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## **EAST AFRICAN STANDARD**

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**Test methods for fish and fishery products — Part 5: Determination of selenium**

**EAST AFRICAN COMMUNITY**

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## Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to meet the above objectives, the EAC Partner States have enacted an East African Standardization, Quality Assurance, Metrology and Test Act, 2006 (EAC SQMT Act, 2006) to make provisions for ensuring standardization, quality assurance, metrology and testing of products produced or originating in a third country and traded in the Community in order to facilitate industrial development and trade as well as helping to protect the health and safety of society and the environment in the Community.

East African Standards are formulated in accordance with the procedures established by the East African Standards Committee. The East African Standards Committee is established under the provisions of Article 4 of the EAC SQMT Act, 2006. The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

Article 15(1) of the EAC SQMT Act, 2006 provides that "Within six months of the declaration of an East African Standard, the Partner States shall adopt, without deviation from the approved text of the standard, the East African Standard as a national standard and withdraw any existing national standard with similar scope and purpose".

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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## Introduction

This method is applicable to fish tissues and various fish products. Selenium levels in the range between 0.05 µg and 800 µg may be determined. Higher selenium levels can be measured by making the appropriate sample dilution.

In the preparation of this East African Standard, the following sources were consulted extensively:

KS 1754-5:2003, *Test methods for fish and fishery products — Part 5: Determination of selenium*

CAC/RCP 52:2003(Rev. 4:2008), *Code of practice for fish and fishery products*

IS 4303-1:1975, *Code of hygienic conditions for fish industry — Part 1: Pre-processing stage*

IS 4303-2:1975, *Code of hygienic conditions for fish industry — Part 2: Canning stage*

Codex Alimentarius website: [http://www.codexalimentarius.net/mrls/vetdrugs/jsp/vetd\\_q-e.jsp](http://www.codexalimentarius.net/mrls/vetdrugs/jsp/vetd_q-e.jsp)

USDA Foreign Agricultural Service website: <http://www.mrlatabase.com>

USDA Agricultural Marketing Service website: <http://www.ams.usda.gov/AMSV1.0/Standards>

European Union: [http://ec.europa.eu/enterprise/sectors/pharmaceuticals/veterinary-use/maximum-residue-limits/index\\_en.htm](http://ec.europa.eu/enterprise/sectors/pharmaceuticals/veterinary-use/maximum-residue-limits/index_en.htm)

Assistance derived from these sources is hereby acknowledged.

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## Test methods for fish and fishery products — Part 5: Determination of selenium

### 1 Scope

This method is applicable to fish tissues and various fish products.

### 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

CAC/GL 21, *Principles for the establishment and application of microbiological criteria for foods*

CAC/RCP 1, *Recommended international code of practice — General principles of food hygiene*

CAC/GL 30, *Principles and guidelines for the conduct of microbiological risk assessment*

CAC/GL 31, *Guidelines for the sensory evaluation of fish and shellfish in laboratories*

CAC/GL 48, *Model certificate for fish and fishery products*

CAC/RCP 52:2003(Rev. 4:2008), *Code of practice for fish and fishery products*

EAS 38, *Labelling of prepackaged foods — Specification*

### 3 Principle

The sample is digested using nitric, perchloric and sulfuric acids. Interfering elements are masked with disodium EDTA. The selenium is complexed with 2,3-diaminonaphthalene, extracted into cyclohexane, and determined fluorometrically.

### 4 Interferences

Elements such as iron, copper and vanadium interfere. These are removed by using disodium EDTA.

### 5 Sampling procedure and storage

#### 5.1 Commercial shipment

Take a representative sample from the product lot and store as to maintain sample integrity.

#### 5.2 Survey samples

Fish may be either pooled or individual. For species normally greater than 30 cm in length, an individual fish may be used as a sample. For species less than 30 cm in length, a pooled sample is required. Store as to maintain integrity.

### 6 Sample preparation

#### 6.1 Commercial shipment

Sample preparation should take into account the type of product and how it is used and prepared by the consumer.

**6.1.1** For fish and fish products that contains no free liquid, comminute the sample until homogeneous.

**6.1.2** For products that are packed in water, brine or similar medium that is normally discarded by the consumer; open the package and drain the product on an appropriate size sieve for 1 min to 1½ min. Comminute the part of the sample retained by the screen until a homogeneous blend is obtained.

**6.1.3** For products that are packed in a medium that may be or is normally used by the consumer, e.g. fish canned in its own juice or oil; transfer the entire contents of the package into a homogenizer and blend for one minute or until a homogeneous mix is obtained.

## **6.2 Survey samples**

**6.2.1** For individual fish, weigh and measure the fork-length, i.e. from the nose to the fork of the tail, for size correlation.

**6.2.2** For a pooled sample, determine the average values for length and weight of the fish.

**6.2.3** Pass the skinned fillets through a commercial meat grinder a sufficient number of times to obtain a homogeneous blend (e.g. three times).

**6.3** Collect the homogenized sample into a thoroughly cleaned, sealable plastic pot or glass bottle. Store the sample in a refrigerator or freezer until required. Ensure that the prepared sample is still homogeneous prior to weighing. If liquid separates from the sample, thoroughly reblend before use.

## **7 Apparatus**

**7.1 Fluorometer**, filter fluorometer or spectrofluorometer capable of excitation at 366 nm and detection of fluorescence at 525 nm.

**7.2 Cuvets or pyrex culture tubes**, 12 mm x 75 mm, matched.

**7.3 Wrist-action shaker**

## **8 Reagents**

**8.1 Nitric acid (HNO<sub>3</sub>)**

**8.2 Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>)**

**8.2.1 Sulphuric acid solution (5 N)**. Dilute 140 ml H<sub>2</sub>SO<sub>4</sub> to 1 L with distilled water.

**8.3 Perchloric acid (HClO<sub>4</sub>)**, 70 %.

**8.4 Ammonium hydroxide (NH<sub>4</sub>OH)**

**8.4.1 Ammonium hydroxide solution (ca 6N)**. Dilute 400 mL NH<sub>4</sub>OH to 1 L with distilled water.

**8.5 Cyclohexane**

**8.6 Disodium ethylenedinitrilotetraacetate (EDTA)**

**8.6.1 EDTA solution (0.02 M)**, dissolve 7.445 g Na<sub>2</sub>H<sub>2</sub> EDTA.2H<sub>2</sub>O in distilled water and dilute to 1 l.

**8.7 2,3-Diaminonaphthalene (DAN)**

**8.7.1 2,3-Diaminonaphthalene solution (1 mg/ml)**, pulverize DAN in a mortar to pass through an 80-mesh sieve. Insert a glass wool plug in the stem of a 250 ml separatory funnel and add 100 ml 5 N H<sub>2</sub>SO<sub>4</sub>. Transfer 0.10 g DAN to separatory funnel and dissolve in the acid. Add 30 ml cyclohexane and shake 5 minutes. Let phases separate 5 min, drain lower phase into another separator and discard cyclohexane (upper) phase. Repeat cyclohexane extraction twice more and, after third

extraction, drain lower phase into a low-actinic glass stoppered bottle, add 1 cm of hexane and store in the cold.

## 8.8 Selenious acid ( $\text{H}_2\text{SeO}_3$ )

**8.8.1 Selenium standard solution (0.1 mg/ml).** Dissolve 0.163 4 g  $\text{H}_2\text{SeO}_3$  in distilled water and dilute with 0.1 N  $\text{H}_2\text{SO}_4$  to 1 l. Make further dilutions in 0.1 N  $\text{H}_2\text{SO}_4$  as required.

## 9 Procedure

**9.1** Place an accurately weighed sample containing 1.0 g dry matter and 800  $\mu\text{g}$  Se with 3 glass beads into a suitable container (100 ml Kjeldahl flask or 125 ml Erlenmeyer flask). Prepare appropriate working standards (such as 0, 0.2, 0.4, 0.6, and 0.8  $\mu\text{g}$  Se) and carry through entire procedure with sample and reagent blank.

**9.2** Add 6.0 ml conc  $\text{HNO}_3$  and heat cautiously until organic matter is in solution. Take care to prevent severe foaming or bumping.

**9.3** Add 2.0 ml 70 %  $\text{HClO}_4$  and 5.0 ml conc  $\text{H}_2\text{SO}_4$  and return to hot plate. Heat until solution turns greenish-yellow, then colourless. (If charring occurs, repeat analysis with new sample, using higher  $\text{HNO}_3/\text{HClO}_4$ /sample weight ratio. If this fails, add small amounts  $\text{HNO}_3$  at first signs of darkening.)

**9.4** Remove flask from heat, swirl contents carefully up the neck, replace flask on heater and continue heating until solution becomes colourless and white fumes appear.

**9.5** Remove flask from heat, let cool, add 1.0 ml 30 %  $\text{H}_2\text{O}_2$ , rinsing walls of flask, and swirl until fuming ceases. Resume heating until contents boil briskly and white fumes appear. Repeat addition of  $\text{H}_2\text{O}_2$  and heating twice more and continue final heating 10 min after appearance of white fumes. Let cool.

**9.6** Add 25 ml  $\text{H}_2\text{O}$ , rinsing walls and mixing thoroughly. Transfer quantitatively to 250 ml stoppered Erlenmeyer flask, using 3 ml x 10 ml  $\text{H}_2\text{O}$  rinses.

**9.7** Add successively with mixing, 10.0 ml EDTA solution, 25.0 ml 6 N  $\text{NH}_4\text{OH}$ , and 5.0 ml DAN solution.

**9.8** Quickly bring contents to a brisk boil with a Bunsen burner, transfer to a hot plate and continue boiling for two minutes.

**9.9** Let reaction mixture stand at room temperature for a definite interval between 1½ h and 2 h. Use same interval for sample, standards and blank.

**9.10** Accurately add a suitable quantity of cyclohexane (6 ml to 10 ml), stopper flask, and place on shaker for 5 min.

**9.11** Allow layers to separate. Draw upper cyclohexane layer into fluorometer cuvet using a Pasteur pipette. If necessary, centrifuge the solution to remove suspended water droplets.

**9.12** Zero fluorometer against reagent blank and read fluorescence of sample and standards.

## 10 Calculations

**10.1** Prepare a calibration curve of fluorescence versus nanograms Se in standards.

**10.2** Determine Se content by comparing sample reading with calibration curve. Take into account the sample weight and the dilution factor. Express the result in terms of total selenium on a wet weight basis (ppm).

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