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EDICT OF GOVERNMENT

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EAS 766 (2011) (English): Antibacterial solid toilet soap – Specification

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EAST AFRICAN STANDARD

Antibacterial solid toilet soap — Specification

EAST AFRICAN COMMUNITY

First Edition 2011

Foreword

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

In order to achieve this objective, the Community established an East African Standards Committee mandated to develop and issue East African Standards.

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.

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.

EAS 766: 2011 was prepared by Technical Committee EAS/TC 000, *TC title*, Subcommittee SC 0, *SC title*.

Antibacterial solid toilet soap — Specification

1 Scope

This draft East African standard specifies the requirements and methods of sampling and test for antibacterial solid toilet soap.

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

IS 286, *Methods of sampling and test for soaps*

IS 1070, *Reagent grade water*

IS 4955, *Household laundry detergent powders*

IS 7597, *Glossary of terms related to surface active agents*

IS 13424, *Safety evaluation of bathing bar and toilet soap - Methods of test*

3 Terms and definitions

For the purposes of this standard the terms and definitions given in IS 7597 and the following apply.

4 Requirements

4.1 Description

Antibacterial solid toilet soap shall be a high grade, thoroughly saponified, milled soap or homogenized soap or both, white or coloured, perfumed, and compressed in the form of firm and smooth cakes, tablets or bars and shall possess good cleaning, lathering and antibacterial properties.

4.2 General requirements

4.2.1 Perfume, moisture, normal colouring matters, preservatives acceptable in toilet soaps may be added;

4.2.2 The soap shall contain permitted antibacterial agent as in Annex .....

4.2.3 The label shall clearly state the antibacterial agent used and its level.

4.2.4. Antibacterial solid toilet soap shall pass the test for dermatological safety when evaluated using IS 13424.

4.2.5 The soap shall pass the antibacterial activity test when determined by the method given in Annex A.

4.3 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 toilet soap, solid cake

1	Total fatty matter, percent by mass, min	76.0	
2	Rosin acids, percent by mass, of total fatty matter, *max*	3.0	
3	Free caustic alkali, as sodium hydroxide (NaOH), percent by mass, max	0.05	
4	Free carbonated alkali, as sodium carbonate (Na_2CO_3), percent by mass, max	1.0	
5	Matter insoluble in alcohol, percent by mass, max	2.5	
6	Antibacterial agent TricIosan (TCN) and Trichlorocarbanilide (TCC)	shall not exceed 1 % by mass either singly or in combination	Annex B
7	Chloroaniline content	shall not exceed 10 ppm	Annex C
8	Synthetic detergent	Shall be absent	IS 4955
9	Phosphate	Shall be absent	IS 286

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 product shall be packed in a suitable, well-closed container, to protect the integrity of the product during transportation and sale.

6.2 Marking

The packages shall be securely closed and marked with the following particulars:

a) name and physical address manufacture, supplier or importer and/or trade mark;
b) net weight ;
c) batch number; and
d) date of manufacture ;and

The following identified critical ingredients in descending order of quantity; percent by mass.

i) Total Fatty Matter (TFM);

ii) matter insoluble in alcohol; and
iii) antibacterial agent.

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 IS 286.

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
(normative)

Determination of antibacterial activity

A.1 General
Two methods have been prescribed, namely, serial dilution method and substantivity test. The serial dilution test shall be the screening test and the substantivity test shall be the absolute test.

A.2 Serial dilution test
A.2.1 Outline of the method

Antibacterial activity is determined by serial dilution method by comparing the effectiveness of antibacterial chemicals present in 10 micrograms of soap per milliliter specified as the maximum inhibitory concentration.

A.2.2 Apparatus

A.2.2.1 **Culture tube**, rimless, 150 mm x 18 mm

A.2.2.2 **Sterilized pipettes**, 10 mL, 5 mL and 1 mL capacities

A.2.2.3 **Loop,** made of stainless steel or platinum wire

A.2.2.4 **Conical flasks**, 250 mL capacity.

A.2.3 Nutrient Broth

A.2.3.1 Dissolve 5 g of beef extract, 5 g of sodium chloride, 10 g of peptone in one litre of distilled water by warming over a water bath. Cool and adjust the pH to 7.2 to 7.6 with sodium hydroxide solution. Distribute 9 mL each to the culture tubes. Plug the tube with non-absorbent cotton wool and sterilize in an autoclave for half an hour at 1 kg/cm^2 pressure.

A.2.3.2 Take 99 mL and 90 mL of distilled water in 250-mL conical flasks. Plug them with non-absorbent cotton wool and sterilize in an autoclave.

A.2.3.3 Get a pure stain of *Staphylococcus aureus,* ATCC 6538 P. Maintain on nutrient agar medium. Transfer to a fresh slant every month and keep in the cold. Use a 24 h nutrient broth culture for the experiment.

A.2.4 Procedure

A.2.4.1 Aseptically transfer 1 g of the soap sample to the flask containing 99 mL of water. Dissolve by slight warming not exceeding 60 °C. Transfer 10 mL of this solution to another flask containing 90 ml of water. Take 1 mL of this solution and add 9 mL of nutrient broth in a culture tube. This gives a concentration of 100 µg/mL.

A.2.4.2 To three tubes containing 9 ml nutrient broth add 1 mL each of the above solution to get a concentration of 10 µg/mL soap per ml of nutrient broth in each tube. Inoculate the tubes with a loopful of the 24 h culture of *Staphylococcus aureus* and keep them in an incubator maintained at 37 °C +- 2 °C. Keep a control tube of nutrient broth containing the same concentration of soap.

A.2.4.3 If after 24 h incubation period, the liquid in all the three tubes is as clear as the control, the soap sample passes the test. Any turbidity more than the control shows the growth of bacteria.

A.3 Substantivity test

A.3.1 Basic principles

For a soap to have antibacterial activity, it shall satisfy two criteria:

a) it shall show, antibacterial activity on the skin even after the soap is rinsed away, that is, the germicide should be retained on the skin under the conditions of use; and
b) the antibacterial activity should be retained on the skin for some period so as to provide protection to the skin.

The test devised gives a measure of both these properties. The test involves application of soap solution on the forearm, rinsing it off in running water and allowing it to dry. A mixed culture of skin flora isolated from five individuals (see A.3.2.1) is applied immediately in prescribed areas and assayed by swabbing at 0 and 10 min. The percent reduction in survivors in 10 min is determined. Similarly the soap solution after rinsing is allowed to remain on the skin for 2 h. The test micro-organisms are applied to the skin at this time in prescribed areas and assayed by swabbing at 0 and 10 min. The percent reduction in survivors is determined. If the reduction in survivors at this time is greater than 45 %, the germicide is said to be substantive.

A.3.2 Method

A.3.2.1 Test micro-organisms

The test organisms consist of a mixed skin flora, prepared by collecting washings from the arms and forearms of at least five individuals using 50 mL of sterile water in each case. Ten mL aliquot of each washing is individually inoculated into flasks containing 90 mL of sterilized nutrient broth. Culture is allowed to grow overnight at 30 °C and flask showing turbidity are pooled together. The mixed culture is transferred through broth and grown as above at least three times and finally maintained as Tryptone-Agar-Glucose Yeast Extract (TGYE) agar, Trypticase Soy Agar (TSA), Nutrient Agar (NA) or similar agar slants. For a test culture, an overnight slant culture is suspended into sterile saline and adjusted to a cell population of 1 x 107 cells per mL.

A.3.2.2 Test procedure

A.3.2.2.1 A number of 4 cm^2 areas (2 cm x 2 cm) are marked out on the innerside of the forearm. 0.1 mL aliquot of an 8 % soap solution with germicide is applied onto individual squares and allowed to dry for 1 min. The areas are then washed with a gentle flow of tap water for two min, dried by blowing warm air. The retentivity of the germicide on skin and its antibacterial action are then assayed by applying 0.1 mL of mixed skin flora (107 cells/mL) onto four such squares at 0 h. Two of the squares are swabbed immediately using standard sterile cotton swab on a stick. Swabs are placed in 5 mL saline solutions. Contents are shaken well in a vortex mixer and ten fold dilutions are prepared. Bacterial cells are assayed on TGYE agar, TSA or NA plates to determine the initial count. After 10 min, two other squares are swabbed and assayed in a similar manner.

A.3.2.2.2 In another set of tests, soap solutions are applied to the four more squares, rinsed and dried. After allowing 2 h interval, 0.1 mL of culture is applied as above to four squares. Two of the squares are swabbed and assayed at 0 h and remaining two after 10 min. Survivals at 0 h and after 2 h are determined.

A.3.2.2.3 The soap shall be considered to have passed the test if the percent kill is greater than or equal to 45 % after two hours challenge.

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 isopropanol into a 500 ml volumetric flash and make upto mark with iso-octane and mix well. Assemble milllipore 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 ~m 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.3.2 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 Jim 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

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

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

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 zone of inhibition

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-(naphthy 1) ethylenediamine hydrochloride to produce co loured compounds which are estimated spectrophotometrically.

C.2 Safety precautions

Dimethyl sulfoxide (DMSO) is readily absorbed into the skin. Inhalation or skin penetration must be avoided. DMSO should never be pi petted using mouth. Always use pipette bulb. The standard chloroanilines and N-1-(naphthyl)-ethylenediamine hydrochloride must 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 % 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 Il-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 HCI.

Cool the mixture and add DMSO.

C.3.9 4-Chloroaniline 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, I 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 50 011 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 2 011 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 (Jlg) 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 Jlg of mixed chloroanilines by dividing sum of absorbances of all different aliquots of the standard by sum of Jlg of chloroanilines in all different aliquots of standard.

C.6 Determination of chloroanilines

C.6.1 Weigh to the nearest mg 3.0-l5 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 Ittass to a previously weighed 250 011 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 w'ater bath for exactly 10 min (measure with a stop watch). Then add 2 m I 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.

Chloroaniline content (in ppm) = 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= Mass of the sample/Average absorbance of 1 µg mixed chloroanilines (AA)

where

AA is the sum of the 00 of the standards/Sum of concentration of standard chloroanilines in µg

Weight of soap actually used, in g = M2M/ (M + M1)

