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EAST AFRICAN STANDARD

Antibacterial liquid toilet soap — Specification

Part 2: Liquid

EAST AFRICAN COMMUNITY

Second Edition 2013

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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

Human skin provides a favourable environment for the existence and multiplication of a variety of microbes. The conventional toilet soap washes away the germs but does not kill them. The function of an antibacterial or antiseptic toilet soap is not only to clean the skin, but also to reduce drastically the bacterial count on the skin. This prevents skin infections and perspiration odour caused by the decomposition of perspiration by bacteria.

Antibacterial toilet soap is a toilet soap that has antibacterial agents incorporated into it. It not only cleans the skin, but also reduces drastically the bacterial count on the skin. This prevents skin infections and perspiration odour caused by the decomposition of sweat by bacteria. The antibacterial toilet soap is specially effective against *staphylococcus* and similar bacteria which have the habit of residing in the under layers of the skin. The antibacterial soaps have to be used regularly to be effective.

In this revision, hexachlorophene has not been permitted to be used as antibacterial agent. The Trichlorocarbanilide (TCC) on heating decomposes to chloroanalines which are harmful to skin hence the limit and method for determination of chloroanaline is included. Use of other antibacterial agents not included in Annex A will be considered when need arises as long as their safety is assured.

This edition cancels and supersedes EAS 766:2011, Antibacterial toilet soap — Specification.

Antibacterial toilet soap — Specification Part 2: Liquid

1 Scope

This East African Standard specifies the requirements, sampling and methods of test for antibacterial liquid toilet soap. It includes antibacterial (bacteriostatic) and antifungal (Fungal static).

The standard does not cover synthetic hand wash liquid detergents, shampoo and products for specific purposes such as those for industrial and surgical uses.

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.

DEAS 794 Determination of the microbial inhibition of cosmetic soap bars and liquid hand and body washes — Test method

ISO 456, *Surface active agents — Analysis of soaps — Determination of free caustic alkali*

ISO 685, *Analysis of soap — Determination of alkali content and total fatty matter content*

ISO 673, *Analysis of soap — Determination of matter insoluble in ethanol*

ISO 862, *Surface active agents — Vocabulary*

EAS 377-2 *Cosmetics — List of substances which must not form part of the composition of any cosmetic product*

EAS 377-3, *Cosmetics — List of substances which cosmetics must not contain except subject to the restrictions and conditions laid down*

EAS 127, *Synthetic laundry detergents for household use — Specification*

3 Definitions

For the purposes of this standard the terms and definitions given in ISO 862 apply.

4 Requirements

4.1 The antibacterial soap shall consist of essentially of an aqueous solution of potassium soaps, sodium soaps or both, made from oils, fatty acids or their mixture. It shall be a homogeneous, clear, translucent or opaque liquid with good lathering and cleaning properties. It may contain permissible synthetic detergents.

4.2 The antibacterial soap shall quickly form a satisfactory lather while in use.

4.3 The antibacterial soap shall remain as homogeneous stable product and shall show no sign of separation or sedimentation when kept at 5°C for 24h.

4.4 The antibacterial soap shall contain permitted antibacterial agent as per Annex A. The label shall clearly state the antibacterial agent used and its level.

4.5 The soap shall pass the antibacterial activity test when determined by the method given in DEAS 794.

4.6 The antibacterial toilet soap shall not contain any material listed as a prohibited material in EAS 377, Part 2 and 3 and subsequent amendments**.**

4.7 Antibacterial solid toilet soap shall pass the test for dermatological safety.

4.8 Specific chemical requirements

Antibacterial toilet soap, solid cake shall also comply with the specific chemical requirements specified in Table 1.

Table 1 — Specific chemical requirement for antibacterial liquid toilet soap

	Characteristic	Requirement	Method of test
1	Total fatty matter, percent by mass, min	15.0	ISO 685
2	Free caustic alkali, (K_2O), % by mass, max.	0.03	ISO 456
3	Synthetic detergents, percent by mass, max.	2.0	EAS 127
4	Matter insoluble in alcohol, percent by mass, max	5	ISO 673
5	Antibacterial agent Triclosan (TCN) and Trichlorocarbanilide (TCC), percent by mass, max	1 either singly or in combination	Annex B
6	Chloroaniline content, ppm, max	10	Annex C
7	Phosphate	absent	EAS 127
NOTE: Trichlorocarbanilide (TCC) is not heat stable and decomposes into chloro anilines on prolonged heating above 60 °C. If TCC is used in soap, the manufacturer should take care that such soap is not subjected to temperature above 60°C during the entire manufacturing process or during storage.			

5 Environmental precautions

Environmental precaution shall be adhered to during production and disposal as per the provisions of the relevant national legislations on prevention and control of pollution of water and air.

6 Packing and marking

6.1 Packing

The antibacterial soap shall be packed in protective containers that will not allow for damage of the product or its contamination.

6.2 Marking

Each container shall be marked legibly and indelibly with the following particulars:

a) name and address of manufacture, supplier or importer and/or trade mark if any

b) net weight of each container;

c) batch number or lot number;

d) date of manufacture and best before date;

e) list of ingredients in descending order of quantity

f) antibacterial agents used and their levels;

g) country of origin

7 Sampling

7.1 Preparation of test samples

For the purpose general precautions, scale of sampling and preparation of test samples shall be as prescribed in the relevant ISO standard.

7.2 Number of tests

7.2.1 Tests for determination of total fatty matter and free caustic alkali and matter insoluble in alcohol shall be conducted on each of the individual samples separately.

7.2.2 Tests for determination of all the remaining characteristics shall be conducted on the composite sample.

7.3 Criteria for conformity

7.3.1 For each of the characteristics which has been determined on the individual samples (see *7*.2.1) the mean (*X)* and the range *(R)* of the test results shall be calculated as follows:

Mean (X) = sum of test result/number of test result

Range *(R)* = the difference between the maximum and the minimum value of test results. The lot shall be deemed as conforming to the requirements given in 7.2.1 if the expression $(X - 0.6\ R)$ is greater than or equal to minimum value given in Table 1 and $(X + 0.6\ R)$ is less than or equal to maximum value given in Table 1.

7.3.2 For declaring the conformity of a lot to the requirements of other characteristics determined on the composite sample, the test results for each of the characteristics shall satisfy the relevant requirement.

Annex A
(informative)

Permitted antibacterial agents

The following is the list of antibacterial agents used generally in antibacterial soap:

A.1 Triclosan (TCN)

A.2 Trichlorocarbanilide (TCC)

A.3 Zinc oxide

A.4 Chloro xylenols

A.5 Plant extracts

A.6 Any other internationally accepted antibacterial agent

Annex B
(normative)

Determination of TCC and TCN in soaps by HPLC

B.1 Principle

TCC and TCN are antibacterial agents, which are separated from other components in soap by normal phase or reverse phase liquid chromatography, detected spectrophotometrically and quantified by comparison with standard TCC and TCN. The method can estimate as low as 1 ppm of the above compounds:

Procedures for both normal and reverse HPLC has been described and provide the option to use either method whichever is available to the users. Both methods are comparable.

B.2 Normal phase HPLC

B.2.1 Reagents

B.2.1.1 Iso-octane, HPLC grade.

B.2.1.2 Iso-propanol (2-propanol), HPLC grade

B.2.1.3 Hexane, HPLC grade.

B.2.1.4 Standard TCC, 99 % pure

B.2.1.5 Standard TCN, 99 % pure

B.2.2 Apparatus

B.2.2.1 High Performance Liquid Chromatograph consisting of a pump, a sample injector of fixed volume with UV detector having variable wavelengths and a recorder.

B.2.2.2 Standard volumetric flasks

B.2.2.3 Pipettes

B.2.2.4 Magnetic stirrer

B.2.2.5 Millipore filter apparatus with 0.5 µ filter

B.2.2.6 Column

B.2.2.6.1 Silica column, stainless steel 25 cm x 0.46 cm packed with Normal phase-silica 5 µ (Lichrosorb Si -60)

B.2.2.6.2 Cyano column, stainless steel 25 cm x 0.40 cm packed with (Lichrospher 100) cyano 5 µ.

NOTE Either, of the above columns can be used depending on the availability.

B.2.2.7 Mobile phase

B.2.2.7.1 For silica column — Transfer 20 ml of iso-propanol into a 500 ml volumetric flash and make upto mark with iso-octane and mix well. Assemble millipore filter apparatus and filter the solvent system prior to use.

B.2.2.7.2 For cyano column — Transfer 50 ml of HPLC grade iso-propanol (2-propanol) into a 500 ml volumetric flask, fill upto the mark with hexane and mix well assemble milllipore filter apparatus and filter the solvent system prior to use.

B.2.2.7.1 For silica column — Transfer 20 ml of iso-propanol into a 500-ml volumetric flask and make up to mark with iso-octane and mix well. Assemble millipore filter apparatus and filter the solvent system prior to use.

B.2.2.7.2 For cyano column — Transfer 50 ml of HPLC grade iso-propanol (2-propanol) into a 500-mL volumetric flask, fill up to the mark with hexane and mix well. Assemble millipore filter apparatus and filter the solvent system prior to use.

B.2.2.8 HPLC conditions

Detector wavelength flow rate:	280 nm
Flow rate:	0.5 ml/min
Injection volume:	20 µl

Retention time

Silica column
TCN - 7.5 min
TCC - 19.2 min

Cyano column
TCN - 4.0 min
TCC - 7.5 min

B.2.3 Procedure

B.2.3.1 Standard preparation (see note under B.3.4)

Weigh accurately 25 mg of triclosan (TCN) and 25 mg of TCC into a 100-ml volumetric flask and make up to volume with the mobile phase and mix well. Pipette 1.0 ml of this solution in a 50 ml volumetric flask and dilute with mobile phase. Final concentration of TCC and TCN is 250 µg/50 ml (5.0 ppm).

B.2.3.2 Sample preparation

Weigh accurately 1 g of homogenized sample into a 100-ml standard flask, and dilute to the mark with mobile phase. Pipette 10 ml of the supernatant liquid to a 50-ml volumetric flask, dilute with mobile phase, to the mark, and filter through 0.45 µm filter.

B.2.3.3 Chromatography

Equilibrate the column, maintained at a temperature of 30 °C, with the mobile phase with a flow rate of 0.5 ml /min for iso-octane - iso-propanol mobile phase and 1.0 ml/min for Hexane - iso-propanol mobile phase for 30 min. Set the wavelength at 280 nm. Inject 20 µl of standard solution and then sample solutions.

Measure area of the peaks of respective retention time for standard and sample.

B.2.4 Calculation

$$\text{TCN, percent by mass} = \frac{\text{Area of sample for TCN x Concentration of standard TCN}}{\text{Area of standard TCN x Concentration of sample}} \text{ x } 100$$

$$\text{TCC, percent by mass} = \frac{\text{Area of sample for TCC x Concentration of standard TCC}}{\text{Area of standard TCC x Concentration of sample}} \text{ x } 100$$

B.3 Reverse phase

B.3.1 Reagents

B.3.1.1 *Methanol* — HPLC grade.

B.3.1.2 *Sodium Dihydrogen Phosphate*

Monohydrate — Chemical grade.

B.3.1.3 Standard TCC

B.3.1.4 Standard TCN (TCS)

B.3 Reverse phase

B.3.1 Reagents

B.3.1.1 Methanol, HPLC grade.

B.3.1.2 Sodium Dihydrogen Phosphate Monohydrate, Chemical grade.

B.3.1.3 Standard TCC

B.3.1.4 Standard TCN (TCS)

B.3.2 Apparatus

B.3.2.1 Column

Octyldimethylsilyl (C-DB)

Supercosil LC-8-DB - 15 cm x 4.6 mm. 5 µ

B.3.2.2 Mobile phase

MeOH/0.01 M Phosphate buffer 62:38 *v/v*

0.01 M Phosphate buffer: Dissolve 1.38 g sodium dihydrogen phosphate monohydrate in 1 000 ml of distilled water. Prepare to pH 3.0 by 10 % phosphate solutions.

B.3.3 Procedure

B.3.3.1 Standard preparation (see Note under B.3.4)

B.3.3.1.1 Weigh accurately about 90 mg of TCN. Dissolve in methanol and make up to 1 000 ml volumetric flask with methanol.

B.3.3.1.2 Weigh about 110 mg of TCC, dissolve well with methanol, and make up the volume to 1 000 ml.

B.3.3.1.3 Accurately pipette 10 ml of the solution prepared in (B.3.3.1.1) into the (B.3.3.1.2) volumetric flask containing TCC. And make up to the volume with methanol. Then accurately pipette 5 ml of the solution into a 50-ml volumetric flask. Make up to the volume with methanol. Filter this standard solution through 0.45 µm filter.

B.3.3.2 Sample preparation

Weigh accurately about 1.0 g of product, dissolve in methanol and make up to 100 ml in a volumetric flask with methanol. Filter this sample solution through 0.45 μm filter.

B.3.3.3 HPLC conditions

Detector wavelength	280 nm
Column temperature	35 ºC
Flow rate	1.0 ml/min
Injection volume	10 µl

Prepare the standard solution and the sample solution at the same time. Inject the standard solution three times and calculate the average of each ingredients peak count. Inject 10 µg the sample solution and determine each ingredients percentage by the calculation shown.

B.3.4 Calculations

TCN, percent by mass = $\frac{(M_s \times A_r \times F)}{(A_s \times M_t \times 100)}$

TCC, percent by mass = $\frac{(M_s \times A_r \times F)}{(A_s \times M_t \times 100)}$

where

A_r is the peak area of the test sample,

A_s is the averaged peak area of the standard,

F is the purity of standard (percent).

M_s is the mass, in grams, of the standard, and

M_t is the mass, in grams, of the test sample,

NOTE Both TCC and TCN are photosensitive, hence standards should be freshly prepared.

Annex C
(normative)

Determination of chloroaniline

C.1 Principle

The chloroanilines are extracted from soap with dimethyl sulfoxide and diazotized with nitrous acid. The reaction products are then coupled with N-1-(naphthyl) ethylenediamine hydrochloride to produce coloured compounds which are estimated spectrophotometrically.

C.2 Safety precautions

Dimethyl sulfoxide (DMSO) is readily absorbed into the skin. Inhalation or skin penetration shall be avoided.

DMSO should never be pipette using mouth. Always use pipette bulb. The standard chloroanilines and N-1-(naphthyl) – ethylenediamine hydrochloride shall not be allowed to come into contact with the skin. If they should, then wash the contaminated parts thoroughly with soap and water.

A supply of diluted sodium hypochlorite should be at hand at all times to deal with accidental spillages of chloraniline solution. Spillage on laboratory surface should be treated immediately with the sodium hypochlorite solution, followed by water.

C.3 Reagents

C.3.1 Dimethyl Sulphoxide(DMSO), AR grade.

C.3.2 Hydrochloric Acid – Concentrated(specific gravity — 1.18).

C.3.3 Sodium Nitrite – 0.4 percent w/v analytical grade, freshly prepared (aqueous).

C.3.4 Ammonium Sulphamate, 2 % w/v solution freshly prepared, (aqueous).

C.3.5 N-1-(naphthyl) Ethylene, 0.1 % w/v solution diamine hydrochloride freshly prepared (aqueous).

C.3.6 n-Butanol, AR grade

C-3.7 Sand, acid purified 40 — 100 micron mesh.

C.3.8 Solvent mixture

DMSO	5 volumes
n-Butanol	2 volumes
distilled water	2 volumes
hydrochloric acid	1 volume

Mix n-butanol, water and HCL,. cool the mixture and add DMSO.

C.3.9 4-Chroroaniline and 3, 4-Dichloroaniline, AR grade.

C.4 Apparatus

C.4.1 Spectrophotometer, suitable for use at 554 nm

C.4.2 Cuvettes — Glass (matched pair) 10 mm

C.4.3 Water bath — Thermostatically controlled at 25 °C

C.4.4 Stop watch

C.4.5 Standard laboratory glassware

C.4.6 Filter Paper, Whatman No. 541

C.5 Procedure

C.5.1 Preparation of Calibration Curve

C.5.1.1 Dissolve 0.349 8 g of 3, 4-dichloroaniline and 0.2753 g of 4-chloroaniline in solvent mixture (see C.2.8) in a 250 ml amber volumetric flask.

Dilute to mark with solvent mixture. 1 ml = 2.5 mg mixed chloroanilines (stock solution).

C.5.1.2 Dilute this stock solution with solvent mixture as given below:

a) Take 5 ml of stock solution and dilute it to 250 ml with solvent mixture

   1 ml = 50 µg mixed chloroanilines.

b) Take 5 ml of the above solution [see C.5.1.2(a)] and further dilute to 250 ml with solvent mixture.

   1 ml = 1 µg mixed chloroanilines.

Use this solution for preparation of calibration curve.

Transfer using a burette 0, 1 ml, 2 ml, 5 ml, 10 ml, 20 ml, 40 ml into 50 ml amber volumetric flasks.

C.5.1.3 From a burette, add sufficient solvent mixture to make total volume to 40-mL in each flask. The flasks are incubated in a water bath at 25 °C for 20 min: After exactly 20 min, add 2-mL of reagent (see C.3.3) into each flask and return them to the water bath for exactly 10 min (measure with a stop watch).

Then add 2-mL of reagent (see C.3.4) into each flask and return them to the water bath for exactly 10 min. Swirl the flask occasionally.

Then add 2-ml of reagent (see C.3.5) into each flask and remove them from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 min. Measure absorbance at 554 nm against the blank solution as prepared in C.5.1.4.

C.5.1.4 In preparing the blank solution, take 40 ml of solvent mixture in a 50 ml amber volumetric flask. Incubate the flask in a water bath at 25 °C for 20 min. After exactly 20 min, add 2 ml of reagent (see C.3.3) into the flask and return it to the water bath for exactly 10 min. Then add 2 ml of reagent (see C.3.4) into the flask and return it to the water bath for exactly 10 min (swirl the flask occasionally). Then add 2 ml of reagent (see C.3.5) into the flask and remove it from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 min. Use this blank solution for preparation of calibration curve only.

C.5.1.5 Prepare a graph by plotting weight (µg) of chloroanilines contained in each 50 ml flask against absorbance. The linear calibration will pass through the origin/or determine the average absorbance (*AA*) of 1 µg of mixed chloroanilines by dividing sum of absorbances of all different aliquots of the standard by sum of µg of chloroanilines in all different aliquots of standard.

C.6 Determination of chloroanilines

C.6.1 Weigh to the nearest mg 3.0 - 15 g of finely grated soap add 10.0 g - 15.0 g of acid purified sand. Transfer quantitatively the sample and the sand into a mortar and grind the mixture thoroughly with a pestle to give a homogenous mass. Transfer the mass to a previously weighed 250 ml flat bottom flask quantitatively and reweigh. Add DMSO (100 ml), stopper firmly and attach the flask to an automatic shaker. Shake for 1 h. Filter the DMSO extract through Whatman No. 541 into a 250 ml amber volumetric flask. Wash the flask and filter paper with small aliquots of DMSO. Allow the filtrate to drain completely, dilute to volume with DMSO and mix. Transfer 20 ml DMSO extract into a 50 ml amber volumetric flask. Add 20 ml of solvent mixture. The flask is incubated in a water bath at 25 °C for 20 min. After exactly 20 min, add 2 ml of reagent (see C.3.3) into the flask and return it to the water bath for exactly 10 min (measure with a stop watch). Then add 2 ml of reagent (see C.3.4) into the flask and return it to the water bath for exactly 10 min (swirl the flask occasionally). Then add 2 ml of reagent (see C.3.5) into the flask and remove it from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 min. Read the absorbance at 554 nm against blank (prepared as below).

C.6.2 Prepare the blank solution by mixing 20 ml of DMSO extract of sample and 20 ml of solvent mixture in a 50 ml amber volumetric flask. Incubate the flask in a water bath at 25 °C for 20 min.

After exactly 20 min, add 2 ml of distilled water into the flask and return it to the water bath for exactly 10 min. Then add 2 ml of reagent (see C.3.4) into the flask and return it to the water bath for exactly 10 min (swirl the flask occasionally). Then add 2 ml of reagent (see C.3.5) into the flask and remove it from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 min. Use this solution as a blank for reading sample only.

C.6.3 Deduce the amount of chloroanilines (µg) from the calibration graph curve.

NOTE The determination should be completed in one day.

C.7 Calculations

Determine the amount of mixed chloroanilines in the aliquot of test solution from the calibration graph.

$$\text{Chloroaniline content (in ppm)} = \frac{250(M + M_1)M_3}{20M_2M}$$

where

M is the mass, in grams, of soap

M_1 is the mass, in grams, of sand

M_2 is the mass, in grams, of soap and sand transferred to the flask

M_3 is the mass, in micrograms, (µg) of mixed chloroanilines found from calibration graph/or it can be calculated as given below:

$$M_3 = \frac{\text{Mass of the sample}}{\text{Average absorbance of 1 } \mu\text{g mixed chloroanilines (AA)}}$$

where

$$\text{AA} = \frac{\text{Sum of the OD of the standards}}{\text{Sum of concentration of standard chloroanilines in } \mu\text{g}}$$

$$\text{Weight of soap actually used, in g} = \frac{M_2M}{(M + M_1)}$$