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EAST AFRICAN STANDARD

Shark liver oil for veterinary use — 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

The increasing awareness of the importance of adopting scientific methods in poultry and livestock management has promoted the development of better feeds for poultry and cattle. The administration of vitamins to livestock has become a regular practice which holds a promise of further expansion. Shark liver oil is the most important source of natural vitamin A. The production of shark liver oil has now established itself as an important industry which caters to the increasing needs of vitamin A in animal nutrition. As shark liver oil is deficient in vitamin D, it may be fortified with synthetic vitamin D which is oil soluble. With the formulation of this Standard, it is expected that shark liver oil for veterinary use fortified with vitamin D and of a uniform quality would be available.

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

IS 3336:1965(R2005), *Specification for Shark Liver Oil for Veterinary Use*

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.mrldatabase.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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Shark liver oil for veterinary use — Specification

1 Scope

This standard prescribes the requirements and the methods of test for shark liver oil for veterinary use fortified with vitamin D. This standard does not cover shark liver oil meant for human consumption.

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*

ISO 16035, *Animal and vegetable fats and oils — Determination of low-boiling halogenated hydrocarbons in edible oils*

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 101 *Food stuffs — Methods of determining of arsenic content*

EAS 103, *Schedule for permitted food additives*

EAS 123, *Distilled water — Specification*

EAS 291, *Animal and vegetable fats and oils — Sampling*

EAS 305, *Animal and vegetable fats and oils — Sample preparation*

EAS 306, *Animal and vegetable fats and oils — Determination of peroxide value*

EAS 307, *Animal and vegetable fats and oils — Determination of acid value and acidity*

EAS 308, *Animal and vegetable fats and oils — Determination of unsaponifiable matter*

EAS 309, *Animal and vegetable fats and oils — Determination of iodine value*

EAS 310, *Animal and vegetable fats and oils — Determination of refractive index*

EAS 311, *Animal and vegetable fats and oils — Determination of moisture and volatile matter*

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EAS 312, *Animal and vegetable fats and oils — Determination of insoluble impurities*

EAS 313, *Animal and vegetable fats and oils — Determination of saponification value*

EAS 314, *Animal and vegetable fats and oils — Determination of lead*

EAS 315, *Animal and vegetable fats and oils — Determination of copper, iron and nickel*

EAS 318, *Animal and vegetable fats and oils — Determination of soap content*

EAS 303, *Animal and vegetable fats and oils — Determination of solid fat content — Pulsed nuclear magnetic resonance method*

EAS 319, *Animal and vegetable fats and oils — Determination of melting point in open capillary tubes (slip point)*

3 Definitions

For the purpose of this standard the following definitions shall apply:

3.1

Acid value

The number of milligrams of potassium hydroxide required to neutralize the free fatty acid present in one gram of oil under the prescribed conditions.

3.2

Iodine value (Wijs)

The number of grams of iodine absorbed per 100 grams of the oil, when determined by using Wijs solution.

3.3

Saponification value

The number of milligrams of potassium hydroxide required to saponify completely one gram of the oil.

3.4

Unsaponifiable matter

The fraction of substances in the oil which is not saponified by caustic alkali, but is soluble in ordinary fat solvents.

4 Types

The shark liver oil for veterinary use shall be of two types, namely, Shark Liver Oil (D₂) for Veterinary Use, and Shark Liver Oil (D₃) for Veterinary Use.

5 Requirements

5.1 Preparation — The material shall be obtained from fresh or preserved shark livers. The oil may be diluted by using refined groundnut or cottonseed oil.

5.1.1 The synthetic vitamin D₂ or vitamin D₃ used for fortification shall conform to Pharmacopoeia grades.

5.2 Description — The material shall be of pale yellow to brownish yellow colour, clear, and the odour shall be characteristically fishy but not rancid.

5.3 Preservatives — No preservatives other than suitable oil soluble anti-oxidants shall be used.

5.4 Solubility — The material shall be slightly soluble in alcohol and miscible with ether, chloroform and light petroleum.

5.5 The material shall also comply with the requirements given in Table 1

Table 1 — Requirements for shark liver oil

S/No.	Characteristic	Requirements	Method of test
(1)	(2)	(3)	(4)
(i)	Iodine value (Wijs)	90 to 150	EAS 309
(ii)	Acid value, Max	3.0	EAS 307
(iii)	Saponification value	150 to 200	EAS 313
(iv)	Unsaponifiable matter, % w/w, Max	7.0	EAS 308
(v)	Vitamin A, IU/gram	1000 to 6000	
(vi)	Vitamin D ₂ , or vitamin D ₃ , IU/gram	100 to 600	

5.6 The level of contaminants in sardine oil shall conform to the limits specified in Table 2.

Table 2 — Limits for contaminants in shark liver oil

Contaminants	Maximum level	Test method
Insoluble impurities, % m/m	0.05	EAS 312
Soap content, % m/m	0.005	EAS 318
Iron, mg/kg	1.5	EAS 315
Copper, mg/kg	0.1	EAS 315
Lead, mg/kg	0.1	EAS 314
Arsenic, mg/kg	0.1	EAS 101
Nickel, mg/kg	0.1	EAS 315

5.7 Pesticide residues

The maximum levels of pesticide residues in edible fats and oils shall conform to the internationally accepted levels recommended by Codex Alimentarius Commission.

6 Packing

The material shall be packed in well-filled, well-closed amber coloured glass bottles, tins, galvanized steel drums or steel barrels.

7 Marking

7.1 Each container shall be clearly marked with the following particulars:

- a) Name of the product with brand name, if any;
- b) Vitamin A, IU/g;
- c) Vitamin D₂ or vitamin in D₃, IU/g;
- d) Name and address of the manufacturer;
- e) Batch number and date of manufacture; and
- f) Quantity in litres.

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7.2 Each container may also be marked with a Certification Mark.

8 Sampling

Representative samples of the material shall be drawn by the method prescribed in Annex B.

9 Tests

9.1 Tests shall be carried out as prescribed in the relevant annexes and the standards identified in Table 1 and Table 2.

9.2 Quality of reagents

Unless specified otherwise, pure chemicals and distilled water (see EAS 123) shall be used in tests.

NOTE 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

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Annex A (normative)

Estimation of vitamin A

A.1 Methods

The determination of vitamin A may be carried out either by the spectrophotometric method (A.2) or by the Carr-Price method (A.3). In case of dispute, the spectrophotometric method shall be used.

A.2 Spectrophotometric method

A.2.1 The vitamin content of the material shall be estimated by the spectrophotometric method. The potency of a preparation of vitamin A is calculated from measurements of its ultra-violet absorption spectrum and expressed in terms of the unit which is 0.344 µg of all-trans vitamin A acetate.

A.2.2 Apparatus

A.2.2.1 Spectrophotometer — any properly calibrated spectrophotometer with a source of ultra-violet light, capable of measuring absorptions in the ultra-violet region.

A.2.2.2 Cells for measuring absorbance in ultra-violet light — Matched quartz cells with one centimetre internal light path shall be used.

A.2.3 Reagents

A.2.3.1 Cyclohexane — Spectroscopic quality.

A.2.3.2 Light petroleum — with boiling range 40 °C to 60 °C, purified as described in A.2.3.2.1.

Light petroleum is allowed to stand in amber-coloured Winchester bottles with approximately 5 percent solution of potassium permanganate (KMnO₄) with occasional shaking for 2 to 3 days. The petroleum and the permanganate layers are separated in a big size separating funnel and the petroleum is then washed with water (3 or 4 times) for removal of any residual permanganate. The petroleum is then distilled and allowed to stand on dehydrated calcium chloride for 4 to 5 days with occasional shaking of the amber-coloured Winchester bottles. Before use for spectroscopy, the petroleum is again freshly distilled.

A.2.4 Procedure

A.2.4.1 Saponification — Weigh accurately from the vitamin A reference standard capsules 0.5 to 1.0 g and transfer it to the saponification flask. Reflux for 30 minutes with 40 ml of ethyl alcohol (95 percent by volume) and 7 ml of potassium hydroxide solution using all-glass apparatus (rubber stoppers and corks should not be used). The refluxing during saponification shall be carried out, preferably in the presence of an inert gas, such as nitrogen in order to avoid the deterioration of vitamin A due to oxidation. During saponification the reaction flask shall be wrapped with a blue cloth. Cool, add 30 ml of water and extract three times with 50-ml portions of ether in a separating funnel. Combine the ether extract in another separating funnel, add 100 ml of water through the ether layer without agitation. When good separation has taken place, after two minutes, remove the aqueous layer. Repeat the washing twice again without agitating. Shake vigorously with 30 to 50-ml portion of water, allow to separate, remove and discard the aqueous layer. If a somewhat resistant emulsion forms, add a few millilitres of saturated sodium chloride solution to eliminate or decrease this emulsion before discarding the aqueous portion. Wash with two additional portions of 30 to 50 ml of water. Again pour two portions of 100 ml of water through the ether layer and see that the final water wash is not alkaline to phenolphthalein. Dry the ethereal extracts with anhydrous sodium sulphate, filter quantitatively by repeated number of small washings over a sintered funnel and reduce the filtrate in volume under suction.

A.2.4.2 Determination — Dissolve an accurately weighed quantity of the material in sufficient cyclohexane or light petroleum so as to give a solution containing between 9 and 15 International Units per millilitre. Determine the wave length of maximum absorption.

E-2.4.2.1 Determine the wave length of maximum absorptions and measure the extinction coefficients. The E value corrected for irrelevant absorption shall be:

For cyclohexane

$$E_{\text{corr}} = 7(E_{326.5\mu\text{m}} - 0.422E_{312.5\mu\text{m}} - 0.578E_{336.5\mu\text{m}})$$

For light petroleum

$$E_{\text{corr}} = 7(E_{324\mu\text{m}} - 0.399E_{309.5\mu\text{m}} - 0.601E_{333.5\mu\text{m}})$$

A.2.4.2.2 Calculate E values for 1 percent concentration and 1 cm cell depth of the absorption cell and the vitamin content as given below:

$$E_{1\text{cm}}^{1\%} \text{ (corrected) at } 326.5 \text{ m}\mu\text{m} \text{ for cyclohexane}$$

$$E_{1\text{cm}}^{1\%} \text{ (corrected) at } 324 \text{ m}\mu\text{m} \text{ for light petroleum}$$

$$\text{Vitamin A content in IU} = E_{1\text{cm}}^{1\%} \text{ (corrected) at } 326.5 \text{ m}\mu\text{m} \times 1910 \text{ for cyclohexane}$$

$$\text{Vitamin A content in IU} = E_{1\text{cm}}^{1\%} \text{ (corrected) at } 324 \text{ m}\mu\text{m} \times 1825 \text{ for light petroleum}$$

A.3 Carr-Price method

A.3.1 Apparatus

A.3.1.1 Photo-electric calorimeter — with a direct reading deflection type galvanometer, suitable for measuring transmittance or absorbance at 620 m μ m.

A.3.2 Reagents

A.3.2.1 United States Pharmacopoeia (USP) Vitamin A Reference Standard — This is a solution of crystalline vitamin A acetate in cottonseed oil encapsulated in gelatin. Each capsule contains 250 mg of the solution equivalent to 0.75 mg or 2 500. International Units of vitamin A.

NOTE In case USP Reference Standard is not available, crystalline all-trans vitamin A acetate may be used for the preparation of these standards.

A.3.2.2 Absolute Alcohol or Isopropanol — of such spectral purity that when measured in 1-cm quartz cell against water, it shall show absorbance not greater than 0.01 between 350 and 320 m μ m and not greater than 0.05 at 300 m μ m.

A.3.2.3 Potassium Hydroxide Solution — 50 percent (w/v).

A.3.2.4 Diethyl Ether — peroxide free (redistilled over reduced iron).

A.3.2.5 Sodium Sulphate — anhydrous, granular. It shall not absorb vitamin A under conditions of use, and 10 percent (w/v) solution shall not be acidic to methyl red indicator solution.

A.3.2.6 Antimony Trichloride Reagent — prepared by dissolving 125 g of antimony trichloride in 300 to 400 ml of chloroform (A.3.2.7). Add 5 g of calcium chloride and filter while hot. Dilute the filtrate to 500 ml with chloroform:

A.3.2.7 Chloroform — Allow chloroform to stand on anhydrous calcium chloride for a few days. Distill, discarding the first and the last 10 percent.

A.3.3 Procedure

A.3.3.1 Saponification — Follow the procedure in A.2.4.1.

A.3.3.2 Preparation of the calibration curve — Evaporate a suitable aliquot of the ether solution of the unsaponifiable extract to about 5 ml. Evaporate off the remaining ether at low heat under reduced pressure. Take up the residue in sufficient chloroform to give a concentration having an absorbance of about 0.8 in the photo-electric colorimeter. From this solution make a series of dilutions in chloroform to give absorbance values of 80, 60, 40, and 20 percent of the original absorbance. Determine absorbance of the blue colour formed when 1-ml aliquot of each of these five solutions plus 1 ml of chloroform is treated with the volume of the antimony trichloride reagent that is suitable for the operation and hereinafter referred to as the fixed volume. The blue colour produced being transient, readings shall be taken within 15 seconds of its formation. The blank is adjusted to 100 percent transmittance using a tube, containing 2 ml of chloroform and the fixed volume of the antimony trichloride reagent.

Using a rectangular co-ordinate paper, plot the five absorbances obtained against known quantities of vitamin A and draw up the best smooth curve from the origin through these points. Do not attempt to draw straight line unless the curve is in fact a straight line with the origin at zero. For those instruments that provide other than straight-line curve, check this curve at frequent intervals. For those instruments that do provide straight-line calibration curve, make one reading of the reference solution with each set of sample readings to establish the curve. In the latter case re-establish the calibration curve whenever variation in the reagent or other variables in procedure occur.

A.3.3.3 Determination — Weigh accurately a quantity of the material containing 20 to 45 IU of vitamin A (not more than 5 g of the material) and then proceed as in A.3.3.1 and obtain the residue after evaporating the ether under moderate heat and reduced pressure. Dissolve the residue in a definite volume of chloroform so that 2 ml of the chloroform solution with the fixed volume of the antimony trichloride reagent would give an absorbance of about 0.5 to 0.2. Before using the antimony trichloride reagent, one percent (w/v) of acetic anhydride shall be added in order to avoid the formation of turbidity. Set the instrument at 100 percent transmittance with 2 ml of chloroform and the fixed volume of the antimony trichloride as blank. Place the tube containing 2 ml of the chloroform solution of the residue and add rapidly the fixed volume of the antimony trichloride solution. Record the maximum colorimeter reading within 15 seconds. Determine vitamin A in the tube from the standard curve and calculate units of vitamin A per gram of the sample.

Annex B
(normative)**Sampling shark oil for veterinary use****B.1 General**

It is very difficult to lay down detailed directions for sampling shark liver oil for veterinary use, that will encompass all conditions and circumstances which may confront the individual charged with the responsibility of taking the samples. There are many instances in which the experience and judgement of the individual should prevail. There are, however, certain general rules relating to the drawing, preparation, storage and handling of samples which should always govern if the sample is to be representative. These are described under A.2 to A.9.

B.2 General precautions in sampling

B.2.1 All sampling instruments should preferably be made of stainless steel; if made of copper, brass or bronze, these should be nickel-plated.

B.2.2 All sampling apparatus shall be clean and dry when used.

B.2.3 Samples shall not be taken in an exposed place. The samples, the material being sampled, the sampling instruments and containers for sample shall be protected from adventitious contamination. The test samples shall be placed in suitable clean and dry containers.

B.2.4 Samples shall be taken and stored in amber-coloured glass bottles in such a manner that they are protected from light and temperature fluctuations and other abnormal conditions.

B.2.5 Sample containers shall be so filled that the air space above the liquid level shall be not more than 5 percent of the capacity of the sample containers.

B.3 Sampling instruments

B.3.1 Sampling tubes — The recommended forms of sampling tubes are (a) closed-type sampling tubes, undivided or divided and (b) open type sampling tube.

B.3.1.1 Closed-type sampling tube, undivided (Figure 1) — It consists of two concentric metallic tubes closely fitted into each other throughout their entire length, so that one tube may be rotated within the other. Longitudinal openings of about one-third the circumference are cut in both tubes. In one position the openings in the two tubes coincide; the sampling tube is open when in this position and admits the material. By turning the inner tube through an angle of 180°, it becomes a sealed container. The inner tube may have a diameter of 20 to 40 mm and is undivided along its length to serve as a single container.

The two concentric tubes are provided with ports at their bottom ends, so placed that it becomes possible to drain the material contained in the instrument through them, when the longitudinal openings coincide. The length of the instrument should be such as to enable it to reach the bottom of the container being sampled.

The instrument is inserted closed, the material is admitted by opening it, and finally it is closed and withdrawn.

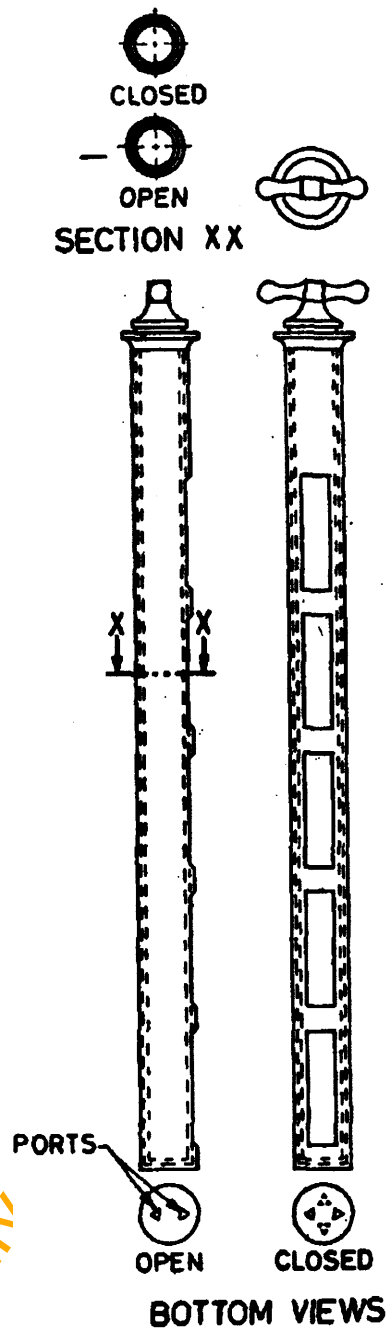


Figure 1 — Closed type sampling tube, undivided

B.3.1.2 Closed-type sampling tube, divided (Figure 2) — It is also of metal and has D-shaped cross-section. It is provided with compartments along its length and is opened and closed by means of a closely-fitting shutter which moves up and down throughout the entire length. It may be from 25 to 60 mm wide.

The instrument is inserted closed, the shutter is pulled out to admit the material, and the tube is then closed and withdrawn.

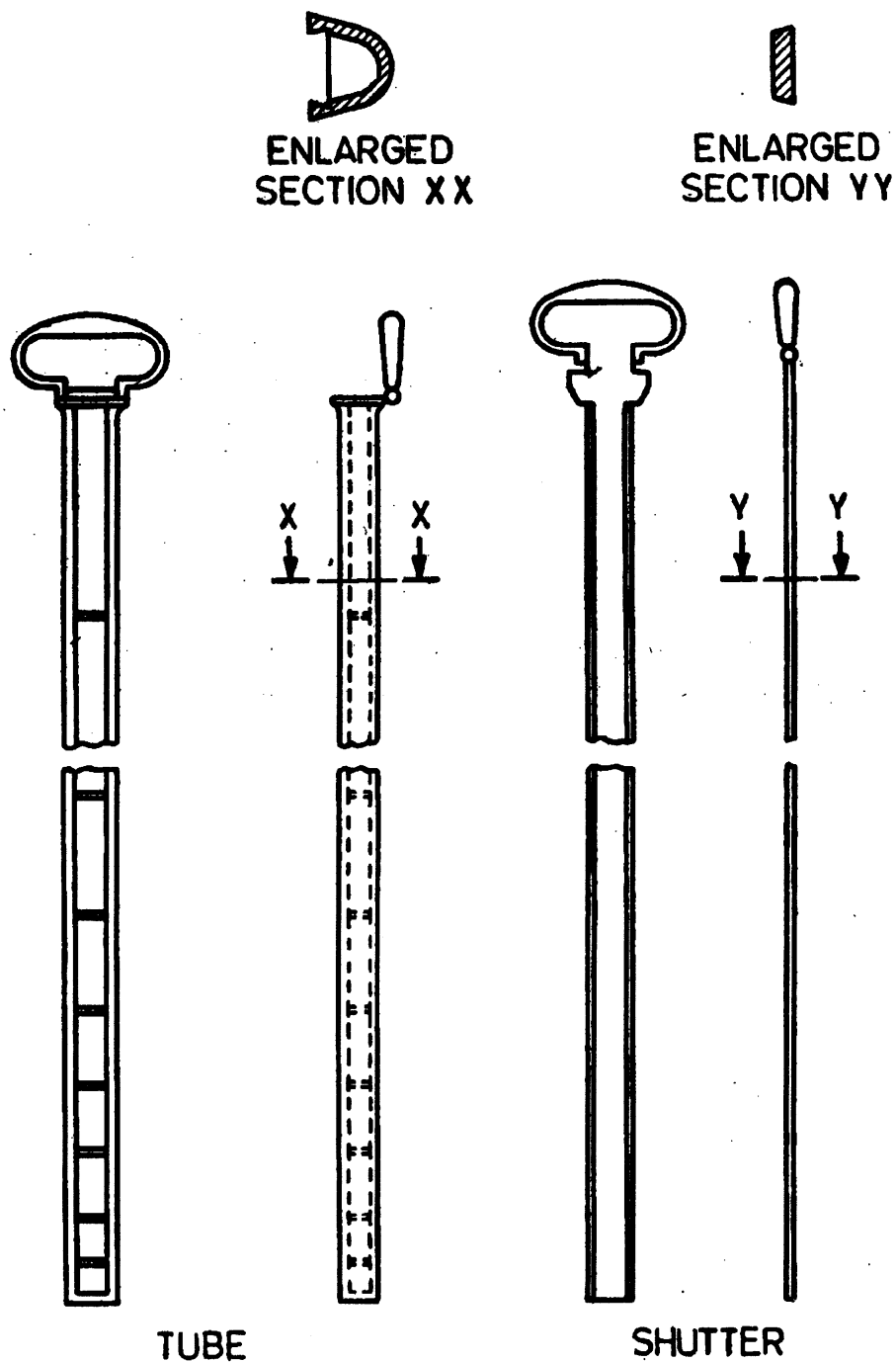


Figure 2 — Closed-type sampling tube, divided

B.3.1.3 Open-type sampling tube (Figure 3) — It is made of metal or thick glass, and may be of 20 to 40 mm diameter and 400 to 750 mm length. The upper and lower ends are conical and narrow down to 6 to 12 mm diameter. Handling is facilitated by two rings at the upper end. For taking a sample, the instrument is first closed at the top with the thumb or a stopper and lowered until the desired depth is reached. It is then opened for a short time to admit the material and finally closed and withdrawn.

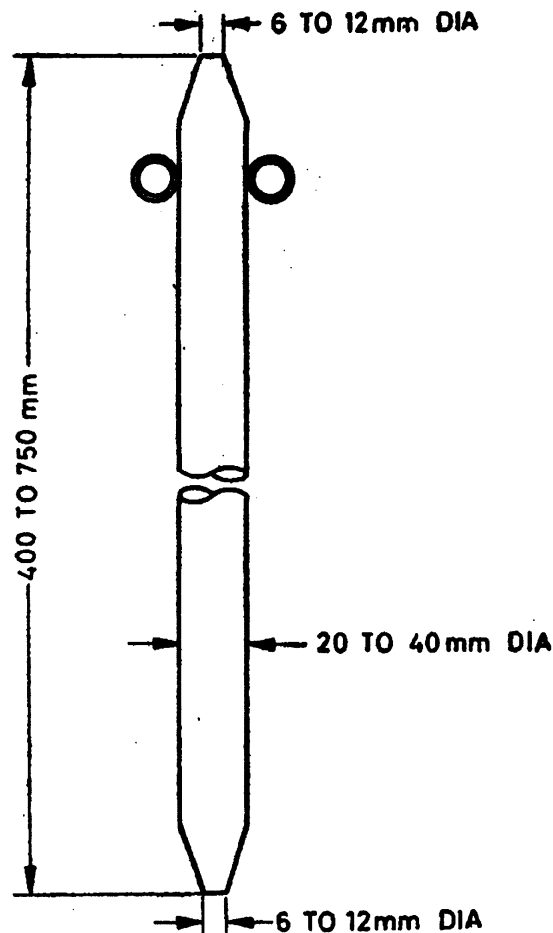


Figure 3 — Open-type sampling tube

B.4 Sample containers

B.4.1 The samples shall be packed in clean dry containers, preferably amber-coloured glass bottles. The sample containers should be almost but not completely filled. Glass bottles of 500-ml capacity are recommended for shark liver oil for veterinary use.

B.4.2 All sample containers shall be fitted with suitable tight stoppers. Rubber stoppers should not be used to close the containers. In the case of glass containers, glass stoppers or new good quality velvet corks should be used and, in the case of tin container, tin caps should be suitably soldered on the top.

B.4.3 The sample containers shall be sealed with scaling wax in such a manner that it is not possible to remove the contents and the label without breaking the imprint of the seal.

B.5 Scale of sampling

B.5.1 Lot — All the containers in a single consignment of one type of material drawn from a single batch of manufacture shall constitute the lot. If a consignment is declared to consist of different batches of manufacture, the batches shall be marked separately and the groups of containers in each batch shall constitute separate lots.

B.5.2 Gross sample — The general procedure for taking a gross sample is to draw a number of portions from all or several containers (B.5.2.1), and mix them. Representative portions of the gross sample shall be transferred to air-tight containers of suitable size for the test samples as described under B.7.

B.5.2.1 Gross Sample from Containers -When sampling from drums, barrels, etc, the container from which the samples are drawn shall be selected at random from the lot. The following schedule is recommended for the number of containers to be sampled:

Number of containers in the lot	Number of containers to be sampled
1 to 4	Each container
5 to 100	At least 20 percent, with a minimum of 4 containers
More than 100	At least 10 percent, with a minimum of 20 containers

B.6 Procedure

B.6.1 Before drawing samples, mix thoroughly the contents of each container in the gross sample, whether it is a drum, bottle, can or any other container, by shaking or stirring. Draw samples by inserting the sampling instrument through the bung hole or other opening from different portions of each container constituting the gross sample.

B.7 Test and referee samples

B.7.1 **Size of test samples** — The minimum size for each test sample shall be 500 ml.

B.7.2 **Preparation of test sample** — Normally, all the samples drawn as described under A.6 shall be put into a clean dry receptacle, and the contents of this receptacle shall be thoroughly mixed and at least four uniform samples (test samples) shall be drawn therefrom. One test sample shall be sent to the purchaser and one to the supplier.

B.7.2.1 The supplier shall have the right to be represented at the time of sampling.

B.7.2.2 The material left over after the preparation of the test samples shall be at the disposal of the supplier.

B.7.3 **Referee sample** — Two of the test samples bearing the seals of the purchaser and the supplier shall constitute the referee samples, to be used in case of dispute between the purchaser and the supplier, and shall be kept at a place agreed to between the purchaser and the supplier.

B.8 Test for acceptance

B.8.1 **Examination and Tests** - The purchaser may separately examine test samples of each of the separate qualities (A.5.2.1) for compliance with the requirements of the individual specification, or he may prepare, for the purpose of such examination and at any stage of the progress of the examination, a composite sample representing the whole of the consignment, by mixing the test samples.

B.8.2 **Criterion for Judgement** — The lot shall be considered as conforming to the requirements of this standard if the test sample satisfies all the requirements and passes all the tests. If two or more qualities are examined from a consignment and if one or more of them do not comply with the requirements of the specification for that particular material, the purchaser shall have the right to accept only that portion which complies with the requirements, or accept or reject the whole of the consignment.

B.9 Marking of sample containers

Each sample container after filling shall be sealed and marked with full details of sampling, the number of containers sampled, the date of sampling, and other particulars of the consignment.

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