



EAST AFRICAN STANDARD

Canned Salmon — Specification



EAST AFRICAN COMMUNITY

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 East African Standard for canned salmon defines minimum acceptability for taint, decomposition, unwholesomeness and other requirements, other than weight, and describes methods for determining that acceptability.

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

CODEX STAN 3:1981(Rev. 2:1995), *Standard for Canned Salmon*

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

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

Assistance derived from these sources is hereby acknowledged.

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Canned salmon — Specification

1 Scope

This standard applies to canned salmon in hermetically sealed containers. It is intended to be used for the inspection of canned salmon prepared from any of the following species:

- (a) *Oncorhynchus nerka* (sockeye salmon, red sockeye salmon, red salmon)
- (b) *Oncorhynchus tshawytscha* (spring salmon, king salmon, chinook salmon)
- (c) *Oncorhynchus kisutch* (coho salmon, medium red coho salmon)
- (d) *Oncorhynchus gorbuscha* (pink salmon)
- (e) *Oncorhynchus keta* (chum salmon, keta salmon)
- (f) *Oncorhynchus mykiss* (steelhead salmon, deep sea trout)
- (g) *Salmo salar* (salmon, Atlantic salmon)

Canned salmon shall be prepared from sound, wholesome raw material processed using good manufacturing practices.

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*

CD/K/572:2010, *Fish and fisheries products — Methods of sampling*

CAC/RCP 52[CD/K/521:2010], *Code of practice for fish and fishery products*

EAS 35, *Edible salt — Specification*

EAS 12, *Drinking (potable water) — Specification*

EAS 38, *Labelling of prepackaged foods — Specification*

EAS 41, *Fruits, vegetables and derived products — Sampling and methods of test*

EAS 103, *Schedule for permitted food additives*

EAS 123, *Distilled water — Specification*

ISO 4831, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique*

ISO 4832, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique*

ISO 4833, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of microorganisms — Colony-count technique at 30 degrees C*

ISO 6579, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Salmonella spp.*

ISO 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 6887-3, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 6888-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 1: Technique using Baird-Parker agar medium*

ISO 6888-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 2: Technique using rabbit plasma fibrinogen agar medium*

ISO 6888-3, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 3: Detection and MPN technique for low numbers*

ISO 7251, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique*

ISO 7937, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of Clostridium perfringens — Colony-count technique*

ISO 13720, *Meat and meat products — Enumeration of Pseudomonas spp.*

ISO 17239, *Fruits, vegetables and derived products — Determination of arsenic content — Method using hydride generation atomic absorption spectrometry*

ISO 6634, *Fruits, vegetables and derived products — Determination of arsenic content — Silver diethyldithiocarbamate spectrophotometric method*

ISO 21567, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp.*

ISO/TS 21872-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of potentially enteropathogenic Vibrio spp. — Part 1: Detection of Vibrio parahaemolyticus and Vibrio cholerae*

ISO/TS 21872-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of potentially enteropathogenic Vibrio spp. — Part 2: Detection of species other than Vibrio parahaemolyticus and Vibrio cholerae*

ISO 11290-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 1: Detection method*

ISO 11290-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 2: Enumeration method*

3 Description

3.1 Product definition

Canned Salmon is the product prepared from headed and eviscerated fish of any of the species listed in Clause 1 from which the fins and tails have been removed, and to which salt, water, salmon oil and/or other edible oils may have been added. Canned salmon may be prepared from fish which is fresh, frozen, cooked or smoked.

The name of the product shall be that recognized in common usage and in accordance with any requirements of the applicable Codex Alimentarius Recommended International Standard. Descriptive terms shall be used where necessary to accurately describe the contents of the can.

3.2 Process definition

Canned salmon is packed in hermetically sealed containers and shall have received a processing treatment sufficient to ensure commercial sterility.

3.3 Presentation

3.3.1 Regular pack — Canned salmon shall consist of sections which are cut transversely from the fish and which are filled vertically into the can. The sections shall be packed so that the cut surfaces are approximately parallel with the ends of the can.

3.3.2 Chunk — A mixture of pieces of fish most of which have dimensions of not less than 1.2 cm in each direction and in which the original muscle structure is retained.

3.3.3 Flake, flaked or flakes — A mixture of particles of fish in which the muscle structure of the flesh is retained.

3.3.4 Minced, grated or shredded — A mixture of particles of fish that have been reduced to a uniform size and in which particles are discrete and do not comprise a paste.

3.3.5 Any other presentation shall be permitted provided that it:

- (i) is sufficiently distinctive from the forms of presentation aforementioned;
- (ii) meets all other requirements of this standard; and
- (iii) is adequately described on the label to avoid confusing or misleading the consumer.

3.4 Spring Salmon (*Oncorhynchus tshawytscha*) Colour Designations — In addition to the appropriate common name, canned salmon of the species *Oncorhynchus tshawytscha* may be designated as "red", "pink" or "white" to indicate the colour of the flesh in accordance with approved standards or relevant regulations.

4 Essential composition and quality factors

4.1 Salmon

The product shall be prepared from sound fish of the species in Clause 1 and of a quality fit to be sold fresh for human consumption.

4.3 Packing media

The product may be presented in one of the following packing media as appropriate to the species and style of pack, with or without permitted optional ingredients:

- a) **Own juice** — Fish packaged without added liquid.

- b) **Potable water** — In conformity with the requirements of EAS 12.
- c) **Spring water or mineral water** — Potable water from an underground source but not obtained from a public community water supply and which meets the requirements of EAS 13.
- d) **Vegetable broth** — The liquid arising from the cooking of sound wholesome vegetables in water and which may be prepared from one or more types of vegetables.
- e) **Olive oils** — In conformity with CODEX STAN 33, *Standard for olive oils and olive pomace oils*
- f) **Other vegetable oils** — Clear, refined, deodorized, edible vegetable oil in conformity with the relevant East African Standards.
- g) **Sauces** — A thickened liquid made from acceptable food grade ingredients giving a characterizing flavour and odour to the product.
- h) **Marinades** — A thin liquid made from acceptable food grade ingredients, usually containing a sweetener, an acid solution or an alcoholic solution, with or without spices, herbs, seasonings, vegetables and other condiments.
- i) **Fish oils** — Clear, refined, edible fish (marine) oil. The species from which the oil is derived should be noted on the product label.

4.2 Other ingredients

All other ingredients used shall be of food grade quality and conform to all applicable Codex standards.

4.3 Final product

Products shall meet the requirements of this standard when lots examined in accordance with Clause 10 comply with the provisions set out in Clause 9. Products shall be examined by the methods given in Clause 8.

5 Food additives

No additives are permitted in this product.

6 Hygiene and handling

6.1 The final product shall be free from any foreign material that poses a threat to human health.

6.2 When tested by appropriate methods of sampling and examination listed in Clause 2, the product:

- (i) shall be free from micro-organisms capable of development under normal conditions of storage; and
- (ii) shall not contain any other substance including substances derived from microorganisms in amounts which may represent a hazard to health; and
- (iii) shall be free from container integrity defects which may compromise the hermetic seal.

6.3 It is recommended that the product covered by the provisions of this standard be prepared and handled in accordance with the appropriate sections of CAC/RCP 1-1969 and CAC/RCP 52.

6.4 The material shall also satisfy the limits for heavy metals and microbiological activity prescribed in Table 1.

Table 1 — Microbiological and heavy metal limits for canned tuna and bonito

Type of contaminant		Maximum limit (mg/kg)	Method of test
(i)	Microbiological requirements	Shall be commercially sterile	See J.5.1: CD/K/551:2010
(i)	Arsenic	1.0	EAS 41
(iii)	Copper	0.4	EAS 41
(iv)	Tin	250.0	EAS 41
(v)	Mercury	0.5	EAS 41
(vi)	Lead	0.3	EAS 41
(vii)	Cadmium	0.3	EAS 41
(viii)	Zinc	50.0	EAS 41
(ix)	Histamine content, mg/100 crab meat, max	20.0	Annex B

7 Labelling

In addition to provisions of EAs 38 the following specific provisions apply:

7.1 Name of the food

The name of the product shall be the designation appropriate to the species of the fish according to the law, custom or practice in the country in which the product is to be distributed.

7.2 Packing medium

The packing medium shall form part of the name of the food.

7.3 Presentation

The presentation provided for in 3.2 shall be declared in close proximity to the common name.

8 Sampling, examination and analyses

8.1 Sampling

8.1.1 The sampling and tolerance plans in CD-K-572:2010 shall be used to determine the acceptability of the lot. The sampling plans dictate the minimum sample size to be taken. If necessary, in the opinion of the inspector, more than the minimum sample size specified may be taken.

8.1.2 Sampling of lots for the sensory examination of the product shall be in accordance with CD-K-572:2010 except that a lower acceptance number for decomposition shall be used as indicated in the sampling tables.

The tables specify the minimum number of sample units to be used for the following types of inspections:

- a) Level I — Sensory examinations of all products subject to inspection other than lots which are subject to reinspection.
- b) Level II — Sensory examinations of all products which are under reinspection.

8.1.3 The sample unit shall consist of a can of salmon and the entire contents thereof.

8.2 Sensory and physical examination

Samples taken for sensoric and physical examination shall be assessed by persons trained in such examination and in accordance with Annex A and the *Guidelines for the Sensory Evaluation of Fish and Shellfish in Laboratories (CAC/GL 31 - 1999)*.

8.3 Determination of net weight

Net weight of all sample units shall be determined by the following procedures:

- (i) Weigh the unopened container.
- (ii) Open the container and remove the contents.
- (iii) Weigh the empty container, including the end and any wrapping material, after removing excess liquid and adhering meat.
- (iv) Subtract the weight of the empty container and any wrapping material from the weight of the unopened container. The resultant figure is the net content.

8.4 Determination of drained weight for products packed with edible oils other than salmon oil

The drained weight of all sample units shall be determined by the following procedures:

- (i) Maintain the container at a temperature of between 20°C and 30°C for a minimum of 12 hours prior to examination.
- (ii) Open the container and distribute the contents on a pre-weighed circular sieve having a wire mesh with square openings of 2.8 mm x 2.8 mm.
- (iii) Remove all wrapping material and incline the sieve at an angle of approximately 17-20° and allow the fish to drain two minutes, measured from the time the product is poured onto the sieve.
- (iv) Weigh the sieve containing the drained fish.
- (v) Determine the weight of drained fish by subtracting the mass of the sieve from the mass of the sieve with drained product.

8.5 Examination methods

8.5.1 Complete external can examination. Open container and complete net weight determination, according to the defined policies and procedures for these examinations.

8.5.2 Examine appearance of product in container. Carefully remove fish from container to examination tray. Compare product form with standard product form. Inspect container contents for presence of foreign material or other undesirable parts, carefully separating fish as necessary.

8.5.3 Examine container interior for presence of foreign material, smut, struvite, and corrosion or other can interior defects.

8.5.4 Evaluate the colour to determine if there is discoloration indicative of rancidity and decomposition. Assess the odour, flavour and texture as required.

8.5.5 Record any defect for that unit on the appropriate worksheet.

9 Definition of defects

A sample unit will be considered defective when it exhibits any of the properties defined below.

9.1 Taint

A unit will be considered tainted when any of the following conditions are found:

- a) **Rancid** — Odour characterized by the distinct or persistent odour of oxidized oil; or
 Flavour characterized by that of oxidized oil which leaves a distinct bitter aftertaste.
 Discolouration indicative of rancidity.
- b) **Abnormal** — Distinct and persistent uncharacteristic odours or flavours such as iodine, burnt, acrid or metallic and not defined as rancid or decomposed; or
 Flavour or odour resulting from the improper addition or mixing of ingredients.
- c) **Excessive levels of sexual maturity** — Distinct and persistent and objectionable odours or flavours indicative of advanced sexual maturity (late-run fish).

9.2 Decomposition

A unit will be considered decomposed when any of the following conditions are found:

- a) **Odours and flavours** — Persistent, distinct, uncharacteristic and objectionable odour or flavour such as: fruity, vegetable, stale, musty, yeasty, sour, faecal, ammonia, hydrogen sulphide, bilge-like, putrid.
- b) **Discolouration** — Discolouration indicative of decomposition.
- c) **Texture** — Breakdown of muscle structure due to decomposition characterized by:
 - 1) excessively mushy flesh uncharacteristic of the species in the presentation; or
 - 2) excessively tough flesh uncharacteristic of the species in the presentation.

9.3 Unwholesome

- a) **Critical foreign material** — A lot will be considered defective when the following conditions are found:
 - the presence of any material which has not been derived from fish (or packing media) and which poses a threat to human health (such as glass, etc.); or
 - distinct and persistent odour or flavour of any material which has not been derived from fish (or packing media) and which poses a threat to human health (such as solvents, fuel oil, etc.).
- b) **Foreign material** — A unit will be considered defective when the following condition is found:
 - the presence of any material which has not been derived from fish (or packing media) but does not pose a threat to human health (such as insect pieces, sand, etc.).
- c) **Other defects** — A unit will be considered defective when any of the following conditions are found:
 - 1) **Struvite crystals** (magnesium ammonium phosphate crystals) — Any struvite crystal greater than 5 mm in length.
 - 2) **Sulphide blackening** (smut) — Staining affecting greater than 5% of the net contents.

- 3) **Undesirable parts** — Any combination of head parts, heads, tails and viscera exceeding 2% of the net weight of the intended pack.

10 Lot acceptance

A lot shall be considered as meeting the requirements of this standard when:

- (i) not any single instance of critical foreign matter occurs; or
- (ii) the total number of sample units found defective for taint, decomposition or unwholesomeness, individually or in combination, does not exceed the acceptance number for the sample size designated in the sampling plans in CD-K-572:2010; or
- (iii) the total number of sample units found defective for decomposition does not exceed the acceptance number (c) shown in parentheses for the sample size designated in the sampling plans in CD-K-572:2010; or
- (iv) the average net weight and the average drained weight of all sample units examined is not less than the declared weight and provided there is no unreasonable shortage in any individual container;
- (v) the Food Additives, Hygiene and Labelling requirements of Sections 5, 6.1, 6.2, and 7 are met.
- (vi) the total number of sample units found defective for standards of identity (style of presentation) and size designation or count range (if a size designation or count range is declared), does not exceed the acceptance number for the sample size designated in the sampling plans.



Example of canning

Standard



Example of canning



Draft for comments only



Canning salmon

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Standard

Annex A
(normative)

Sensory and physical examination

1. Complete external can examination for the presence of container integrity defects or can ends which may be distorted outwards.
2. Open can and complete weight determination according to defined procedures in Sections 7.3 and 7.4.
3. Examine product for discolouration, foreign and objectionable matter.
4. Assess odour, flavour and texture in accordance with the *Guidelines for the Sensory Evaluation of Fish and Shellfish in Laboratories (CAC/GL 31-1999)*

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Annex B (normative)

Determination of histamine

B.1 Introduction

B.1.1 Principle — Histamine is extracted with methanol and derivatized with o-phthalaldehyde (OPT) to generate the fluorescent product. This method is used to determine the histamine content in raw, pre-cooked, and canned tuna.

B.1.2 Interference — All methods of histamine determination are overwhelmed with interfering substances which have to be removed in order to accurately measure the histamine present. The two naturally occurring substances that cause the most interference are histamine and spermidine since they also react with OPT to form fluorescent products. However, spermidine, the major contaminant in extracts can be separated from histamine on cellulose phosphate cation-exchange columns. There is also variability due to the pH and temperature sensitivity of the o-phthalaldehyde-histamine fluorophor. Because of the ubiquity of interfering fluorophors, all reagents used must be of the highest obtainable purity. Exposure of any of the materials involved to rubber or silicones may produce erratic results. It is recommended that polyethylene labware be used in place of glass, due to an observed loss of fluorescence. All labware should be acid-washed and rinsed in distilled water prior to use. New solution must be prepared after four to seven days, due to an observed increase in blank readings.

B.1.3 Summary of method — The histamine-containing material are homogenized and extracted with methanol. The extract can then be passed through an anion exchange column to remove any remaining interfering substances. The elutant is reacted with the OPT reagent and allowed to stand for 4 minutes. The mixture is acidified with H_3PO_4 and the corresponding fluorescence is read on a calibrated instrument.

B.2 Material required

TD-360 Min-Fluorometer with U.V. optical configuration of (P/N 36000-010) 10 mm x 10 mm Methacrylate fluorescence cuvettes (P/N 7000-959).

B.2.1 Labware — All re-usable labware (glass, polyethylene, Teflon etc.) should be cleaned by soaking in laboratory grade detergent and water for 4 h, rinsed with tap water, deionized water, and methanol. It is recommended that polyethylene ware be used due to absorbency observed when using glass.

B.2.1.1 Assorted Class A calibrated pipettes

B.2.1.2 Graduated cylinder — 100 ml.

B.2.1.3 Assorted Volumetric Flasks — For preparing dilution standards.

B.2.2 Chromatographic Columns (Kontes M.K 422250).

B.3 Reagents and standards

B.3.1 Ion Exchange Resin — Sigma 1 x 8-200, chloride form 100-200 mesh: or BioRad AG1- x 8, 50-100 mesh, chloride form, Cat. No. 140-1431, or equivalent.

B.3.2 Ion Sodium Hydroxide — Dissolve 40 g NaOH in 1 Litre of distilled water.

B.3.3 2.0N Sodium Hydroxide — Dissolve 80 g NaOH in 1 Litre of distilled water.

B.3.4 Histamine Dihydrochloride — MCB X 0440 or J.T. Baker 1-N330.

B.3.5 1.0N Hydrochloric Acid — Add 83 ml concentrated HCL to about 500 ml distilled water. Cool and bring to 1-litre volume with distilled water.

B.3.6 0.1N Hydrochloric Acid — Add 100-mL 1N HCl to about 500-mL distilled water. Cool and bring to 1-Litre volume with distilled water.

B3.7 Methanol Reagent Grade

B3.8 0.1 % o-phthalaldehydol (OPT reagent) — Phthalic dicarboxaldehyde (Aldrich, Milwaukee, WI), or o-phthaldialdehyde (Sigma, St. Louis, MO) $C_6H_4(CHO)_2$. F.W, 134.13. Dissolve 0.10 g OPT in 100-mL methanol. Store in an amber bottle and refrigerate when not in use. Prepare fresh weekly.

B.3.9 3.57N Phosphoric Acid — Add 121.8 ml of 85 % H_3PO_4 to about 500-mL distilled water. Bring to 1- litre volume with distilled water.

B.3.10 Histamine Standard Solution A, 1 mg Hm/ml — Weigh 0.1656 g of histamine dihydrochloride into 100-ml volumetric flask. Dissolve in, and dilute to volume with 0.1N HCl.

B.3.11 Histamine Standard Solution B, 10 µg Hm/ml — Dilute 1.0 ml Solution A to 100 ml with 0.1N HCL.

B.3.12 Histamine Standard Solution A1 (This is our control solution) — Dilute 1.0 ml Solution A to 100 ml with methanol.

B.3.13 Histamine Standard Solution C, 0.1 mg Hm/ml — Dilute 1.0 ml Solution B to 100 ml with 0.1N HCl.

B.3.14 Histamine Standard Solution D, 0.2 M 0.2 M Hm/ml — Dilute 2.0 ml Solution B to 100 ml with 0.1 N HCl.

B.3.15 Histamine Standard Solution E, 0.3 mg Hm/ml — Dilute 3.0 ml Solution B to 100 ml with 0.1N HCl.

NOTE Prepare Solution A and B monthly. Prepare Solutions C, D, E, and A1 weekly. Refrigerate solutions when not in use.

B.4 Preparation

B.4.1 Resin preparation

B.4.1.1 Place 20 g of ion exchange resin in a beaker.

B.4.1.2 Add 2 N sodium hydroxide to the resin in a ratio of 15 ml per gram of resin.

B.4.1.3 Mix well and allow the resin to settle for a minimum of 15 minutes, but no more than 30 minutes. Decant liquid and repeat with additional 2 N sodium hydroxide.

B.4.1.4 Wash resin thoroughly with distilled water to remove traces of the sodium hydroxide until pH is less than or equal to 8.5.

B.4.1.5 Slurry resin with distilled water and transfer to a funnel containing a fluted filter paper. Thoroughly wash with distilled water.

B.4.1.6 Transfer resin to a suitable container and make sure the distilled water level is above the resin level at all times.

B.4.2 Column preparation

B.4.2.1 Slurry sufficient prepared resin into each column to form a bed 8 cm in height. Maintain a liquid level above the top of the resin at all times.

B.4.2.2 Refill columns with fresh resin at least twice per week.

B.5 Instrument set-up

B.5.1 Check that light source and filter holder are installed in your TD-360 Mini-Fluorometer. Turn on the instrument and allow to warm-up. For additional assistance, refer to your TD-360 Operating Manual.

B.5.2 Blank with a reagent blank — Calibrate instrument with the prepared histamine standard Solution E. Enter standard value of 3 000 mg/lm. Remember later to divide all reading by 10 000 to get mg Hm/ml of sample.

B.5.3 Analyze Histamine Standard Solutions C and D like you would a sample. You now have a standard curve for your samples.

B.6 Procedure

B.6.1 Sample preparation

B.6.1.1 Blend fish in a warring blender with an equal weight of deionized water to produce a 1:1 slurry.

B.6.1.2 Transfer 10.0 g of the slurry to a 150-ml beaker. Add 40.0 ml of methanol and mix thoroughly.

B.6.1.3 Using Whatman No.1 filter paper, or equivalent, filter the mixture into a suitable container. If the filtrate is to be saved for later analysis, refrigerate in a closed container.

NOTE Evaporation of methanol from the filtrate can cause erroneous results.

B.6.2 Histamine Elusion

B.6.2.1 Pass 15-20-ml distilled water through the exchange column and discard.

B.6.2.2 Place a 50-ml volumetric flask containing 5 ml in HCl at the column outlet.

B.6.2.3 Pipette 1.0 ml of filtrate (methanol extract) onto the resin bed with 5.10 ml distilled water.

B.6.2.4 Immediately initiate column flow. Flow should be maintained at a rate grater than 3 ml/min.

B.6.2.5 When liquid level is slightly above the resin, add about 5-ml distilled water and allow it to flow through the resin. Repeat with distilled water in larger increments until total water through column is about 40 ml.

B.6.2.6 Discontinue Column Flow

B.6.2.7 Remove volumetric flask and bring to 50-ml volume with distilled water. Store column effluent in the refrigerator if necessary to postpone determination for more than 2 h.

B.6.3 Controls and blanks

B.6.3.1 At the beginning of a set of analysis, and again at the end, pass 1 ml of Solution A1 through one of the columns and proceed through the procedure as though it were a fish extract. Fluorescence readings should be very similar to Solution D reading. If readings are not within 20 per cent of Solution D, all analysis performed at the same time are suspect and should be repeated.

B.6.4 Histamine determination

B.6.4.1 Into separate 25-ml glass stoppered flask, pipette 5.0 ml of 0.1 HCl (Blank); Solutions C, D and E: and each diluted column effluent.

B.6.4.2 Add 10 ml 0.1N HCl to each flask.

B.6.4.3 Add 3 ml in NaOH. Mix thoroughly.

B.6.4.4 Within 5 minutes, add 1 ml OPT solution and mix thoroughly.

B.6.4.5 After exactly 4 minutes, add 3 ml 3.57 N H₃PO₄ and mix immediately.

B.6.4.6 Let solutions stand for 15-20 minutes and then determine the fluorescence intensities on the TD-360 Min-fluorometer. If a sample reading is greater than that of Solution E, dilute 25 ml of the column effluent to 100 ml with 0.1N HCl and proceed from B.6.4.1.

CAUTION! Fish with high salt content may cause problems with the resin necessitating more frequent changing of columns.

B.6.4.7 If sample dilution was necessary in B.6.4.6, multiply the obtained result by 4.

B.6.4.8 After all readings are obtained, divide all results by 10, 1 000 to get histamine concentration in mg Hm/ml

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