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中华人民共和国进出口商品检验行业标准

SN 0206—93

出口活鳗鱼中噁喹酸残留量检验方法

Method for determination of oxolinic acid residues in live eel for export

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1 主题内容与适用范围

本标准规定了出口活鳗鱼中噁喹酸残留量检验的抽样、制样和液相色谱测定方法。

本标准适用于出口活鳗鱼中噁喹酸残留量的检验。

2 抽样与制样

2.1 检验批和抽样数量

按出口活鳗鱼同一养殖池塘、同一规格鳗鱼为一检验批。随机抽取活鳗鱼 0.5 kg 作为实验室样品，标明标记并及时送实验室。

2.2 试样制备

送实验室待测活鳗鱼，将其窒息后，开膛，去除内脏，用捣肉机捣碎成浆状，然后按四分法分样、均分成两份，装入洁净容器内，作为试样。密封，并标明标记。

2.3 试样的保存

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

样品经捣碎后，用二氯甲烷提取，除去溶剂后，残渣用稀盐酸溶液和正己烷处理以去除脂肪，然后用二氯甲烷萃取。在减压下除去提取液中有机试剂，然后在重氮甲烷-乙醚溶液中甲基化，吹干，残留物溶于甲醇水溶液中，用液相色谱仪进行测定。

3.2 试剂和材料

除另有规定外，试剂均为分析纯，能蒸馏者须经重蒸馏；水指蒸馏水或相适应的去离子水。

3.2.1 乙醚。

3.2.2 二氯甲烷。

3.2.3 正己烷。

3.2.4 甲醇。

3.2.5 无水硫酸钠。

3.2.6 盐酸溶液：1 mol/L。

3.2.7 海砂：分析用。

3.2.8 重氮甲烷溶液配制：

将装有氢氧化钾水溶液(浓度：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，置于有磁力搅拌器加热板上的水浴中，水温 70 ℃，将搅拌子放入瓶中，接上滴液漏斗和高效冷凝器，

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冷凝器后串连两个 125 mL 的烧瓶。在二个烧瓶中各放 10 mL 乙醚，出口管均应插到乙醚液面以下。在冰水浴中冷却这两个接收瓶。边用磁力搅拌器搅拌，边通过滴液漏斗滴加盛有 21.5 g N-甲基-N-亚硝基-p-甲苯磺酰胺溶解于 140 mL 乙醚的溶液。滴完全部溶液的时间控制在 20 min 以上。当蒸馏液近于无色时，停止蒸馏。将两个接收瓶中的液体合并，在 70 ℃水浴上再蒸馏，其蒸馏液作为重氮甲烷溶液，此溶液应密闭置于冰箱中保存，保存期为一个月。

3.2.9 噁喹酸标准品：纯度>99%。

3.2.10 噁喹酸标准溶液：准确称取 10.0 mg 噁喹酸标准品溶于丙酮中，并在 100 mL 容量瓶中稀释至刻度。准确吸取该溶液 10 mL，用丙酮配成 1.0 μg/mL 的噁喹酸标准工作液。

3.3 仪器和设备

3.3.1 玻璃抽滤器。

3.3.2 砂芯漏斗，2 号或 3 号。

3.3.3 均质器或研钵。

3.3.4 旋转蒸发器。

3.3.5 液相色谱仪并配备紫外检测器。

3.3.6 微量注射器：10 μL 或 50μL。

3.4 测定步骤

3.4.1 提取和净化

称取经捣碎成浆的试样约 5.0 g(精确至 0.1 g)于研钵中，加入少量无水硫酸钠及海砂，经仔细研磨后用 60 mL 二氯甲烷分数次研磨并将试样溶液通过砂芯漏斗过滤。收集滤液于鸡心瓶中，置旋转蒸发器上浓缩，然后用氮气流吹至干。在残留物中加入 30 mL 1 mol/L 盐酸溶液，摇匀后，分二次，每次加入 20 mL 正己烷，振摇 5 min，分层后弃除正己烷层。下层酸性溶液，每次用 30 mL 二氯甲烷萃取二次。萃取液通过无水硫酸钠柱脱水，二氯甲烷萃取液经旋转蒸发器浓缩后，继用氮气流吹至溶剂完全蒸发除去为止。

3.4.2 甲基氮化

残渣用 1 mL 甲醇水(7∶3)溶解。加入 3 mL 重氮甲烷溶液，轻轻摇混后，在室温下反应 1 h。反应完毕后在减压下蒸干溶液，再用 1.0 mL 甲醇水(7∶3)溶解残渣。此溶液供液相色谱仪测定。

3.4.3 测定

3.4.3.1 色谱条件

a. 色谱柱：内径 4.6 mm、长 25 cm 不锈钢柱；

b. 固定相：YWG-G18，粒度 5 μm；

c. 流动相：甲醇：水＝7∶3(*V*/*V*)；

d 检测器波长：254 nm；

e 温度：室温。

3.4.3.2 色谱测定

分别将等体积标准工作液、样液注入液相色谱仪，测量其峰面积(或峰高)。

3.4.4 空白试验

除不加试样外，均按上述测定步骤进行。

3.4.5 结果计算和表述

按下式计算试样中噁喹酸的含量：

$$X = \frac{A \cdot c_s}{A_s \cdot c}$$

式中：*X*——试样中噁喹酸含量，mg/kg；

A ——样液中噁喹酸色谱峰面积(或峰高)，mm^2(或 mm)；

A_s——标准工作溶液中噁喹酸色谱峰面积(或峰高),mm^2(或 mm);

c_s——标准工作溶液中噁喹酸的浓度,μg/mL;

c——最终样液所代表试样的浓度,g/mL。

注:计算结果须将空白值扣除。

4 测定低限、回收率

4.1 测定低限

本方法的测定低限为 0.04 mg/kg。

4.2 回收率

回收率的实验数据:噁喹酸的浓度在 0.04～0.40 mg/kg 范围内,回收率为 92.0%～96.0%。

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进出口商品检验局负责起草。

本标准主要起草人陈志诚、黄伟坤。

主要参考文献:

The Inspection Method of Residues in Livestock and Aqualic Animal Foods, Japan, Vol. Ⅱ (March, 1982).

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

SN 0206—93

Method for determination of oxolinic acid residues in live eel for export

1 Scope and field of application

This standard specifies the methods of sampling, sample preparation and determination by liquid chromatography of oxolinic acid residues in live eel for export.

This standard is applicable to the determination of oxolinic acid residue content in live eel for export.

2 Sampling and sample preparation

2.1 Inspection lot and quantity of the sample taken

An inspection lot of live eel for export shall be of the same specification and bred in the same pond.

Take at random 0.5 kg from each lot as the test sample, label and send to the laboratory in time.

2.2 Preparation of test sample

Slash and eviscerate the eels after having expired, grind the sample in a mincer to mince, then reduce by quartering and divided into two equal portions. Each portion is placed in a clean container as test sample, which is sealed and labeled.

2.3 Storage of 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 factors which may cause the change of the residue content.

3 Method of determination

3.1 Principle

The oxolinic acid residues in eel is extracted with methylene chloride. After expelling the solvent, the residue is treated with dilute HCl and n-hexane to remove the fat. Repeat the extraction with methylene chloride. Remove the organic solvent under reduced pressure, then methylate with diazomethane in ether solution, evaporate to dryness and dissolve the residue in methanol-aqueous solution. The residue content is determined by liquid chromatography.

3.2 Reagents and materials

Unless otherwise specified, all reagents shall be of analytical grade and redistilled if distillabel; "water" is distilled water or corresponding de-ionized water.

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Implemented from Aug. 1, 1993

3.2.1 Ethyl ether.

3.2.2 Methylene chloride.

3.2.3 n-Hexane.

3.2.4 Methanol.

3.2.5 Anhydrous sodium sulfate.

3.2.6 Hydrochloric acid: 1 mol/L.

3.2.7 Sea sand: Reagent grade.

3.2.8 Preparation of diazomethane solution:

Add 35 mL ethanol, 10 mL potassium hydroxide solution (0.6 g/mL) and 10 mL ethyl ether into a double-mouthed distilling flask. Mount the flask in a water bath (70 ℃) on the hot plate with a magnetic stirring device. Place a magnetic stirring rod in the flask. Attach the flask with a dropping funnel and an efficient condenser and connect the latter in series with two 125 mL conical flasks. Place 10 mL of ether in each and let the tips of the outlet tubes dipped below the surface of the ether. Cool both receiving flasks in ice. Add dropwise a solution of 21.5 g N-methyl-N-nitroso-p-toluenesulfonamide in 140 mL of ether through the dropping funnel while stirring the contents of the flask with the magnetic stirring rod. It should take more than 20 min to add all the solution into the flask. When the distillate turns pale yellow, stop the distillation. Combine the contents of the two receivers, redistill the combined distillate in a 70 ℃ water bath, the distillate is the diazomethane solution. The solution shall be kept in an airtight container and preserved in a refrigerator not exceeding one month.

3.2.9 Standard oxolinic acid: Purity $>99\%$.

3.2.10 Oxolinic acid standard solution: Accurately weigh 10.0 mg standard oxolinic acid, dissolve in acetone and transfer with acetone to a 100 mL volumetric flask, and dilute to volume. Accurately pipet 10 mL of this solution, and further dilute with acetone to form a standard working solution with a concentration of 1.0 μg/mL oxolinic acid solution.

3.3 Apparatus and equipment

3.3.1 Glass suction flask.

3.3.2 Filter: Sintered glass, No. 2 or 3.

3.3.3 Homogenizer or mortar.

3.3.4 Rotary evaporator.

3.3.5 Liquid chromatograph equipped with UV detector.

3.3.6 Micro syringe: 10 μL or 50 μL.

3.4 Procedure

3.4.1 Extraction and clean up

Weigh ca 5.0 g (accurate to 0.1 g) paste sample in a mortar, add a small amount of anhydrous sodium sulfate and sea sand, grind with great care. Further grind the ground sample with 60 mL of methylene chloride in several portions and filter the solution through a sintered glass filter into a heart-shaped flask. Concentrate in a rotary evaporator, then evaporate to dryness under a stream of nitrogen. Add 30 mL of 1 mol/L HCl, shake and mix well. Add 20 mL of n-hexane, shake for 5 min, discard the n-hexane layer. Repeat once with 20 mL of n-hexane, discard the n-hexane layer. Extract the lower acid solution twice with 30 mL of methylene chloride each. Combine the extracts and dehydrate by passing through anhydrous sodium sulfate column. Evaporate the methylene chloride extract in a rotary evaporator and remove the solvent completely under a stream of nitrogen.

3.4.2 Methylation

Dissolve the residue with 1 mL of methanol aqueous solution (7 : 3), add 3 mL of diazomethane solution, mix by gently shaking, let react 1 h at room temperature. After the reaction is complete, evaporate to dryness under reduced pressure. Dissolve the residue again in 1 mL of methanol aqueous solution (7 : 3). The solution is ready for liquid chromatography.

3.4.3 Determination.

3.4.3.1 HPLC operating condition:

a. HPLC column: Stainless steel, 250 mm × 4.6 mm(id);

b. Fixed phase: YWG-G18, granule size: 5 μm;

c. Mobile phase: Methanol : water (7 : 3) (*V*/*V*);

d. Detector wavelength: 254 nm;

e. Temperature: Room temperature.

3.4.3.2 Chromatographic determination

Inject separately the standard working solution and the test solution of equal volume into the liquid chromatograph and measure the peak area (or peak height).

3.4.4 Blank test

Proceed by following the above procedure with the omission of the sample weighing.

3.4.5 Calculation and expression of the result

Calculate the content of oxolinic acid in the sample according to the following formula:

$$X = \frac{A \cdot c_s}{A_s \cdot c}$$

where

X—Content of oxolinic acid in the sample, mg/kg;

A—Chromatographic peak area (or peak height) of oxolinic acid in the sample solution, mm^2 (or mm);

A_s—Chromatographic peak area (or peak height) of oxolinic acid in the standard working solution, mm^2 (or mm);

c_s—Concentration of oxolinic acid in the standard working solution, μg/mL;

c—Concentration in term of sample mass in the final test solution, g/mL.

Note: The response value in 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 0.04 mg/kg.

4.2 Recovery

According to the experimental data, when the concentration of oxolinic acid is in the range of 0.04—0.40 mg/kg, the recovery is 92.0%—96.0%.

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 Import and Export Commodity Inspection Bureau of

the People's Republic of China.

This standard was mainly drafted by Chen Zhicheng and Wang Weikun.

Reference:

The Inspection Method of Residues in Livestock and Aqualic Animal Foods, Japan, Vol. Ⅱ (March, 1982).

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