SN

中华人民共和国进出口商品检验行业标准

SN 0289—93

出口禽肉中二甲硝咪唑残留量检验方法

Method for the determination of dimetridazole residues in poultry meat for export

1993-12-28发布　　1994-05-01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甲硝咪唑残留量检验的抽样、制样和气相色谱测定方法。

本标准适用于出口鸡肉中二甲硝咪唑残留量的检验。

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。每件至少取一袋作为原始样品，原始样品总量不得少于 2 kg。放入清洁容器内加封后，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，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

鸡肉中二甲硝咪唑残留量用二氯甲烷提取，用液-液分配法净化。浓缩蒸干后，加抗蚜威(pirimicarb)内标溶液溶解残渣，用配有氮磷检测器的气相色谱仪作定量测定。

3.2 试剂和材料

中华人民共和国国家进出口商品检验局1993-12-28批准　　1994-05-01实施

所用试剂除注明外均为分析纯，水为蒸馏水。

3.2.1 二氯甲烷：重蒸馏。

3.2.2 无水乙醇。

3.2.3 碳酸钠溶液：2 mol/L。

3.2.4 盐酸溶液：1 mol/L。

3.2.5 无水硫酸钠：650℃灼烧 4 h，冷却后贮于密闭容器中。

3.2.6 二甲硝咪唑和抗蚜威标准品：纯度≥99.5%。

3.2.7 二甲硝咪唑标准溶液：准确称取适量二甲硝咪唑标准品，用无水乙醇配成 0.5 mg/mL 标准贮备溶液，根据需要再配成适当浓度的标准工作溶液。

3.2.8 抗蚜威内标溶液：准确称取适量抗蚜威标准品，用无水乙醇配成 0.5 mg/mL 内标贮备溶液，根据需要再用无水乙醇配成适当浓度的内标工作溶液。

3.2.9 混合标准溶液：根据需要吸取适当浓度的二甲硝咪唑标准工作溶液和抗蚜威内标工作溶液，用无水乙醇配成混合标准工作溶液。

3.3 仪器和设备

3.3.1 气相色谱仪：配有氮磷检测器。

3.3.2 振荡器。

3.3.3 旋转蒸发器。

3.3.4 平底漏斗和减压过滤装置。

3.3.5 无水硫酸钠柱：筒形漏斗内装约 5 cm 高的无水硫酸钠。

3.4 测定步骤

3.4.1 提取

称取试样约 10 g(精确到 0.1 g)于锥形瓶内，加 10 g 无水硫酸钠和 20 mL 二氯甲烷，振荡 30 min。在有快速滤纸的平底漏斗上减压过滤。残渣再用 20 mL 二氯甲烷振荡 15 min，在原滤纸上过滤。合并滤液于 250 mL 分液漏斗内。

3.4.2 净化

加 20 mL 盐酸溶液(3.2.4)于滤液中，振荡 1 min，分层后将下层水相转移至另一分液漏斗内。每次用 20 mL 盐酸溶液再提取两次，合并水相。然后对水相每次用 20 mL 二氯甲烷提取三次，弃去有机相。用碳酸钠溶液(3.2.3)调整水相的 pH 至 9(用精密 pH 试纸试验)后，每次用 20 mL 二氯甲烷提取二次，保留并合并有机相。使有机相通过无水硫酸钠柱脱水流入蒸发瓶。用 20 mL 二氯甲烷洗分液漏斗和无水硫酸钠柱，流出液合并入蒸发瓶中。在 35℃水浴中，用旋转蒸发器减压浓缩至近干，在室温下自然挥干。用 1.0 mL 抗蚜威内标工作溶液(3.2.8)溶解残渣，并移入具塞小试管内，供气相色谱测定用。

3.4.3 测定

3.4.3.1 色谱条件

a. 色谱柱：玻璃柱 1 m×2 mm(id)，填充物为 5%(*m*/*m*)DEGS 涂于 Chromosorb W HP(80～100 目)；

b. 色谱柱温度：200℃；

c. 进样口温度：250℃；

d. 检测器温度：280℃；

e. 氮气：纯度≥99.99%，50 mL/min；

f. 氢气：3 mL/min；

g. 空气：50 mL/min。

3.4.3.2 色谱测定

根据样液中二甲硝咪唑含量情况，选定峰高相近的混合标准工作溶液(3.2.9)。混合标准工作溶液

(3.2.9)和样液中二甲硝咪唑和抗蚜威的响应值均应在仪器检测线性范围内。对混合标准工作溶液(3.2.9)和样液进行等体积测定。在上述色谱条件下,二甲硝咪唑保留时间约为 2 min,内标物抗蚜威保留时间约为 3 min。

3.4.4 空白试验

除不加试样外,均按上述测定步骤进行。

3.5 结果计算和表述

用色谱数据处理机或按下式计算试样中二甲硝咪唑残留含量:

$$X = \frac{h \cdot h'_i \cdot c \cdot m_i}{h' \cdot h_i \cdot c_i \cdot m}$$

式中:X ——试样中二甲硝咪唑的含量,mg/kg;

h ——样液中二甲硝咪唑的峰高,mm;

h' ——混合标准工作溶液中二甲硝咪唑的峰高,mm;

h'_i ——混合标准工作溶液中抗蚜威的峰高,mm;

h_i ——样液中抗蚜威的峰高,mm;

c ——混合标准工作溶液中二甲硝咪唑的浓度,μg/mL;

c_i ——混合标准工作溶液中抗蚜威的浓度,μg/mL;

m_i ——样液中加入抗蚜威的量,μg;

m ——所称的试样量,g。

注:空白值应从计算结果中扣除。

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

回收率的实验数据:二甲硝咪唑添加浓度在 0.005～0.07 mg/kg 范围内,回收率为 87.6%～90.7%。

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湖北进出口商品检验局负责起草。

本标准主要起草人姚昌莉、卢康全。

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

Method for the determination of dimetridazole residues in poultry meat for export

SN 0289—93

1 Scope and field of application

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

This standard is applicable to the determination of dimetridazole residues in chicken meat 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, grade and specification, 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. At least one bag shall be taken as the primary sample from each package. 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 the representative sample is taken from each bag of the primary samples, the edible portions of which are ground 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 a clean container, which is then sealed and

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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 the contamination or any factors which may cause the change of residue content.

3 Method of determination

3.1 Principle

Dimetridazole residues are extracted from the test sample with dichloromethane. The extract is cleaned up by liquid-liquid partitioning between acid soluhon-dichloromethane and alkaline solution-dichloromethane. The final dichloromethane extract is evaparated to dryness. The residue is dissolved in pirimicarb internal standard working solution and analyzed by gas chromatography with a N/P detector.

3.2 Reagents and materials

All reagents are of analytical grade unless otherwise specified, water is distilled water.

3.2.1 Dichloromethane: Redistilled.

3.2.2 Absolute ethyl alcohol.

3.2.3 Sodium carbonate solution: 2 mol/L.

3.2.4 Hydrochloric acid solution: 1 mol/L.

3.2.5 Anhydrous sodium sulfate: Ignite at 650℃ for 4 h, and stored in an air-tight glass bottle.

3.2.6 Dimetridazole and pirimicarb standards: Purity≥99.5%.

3.2.7 Dimetridazole standard solution: Accurately weigh a suitable amount of standard dimetridazole, dissolve in absolute ethyl alcohol and prepare a solution of 0.5 mg/mL as the standard stock solution. According to the requirement, prepare a standard working solution of appropriate concentration.

3.2.8 Pirimicarb internal standard solution: Accurately weigh a suitable amount of standard pirimicarb, dissolve in absolute ethyl alcohol to prepare a solutions of 0.5 mg/mL as the standard stock solution. According to the requirement, prepare an internal standard working solution of appropriate concentration.

3.2.9 Mixed standard solution: According to the requirement, mix appropriate volumes of dimetridazole standard solution and pirimicarb internal standard solution to prepare a mixed standard working solution.

3.3 Apparatus and equipment

3.3.1 Gas chromatograph: Equipped with nitrogen/phosphorus detector.

3.3.2 Shaker.

3.3.3 Rotary evaporator.

3.3.4 Buchner funnel with suction device.

3.3.5 Anhydrous sodium sulfate column: A cylindrical-shape funnel, filled with 5 cm height of anhydrous sodium sulfate.

3.4 Procedure

3.4.1 Extraction

Weigh ca 10 g of the test sample (accurate to 0.1 g) into a conical flask, add 10 g of anhydrous sodium sulfate and 20 mL of dichloromethane, place it on a shaker and shake for 30 min. Filter the ex-

tract through a rapid filter paper on a Buchner funnel under reduced pressure. The residue is extracted once more with 20 mL of dichloromethane, by shaking it on a shaker for 15 min. Filter the extract with the same filter. Combine all the filtrates in a 250 mL separatory funnel.

3.4.2 Cleanup

Add 20 mL of hydrochloric acid solution(3.2.4) into the combined filtrate, and shake for 1 min. Let stand to separate. Drain the lower hydrochloric acid solution into another separatory funnel. Repeat the extraction twice, each with 20 mL portions of hydrochloric acid solution(3.2.4). Combine all the hydrochloric acid layers in the separatory funnel. The aqueous phase is reextracted three times with dichloromathane, each with 20 mL, and discard the dichloromethane layer. The aqueous phase is adjusted to pH 9.0, using sodium carbonate solution(3.2.3) and testing with precise pH paper. Then add 20 mL of dichloromathane and shake vigorously for 1 min. Let stand to separate. Reserve the dichloromathane layer. Repeat the extraction once again. Combine the dichloromathane layers and pass it through an anhydrous sodium sulfate column for dehydration into an evaporating bottle. Wash the separatory funnel and anhydrous sodium sulfate column with 20 mL of dichloromathane, and combine the washings into the evaporating bottle. Concentrate the dichloromethane solution almost to dryness in a 35 C water-bath with a rotary evaporator, and then let evaporate to dryness at room temperature. Dissolve the residue in 1.0 mL of internal standard working solution(3.2.8) to form a sample solution, which is transfered to a test-tube(with stopper) for chromatographic determination.

3.4.3 Determination

3.4.3.1 GC operating condition

a. Column: glass, 1 m×2 mm(id), packed with 5% (m/m) DEGS on Chromosorb W HP(80—100 mesh);

b. Column temperature: 200℃;

c. Injection port temperature: 250℃;

d. Detector temperature: 280℃;

e. Nitrogen: Purity ≥99.99%, 50 mL/min;

f. Hydrogen: 3 mL/min;

g. Air: 50 mL/min.

3.4.3.2 GC determination

According to the approximate concentration of dimetridazole in the sample solution, select a mixed standard working solution(3.2.9) with similar peak height to that of sample solution. The responses of dimetridazole and pirimicarb in the mixed standard working solution and sample solution should be in the linear range of the instrumental detection. The mixed standard working solutions should be injected with the sample solution of equal volume. Under the above chromatographic condition, the retention time of dimetridazole is about 2 min, and that of pirimicarb is about 3 min.

3.4.4 Blank test

The operation of the blank test is the same as that described in the method of determination, but without addition of the sample.

3.5 Calculation and expression of result

The calculation of the content of dimetridazole residues in the test sample is carried out by GC data processor or according to the following formula:

$$X = \frac{h \cdot h'_i \cdot c \cdot m_i}{h' \cdot h_i \cdot c_i \cdot m}$$

where

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

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

c_i—the concentration of pirimicarb in the mixed standard working solution, μg/mL;

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

h_i—the peak height of pirimicarb in the sample solution, mm;

h'_i—the peak height of pirimicarb in the mixed standard working solution, mm;

h'—the peak height of dimetridazole in the mixed standard working solution, mm;

m_i—the quantity of pirimicarb added in the sample solution, μg;

m—the mass of 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 0.005 mg/kg.

4.2 Recovery

According to the experimental data, when the concentration of dimetridazole is in the range of 0.005—0.07 mg/kg, the recovery is 87.6%—90.7%.

Additional explanations:

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

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

This standard was mainly drafted by Yao Changli, Lu Kangquan.

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