SN

中华人民共和国进出口商品检验行业标准

SN 0698—1997

出口肉及肉制品中莫能菌素残留量检验方法 液相色谱法

Method for the determination of monensin residues in meats and meat products for export —Liquid chromatographic method

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》的要求进行编写的。其中测定方法是参考国内外有关文献，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肉及肉制品中莫能菌素残留量的最高限量和测定方法的灵敏度而制定的。

本标准的附录A是提示的附录。

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浙江进出口商品检验局负责起草。

本标准主要起草人：陈笑梅、施旭霞。

本标准系首次发布的行业标准。

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1 范围

本标准规定了出口肉及肉制品中莫能菌素残留量检验的抽样、制样和液相色谱测定方法。

本标准适用于出口鸡肉中莫能菌素残留量的检验。

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，则可用灭菌锋利刀在抽出的包件中，每件割取不少于 100 g，混合后置于洁净容器内，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。

2.4 试样制备

从所取全部样品中取出有代表性样品约 1 kg，取可食部分经捣碎机充分捣碎均匀，均分成两份，分别装入洁净容器内作为试样。密封并标明标记。

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

用甲醇-水溶液提取试样中的莫能菌素，过滤。滤液用二氯甲烷进行液液分配提取。提取液经浓缩并经硅胶小柱净化。用二氯甲烷-甲醇洗脱，洗脱液经浓缩后用甲醇定容，供HPLC柱后衍生分析用。莫能菌素与香兰素反应后生成的衍生物，在520 nm波长处被检测，外标法定量。

3.2 试剂和材料

除另有规定外，试剂均为分析纯，水为蒸馏水。

3.2.1 甲醇：色谱纯。

3.2.2 二氯甲烷。

3.2.3 浓硫酸：密度 ρ 约1.84 g/mL。

3.2.4 磷酸：纯度85%。

3.2.5 香兰素：纯度≥99%。

3.2.6 氯化钠溶液：100 g氯化钠溶于1 000 mL水中。

3.2.7 提取液：甲醇-水(850+150)。

3.2.8 无水硫酸钠：650℃灼烧4 h，置于干燥器中备用。

3.2.9 莫能菌素标准品：已知纯度≥80%。

3.2.10 莫能菌素标准溶液：准确称取适量的莫能菌素标准品，用甲醇配成浓度为100 μg莫能菌素/mL的标准储备液，根据需要再用甲醇稀释储备液，配成适当浓度的标准工作液。

3.3 仪器和设备

3.3.1 高效液相色谱仪：配有紫外检测器和柱后衍生化装置。

3.3.2 捣碎机。

3.3.3 超声波浴。

3.3.4 锥形瓶：100 mL。

3.3.5 分液漏斗：250 mL。

3.3.6 旋转蒸发器：备温控水浴。

3.3.7 浓缩瓶：具磨口，100 mL。

3.3.8 硅胶小柱：Sep-Pak PT-硅胶小柱(2g)。

3.4 测定步骤

3.4.1 提取和净化

称取试样约10 g(精确至0.1 g)，置于100 mL锥形瓶中，加75 mL提取液，搅拌后，超声提取5 min。将提取液过滤到分液漏斗中，加50 mL氯化钠溶液，用2×35 mL二氯甲烷萃取。收集二氯甲烷层，过无水硫酸钠柱滤入浓缩瓶中。于45℃浓缩至干。加7 mL二氯甲烷以溶解残留物，过硅胶小柱(小柱预先用3 mL二氯甲烷浸润)，流速小于5 mL/min，再加3 mL二氯甲烷淋洗浓缩瓶，一并过柱，弃去流出液。用10 mL二氯甲烷淋洗小柱，弃去流出液。然后用5 mL二氯甲烷-甲醇(95+5)溶液洗脱柱上的莫能菌素。洗脱液收集于浓缩瓶中，在45℃旋转蒸发至干。用甲醇定容至1.0 mL，溶液供液相色谱测定。

3.4.2 测定

3.4.2.1 液相色谱条件

a) 色谱柱：μBondapak™C_{18}，300 mm×3.9 mm(内径)，5 μm，或相当的色谱柱；

b) 流动相：甲醇-水-磷酸(940+60+1)，0.7 mL/min；

c) 柱后衍生试剂：边搅拌边将20 mL浓硫酸慢慢加到950 mL甲醇中，冷却至室温，加30.0 g香兰素，搅拌至溶解。流速：0.7 mL/min；

d) 柱后反应室：体积>1.4 mL 不锈钢管 300 cm×1 mm(内径)，柱后反应温度：90℃；

e) 检测波长：520 nm；

f) 进样量：100 μL。

3.4.2.2 色谱测定

根据样液中莫能菌素含量的情况选定峰面积相近的标准工作溶液。标准溶液和样液中莫能菌素的响应值均应在仪器检测的线性范围内。标准溶液和样液等体积参插进样测定。在上述色谱条件下莫能菌素衍生物的保留时间约为 6.5 min。标准品色谱图见附录 A 中图 A1。

3.4.3 空白试验

除不加试样外，均按上述操作步骤进行。

3.4.4 结果计算和表述

按式(1)计算试样中莫能菌素的残留含量：

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

式中：X——试样中莫能菌素的残留含量，mg/kg；

A——样液中莫能菌素衍生物的峰面积，mm^2；

A_s——标准工作液中莫能菌素衍生物的峰面积，mm^2；

c——标准工作液中莫能菌素的浓度，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

本方法的测定低限为 0.020 mg/kg。

4.2 回收率

鸡肉中莫能菌素添加浓度及其回收率的实验数据：

在 0.020 mg/kg，回收率为 94.6%；

在 0.050 mg/kg，回收率为 93.6%；

在 0.200 mg/kg，回收率为 95.7%。

附 录 A
（提示的附录）
标准品色谱图

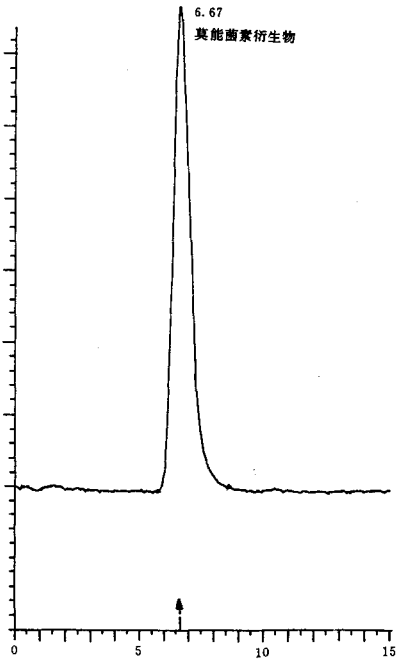


图 A1 莫能菌素标准品衍生物的液相色谱图

Foreword

The standard was drafted in accordance with the requirements of GB/T 1.1—1993"Directives for the work of standardization —Unit 1:Drafting and presentation of standards—Part 1: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 relevant domestic and foreign literatures through research, modification and verification. Besides, methods of sampling and sample preparation are also specified in this stardard.

The limit of determitationin in this standard is defined on the basis of the current international maximum limit for monensin residues in meats and meat products and the sensitivity of this 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 Zhejiang Import and Export Commodity Inspection Bureau of the People's Republic of China.

The main drafters of this standard are Chen Xiaomei and Shi Xuxia.

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 0698—1997

Method for the determination of monensin residues in meats and meat products for export —Liquid chromatographic method

1 Scope

This standard specifies the methods of sampling, sample preparation and determination by high performance liquid chromatography (HPLC) of monensin residues in meats and meat products for export.

This standard is applicable to the determination of monensin 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, 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

The number of packages specified in 2.2 are taken at random and opened one by one. From each package, 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.

In case the meat-pieces are not contained in small bags inside each package, or if there are small bags inside but the content of bag exceeds 2 kg, cut out a part from the meat-piece in each package not less than 100 g with a disinfected sharp knife. Mix the parts of the meat as the mixed primary sample, which shall not be less than 2 kg. Place in a clean container, seal, lable and sent to the laboratory in

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Implemented from Apr. 1, 1998

time.

2.4 Preparation of test sample

The mixed primary sample is reduced to 1 kg. The edible portions are thoroughly ground and homogenized in a meat grinder, then divided into two equal portions. Each portion is placed in a clean container as the test sample, which is sealed, labeled.

2.5 Storage of test sample

The test samples should be stored below −18℃.

Note: In the course of sampling and sample preparation, precautions must be taken to avoid contamination or any factors which may cause the change of residue content.

3 Method of determination

3.1 Principle

Monensin in the test sample is extracted with methanol-water and the extract is filtered and partitioned with dichloromethane. The dichloromethane extract is concentrated and cleaned up by passing through a silica gel cartridge. The analyte on the cartridge is then eluted with dichloromethane-methanol. The eluate is concentrated and made to a difinite volume with methanol. The solution is used for post-column derivatization-HPLC determination. Monensin is derivatized with vanillin, the derivative is detected at 520 nm and quantitated by external standard method.

3.2 Reagents and materials

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

3.2.1 Methanol: HPLC grade.

3.2.2 Dichloromethane.

3.2.3 Concentrated sulfuric acid: Density ρ ca 1.84 g/mL.

3.2.4 Phosphoric acid: Purity≥85%.

3.2.5 Vanillin: Purity≥99%.

3.2.6 Sodium chloride solution: Dissolove 100 g of sodium chloride and make up to 1000 mL with water.

3.2.7 Extraction agent: Methanol-water (850+150).

3.2.8 Anhydrous sodium sulfrate: Ignite at 650℃ for 4 h and keep in a desiccator.

3.2.9 Monensin standard: Known purity≥80%.

3.2.10 Monensin standard solution: Accurately weigh an appropriate amount of monensin standard and dissolve with methanol to prepare a standard stock solution of 100 μg monensin/mL in concentration. According to the requirment, dilute the stock solution with methanol to prepare a standard working solution of suitable concentration.

3.3 Apparatus and equipment

3.3.1 Liquid chromatograph: With UV detector and post-column derivatizer.

3.3.2 Meat grinder.

3.3.3 Ultrasonic bath.

3.3.4 Conical flask: 100 mL.

3.3.5 Separatory flask: 250 mL.

3.3.6 Rotary evaporator: With temperature-controlled water bath.

3.3.7 Evaporating flask: 100 mL, with ground stopper.

3.3.8 Silica gel cartridge: Sep-pak PT-silica gel cartridge (2 g).

3.4 Procedure

3.4.1 Extraction and cleanup

Weigh ca 10 g of the test sample (accurate to 0.1 g) into a 100 mL conical flask. Add 75 mL of the extraction agent, stir and extract for ca 5 min in an ultrasonic bath. Filter the extract into a separatory funnel, add 50 mL of sodium chloride solution and extract with 2×35 mL of dichloromethane. Collect the dichloromethane extracts and let pass through an anhydrous sodium sulfate column into an evaporationg flask, rotary-evaporated to dryness at 45℃. Dissolve the residue with 7 mL of dichloromethane and let the solution pass through a Sep-pak silica gel cartridge, which is prewashed with 3 mL of dichloromethane, at a flow rate of not more than 5 mL/min. Rinse the flask with an additional 3 mL of dichloromethane and pour the rinsings into the cartridge. Discard all the effluents. Wash the cartridge further with 10 mL of dichloromethane and discard the effluent. Then elute the monensin on the cartridge with 5 mL of dichloromethane-methanol (95+5) and collect the eluate in an evaporating flask. Rotary-evaporate the solution to dryness at 45℃. Dissolve and make up to 1.0 mL with methanol, the solution is used for HPLC analysis.

3.4.2 Determination

3.4.2.1 HPLC operating condition

a) Chromatographic column: μBondapak™ C_{18}, 300 mm×3.9 mm (id), 5 μm, or equivalent;

b) Mobile phase: Methanol-water-phosphoric acid (940+60+1), flow rate: 0.7 mL/min;

c) Reagent for post-column derivatization: While stirring gently, add slowly and carefully 20 mL of concentrated sulfuric acid to 950 mL of methanol. Allow the solution to cool to room temperature, add 30 g of vanillin and dissove by stirring for 5—10 min. Flow rate: 0.7 mL/min;

d) Post-column reaction chamber: 1.4 mL, stainless steel chamber, 300 cm×1 mm (id), reaction temperature: 90℃;

e) Detection wavelength: 520 nm;

f) Injection volume: 100 μL.

3.4.2.2 HPLC determination

According to the concentration of monensin in the sample solution, select the standard working solution with the peak area similar to that of sample solution. The responses of monensin derivative in the standard solution and the sample solution should be within the linear range of the instrumental detection. The standard solution should be randomly injected in-between the injections of sample solution of equal volume. Under the above operating condition, the retention time of monensin derivative is about 6.5 min. For the chromatogram of the standard, see fig. A1 in annex A.

3.4.3 Blank test

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

3.4.4 Calculation and expression of result

The calculation of result is carried out according to the formula (1):

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

where

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

A—the peak area of monensin derivative in sample solution, mm^2;

A_s—the peak area of monensin derivative in the standard working solution, mm^2;

c—the concentration of monensin in the standard working solution, $\mu g/mL$;

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

m—the correspoding 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 0.020 mg/kg.

4.2 Recovery

According to the experimental data, the fortifying concentrations of monensin in chicken meat and its correspoding recoveries are:

0.020 mg/kg, the recovery 94.6%;

0.050 mg/kg, the recovery 93.6%;

0.200 mg/kg, the recovery 95.7%.

Annex A

(informative)

Chromatogram of the standard

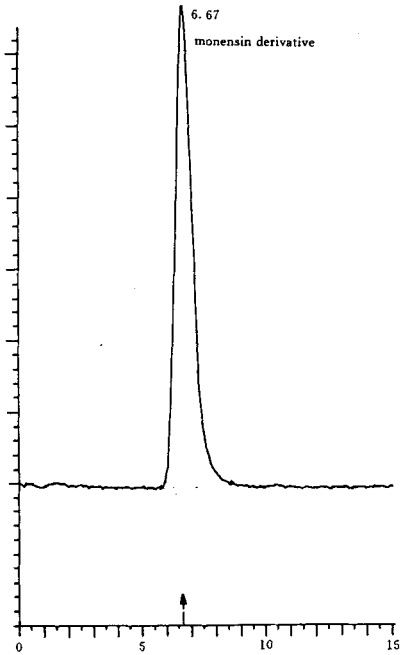


Fig. A1 High performance liquid chromatogram of the derivative of monensin standard