SN

中华人民共和国进出口商品检验行业标准

SN 0705—1997

出口肉及肉制品中乙烯利残留量检验方法

Method for the determination of ethephon residues in meats and meat products for export

1997-12-01发布　　1998-04-01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》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了国内外有关文献,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上对出口肉及肉制品中乙烯利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。

本标准由中华人民共和国陕西进出口商品检验局负责起草。

本标准主要起草人:庄金传、孔祥虹、李剑、何学文。

本标准系首次发布的行业标准。

中华人民共和国进出口商品检验行业标准

出口肉及肉制品中乙烯利残留量检验方法

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Method for the determination of ethephon residues in meats and meat products for export

1 范围

本标准规定了出口肉及肉制品中乙烯利残留量检验的抽样、制样和气相色谱测定方法。

本标准适用于出口猪肉中乙烯利残留量的检验。

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。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，则可用灭菌的锋利刀在抽出的包件中，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

从原始样品中分取出约 1 kg，经充分绞碎、混匀，均分成两份，分别装入清洁的容器内，作为试样。加封并标明标记。

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。

中华人民共和国国家进出口商品检验局1997-12-01批准　　1998-04-01实施

3 测定方法

3.1 方法提要

试样中的乙烯利残留用甲醇提取，提取液经冷冻除去脂肪和蜡质，然后浓缩。待测物再用乙醚提取并经重氮甲烷衍生化，使乙烯利衍生化成二甲基乙烯利后，用配有氮磷检测器的气相色谱仪测定，外标法定量。

3.2 试剂和材料

除另有规定外，所用试剂均为分析纯，水为重蒸馏水。

3.2.1 氢氧化钾。

3.2.2 甲醇：重蒸馏。

3.2.3 N-甲基-N-亚硝基-对甲苯磺酰胺。

3.2.4 无水乙醇。

3.2.5 无水乙醚：重蒸馏。

3.2.6 甲醇-盐酸(38%)溶液(90+10)。

3.2.7 无水硫酸钠：650℃灼烧 4 h，置于密封容器中备用。

3.2.8 乙烯利标准品：纯度≥99%。

3.2.9 乙烯利标准溶液：准确称取适量的乙烯利标准品，用甲醇配制成浓度为 1.0 mg/mL 的标准储备液。根据需要再用甲醇稀释标准储备液以制备适当浓度的标准工作液。

注：配制标准溶液时，均应使用聚乙烯器皿。

3.2.10 重氮甲烷溶液：将盛有氢氧化钾水溶液(0.6 g/mL)10 mL，乙醇 35 mL 及乙醚 10 mL 的混合液的双口蒸馏瓶置于磁力搅拌器加热板上的水浴中。将搅拌子放入瓶中，接上滴液漏斗和高效冷凝器，冷凝器后串联两个 125 mL 的烧瓶作为接收瓶。在第二个烧瓶中放有 10 mL 乙醚，且将入口管插到乙醚液面以下。将这两个接收瓶置于冰水浴中。将水浴加温至 70℃，边用磁力搅拌器搅拌，边从滴液漏斗中滴加 N-甲基-N-亚硝基-对甲苯磺酰胺的乙醚溶液(21.5 g/140 mL)。滴完全部溶液的时间控制在 20 min 以上。当蒸馏液呈淡黄色时，停止蒸馏。将 两个接收瓶中的液体合并，作为重氮甲烷溶液。如果含杂质太多可再重蒸馏。此溶液密闭置于冰箱中保存，保存期为一个月。

3.3 仪器和设备

3.3.1 气相色谱仪并配有氮磷检测器。

3.3.2 超声波浴。

3.3.3 快速混合器。

3.3.4 离心器：6 000 r/min。

3.3.5 聚乙烯烧瓶：100 mL，具塞。

3.3.6 聚乙烯烧杯：250 mL。

3.3.7 微量注射器：10 μL 或 5 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)于聚乙烯烧杯中，加入甲醇-盐酸(90+10)溶液(3.2.6)0.5 mL 和甲醇 40 mL，于超声波浴中提取 5 min。转入离心管离心(6 000 r/min)2 min 后，用聚乙烯吸管将上部清液吸入具塞 100 mL 聚乙烯烧瓶中。残渣再用 40 mL 甲醇按上述步骤提取一次，合并提取液于同一聚乙烯烧瓶中。

3.4.2 净化

将盛有甲醇提取液的聚乙烯烧瓶，放入－18℃以下的冰柜中冷冻 4 h。取出后迅速转入离心管中离心(6 000 r/min)5 min。将上层清液倾入用 二甲基 二氯硅烷处理过的 250 mL 心形瓶中，剩下的残渣用

2 mL 冷冻过的甲醇洗涤，洗液转移到同一个心形瓶中。用旋转蒸发器在 30～35℃水浴上浓缩至约 60 mL。将浓缩液转移到用二甲基二氯硅烷处理过的 100 mL 容量瓶中，用少量甲醇洗涤心形瓶，洗液并入容量瓶内。用甲醇定容。

3.4.3 衍生化

吸取 10.0 mL 上述溶液，放入 15 mL 具塞刻度离心管中，在干燥氮气流下于 30～35℃水浴中浓缩至约 1.5 mL。加入 0.5 mL 甲醇-盐酸溶液(90+10)和 8 mL 无水乙醚，充分混合，放置 10 min。将上层清液倾入另一个具塞刻度管中，用 2×1 mL 无水乙醚洗涤残渣。洗液并入上述清液中。于氮气流下在 30～35℃水浴上浓缩至约 1 mL。在通风柜内，向浓缩液里滴加重氮甲烷溶液(3.2.10)，直至黄色不褪为止。盖严塞子，放置 15 min。用氮气流把过量的重氮甲烷吹除。再用 2×5 mL 水洗涤，每次离心(6 000 r/min)2 min，弃去下层水相。用氮气流在 30～35℃水浴中浓缩至约 0.8 mL，用无水乙醚稀释到 1.00 mL。溶液经用约 0.5 g 无水硫酸钠脱水后供气相色谱测定。

3.4.4 标准溶液的衍生化

取适量的乙烯利标准工作液，置于 100 mL 具塞聚乙烯烧瓶里，加入重氮甲烷溶液，直到黄色不褪为止。盖紧瓶塞，放置 15 min。用氮气流把过量的重氮甲烷吹除，这时溶液变为无色。将烧瓶置于 30～35℃水浴中在氮气流下浓缩至近干。用无水乙醚溶解残渣并稀释至所取标准工作液的同样体积。

3.4.5 测定

3.4.5.1 气相色谱条件

a) 色谱柱：不锈钢柱，2 m×3 mm(内径)，填充物为 3%(*m*/*m*)FFAP 涂于 Chromosorb G，AW，DMCS(60～80 目)；

b) 载气：氮气，纯度≥99.9%，50 mL/min；

c) 氢气：4 mL/min；

d) 空气：175 mL/min；

e) 色谱柱温度：145℃；

f) 进样口温度：230℃；

g) 检测器温度：300℃；

h) 进样量：1 μL。

3.4.5.2 气相色谱测定

根据试样中被测农药含量情况，选定峰高相近的衍生化的标准工作溶液。标准工作溶液和待测样液中农药衍生化物的响应值均应在仪器检测的线性范围内。对标准工作液与样液应等体积参插进样测定。在上述色谱条件下，乙烯利衍生化物(二甲基乙烯利)的保留时间约为 1.6 min。标准品衍生化物的色谱图见附录 A 中图 A1。

3.4.6 空白试验

除不称取试样外，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式(1)计算试样中乙烯利的残留含量：

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

式中：X——试样中乙烯利残留含量，mg/kg；

h——样液中二甲基乙烯利的色谱峰高，mm；

h_s——标准工作液中二甲基乙烯利的色谱峰高，mm；

c——标准工作液中乙烯利的浓度，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：计算结果需扣除空白值。

4 测定低限、回收率

4.1 测定低限

本方法测定低限为 0.01 mg/kg。

4.2 回收率

猪肉中乙烯利的添加浓度及其回收率的实验数据：

0.010 mg/kg 时，回收率为 84.6%；

0.10 mg/kg 时，回收率为 86.1%；

1.0 mg/kg 时，回收率为 90.2%。

附 录 A
（提示的附录）
标准品色谱图

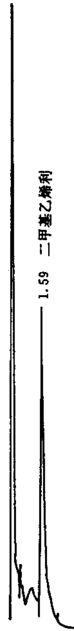

图 A1 乙烯利标准品的衍生物（二甲基乙烯利）气相色谱图

Foreword

This standard was drafted in accordance with the requirements of GB/T 1. 1—1993"Directives for the work of standardization—Unit1:Drafting and presentation of standards—Part1:General rules for drafting 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 of this standard was drafted by referring to the relevant domestic and foreign literatures through research, modification and verification. In addition, methods of sampling and sample preparation are also specified in this standard.

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

Annex A of this standard is an informative annex.

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

The main drafters of this standard are Zhuang Jinshi, Kong Xianghong, Li Jian and He Xuewen.

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

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

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

SN 0705—1997

Method for the determination of ethephon residues in meats and meat products for export

1 Scope

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

This standard is applicable to the determination of ethephon residue content in pork 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 an 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 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 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 disinfected sharp knife. Mix the parts of the meat as the mixed primary sample, which shall be not less than 2 kg. Seal, label and send to the laboratory in time.

2.4 Preparation of test sample

The primary sample is reduced to 1 kg, which is then blended, mixed, divided into two equal por-

Approved by the State Administration of Import and Export commodity Inspection of the People's Republic of China on Dec. 1, 1997

Implemented from Apr. 1, 1998

tions and placed in clean containers as test samples. Seal and label.

2.5 Storage of test sample

The test samples 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 ethephon residues in the test sample are extracted with methanol. The fat and wax are removed by freezing. The extract is concentrated and the analyte is extracted with ethyl ether and derivatized by diazomethane. The resulting dimethyl ethephon is determined by gas chromatoraph with nitrogen-phosphorus detector(GC-NPD), using external standard method.

3.2 Reagents and materials

Unless otherwise specified, all reagents used should be of analytical grade, "water" is redistilled water.

3.2.1 Potassium hydroxide.

3.2.2 Methanol: Redistilled.

3.2.3 N-methyl-N-nitroso-p-toluene sulfonamide.

3.2.4 Anhydrous ethanol.

3.2.5 Anhydrous ethyl ether: Redistilled.

3.2.6 Methanol-hydrochloric acid(38%)solution(90+10).

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

3.2.8 Ethephon standard: Purity≥99%.

3.2.9 Ethephon standard solution: Accurately weigh an adequate amount of ethephon standard and dissolve in methanol to prepare a solution of 1.0 mg/mL in concentration as the standard stock solution. According to the requirement, prepare a standard working solution of appropriate concentration by diluting the stock solution with methanol.

Note: For preparing standard solutions, polyethylene vessels should be used.

3.2.10 Diazomethane solution: Place a double-neck distilling flask containing 10 mL of potassium hydroxide aqueous solution(0.6 g/mL), 35 mL of ethanol and 10 mL of ethyl ether in a water-bath on the hot plate with magnetic stirring device. Place the magnetic stirrer into the flask, join a dropping funnel and a high-performance condenser. Connect the condenser outlet with two 125 mL flasks in series as receiver. Add 10 mL of ethyl ether into the second flask, dip the tube outlet under the ether surface, and place both flasks in ice-bath. Heat the water-bath to 70℃, and drip in the ethyl ether solution of N-methyl-nitroso-*p*-toluene sulfonamide(21.5 g/140 mL)through the dropping funnel within a duration of more than 20 min while stirring. Stop the distillation when the distillate appears pale yellow. Combine the liquids in two receiving flasks as diazomethane solution. If the distillate contains too much impurities, it may be redistilled. Store the solution(sealed)in a refrigerator, it can be used within one month.

3.3 Apparatus and equipment

3.3.1 Gas chromatograph with nitrogen-phosphorus detector.

3.3.2 Ultrasonic bath.

3.3.3 High-speed mixer.

3.3.4 Centrifuge:6 000 r/min.

3.3.5 Polyethylene bottle with stopper:100 mL.

3.3.6 Polyethylene beaker:250 mL.

3.3.7 Micro syringe:10 μL or 5 μL.

3.4 Procedure

3.4.1 Extraction

Weigh ca 20 g(accurate to 0.1 g)of the test sample in a polyethylene beaker,add 0.5 mL of methanol-hydrochloric acid(90+10)solution(3.2.6)and 40 mL of methanol,extract for 5 min in a ultrasonic bath. Transfer the mixture into a 100 mL centrifuge tube and centrifuge for 2 min at 6 000 r/min. Transfer the supernatant with a polyethylene pipet to a 100 mL polyethylene bottle with stopper. Extract the residue once more with 40 mL of methanol as described,and combine the extract in the same bottle.

3.4.2 Cleanup

Freeze the above extract in the polyethylene bottle for 4 h at a temperature below −18℃ in a refrigerator. Take out and transfer quickly the mixture into a 100 mL centrifuge tube and centrifuge for 5 min at 6 000 r/min. Transfer the supernatant into a 250 mL heart-shaped bottle which has been treated with silicon dimethyl dichloride,wash the residue with 2 mL of frozen methanol,and combine the washings into the same heart-shaped bottle. Evaporate the extract to ca 60 mL in a water-bath at 30—35℃ with a rotary evaporator. Transfer the concentrated solution to a 100 mL volumetric flask which has been treated with silicon dimethyl dichloride,rinse the heart-shaped bottle with a little methanol,and transfer all washings to the volumetric flask,make up to volume with methanol.

3.4.3 Derivatization

Pipet 10.0 mL of the above solution into a 15 mL graduated centrifuge tube with stopper,concentrate to 1.5 mL in a wate-bath at 30—35℃ under a dry nitrogen current. Add 0.5 mL of methanolhydrochloric solution(90+10)and 8 mL of anhydrous ethyl ether,mix thoroughly and set aside for 10 min. Pour the supernatant into another graduated tube with stopper,rinse the residue with 2×1 mL of anhydrous ethyl ether,and combine the rinsings with the above supernatant. Concentrate to ca 1 mL in a 30—35℃ water-bath under a nitrogen current to ca 1 mL. In a ventilating cabinet,add to the concentrate slowly the diazomethane solution(3.2.10)until a permanent yellowish color appears. Stopper tightly and set aside for 15 min. Expel the excess diazomethane under a nitrogen current,wash with 2×5 mL of water. After each washing centrifuge for 2 min at 6 000 r/min,and discard the lower water phases. Evaporate the above liquid to ca 0.8 mL in a water-bath at 30—35℃ under a nitrogen current, and dilute to 1.00 mL with anhydrous ethyl ether. Add ca 0.5 g of anhydrous sodium sulfate for dehydration,and the solution is ready for gas chromatographic determination.

3.4.4 Derivatization of standard solution

Pipet a suitable amount of ethephon standard working solution of required concentration into a 100 mL flask with stopper,add the diazomethane solution slowly until permanent yellowish color appears. Stopper the flask and let stand for 15 min. Evaporate under nitrogen current to expel the excess diazomethane until the solution turns colorless. Then place the flask in a 30—35℃ water-bath,evaporate the solution to near dryness under the same nitrogen current. Dissolve the residue and dilute with anhydrous ethyl ether to the same volume as that of the standard working solution taken.

3.4.5 Determination

3.4.5.1 GC operating condition

a) Column: Stainless steel, 2 m×3 mm(id), packed with FFAP 3%(*m*/*m*) on Chromosorb G, AW, DMCS(60—80 mesh);

b) Carrier gas: Nitrogen, purity≥99.99%, 50 mL/min;

c) Hydrogen: 4 mL/min;

d) Air: 175 mL/min;

e) Column temperature: 145℃;

f) Injection port temperature: 230℃;

g) Detector temperature: 300℃;

h) Injection volume: 1 μL.

3.4.5.2 GC determination

According to the approximate concentration of ethephon in the sample solution, select the derivatized standard working solution with similar peak height to that of the derivatized sample solution. The responses of the derivatized ethephon in the standard working solution and sample solution should be within the linear range of the instrumental detection. The standard working solution should be randomly injected in between the injections of the sample solution of equal volume. Under the above operating condition, the retention time of the derivatized ethephon is about 1.6 min. For chromatogram of the derivatized standard, see fig. Al of annex A.

3.4.6 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 the result

The calculation of ethephon content in the test sample is carried out by GC data processor or according to the formula(1):

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

where

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

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

h_s—the peak height of dimethyl ethephon in standard working solution, mm;

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

V—the final volume of sample solution, mL;

m—the corresponding mass of 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, the fortifying concentrations of ethephon in pork and its corresponding recoveries are:

0.010 mg/kg, the recovery 84.6%;

0.10 mg/kg, the recovery 86.1%;

1.0 mg/kg, the recovery 90.2%.

Annex A
(informative)
Chromatogram of the standard

Fig. A1 Chromatogram of dimethyl ethephon standard