每500 mL氢氧化钠溶液(30 g/L)用25 mL三氯甲烷抽提两次后备用。

3.2.7 氯化钠：优级纯，每250 g氯化钠用100 mL正己烷超声波抽提两次，挥干，于600℃灼烧4 h，贮于具塞瓶中。

3.2.8 饱合氯化钠溶液：以优级纯氯化钠配制。每250 mL饱合氯化钠溶液用25 mL三氯甲烷抽提两次后备用。

3.2.9 无水硫酸钠：分析纯，每100 g无水硫酸钠用20 mL正己烷超声波抽提两次后，挥干，于600℃灼烧4 h，储于密封容器中备用。

3.2.10 硫酸钠溶液(40 g/L)：称取无水硫酸钠(3.2.9)4 g溶于100 mL蒸馏水中。每100 mL溶液用10 mL正己烷抽提两次后备用。

3.2.11 三氟化硼-乙醚试剂(470 g/L)：分析纯，北京化工厂。

3.2.12 甲酯化溶液：将三氟化硼-乙醚试剂和甲醇置于－15℃下预冷，将30 mL冷三氟化硼-乙醚试剂和120 mL冷甲醇混和，置0～4℃下储存备用。

3.2.13 2,4-滴标准品：纯度≥99%。

3.2.14 2,4-滴标准溶液：准确称取2,4-滴标准品以无水乙醚(3.2.4)配制成浓度为0.1 mg/mL标准贮备溶液。再以无水乙醚稀释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。

3.3.2 组织捣碎机。

3.3.3 康氏振荡器。

3.3.4 心形瓶：250 mL。

3.3.5 密封试管：15 mL，带螺旋盖(附聚四氟乙烯内衬密封垫)。

3.3.6 恒温水浴箱。

注：全部玻璃器皿经超声波清洗，用前以少量正己烷淋洗。

3.4 测定步骤

3.4.1 提取及净化

称取搅碎混匀的试样约20 g(精确到0.1 g)于500 mL具塞锥形瓶中，加15 mL乙醇，5 mL硫酸-水(1+9)、10 g氯化钠和100 mL三氯甲烷。加塞，振荡提取30 min。通过快速滤纸过滤，滤液收集于

250 mL分液漏斗中，用约 50 mL 三氯甲烷分三次洗涤锥形瓶及滤渣。合并洗液于分液漏斗中，于上述分液漏斗中加 25 mL 氢氧化钠(30 g/L)和 50 mL 蒸馏水，再加入 10 mL 饱和氯化钠溶液〔此时水相 pH 应>12，否则应补加适量氢氧化钠溶液(3.2.6)。对于极易乳化的样品可再补加约 3 g 氯化钠〕，振摇提取 2 min。静置分层，弃去三氯甲烷层。以 25 mL 三氯甲烷洗涤水层二次，弃去三氯甲烷层。再以 25 mL 乙醚洗涤水层，静置分层，将下层水相转移至另一 250 mL 分液漏斗中，弃去乙醚层。于上述水相中，加入 25 mL 硫酸-水(1+9)进行酸化，摇匀〔水相 pH 应<2，否则应适量补加硫酸-水(1+9)〕，然后分别用 50 mL、25 mL、25 mL 三氯甲烷提取水相。将三氯甲烷提取液收集于 250 mL 心形瓶中。于 60℃水浴中通以氮气流将三氯甲烷挥至约 3～5 mL。以数毫升三氯甲烷将其定量转移至密封试管(3.3.5)中。再以氮气流将三氯甲烷完全挥除。

3.4.2 甲酯化

于上述密封试管中，加 1 mL 甲酯化溶液(3.2.12)，将螺旋盖密封。充分振动混匀，于 70℃水浴酯化 1 h，放冷至室温。以约 10 mL 硫酸钠溶液(3.2.10)将甲酯液转移至 25 mL 具塞试管中，分别以 4 mL 正己烷提取两次。用滴管将正己烷层转移至另一 25 mL 具塞试管中，以 2 mL 硫酸钠溶液洗涤正己烷层二次，用滴管吸除水层，以容量瓶定容至 10 mL。吸取上述正己烷溶液 3～5 mL 于 10 mL 具塞试管中，加约 1 g 无水硫酸钠，振摇脱水。供气相色谱分析。

3.4.3 2,4-滴甲酯标准工作溶液的制备

准确吸取适用浓度的 2,4-滴标准溶液 1 mL 于带螺旋帽盖的试管(3.3.5)中。以氮气流将溶剂挥除。按 3.4.2 操作进行甲酯化后供气相色谱分析。

3.4.4 测定

3.4.4.1 色谱条件

a. 色谱柱：玻璃柱，2 m×2 mm(id)，填充物为 2.5%(*m*/*m*)OV-17+3.3%(*m*/*m*)QF-1 涂于 Chromosorb HP (80～100 筛目)；

b. 色谱柱温度：220℃；

c. 进样口温度：280℃；

d. 检测器温度：280℃；

e. 载气：氮气，纯度>99.99%，60 mL/min。

3.4.4.2 色谱测定：分别准确吸取上述液(3.4.2)及标准工作溶液(3.4.3)1～2 μL(等量进样)注入气相色谱仪。实际应用的标准工作溶液及待测样液中，2,4-滴甲酯的响应值应相近并均应在检测器的线性范围内。

在上述条件下 2,4-滴甲酯保留时间约为 2 min。

3.4.5 空白试验

除不加样品外，按上述相同条件和步骤进行。

3.5 结果计算和表述

用色谱数据处理机或按下列公式计算：

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

式中：X——试样中 2,4-滴含量，mg/kg；

h——样液中 2,4-滴甲酯的峰高，mm；

h_s——标准溶液中 2,4-滴甲酯的峰高，mm；

c——标准工作溶液中 2,4-滴浓度，μg/mL；

V——样液定容体积，mL；

m——样品重，g。

注：计算结果需扣除空白值。

4 方法的测定低限、回收率

4.1 测定低限

本方法测定低限为：0.02 mg/kg。

4.2 回收率

回收率的实验数据：2,4-滴浓度在 0.02～0.20 mg/kg 范围，回收率为 70%～97%。

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天津进出口商品检验局负责起草。

本标准主要起草人穆乃强、林安清、王虹。

主要参考文献：

FDA-Pesticide Analytical Manual, Vol. I, 201-11-16, 1985。

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

SN 0195—93

Method for determination of 2,4-D residues in meat and meat products for export

1 Scope and field of application

This standard specifies the method of sampling, sample preparation and determination by gas chromatography of 2,4-D residues in meat and meat products for export.

This standard is applicable to the determination of 2,4-D residue in frozen cuts of meat, canned steamed pork and corned beef 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.

Meat and meat products (except canned foods): From each, at least 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 the laboratory in time.

In case the frozen meat-pieces are not contained in small bags inside each package, or if there are small bags inside the package but the content of the 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 with alcohol). Mix the parts of the meat as the mixed primary sample, which shall not be less than 2 kg. Sealed, labeled and sent to the laboratory in time.

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Canned foods: One can shall be taken at random from each package.

Samples taken should be labeled and sent to laboratory in time.

2.4 Preparation of test sample

Meat and meat products (except canned foods): The combined primary sample is reduced to 1 000 g which is blended, mixed and divided into two equal portions, each portion is placed in a clean vessel as a test sample, which is then sealed and labeled.

Canned foods: Open all the cans sample, blend, mix and reduce to 1 000 g, which is divided into two equal portions, each portion is placed in a clean vessel as a test sample, which is then sealed and labeled.

2.5 Storage of sample

The test sample should be stored at −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 chlorophenoxy acid (2,4-D) residues and its Na salts are extracted from the tissue with chloroform under acidified conditon, then transferred into alkaline solution. The alkaline solution is washed with organic solvents and acidified, 2,4-D are then reextracted with chloroform and methylated after the chloroform has been evaporated. Determination is made by GC with electron capture detector and quantified by using the external standard.

3.2 Reagents and materials

3.2.1 Chloroform: Analytical grade, redistilled.

3.2.2 Ethanol: Analytical grade, redistilled.

3.2.3 n-Hexane: Analytical grade, redistilled.

3.2.4 Ethyl ether, anhydrous: Analytical grade, redistilled.

3.2.5 Sulfuric acid-water (1+9): Prepare the solution with GR grade sulfuric acid. Each 500 mL of sulfuric acid water (1+9) is extracted with 50 mL of chloroform twice.

3.2.6 Sodium hydroxide solution (30 g/L): Prepare the solution with GR grade of sodium hydroxide. Each 500 mL of sodium hydroxide solution is extracted with 25 mL of chloroform twice.

3.2.7 Sodium chloride: GR grade, wash 250 g of sodium chloride with 100 mL of n-hexane twice by ultrasonic cleaner. Evaporate to expel the solvent and ignite at 600℃ for 4 h, store in a stoppered glass jar.

3.2.8 Saturated sodium chloride solution: Prepare with GR grade sodium chloride. Each 250 mL of saturated sodium chloride solution is extracted with 25 mL of chloroform twice.

3.2.9 Anhydrous sodium sulfate: Analytical grade, wash 100 g of anhydrous sodium sulfate with 20 mL of n-hexane twice by ultrasonic cleaner. Evaporate to expel the solvent, ignite at 600℃ for 4 h, and store in air-tight container.

3.2.10 Sodium sulfate solution (40 g/L): Weigh 4 g of anhydrous sodium sulfate and dissolve in 100 mL water. Each 100 mL of this solution is extracted with 10 mL of n-hexane twice.

3.2.11 Boron trifluoride-ether reagent (470 g/L): Analytical grade.

3.2.12 Methylation solution: Pre-cool the boron trifluoride-ether reagent and methanol at −15℃, add 30 mL of cold boron trifluoride-ether reagent into 120 mL of cold methanol and mix thoroughly,

stopper, store at 0—4℃.

3.2.13 2,4-D standard: Purity≥99%.

3.2.14 2,4-D standard solution: Prepare 0.1 mg/mL standard stock solution with ethyl ether, then dilute to suitable concentration with ethyl ether as standard working solution.

3.3 Apparatus and equipment

3.3.1 Gas chromatograph equipped with electron capture detector.

3.3.2 Blender.

3.3.3 Shaker.

3.3.4 Heart shape flask: 250 mL.

3.3.5 Test tube: 15 mL, teflon-lined screw cap.

3.3.6 Thermostatic water bath.

Note: All the glasswares should be washed by ultrasonic cleaner, rinse the glasswares with a little n-hexane before use.

3.4 Procedure

3.4.1 Extraction and clean up

Weigh ca 20 g(accurate to 0.1 g)of test sample in a 500 mL conical flask with stopper, add 15 mL of ethanol, 5 mL of sulfuric acid-water(1+9), 10 g of sodium chloride and 100 mL of chloroform. Stopper tightly, shake for 30 min. Then filter the extract through rapid filter paper into a 250 mL separatory funnel, rinse the conical flask and filter cake with 50 mL of chloroform. Transfer all washings into the separtory funnel. Add 25 mL of sodium hydroxide solution (30 g/L) and 50 mL of distilled water. Add 10 mL saturated sodium chloride solution (pH of the aqueous phase should be >12, otherwise, additional sodium hydroxide solution is needed. Additional 3 g of sodium chloride should be added to the easily emulsified sample), shake for 2 min. Let stand to separate, discard the chloroform layer. Wash the aqueous phase with 25 mL portions of chloroform twice, discard the chloroform layer. And then wash the aqueous phase with 25 mL of ether, discard the ether layer. The above aqueous phase is acidified with 25 mL of sulfuric acid-water (1+9) and mix thoroughly (pH of the aqueous phase should be<2, otherwise, additional sulfuric acid-water<1+9>is needed). Then extract the aqueous phase successively with 50, 25 and 25 mL chloroform. Collect the extract of chloroform into 250 mL heart shape flask. Evaporate the chloroform to ca 3—5 mL in a water bath at 60℃ under nitrogen flow. Then transfer quantitatively to the tube (3.3.5) with a few mL of chloroform. Expel the chloroform completely under nitrogen flow.

3.4.2 Methylation

Add 1 mL methylation solution (3.2.12)into the above tube and seal tightly, mix thoroughly, place the tube in water bath for 1 h at 70℃. Cool to room temperature, transfer the methylated solution with ca 10 mL of sodium sulfate solution to a 25 mL tube with stopper. Extract with n-hexane twice each 4 mL. Transfer the n-hexane layer to another 25 mL tube. Extract the n-hexane layer with each 2 mL of sodium sulfate solution twice and discard the aqueous layer. Dilute the hexane layer to 10 mL in a volumetric flask. Pipet 3—5 mL n-hexane layer in to a 10 mL tube with stopper, add ca 1 g of anhydrous sodium sulfate, mix well. The solution is ready for gas chromatographic determination.

3.4.3 Preparation of methlylated 2,4-D standard working solution

Accurately pipette 1 mL of 2,4-D standard solution of suitable concentration to tube with screw cap (3.3.5), remove the solvent under nitrogen flow, proceed as section 3.4.2.

3.4.4 Determination

3.4.4.1 GC operating conditions

a. Column: glass, 2 m × 2 mm (id), packed with 2.5%(*m*/*m*) OV-17 + 3.5%(*m*/*m*) QF-1 on Chromosorb HP (80—100 mesh);

b. Column temperature: 220℃;

c. Injection port temperature: 280℃;

d. Detector temperature: 280℃;

e. Nitrogen: purity ≥ 99.99%, 60 mL/min.

3.4.4.2 GC determination

Inject accurately equal amount (1—2 μL) of the methylated standard working solution (section 3.4.3) and sample solution (section 3.4.2) into gas chromatograph respectively. The responses of the 2,4-D methyl in the standard working solution and sample solution should be similar and must be within the linear range of the detector.

The retention time of 2,4-D methyl is ca 2 min under the above conditions.

3.4.5 Blank test

The operation of blank test is the same as above, but with omission of sample addition.

3.5 Calculation and expression of result

Calculate the content of 2,4-D 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 2,4-D in test sample, mg/kg;

h—peak height of 2,4-D methyl in sample solution, mm;

h_s—peak height of 2,4-D methyl in standard solution, mm;

c—concentration of 2,4-D in standard solution, μg/mL;

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

m—mass of test sample, g.

Note: The blank value shall 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, when the concentration of 2,4-D is in the range of 0.02—0.20 mg/kg, the recovery is 70%—97%.

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

This standard was mainly drafted by Mu Naiqiang, Lin Anqing, Wan Hong.

Reference:

FDA-Pesticide Analytical Manual, Vol. I, 201-11-16, 1985.

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