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## **EAST AFRICAN STANDARD**

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**Test methods for fish and fishery products — Part 7: Determination of lead by atomic absorption spectrophotometry**

**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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East African Community

P O Box 1096

**Arusha**

Tanzania

Tel: 255 27 2504253/8

Fax: 255-27-2504481/2504255

E-Mail: [eac@eachq.org](mailto:eac@eachq.org)

Web: [www.each.int](http://www.each.int)

## Introduction

This procedure is applicable to the determination of lead in fish at levels of 0 µg/ml to 10 µg/ml. Lead content is determined by atomic absorption spectrophotometer at 283.3 nm. This method is reliable and procedures are easy to follow, it will thus go a long way in improving the quality of fishery products reaching the consumer.

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

KS 1754-7:2003, *Test methods for fish and fishery products — Part 7: Determination of lead by atomic absorption spectrophotometry*

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.mrlidatabase.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 7: Determination of lead by atomic absorption spectrophotometry

### 1 Scope

This procedure is applicable to the determination of lead in fish and fishery 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/RCP 52:2003(Rev. 4:2008), *Code of practice for fish and fishery products*

CAC/GL 53, *Guidelines on the judgement of equivalence of sanitary measures associated with food inspection and certification systems*

EAS 38, *Labelling of prepackaged foods — Specification*

### 3 Principle

The samples are dry-ashed at 500 °C, then dissolved in 1N HCl. Lead content is determined by atomic absorption spectrophotometry at 283,3 nm.

### 4 Interferences

Large excesses of other elements may interfere with the lead signal (e.g. 10 000 mg/l Fe enhances the lead signals).

### 5 Sampling procedure and storage

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

### 6 Sample preparation

Comminute the sample until homogeneous and place the homogenate in an acid-washed sealable plastic or glass container. Store in a freezer until required. Ensure the sample is homogeneous prior to weighing. If liquid separates from the sample, reblend before use.

### 7 Apparatus

**7.1 Atomic absorption spectrophotometer double beam**, equipped with a deuterium background corrector and capable of absorbance measurements at 283.3 nm and 217 nm, and having a range of 0 to 10 µg/ml.

**7.2 Hollow cathode or electrodeless discharge lead lamp**

**7.3 Crucible**, Porcelain, ca 50-mL capacity and 5-cm deep, or tall form Vycor or quartz beaker, 100 mL (*Kontes Glass Co., K319000*).

**7.4 Muffle Furnace**, capable of ashing samples at 500 °C.

**8 Reagents**

**8.1 Hydrochloric Acid, 1 N** — Dilute 82 mL HCl to 1 L with H<sub>2</sub>O.

**8.2 Nitric Acid** — Add 128 ml redistilled HNO<sub>3</sub> to 500 to 800 mL distilled or deionized H<sub>2</sub>O and dilute to 2 l. Redistilled HNO<sub>3</sub> (*G. Frederick Smith Chemical Co., No. 53*) may be diluted and used without redistillation.

**8.3 Lead Standard Solutions**

**8.3.1 Stock Solution, 1000 µg/ml** — Dissolve 1.5985 g recrystallized Pb(NO<sub>3</sub>)<sub>2</sub> in ca 500 ml 1 N HCl in 1 L volumetric flask and dilute to volume with 1 N HNO<sub>3</sub>.

**8.3.2 Working Solution 10 µg Pb/ml** — Pipet 10 ml stock solution into 1-l volumetric flask, add 82 ml HCl, and dilute to volume with H<sub>2</sub>O.

**8.4 Buffer solution** — Disperse 163 g EDTA in 200 ml H<sub>2</sub>O in 2 l volumetric flask and add enough NH<sub>4</sub>OH to dissolve. Dilute 60 ml 70.5% HClO<sub>4</sub> (CAUTION: See Clause 11) by pouring carefully into ca 500-ml H<sub>2</sub>O and cool. Dissolve 50 g La<sub>2</sub>O<sub>3</sub> in HClO<sub>4</sub> solution. Add 8 drops methyl orange indicator to ammoniacal EDTA solution and add La<sub>2</sub>O<sub>3</sub> to EDTA solution while stirring vigorously. If necessary, add NH<sub>4</sub>OH to maintain alkyl of above solution to methyl orange. Dilute to 2 l.

**9 Procedure**

**9.1** Test purity of reagents as follows: Evaporate 4-mL HNO<sub>3</sub> (CAUTION: See Clause 11) in crucible to dryness on hot plate or steam bath, dissolve residue in 1 N HCl and transfer to 25 ml volumetric flask. Heat residue again successively with two 5 ml portions 1 N HCl and add to flask. Cool, dilute to volume with 1 N HCl and mix. Proceed with determination. Total reagent blank should be < 10 µg Pb (equivalent to 0.4 ppm in sample) for determinations at levels > 1 ppm.

For determinations at < 1 ppm, follow the procedure as outlined in Annex A to attain blank at < 50% of limiting level of concern.

**9.2** Weigh ca 25 g (to nearest 0.1 g) sample into crucible. Dry for 2 h at 135 to 150 °C. Transfer to a cold temperature-controlled furnace and slowly raise temperature to 500 °C. Set control and maintain temperature at 500 °C as temperatures of 550 °C and higher may cause loss of Pb. Ash overnight (16 h).

**9.3** Remove sample, let cool to room temperature, cautiously add 2 ml HNO<sub>3</sub> and swirl.

**9.4** Evaporate carefully "just" to dryness on "warm" hot plate or steam bath. Transfer to cooled furnace, slowly raise temperature to 500 °C, hold at 500 °C for 1 h.

**9.5** Remove dish and cool.

**9.6** Repeat HNO<sub>3</sub> ashing, if necessary, to obtain clean, practically C-free ash.

**9.7** Add 10 ml 1 N HCl and dissolve ash by heating cautiously on hot plate. Transfer to 25 ml volumetric flask.

**9.8** Heat ash residue again successively with two 5-mL portions of 1 N HCl and add to flask.

**9.9** Cool, dilute to volume with 1 N HCl and mix.

## 10 Calculations

**10.1** Transfer 0, 1, 3, 5, 15, 25 and 50-ml Pb working solutions to separate 50-mL volumetric flasks and dilute to volume with 1 N HCl (0, 0.2, 0.6, 1.0, 3.0, 5.0 and 10.0 µg Pb/ml respectively).

**10.2** Set spectrophotometer to previously established optimum conditions for maximum signal at 283.3 nm. Use air-C<sub>2</sub>H<sub>2</sub> flow rates recommended by manufacturer for standard conditions for Pb.

**10.3** (a) For digital concentration readout, calibrate in concentration mode with solutions containing 0.2 and 10.0 µg Pb/ml. Record concentration directly after calibration of instrument.

(b) For strip-chart readout, set amplification to give > 1% absorption reading for 0.2 µg/ml working solution and prepare standard curve of absorption against µg Pb/ml.

**10.4** Use aliquot of prepared sample 10.2 to 10.9 and proceed as outlined in (a) or (b):

(a) Clear solutions — Determine absorption of sample and standard solution as outlined in 10.3 (a) or (b) using the following sequence 3 times:

Read the standard solution first; then, sample solution, alternating until all samples and standard solutions have been read. When many samples are to be analyzed, standard solutions may be read after a series of 3 samples instead of after each:

$$\text{ppm Pb} = [(\mu\text{g Pb/ml sample solution}) \times 25]/\text{g sample}$$

(b) Cloudy solutions — Proceed as for clear solutions, but add 1 ml buffer solution, 9.4, to aliquots of sample and standard solutions before reading.

If additional dilutions are necessary or buffer is added:

$$\text{ppm Pb} = (\mu\text{g Pb/ml diluted sample}) \times (\text{ml diluted sample/ml original aliquot}) \times (25/\text{g sample})$$

## 11 Remarks

**11.1 Glassware** — Wash all glassware with water, soak in nitric acid (1 + 2) followed by distilled water rinses.

**11.2 Perchloric Acid (HClO<sub>4</sub>)** — Contact with oxidizable or combustible materials or with dehydrating or reducing agents may result in fire or explosion. Persons using this acid should be thoroughly familiar with its hazards. Safety practices should include the following:

(a) Remove spilled HClO<sub>4</sub> by immediate and thorough washing with large amounts of water.

(b) Hoods, ducts, and other devices for removing HClO<sub>4</sub> vapour should be made of chemically inert materials and so designed that they can be thoroughly washed with water. Exhaust systems should discharge in safe location and fan should be accessible for cleaning.

(c) Avoid use of organic chemicals in hoods or other fume removal devices used for HClO<sub>4</sub> digestions.

(d) Use goggles, barrier shields, and other devices as necessary for personal protection; use polyvinyl chloride, not rubber, gloves.

(e) In wet combustions with HClO<sub>4</sub>, treat sample first with HNO<sub>3</sub> to destroy easily oxidizable organic matter unless otherwise specified. Do not evaporate to dryness.

(f) Contact of HClO<sub>4</sub> with strong dehydrating agents such as P<sub>2</sub>O<sub>5</sub> or concentrated H<sub>2</sub>SO<sub>4</sub> may result in formation of anhydrous HClO<sub>4</sub>, which reacts explosively with organic matter and with

reducing agents. Exercise special care in performing analyses requiring use of  $\text{HClO}_4$  with such agents. Extremely sensitive to shock and heat when concentration is  $> 72\%$ .

Also observe precautions outlined in (1) "Perchloric Acid Solution". Chemical Safety Data Sheet SD-11 (1965). Manufacturing Chemists Association of the US, 1825 Connecticut Ave., NW, Washington, DC 20009; (2) "Applied Inorganic Analysis", W.F. Hillebrand, G.E.F. Lundell, H.A. Bright and J.I. Hoffman, 2nd ed. (1953), pp. 39-40, John Wiley and Sons Inc., New York, NY; (3) "Notes on Perchloric Acid and its Handling in Analytical Work", Analyst 84, 214-216 (1959); (4) "Perchlorates", ACS Monograph No. 146, J.C. Schumacher, ed., Reinhold (1960).

**11.3 Nitric acid** — Reacts vigorously or explosively with aniline,  $\text{H}_2\text{S}$ , flammable solvents, hydrazine, and metal powders (especially Zn, Al, and Mg). Gaseous nitrogen oxides from  $\text{HNO}_3$  can cause severe lung damage. Copious fumes are evolved when concentrated  $\text{HNO}_3$  and concentrated HCl are mixed. Avoid premixing. Use effective fume removal device when fumes are generated. Handle with disposable polyvinyl chloride, not rubber, gloves.

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**Annex**  
(informative)

**Analytical considerations**

The analyst should decide whether nature of determination requires unusual care in purification of reagents, or whether blank determination will suffice. The smaller the amount of Pb to be detected, the greater the care required in reduction of blank.

To test suitability of reagents, place 10 to 15 g solid reagents dissolved in redistilled H<sub>2</sub>O or 15 to 20 ml concentrated acids previously neutralized with redistilled NH<sub>4</sub>OH in separator and add enough Pb-free citric acid to prevent precipitation by HN<sub>4</sub>OH of Fe, Al, alkaline, earth phosphates, or other substances. Make solution ammoniacal and add 2 to 3 ml 10% KCN solution. Shake solution with ca 5 ml dithizone solution, (5 to 10 mg/l). If lower layer is green, transfer it to another separator and extract excess dithizone with NH<sub>4</sub>OH (1 + 99) to which has been added drop of KCN solution. If CHCl<sub>3</sub> layer is colourless, consider test negative for use with dithizone methods.

When special purification becomes necessary, redistil H<sub>2</sub>O (distilled H<sub>2</sub>O stored in Sn-lined tanks usually contains Pb and Sn), HNO<sub>3</sub>, HCl, HBr, Br, and CHCl<sub>3</sub> in all-glass (pyrex) or quartz stills (preferably quartz). Prepare NH<sub>4</sub>OH by distilling ordinary reagent into ice-cold redistilled H<sub>2</sub>O. If stills are new, steam them out with hot HCl or HNO<sub>3</sub> vapors to remove "surface" Pb. (Subsequent distillates may not be totally Pb-free). Pb(NO<sub>3</sub>)<sub>2</sub> may be purified as follows: Dissolve 20 to 50 g in minimum of hot H<sub>2</sub>O and cool with stirring. Filter crystals with suction on small buchner, redissolve, and recrystallize. Dry crystals at 100 to 110 °C to constant weight. Cool in desiccator and store in tightly stoppered bottle. (Product has no H<sub>2</sub>O of crystallization and is not appreciably hygroscopic).

Purify citric acid, NaOAc or NH<sub>4</sub>OAc, Al(NO<sub>3</sub>)<sub>3</sub>, Ca(NO<sub>3</sub>)<sub>2</sub>, and Na<sub>2</sub>SO<sub>4</sub> by precipitating Pb from their aqueous solutions with H<sub>2</sub>S, using 5 to 10 mg CuSO<sub>4</sub> as coprecipitant (citric acid and Al(NO<sub>3</sub>)<sub>3</sub> solutions require adjustment with HN<sub>4</sub>OH to pH 3.0 to 3.5, bromophenol blue indicator). Filter (fritted glass filter is most convenient), boil filtrates 20 min to expel excess H<sub>2</sub>S, and refilter if necessary to obtain brilliantly clear solutions. Purify other reagents by recrystallization.

Store redistilled acids or purified solutions of reagents in teflon or conventional polyethylene containers carefully cleaned of surface Pb with hot HNO<sub>3</sub>. Paraffin-lined bottles may be used for alkaline reagents. Carefully clean new glass, plastic and chemical ware with hot 10% NaOH solution followed by hot HNO<sub>3</sub> and use only for Pb determinations.

In preparation of samples for analysis, avoid Pb contamination. If mixing or grinding is necessary, use porcelain mortar if possible. Avoid use of metal food grinders unless previous experiment has shown that no contamination of sample with Pb or Sn results. If product to be analyzed cannot be thoroughly mixed in its own container, or if composite sample of number of containers is desired, empty into large glass jar or porcelain dish and mix thoroughly with wooden spoon or porcelain spatula. If liquid portion of sample cannot be incorporated into ground solid material to obtain homogeneous mixture, analyze separately. If food is packed in tins having soldered seams (sardines and meats), open tins from bottom to avoid contaminating sample with bits of solder. Avoid sifting in preparation of samples to prevent metallic contamination or segregation of Pb.

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