# PS: 1721-2009 (R)

# PAKISTAN STANDARD SPECIFICATION

# FOR

# TOOTH PASTE (1ST REVISION)



**PAKISTAN STANDARDS AND QUALITY CONTROL AUTHORITY,**
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Karachi.

# PAKISTAN STANDARD
# FOR
# TOOTH PASTE (1ST Revision)

## 0. FOREWORD

0.1 This Pakistan Standard was adopted by the Pakistan Standards and Quality Control Authority on 11-02-2009 approved by the Chemical National Standard Committee and the draft finalized by the after the Cosmetics and Toilet Goods Technical Committee.

0.2 This Pakistan Standard, for Tooth Paste, was first published in 1985, which classifying toothpaste as opaque and non-opaque type. The Technical Committee, responsible for the preparation of this standard, reviewed it and decided; the classification has been done in to fluoridated & non-fluoridated types, included the requirements for microbiological properties and also include the important requirements for packing and labeling as per PS: ISO 22715

0.3 Tooth-paste, cream or gel when used with tooth brush in normal manner, assists in the removal of usual daily accumulations of debris and deposits from the exposed surface of the teeth without causing injury to the teeth and mucous membrane of the mouth. In addition, it may remove stains and odour. But it shall not scratch the enamel of the teeth.

0.4 While no stipulations have been made regarding the composition of tooth-pastes, cream or gel if it is essential that the toothpaste, cream or gel formulation does not contain any ingredient in sufficient concentration to cause a toxic or irritating reaction when used in mouth nor shall it be harmful in normal use, keeping in mind that small amounts may be ingested inadvertently.

0.5 In the preparation of this standard specification the views of the representative from the manufacturers, research institutions and consumers were sought. In addition the valuable assistance derived from the following International standards.

*ISO – 11609 – 1995 - Dentistry – Tooth pastes - Requirements, test methods & marking, BS– 5136 – 1981 – Specification for tooth pastes, MS – 527 - 1986 – ' tooth pasts', SLS – 275 – 1974 –"Tooth paste", ISO – 11969 – Water quality – Determination of Arsenic, ISO – 22715 – Cosmetics – Packaging and labeling.*

0.6 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with PS:103–1991(1st Revision) " Methods and rules for rounding off numerical value". The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

## 1. SCOPE

1.1 This Pakistan Standards specification prescribes the requirements, test methods and sampling, for physical and chemical properties and for the marking and labeling of tooth-paste for daily use by the Public with a tooth brush to promote oral hygiene. The tooth paste with the intended use other than general purpose (as defined under this standard) shall be allowed to subject to be subject under Drug Act 1996.

## 2. TYPES

2.1 The tooth-paste shall be classified into two type's i.e.

Type-1 – Non Fluoridated
Type-2 – Fluoridated

## 3 TERMS AND DEFINITION

For the purpose of this Pakistan Standards specification, the following definitions apply

**Dentifirice**: Any substance or combination of substances specially prepared for the public for cleaning the accessible surface of teeth.

**Toothpaste**: Any semi solid dentifrice preparation presented in the form of a paste, cream or gel.

**Composition**: - The tooth-paste shall not contain sucrose or other readily fermentable carbohydrates

***Toxicity***: - The tooth-paste shall not contain ingredients in sufficient concentration to cause a toxic or irritant reaction when used in the oral cavity, nor shall it be otherwise harmful in normal use.

**Fluoridated*: - Tooth*** pastes containing fluorides have been demonstrated effective in caries control but excessive fluorides contents can cause fluoride toxicity know as fluorosis.. Therefore fluoride ion in fluoridated toothpastes should not exceed the limit prescribed in this standard, see table 1

***Extrusion from tube***: - The bulk of the tooth-paste shall extrude from the tube at 25 ± 2°C in the form of a continuous mass with the application of normal force starting from the end of the tube. When cooled to a temperature of 5 °C for 1 hour, after taking out and pressing tube, the paste shall be found extrudable from the tube.

***Consistency****:* - The tooth-paste shall be free of lumps or particles which are palpable in the mouth as separates or discrete particles.

***Stability*: -** The tooth-paste shall be not segregate, ferment or physically deteriorate during normal conditions of storage and use. Test on stability done in accordance with appendix A

***Tube/Container:*** - The toothpaste shall be packed in collapsible tubes or any other suitable material which shall not corrode, deteriorates, or cause contamination of the tooth-paste during normal conditions of storage and use. The paste shall be examined visually by extruding part of the contents. The internal surface of the tube shall be examined after slitting it open and removing the remaining contents. Their should be no sign corrosion, chemical attack or damage.

**pH:-** The pH value of the Tooth Paste, determined at a temperature of 23 ± 1 °C in accordance with A-5 shall also comply with the requirements given in Table 1

**Alkalinity**:- When tested in accordance with A-5 the Tooth paste shall have a pH below 10.5.

**Abrasivity**: - Property of a material to wear away other material by friction and shall comply with the requirement given in Table 1.

**Microbial property**: - Testing of Microbiological contamination shall be carried out accordance to A- & A-11, shall also comply with the requirements given in Table 1.

**Heavy Metals**: - The maximum concentration of Heavy metals (Arsenic and Lead) in Tooth Paste shall not exceed the limits set in the table one and tested accordance with B-5 and B-9 respectively,

**Shelf Life/Date of Expiry**:- Should be declared by the manufacturer, time from the date of manufacture, during which a material retains the properties necessary for its prescribed purpose , 30 months after the manufacture. Should be mentioned on tube and carton.



## 4. REQUIREMENTS

### 4.1 Composition

4.1.1 A toothpaste shall not contain mono or disaccharides, for example, sucrose and other readily fermentable carbohydrates. A list of ingredients conventionally used in the manufacture of tooth paste

is given in Annex-B for information only. The dyes used in manufacture of toothpaste shall comply with the provisions of PS: 656 subject to provisions of Drugs Act 1996 issued by Government of Pakistan.

4.1.2 The Toothpaste shall not contain any type of steroids, manufacturers/Importer would be required to provide the declaration that their product (Toothpaste, Cream or gel) is free form steroids, on yearly basis.

Ingredients other than dyes shall comply with the provisions of PS: 656

4.1.3 The toothpaste shall also comply with the requirements given in Table 1 when tested according to the methods given in Annex B Reference to the relevant clauses of Annex B are given in col 5 of Table 1.

**TABLE –1**
**REQUIREMENT FOR TOOTH-PASTE**

| S.# | CHARACTERISTIC | REQUIREMENT | | Method Of Test (Ref. To Cl No. In Appendix B) |
|---|---|---|---|---|
| | | Non – Fluoridated | Fluoridated | |
| 1 | Hard and sharp-edged abrasive particles | Grit free | Grit free | Clause, A-2 |
| 2 | Spreadibility, cm, max | 8.5 | 8.5 | Clause, A-3 |
| 3 | Fineness<br>a) Particles retained on 150 micron PS Sieve (100 mesh), percent by mass, Max.<br>b) Particles retained on75 micron BS Sieve (200 mesh), percent by mass, Max. | 0.5<br>2.0 | 0.5<br>2.0 | Clause, A-4 |
| 4 | pH of aqueous suspension at 25$^0$C | 5.5 to 10 | 5.5 to 10 | Clause, A-5 |
| 5 | Stability at 25 ± 2 $^o$C | Should not detoriated | | Clause, A-6 |
| 6 | Fluoride ion (if present) ppm fluorine (1000 ppm fluorine correspond to 0.76% MFP, 0.2% NaF, 0.4% $SnF_2$ ) max | 50 | 1000 to 1500 | Clause, A-7 0r A-7.6 |
| 7 | **Heavy Metal**<br>a) Lead, (as Pb) mg/k, Max<br>b) Arsenic (as $As_2O_3$), mg/k, max. | <br>20<br>2 | <br>20<br>2 | <br>A-8<br>A-9 |
| 8 | **Microbial Property**<br>a) Total aerobic Bacteria per gram, max.<br>b) Gram Negative Pathogen per 10 grams | <br>800<br>Absent | <br>800<br>Absent | <br>A-10<br>A-11 |

*Note:* - If all the raw materials requiring test for heavy metals and arsenic have been so tested and comply with the requirement then manufacturer may not test the finished cosmetic for heave metals and arsenic.

## 5. PACKING AND MARKING

5.1 **Packaging: -** Tooth paste shall be packed in collapsible or in any other suitable container. When packed the tube shall be properly crimped and shall have a leak proof cap. There are two types of packaging i.e. Primary packaging and secondary packaging.

5.1.1 **Primary Packaging: -** Packaging designed to come into direct contact with the contents

5.1.2 **Secondary Packaging:-**Packaging designed to contain one or more primary packages and including any protective materials, if present

*NOTE: - for labeling purposes, the outermost packaging is considered to be the secondary packaging that holds or contains the package at the point of sale.*

**5.3** **Labeling: -**Information to be provided on the packaging**.**

- List of critical ingredients should be mentioned on the tube and carton. Minimum information to be provided on packaging in the general case
- Name and Address of the person responsible for placing the products on the market. It can be the manufacturer or his authorized Agent.

**5.4** **Marking: -** The Tubes and the cartons shall be legibly marked with the following information.

a. Name and type of toothpaste.

b. Name and address of the manufacturer or responsible distributor.

c. Net mass in gram, and/or net volume, in milliliter (ml)

d. Batch/or lot number

e. Fluoride ion content in ppm for Type 2:

f. Date of Manufacturing & Date of expiry.

g. Key ingredients of formulation; and

## 6. SAMPLING

Representative test samples of the material shall be drawn as prescribed in PS: 1720 – 1985 "Methods of Sampling cosmetics and toilet goods"

## APPENDIX – A.

## METHODS OF TEST FOR TOOTH-PASTE

### A-1. QUALITY OF REAGENTS

A-1.1 Unless specified otherwise, pure chemicals and distilled water (see PS: 593 – 1966) shall be employed in tests.

*NOTE: - "Pure Chemical" shall mean chemicals that do not contain impurities which affect the results of analysis.*

### A-2. TEST FOR HARD AND SHARP-EDGED ABRASIVE PARTICLES

A-2.1 ***Procedure***: - Extrude the paste about 15 to 20 cm on length each from at least ten collapsible tubes on a butter paper. Test the paste by pressing it along its entire length by a finger for presence of hard and sharp-edged abrasive particles. In all the cases, the material shall be free from hard and sharp-edged abrasive particles which will be distinguished readily from other particles.

### A-3 DETERMINATION OF SPREADABILITY

Weigh about 1 g of toothpaste at the centre of the glass plate (10 x 10 cm) and place another glass plate over it carefully. Place 2 kg weight at the centre of the plate (avoid sliding of the plates). Measure the diameter of the paste in cms, after 30 minutes. Repeat the experiment thrice and report the average.

### A-4 DETERMINATION OF FINENESS:

A-4.1 On 150 – micron PS Sieve

A-4.1.1 ***Procedure*: -** Place about 10g of the tooth-paste, accurately weighed, in a 100-ml beaker. Add 50 ml of water and allow to stand for 30 minutes with occasional stirring until the tooth-paste is completely dispersed. Transfer to a 150-micron equivalent to BS: Sieve and wash by means of a slow stream of running tap water and finally with a fine stream from a wash bottle until all the matter that can pass through the sieve. Let the water drain from the sieve and then dry the sieve containing the residue in an oven. If there is any residue on the sieve, carefully transfer it to a tarred watch glass and dry it to constant mass in an oven at 105 ± $2^0$C.

A-4.1.2 **Calculation**

$M_1$
M
Material retained on 150 – micron
PS: Sieve, percent by mass

Where
$M_1$ = mass in g of residue retained on the sieve, and
M = mass in g of the material taken for the test.

A-4.2 On 75 Micron BS Sieve

A-4.2.1 ***Procedure: -*** Weigh accurately about 10 g of the tooth-paste and processed as in A-2.1.1, using a 75-micron. BS Sieve. If there is any residue on the sieve carefully transfer it to be tarred watch glass and dry it to constant mass in an oven at 105 ± $2^0$C.

A-4.2.2 *Calculation: -* Calculate as in A-2.1.2.

A-5. **DETERMINATION OF pH**

A-5.1 ***Procedure*:** - Take 10g of the tooth-paste in a 150-ml beaker and add 100 ml of freshly boiled and cooled water (at $25^0$C). Stir well to make a thorough suspension. Determine the pH of the suspension with 5 minutes, using a pH meter.

A-6 **TEST FOR STABILITY**

A-6.1. **PROCEDURE**

A-6.1.1 Transfer suitable quantity of the composite sample into each of four glass tube. Stopper them and proceed as follow:

Heat two of the test-tubes for 72 hours in an oven maintained at 45 O± 1°C. Allow to cool to 25 ± 2°C and examine the contents. Cool the other two tubes 0 ± 1°C, maintain at this temperature for 1 hour, allow to attain to 25 ± 2°C, and examine the contents. on visual examination the paste should remain homogeneous and should not show any signs of fermentation or other deterioration or both.

A-7 **DETERMINATION OF FLUORIDE ION CONTENT**

A-7.1 **Apparatus**

a) *Gas chromatograph:-* A suitable gas chromatograph equipped with a flame ionization detector, and having a column of length about 1.5 m and nominal outside diameter 6 mm packed with 10% methyl silicone (100%) on 80-100 mesh flux calcined diatomite, acid washed.

b) Gas chromatograph:- operating conditions

*1) Temperatures*

Detector $100^0$C
Injection $80^0$C
Oven $50^0$C

2) Carrier gas (nitrogen) flow rate, 20 ml/min

c) Micro syringe, 1 µl

A-7.2 **Reagents**

a) Hydrochloric acid, concentrated
b) Nitrogen. A source of pure nitrogen gas.
c) n-pentane solution in benzene. 1.6 g of n-pentane made up to 1.l with benzene.
d) Standard fluoride solution. An aqueous solution of sodium fluoride (NaF) containing 1.0 mg of $F^-$ / ml.
e) Trimethylchlorosilane.

***NOTE:*** *- Unless inconsistent with the text, use only reagents of analytical reagent grade and distilled water.*

A-7.3 **Determination of the Response Factor of the Apparatus**

a) Pipette 5 ml of the standard fluoride solution into a 50 ml plastics flask. Using a measuring cylinder, add 2 ml of the hydrochloric acid, stopper the flask, and mix the contents. Add from a burette 2.0 ml of the trimethylchlorosilane.

b) Securely stopper the flask, mix well by shaking and allow stand for 15 min to cool. Using a safety pipette, pipette 10 ml of the benzene solution of n-pentane into the flask, and stopper Mix thoroughly by shaking vigorously for 2 min. Transfer the contents of the flask to a centrifuge tube, stopper, and centrifuge at 2500 r.p.m. for 5 min. Inject 1.0 µl of the upper layer on to the column and allow the chromatogram to develop.

c) Draw tangent base lines to the peaks of interest, and measure the heights of the trimethyl fluorosilane (hf) and n-pentane (hp) peaks. (Alternatively determine the areas of the relevant peaks.).

d) Calculate the response factor (k) as follows:-

$$K = 3.2 \times \frac{hf}{hp}$$

A-7.4 **Procedure: -** Weigh out accurately about 3.5 g of the composite sample into a centrifuge tube. Add 10 ml of water and 4-6 small glass beads. Stopper and shake vigorously until the paste is dispersed and removed from the side of the tube. Centrifuge at 2000 r.p.m. for 10 min.

Decant the upper aqueous layer into a 50 ml plastics flask. Redisperes the residue in the tube with 5 ml of water and centrifuge. Decant the aqueous layer into the plastics flask.

Cautiously add 5 ml of the hydrochloric acid to the residue in the centrifuge tube. When any effervescence has subsided, stopped and shake vigorously to disperse. Centrifuge and decant the solution into the plastics flask. Redispress the residue (if any) and wash the tube within 3 ml of the hydrochloric acid. Centrifuge and decant into the plastics flask. Wash the tube and residue (if any) with 5 ml water, centrifuge, and decant into the plastics flask. Wash the tube and residue (if any) with a further 5 ml of water, centrifuge if necessary, and add the washings to the plastics flask. Stopper the plastics flask; shake to mix, then heat in a boiling water bath for 1 min. Cool to room temperature in a cold water bath.

Add to the contents of the flask from a burette 2.0ml of the trimethyl chlorosilane. Stopper, shake well, and allow to stand for 15 min. Using a safety pipette, pipette 10 ml of the benzene solution of n-pentane into the flask. Shake vigorously for at least 2 min.

Pour the contents of the flask into a centrifuge tube, stopper, and centrifuge at 2500 rpm. for 10 min.

***NOTE:-*** *Some pastes develop emulsions that require at least one decanting and a second centrifuging in order to separate the organic layer.*

Inject 1 µl of the organic phase onto the column and allow the chromatogram to develop, the proceed as in A-7.3(c).

A-7.5 **Calculations:** Calculate the total fluoride content as follows:-

$$\text{Total fluoride content, as F \% (by mass)} = \frac{1.6 \times h\,f}{m \times h\,`p \times K}$$

where

h `f = height of trimethy lfluorosilane peak
h `p = height of n-pentane peak
m = mass of sample used, g
k = response factor [from A-7.3(d).]

**ALTERNATE METHOD**

**A 7.6 DETERMINATION OF FLUORIDE ION**

A-7.6.1 **Principle**

Sodium monofluorophosphate or fluoride ions are extracted with water from toothpaste and extract is fused with sodium carbonate to convert it into sodium fluoride and then determined potentiometrically with the help of fluoride ion sensitive electrode.

A-7.6.2 **Apparatus**
Potentiometer with pH meter

A-7.6.3 A Fluoride ion sensitive Electrode

A-7.6.4 A saturated Calomel Electrode as Reference Electrode.

A-7.6.5 **Reagents**

A-7.6.6 Sodium Fluoride (Analar)

A-7.6.7 Triehanolamine Pure

A-7.6.8 EDTA (AR) (disodium salt, dehydrate)

A-7.6.9 Sodium Carbonate (AR)

A-7.6.10 Hydrochloric Acid (AR)

A-7.6.11 Hydrochloric Acid (AR)

A-7.6.12 Standard Solutions and reagent Solutions

A-7.6.13 Triethanolamine Buffer Solution

149.0 g of triethanolamine pure is dissolved in 600 ml of distilled water and with concentrated hydrochloric acid; adjust the pH to 7.0 using pH meter. The solution is cooled to room temperature and the pH tested; if necessary it is re-adjusted; then diluted to 1000 ml with distilled water.

A-7.6.14 0.1 M EDTA

Weigh accurately 37.224 g of EDTA and dissolve in distilled water and make up to 1000 ml in a volumetric flask.

A-7.6.15 Standard Sodium Fluoride Solution (0.01 mgF- per ml)

A-7.6.16 Standard Sodium Fluoride Solution (0.01 mg F- per ml)

Dry the sodium fluoride at 110 $^{o}$C for 4 hours and transfer accurately 0.222 g to 100 ml volumetric flask. Add distilled water to dissolve the sodium fluoride and make up to the mark (Solution-A). Take 10 ml of this Solution –A in 1000 ml volumetric flask and make up this volume to the mark (Solution-B). Each ml of Solution-B contains 0.01 mg F- Transfer Solutions A and B to polythene bottles for storing.

A-7.6.17 Preparation of Standard Solutions of Sodium Fluoride

Take 1,2,5,10,20 and 25 ml of Solution –B (above, A-7.9.15) in 100 ml volumetric flask marked A, B, C, D, E and F respectively. To each and 25 ml of EDTA and 10 ml of triethanolamine hydrochloride buffer solution and make up the volume to 100 ml with distilled water. Now the solutions A, B, C, D, E and F are containing 0.01, 0.02, 0.05, 0.1, 0.2 and 0.25 mg of F per 100 ml respectively. Transfer the solutions to 150 ml polythene beaker for milli-Volt (mV) measurement.

**A-7.6.18 Test Solution**

Weigh accurately 5.0 g of toothpaste in a 150 ml beaker and add 50 ml of distilled water. Stir over a magnetic stirrer at about 40 $^{o}$C for half hour and cool. Centrifuge the solution for 10 minutes at 15000 rpm, wash and collect the washings. Transfer the supernatant liquid and the washing. Transfer the supernatant liquid and the washing to 100 ml volumetric flask and make up to the mark. Transfer the solution to a polythene bottle. Take 5 ml of supernatant solution in a 25 ml capacity platinum crucible and add 1 ml of 10 percent solution of sodium carbonate. Heat the crucible over a flame to dryness. Transfer the crucible in muffle furnace (600 $^{0}$C). After cooling the crucible in desiccator, add 25 ml of MEDTA and boil for some time. Cool and transfer to a 100 ml volumetric flask (if necessary filter). Add 10 ml of triethanolamine hydrochloride buffer solution and make up to 100 ml. Transfer the solution to a 150 ml polythene beaker for milli-Volt (mV) measurement.

The standard solutions and the test solutions are stirred over the magnetic stirrer and the steady milli-Volt (mV) (potential a difference) is measured.

The reading for standard and test solutions is taken simultaneously.

**A-7.6.19 Calculation**

A graph is plotted for concentration of F-against potential my in a semi-logarithmic paper. The potential my is plotted on X axis and mg of F- on Y axis (on logarithmic scale).

$$\text{Concentration of F- in toothpaste, parts per million} = \frac{2a \times 10000}{M}$$

A-8 **DETERMINATION OF LEAD CONTENT**

A-8.1 ***Principle:*** Lead in toothpaste is extracted with acid after low temperature ashing. The ammonium pyrrolidine dithiocarbamate (APDC) complex is formed, extracted into 4-methylpentan-2one and the lead is determined by atomic absorption spectroscopy.

A-8.2 ***Precautions***: - All apparatus and reagents shall be lead free. Contamination, particularly from atmospheric dust, shall be prevented. To avoid volatilization of lead, it is essential that ashing be carried out at a low temperature; 450$^{0}$C is recommended, 500$^{0}$C maximum.

A-8.3 ***Apparatus*: -** The following apparatus is required.

A-8.3.1 Borosilicate glass beakers, 100ml capacity, or silica evaporating dishes.

A-8.3.2 Conical flasks, 100ml capacity.

A-8.3.3 Separating funnels, 100 ml capacity.

A-8.3.4 Muffle furnace.

A-8.3.5 Atomic absorption spectrophotometer and lead hollow cathodes lamp.

A-8.3.6 Pipette, Pasteur type.

A-8.3.7 Pipette, 10 ml.

A-8.4 ***Reagents.*** The following reagents shall be of analytical grade.

A-8.4.1 Acetone.

A-8.4.2 Ethanol.

A-8.4.3 4-methylpentan-2-one (isobutyl methyl ketone).

A-8.4.4 Nitric acid, concentrated.

A-8.4.5 Nitric acid, 5 N.

A-8.4.6. Ammonia solution, 5N.

A-8.4.7 APDC 1 % solution. Wash 1.5 g of ammonium pyrrolidine dithiocarbamate (APDC) with 20 ml of acetone in a P 4 sintered glass crucible. Dry, weigh 1 g and dilute to 100 ml with water.

A-8.4.8 Standard lead solution 1000 mg/kg (1000 p.p.m.) of lead.

A-8.5 ***Procedure:-***

A-8.5.1 ***Destruction of Organic matter: -*** Accurately weigh about 2g of paste into a 100 ml beaker. Slurry with 10 ml to 15 ml of ethanol and evaporate to dryness on a water bath. Place in the muffle furnace at 100 $^0$C and slowly raise the temperature by 50$^0$C steps to 450$^0$C to avoid ignition. Complete the ashing at 450$^0$C for between 30 minutes and 1 hour.

A-8.5.2 ***Acid extraction of the ash.*** Add 5 ml of water and 10 ml of 5N nitric acid to the cooled ash and boil gently for 5 min. Filter the solution into a 100 ml conical flask, rinsing with two 5 ml portions of water and retaining as much as possible of any insoluble matter in the beaker or dish. Add 10 ml of 5N nitric acid to the beaker and boil gently for 5 min. Filter into the same conical flask, rinsing with two 5 ml portions of water.

A-8.5.3 ***Determination of lead.*** Adjust the pH value of the acid solution to between 3 and 4, using the ammonia solution, transfer to separating funnel and cool to room temperature.

**NOTE: -** *The effect of the pH value on extraction of lead is shown in table 2.*

**Table 2**

**Effect of pH value on extraction of lead**

| pH | Peak height (mm) | |
|---|---|---|
| | **0.5 µg / ml** | **1.0 µg / ml** |
| 1 | 55 | 100 |

| | | |
|---|---|---|
| 2 | 55 | 122 |
| 3 | 72 | 142 |
| 4 | 68 | 136 |
| 5 | 66 | 108 |
| 6 | 54 | 115 |
| 7 | 44 | 92 |
| 8 | 50 | 90 |

Add 2ml of APDC solution mix and set aside for 5 min. add 10ml of 4-methylpentan-2-one, measured accurately using a pipette, and shake vigorously for 1 min. Allow to separates and remove the clear organic layer with a Pasteur pipette. Aspirate the organic layer into the atomic absorption spectrophotometer fitted with a lead hollow cathodes lamp set at 283. 3 nm.

A-8.5.4 ***Calibration curve.*** Submit aliquot portions of standard lead solution containing 0 to 20 µg of lead to the procedure in 5.2 and 5.3. Construct a new curve for each set of measurements.

A-8.5.5 *Calculation:* Relate the test result to the calibration curve and calculate the lead content in mg/kg (ppm).

A-9 **DETERMINATION OF ARSENIC**

**Out line of the method**
Arsenic present in a solution of the material is reduced to gaseous arsenic (III) hydride ($AsH_3$) by reaction with sodium tetrahydroborate in a hydrochloric acid medium.

The absorbance is determined at a wavelength at 193.7 nm.

A- 9.1. **Reagents**

A-9.1.1 Concentrated sulfuric Acid ($H_2SO_4$) (See PS: 38)

A-9.1.2 Concentrated Hydrochloric Acid (HCl) (See PS: 37)

A-9.1.3 Hydrogen peroxide ($H_2O_2$) 30% (m/m).

A-9.1.4 Sodium hydroxide (NaOH)

A-9.1.5. ***Sodium tetrahydroborate solution.***

Dissolve 1 g of sodium hydroxide in about 20 ml of water. Add 3 g of sodium tetraborate ($NaBH_4$). Dilute to 100 ml with water. Prepare the solution on the day of use.

A-9.1.6 ***Potassium iodide – ascorbic acid solution:***

Dissolve 3 g of potassium iodide (KI) and 5 g of L (+) – Ascorbic acid ($C_6 H_8 O_6$) in 100 ml of water.

Prepare the solution on the day of use.

A-9.1.7 **Arsenic stock solution** – corresponding to 1000 mg of as per litre.

Place 1.320 g of arsenic (III) Oxide ($As_2O_3$), in a volumetric flask of nominal capacity 1000- ml. Add 2 g of sodium hydroxide and dissolve in a small quantity of water. Dilute to volume with water.

A-9.1.8 **Arsenic standard solution 1**, corresponding to 10 mg of arsenic per litre.

Pipette 10 ml of arsenic stock solution into a volumetric flask of nominal capacity 1000 ml. Add 20 ml of hydrochloric acid and dilute to volume with water.

A-9.1.9. **Arsenic standard solution 2,** corresponding to 0.1 mg of arsenic per liter.

Pipette 10 ml of arsenic standard solution1 into a volumetric flask of nominal capacity 1000 ml. Add 20 ml of hydrochloric acid and dilute to volume with water.

Prepare the solution on the days of use.

A-9.2 **Apparatus**

Usual Laboratory Apparatus and

A-9.2.1 **Atomic absorption spectrometer**, fitted with a hydride system, and suitable radiation source for the determination of arsenic, for example electrode-less discharge lamp or a hollow cathode lamp with a back ground facilities, if necessary.

A-9.2.2 **Gas Supply**, with argon or nitrogen.

A-9.2.3 **Glassware,** to be cleaned immediately before use with warm, dilute nitric acid (10 % v/v) and rinsed with water.

A-9.3 **PROCEDURE**

A-9.3.1 **Blank solution**

Pipette 2 ml of hydrochloric acid into a volumetric flask of nominal capacity 100 ml and dilute to volume with water.

Test the blank in exactly the same way as the sample.

A-9.3.2 **Calibration Solutions.**

Using arsenic standard solution 2, prepare at least five calibration solution covering the expected working range.

For example, for the range 1 µg/l to 10 µg/l, pipette 1 ml, 3 ml, 5 ml, 6 ml and 10 ml of arsenic standard solution 2 into a series of 100 ml volumetric flasks, to each of these flaks, add 2 ml of hydrochloric acid and dilute to volume with water. These solutions correspond to arsenic concentrations of 1 µg/l, 3µg/l, 5 µg/l, 8 µg/l and 10µg/l, respectively.

Prepare the calibration solutions daily.

Treat the calibration solutions in exactly the same way as the sample.

A-9.3.3 **Pretreatment**

Most of the organically bound arsenic compounds are decomposed by the digestion procedure described in A-9.3.1. If it is known that the sample to be analyzed does not contain organic arsenic compounds, it is permissible to omit the digestion process. In this case, proceed to A-8.3.2.

Place 50 ml of the sample in a round bottomed flask.

A-9.3.4 **Method of digestion**

WARNING - *Fumes produced by heating concentrated sulfuric acid are irritant and this operation must therefore be carried out in a fume chamber.*

Add 5 ml of sulfuric acid and 5 ml of hydrogen peroxide to the rounded-bottomed flask. Add some anti-bumping beads and connect the flask to the apparatus, Heat the contents of the flaks to boiling and collect the condensate in the condensate reservoir. Continue heating until fumes of

sulfuric acid appear. Examine the appearance of the sample. If it is turbid and almost colourless, add a further 5 ml portion of hydrogen peroxide and continue boiling as described in the previous paragraph. When the appearance of the sample is colourless and not turbid, cool the flask and contents, return the condensate to the round-bottomed flask and proceed to A-9..3.2.

Take care to ensure that the sample is never evaporated to complete dryness.

A-9.3.5 **Reduction from As (V) to As (III)**

Add 20 ml of hydrochloric acid (4.2) and 4 ml of potassium iodide-ascorbic acid and solution to the round-bottomed flask containing the digested sample or non-digested sample. Heat gently for 15 min at $50^0$C. Cool the sample solution and transfer it quantitatively to a volumetric flask of nominal capacity 100ml. Dilute to volume with water.

A-9.3.6 **Calibration and determination**

Depending on the hydride system used, greater or smaller volumes than those described below are permitted. However, the quantity ratios defined shall be maintained. Set all instrumental parameters of the atomic ratios defined shall be maintained. Set all instrumental parameters of the atomic absorption spectrometer in accordance with the manufacturer's operating manual (wavelength; 1937 nm) and optimize the position of the absorption cell in order to obtain maximum transmission of the light beam. Pass a stream of argon of nitrogen through the system and set the instrument to zero.
Measure the absorption given by the solutions in the following order.

- blank solution,
- calibration solutions,
- samples, prepared as follows.

Transfer an appropriate volume of the sample solution to the reaction vessel. Connect the reaction vessel to the hydride system. Pass argon or nitrogen through the solution until the absorption signal of the atomic absorption spectrometer returns to zero.

For 20 ml of the sample solution, add 5 ml ± 0.1 ml of sodium tetrahydroborate solution to the solution and record the signal. Repeat the procedure using separate portions of each solution. Use the mean of these results. Establish the calibration curve using means of values obtained with the blank and calibration solutions.

**NOTES**:

* *It is good practice to check the blank and calibration points from time to time.*

* *With unknown samples, it is recommended to check the validity of the method by adding a know volume of arsenic to at least one sample of recovery tests are not satisfactory, the procedure of standard additions should be used.*

A-9.3.7 **Calculation of the results using the standard calibration method.**

Calculate the arsenic concentration in the solution by comparing the absorption response of the sample solution with those of known standard concentrations obtained from the calibration procedure A-9.4

All dilution steps shall be taken into account.

A-9.4 **Expression of results**

Express the results, in micrograms per liter, to two significant figures and one decimal place.

A-9.4.1 The test report shall contain the following information:

a) A reference to this Pakistan Standard.
b) Complete identification of the sample.
c) Expression of the results as indicated in clause A-9.6.
d) Any details not specified in this Pakistan Standard or which are optional, as well as any factor which may have affected the results.

## A-10 METHODS OF TEST FOR MICROBIAL PURITY

### A-10.1 Determination of Total Aerobic Bacteria

#### A-10.1.1 Outline of the Method

The test consists of plating a know dilution of the sample on Soybean casein Digest Agar Medium suitable for the growth of total Aerobic Bacteria and incubating them for a specified period to permit the development of visual colonies for counting.

#### A-10.1.2 Apparatus and Equipment

A-10.1.2.1 Conical flasks with glass-beads – 100, 250 and 500 ml.

A-10.1.2.2 Pipettes – Sterile -1.5 and 10 ml graduated.

A-10.1.2.3 Test tubes – Without rim.

18 mm diameter X 150 mm height (with Durham's tubes),
25 mm diameter X 150 mm height, and
38 mm diameter X 200 mm height.

A-10.1.2.4 Petri dishes – Sterile 15 X 100 mm.

A-10.1.2.5 Standard Streaking Loop, Straight inoculating wire.

A-10.1.2.6 Analytical Balance.

A-10.1.2.7 Autoclave for steam sterilization at 121$^0$C at 103 kN / m$^2$ (15 1b per square inch) pressure.

A-10.1.2.8 Oven Temperature up to 250$^o$C.

A-10.1.2.9 Incubator 30 to 35$^o$C and 37 ± 2$^o$C.

A-10.1.2.10 Water-baths 45 ± 2$^0$C and boiling water-bath.

A-10.1.2.11 Colony counter

A-10.1.2.12 Laboratory microscope

#### A-10.1.3 Diluents and Media

Diluent's – Fluid Casein Digest – Soy-Lecithin Polysorbate 20 Medium.

| | |
|---|---|
| Pancreatic digest of Casein | 20 g |
| Soy-lecithin | 5 g |
| Polysorbate 20 | 40 ml |
| Distilled Water | 960 ml |

Dissolve the Pancreatic Digest or Casein and Soy-Lecithin in 960 ml of water, heating in a water bath for about 30 minutes to solution. Add 40 ml of Polysorbate 20. Mix and dispence in 90 ml quantity with few glass-beads in conical flasks of 250 ml capacity.
Autoclave at 121°C with 103 kN/ $m^2$ (15 Ib per square inch) pressure for 15 minutes.

*NOTE – Any other suitable diluent can be used provided it conforms to the test in D-9.3.*

Medium – Soybean Casein Digest Agar Medium with 0.002 percent TTC.

| | |
|---|---|
| Pancreatic digest of casein. | 15.0 g |
| Papain digest of soybean meal | 5.0 g |
| Sodium chloride | 5.0 g |
| Agar | 15.0 g |
| Distilled water | 1000 ml. |
| pH after sterilization | 7.3 ± 0.2 |

Digest in water – bath and dispense as desired in conical flasks or test tubes. Autoclave at $121^0$C with 103 kN/$m^2$ (15 lb per square inch) pressure for 15 minutes. Add separately sterile TTC solution in the sterile medium aseptically to get 0.002 percent concentration.

A-10..1.4 **Preparation of Sterile TTC Solution.**

| | |
|---|---|
| 2,3,5-Triphenyl-2H-tetrazolium chloride | 0.02 g |
| Distilled water | 10 ml |

Autoclave at $121^0$C with 103 kN /$m^2$ (15 Ib per square inch) pressure for 15 minutes.

A-10.1.5 **Procedure**

Weigh and transfer aseptically 10 g of sample into a sterile conical flask containing diluent. Shake well to get homogeneous sample preparation. Make further dilutions if required in sterile diluent to yield colonies between 30 and 300 per plate.

Pipette out 1 ml portions in duplicate of sample dilution on Petri dishes. Promptly add to it 15 to 20 ml of agar medium that previously has been melted and cooled to approximately 45 ± $2^0$C in a water-bath. Cover the Petri dishes; mix the sample with the agar by tilting or rotating the dishes and allow the contents to solidify at room-temperature.

Invert the Petri dishes and incubate at 30 to $35^0$C for a minimum period of 72 hours up to 5 days. Following incubation, examine the plates for growth, count the number of colonies under Colony Counter and express the Average Number of Colonies X appropriate Dilution factor as the Total Aerobic Bacteria per gram for the sample. If no bacterial colonies are recovered from the Petri dishes representing the initia 1: 10 dilution of the sample, express the results as 'less than 10. Total Aerobic Bacteria per gram' for the sample.



## B-11 Salmonellae and Escherichia Coli

### B-11.1 Outline of the Method

The test consists of enrichment of above bacteria from a sample in a suitable culture medium and then in selective culture media individually and after incubation streaking over on selective agar plates for identification.

### B-11...2 Apparatus and Equipment

As per B-10.1.2 except colony counter.

B-11...3 **Media and Reagents.**

**a) Lactose broth**

| | |
|---|---|
| Beef Extract | 3.0 g |
| Pancreatic digest of gelatin | 5.0 g |
| Lactose | 5.0 g |
| Soy-Lecithin | 5.0 g |
| Tween 20 | 40 ml |
| Distilled water | 960 ml |
| pH after sterilization | 6.9 ± 0.2 |

Mix well and dispense in 90 ml in conical flasks of 250 ml capacity.

Autoclave at 121$^0$C with 103 kN / m$^2$ (15 Ib per square inch) pressure for 15 minutes.

**b) Selentite Cystine broth**

| | |
|---|---|
| Pancreatic digest of casein | 5.0 g |
| Lactose | 4.0 g |
| Sodium Phosphate | 10.0 g |
| Sodium acid selenite | 4.0 g |
| L-cystine | 10 mg |
| Distilled water | 1 000 ml |
| Final pH | 7.0 ± 0.2 |

Mix and heat to effect solution. Heat in flowing steam for 15 minutes. Dispense in sterile 10 ml tubes aseptically. Do not autoclave for sterilization.

**c) Tetrathionate broth**

| | |
|---|---|
| Pancreatic digest of casein | 2.5g |
| Peptic digest of animal tissue | 2.5 g |
| Bile salts | 1.0 g |
| Calcium carbonates | 10.0 g |
| Sodium thiosulphate | 30.0 g |
| Distilled water | 1000 ml |

Heat the solution of solids to boiling. On the day of use, add a solution prepared by dissolving 5 g of potassium iodide and 6 g of iodine in 20 ml of water. Then add 10 ml of solution of Brilliant Green (1: 1000), and mix. Do not heat the medium after adding the Brilliant Green solution.

**d) Brilliant Green Agar**

| | |
|---|---|
| Yeast extracts | 3.0 g |
| Peptic digest of animal tissue | 5.0 g |
| Pancreatic digest of casein | 5.0 g |
| Lactose | 10.0 g |
| Sodium chloride | 5.0 g |
| Sucrose | 10.0g |
| Phenol red | 80 mg |
| Agar | 20.0 g |
| Brilliant green | 12.5 mg |

| | |
|---|---|
| Distilled water. | 1 000 ml |

Boil the solution of solids for I minute. Sterilize just prior to use, melt the medium, pour into sterile Petri dishes, and allow it to cool.

**e) Bismuth Sulphite Agar**

| | |
|---|---|
| Beef extract | 5.0 g |
| Pancreatic digest of casein | 5.0 g |
| Peptic digest of animal tissue | 5.0 g |
| Dextrose | 5.0 g |
| Sodium phosphate | 4.0 g |
| Ferrous sulphate | 300 mg |
| Bismuth sulphate indicator | 8.0 g |
| Agar Brilliant green | 25 mg |
| Distilled water | 1 000 ml |
| Final pH | 7.6 ± 0.2 |

Heat the mixture of solids and water in a boiling water-bath, with swirling just to the boiling point. Do not over-heat or sterilize, Transfer at once at water-bath maintained at $50^0$C and pour into sterile Petri dishes as soon as the medium has cooled up to $50^0$C.

**MacConkey`s broth**

| | |
|---|---|
| Peptone | 20.0 g |
| Sodium chloride | 5.0 g |
| Sodium taurocholate | 5.0 g |
| Distilled water | 100 ml |
| Bromocresol purple | 10 mg |
| Lactose | 10 g |

Dissolve the peptone, the sodium chloride, and the sodium taurocholate in distilled water with the aid of water-bath. Adjust to pH 8.0 and boil for twenty minutes. Cool, filter and adjust to pH 7. 4 ± 0.2. Add the Lactose and indicator solution, mix and distribute in tubes containing inverted Durham`s tubes. Autoclave at $121^0$C for 103 kN / $m^2$ (15 Ib per square inch) pressure for 15 minutes.

**f) MacConkey`s Agar**

| | |
|---|---|
| Pancreatic digest of gelatin | 17.0 g |
| Pancreatic digest of casein | 1.5 g |
| Peptic digest of animal tissue | 1.5 g |
| Lactose | 10.0 g |
| Bile salts mixture | 1.5 g |
| Sodium chloride | 5.0 g |
| Neutral red | 30 mg |
| Crystal violet | 1.0 mg |
| Distilled water | 1 000 ml |
| pH after sterilization | 7.1 ± 0.2 |

Boil the mixture of solids and water for 1 minute to effect solution. Autoclave at $121^0$C with 105 kN /$m^2$ (15 Ib per square inch) pressure for 15 minutes. Pour 15 to 20 ml in sterile Petri dishes aseptically.

**g) Tripple Sugar-Iron-Agar**

| | |
|---|---|
| Pancreatic d digest of casein | 10.0 g |
| Pancreatic digest of animal tissue | 10.0 g |
| Lactose | 10.0 g |
| Sucrose | 10.0 g |
| Dextrose | 1.0 g |
| Ferrous ammonium sulphate | 200 mg |
| Sodium chloride | 5.0 g |
| Sodium thiosulphate | 200 mg |
| Agar | 13.0 g |
| Phenol red | 25 mg |
| Distilled water | 1000 ml |

After mixing and digesting on a boiling water-bath distribute 10 ml in test tubes of 18 X 150 mm size. Autoclave at $121^0$C with 103 kN / $m^2$ (15 1b per square inch) pressure for 15 minutes. Cool approximately at $45^0$C and put in slanting position to solidify.

## B-11.4 Gram Staining Method

### B-11.4.1 Outline of the method

The test consists essentially of applying crystal violet to a fixed smear from the culture, removing the primary stain after a suitable staining period and then applying iodine as a mordant. The mordant in turn is removed and decolorized is added to remove the primary stain where possible. Saffranin is then added as a counter stain.

Organisms are judged to be gram-positive under oil immerstan lens of microscope, if they retain the primary stain after decolourization, Gram- negative organisms are decolorized and appear pink to red because they take up the counter stain.

### B-11.4.2 Gram stain reagents

**Stain 1**

| | | |
|---|---|---|
| A) | Crystal violet (90 percent dye content) | 2 g |
| | Ethanol (95 percent) | 20 ml |
| B) | Ammonium oxalate | 0.8 g |
| | Distilled water | 80 ml |

Mix A and B

**Stain 2**

| | |
|---|---|
| Iodine | 1 g |
| Potassium lodide | 2 g |
| Ethanol (95 percent) | 25 ml |
| Distilled water | 100 ml |

**Stain 3**

5 ml of Stain-2 added to 95 ml of ethanol (95 percent).

**Stain 4**

| | |
|---|---|
| Saffranin | 0.25 g |
| Ethanol (95 percent) | 10 ml |
| Distilled water | 100 ml |

B-11.4.3 **Procedure for preparation of identification plates.**

Place a small loop full of distilled water on a clean microscope slide. Touch a sterile inoculating loop to a colony, rub it into the droplet to obtain and even suspension of micro-organisms and spread the suspension over the surface of the slide to obtain correct density. Let the smear dry at room-temperature. Heat fix by passing the slide 3 times through a low gas flame. Cool to room-temperature before staining.

Flood the stain-l on fixed smear for 1 minute. Wash excess of stain with tap water. Flood the smear with stain-2 by gentle washing in cold tap water. Flood off stain-3 until solvent runs colourlessly from the slide for 30 to 60 seconds. Counter stain with stain-4 for 30 to 60 seconds. Wash off excess stain with cold tap water. Blot off excess water with paper, air dry and examine under oil immersion lens.

*NOTE :- All the above media and reagents in dehydrated form are commercially available.*

B-11.5 **Procedure**

Weigh and transfer aseptically 10 g of sample into a sterile conical flask containing 90 ml of Lactose broth. Mix well and incubate at 37 ± $2^0$C for 18 24 hours. Examine the broth for growth, and if the growth is present, mix by gently shaking. Transfer aseptically I ml portions with the help of sterile pipette into:

a) 10 ml selenite cystine broth.
b) 10 ml tetrathionate broth.

Incubate at 37 ± $2^0$C for 18 to 24 hours.

c) 10 ml Sterile MacConkey`s broth tubes having durhams tube.

Incubate at 37 ± $2^0$C for 48 hours.

B-11.6 **Identification Test for Salmonellae**

By means of an inoculating loop streak portions from broth (a) and (b), on the surface of Brilliant Green Agar and Bismuth Sulphite Agar plates. Cover and invert the petridishes and incubate at 37 ± $2^0$C for 48 hours. Upon examination. If none of the colonies conforms to the description given in Table 2, the sample meets the requirement for the absence of Salmonellae.

Gram stain the portion of suspected colony and if the colonies of Gram-Negative rods are found, proceed with further identification by transferring representative suspect colonies individually.

***Table 2***

***- Morphological Characteristics of Salmonellae***

| Medium | Description of Colony |
|---|---|

| | |
|---|---|
| Brilliant green agar | Small transparent, colorless of pink to white opaque Frequently surrounded by pink to red zone) |
| Bismuth sulphite agar | Black or Green |

by means of inoculating wire, to a butt-slant tube of triple-sugar iron agar by first streaking the surface of the slant and then stabbing the wire well beneath the surface. Incubate at 37 ± $2^0$C for 18 to 24 hours. If examination discloses, no evidence of tubes having alkaline (red) slants and acid (yellow) butts (with or without concomitant blackening of butt from hydrogen sulphide production, the sample meets the requirements for the absence of salmonellae.

B-11...6.2 **Identification Test for Escherichia coli.**

After incubation of MacConkey`s broth tubes if the contents do no9t show acid and gas, the sample meets the requirement of the test of the absence of Escherichia coli.

If there is acid and gas in the MacConkey`s broth tube, streak a portion of the above growth on the surface of MacConkey`s Agar plate. Cover and invert the Petri dishes and incubate at 37 ± $2^0$C for 18 to 24 hours. Upon examination if none of the colonies conforms to the description of Table 3 for the medium, the sample meets the requirements of the test for the absence of Escherichia coli.

**Table 3**
**Morphological Characteristics of Escherichia Coli.**

| Medium | Descriptions of Colony |
|---|---|
| MacConkey`s Agar | Brick – red may have surrounding zone of precipitated bile. |

If colonies matching the description in Table 2 are found, and are Gram negative proceed with further identification by transferring the suspect colonies individually by means of inoculating loop to the surface of Levine Eosin – Methylene Blue Agar plates. Divide the surface of the Agar plate, if multiple colonies are observed. Incubate at 37 ± $2^0$C for 18 to 234 hours, Upon exami8nation, if more of the colonies exhibits both a characteristic metallic sheet under reflected light and a blue-black appearance under transmitted light, the sample meets the requirements of the test for the absence of Escherichia coli. The presence of Escherichia coli may be confirmed by further suitable cultural and biochemical tests.

B-11.7 **Validation of Test Procedures for D-9.1 and D-9.2**

The validity of the results of the test set forth in this chapter rests largety upon the adequacy of a demonstration that the test samples to which they are applied do not; of themselves inhibit the multiplication, under the test conditions, of micro organism that may be present.

This can be done by adding 1 ml of not less than 10 – 4 dilution of 24 hours broth culture of:

1. Staphylococcus aureus ATCC 6538 –P
2. Pseudomonas aeruginosa ATCC 9027
3. Salmonella abony NCTC 6017
4. Escherichia Coli. ATCC 8739

to the first dilution (In Fluid Casein Digest Soy-Lecithin Polysorbate 20 Medium and Lactose broth with 0.5 percent of Soy-Lecithin and 4 percent of Tween 20) of the test sample; and following the test procedure.

If inspite of incorporation of inactivating agents and a substantial increase in the volume of diluent, it is still not possible to recover the viable cultures described above, it can be assumed that failure to isolate the inoculated organisms is attributable to the bactericidal activity of the product. The sample is not likely to be contaminated with the given species of micro-organisms. Monitoring should be continued in order to establish the spectrum of inhibition and bactericidal activity of the sample.

To prove the growth promoting ability of different culture media, organisms without sample preparation is run in the same manner.

## ANNEXURE-B
**(For Information Only)**
(Clause 4.1)

### INGREDIENTS CONVENTIONALLY USED IN THE MANUFACTURE OF TOOTHPASTE

B.1 The material used fall into the following categories:

A. Polishing agents
B. Soap or detergent
C. Humectant
D. Binding agent
E. Other Substances

**B.1.1 Polishing Agents**
The principal polishing agents generally used are one or more of those given below:

a. Precipitated calcium carbonate
b. Magnesium carbonate
c. Dicalcium phosphate
d Insoluble sodium metaphosphate
e Hydrated alumina
f Aluminium hydroxide
g Aluminium silicate
h Alumina
i Alumina fumed
j Calcium phosphate
k Calcium pyrophosphate
l Dicalcium phosphate dihyrate
m Kaolin
n Magnesium silicate
o Potassium metaphosphate
p Silica gel or precipitated
q Silica fumed
r Silica hydrated
s Sodium aluminium silicate
t Sodium bicarbonate
u Sodium Metaphosphate

c Sodium sulphoricinoleate
d Sodium lauryl Sulphate
e Sodium alkyl sulphoacetate
f Sodium salt of sulphated monoglyceride
g Sodium lauryl sarcosinate

h Sodium alpha olefin sulphonate
i Coco-amido propyl betaine

j Sodium dodecyl benzene sulphonate
k Sodium lauryl sulphoacetate
l Coconut monoglyceride sulphonates
m Dioctylsodium-sulphosuccinate
n Magnesium lauryl Sulphate
o Sodium alkyl benzene sulphonate
p Sodium alkyl Sulphate
q Sodium lauryl ether Sulphate
r Coco betaine

**B.1.3 Humectants**
a. Glycerol
b. Sorbitol
c. Maltitol
e Mannitol
f Polyethylene glycol
g Propylene glycol
h Lactitol
i Xylitol

**B.1.4 Binding Agents**
The binding agents generally used are one or more of those given below:

v Hydroxyapatite
w Ziroconium silicate
x Sodium polymetaphosphate

**B.1.2 Soap or Detergents**

The soap or detergent (s) generally used are one or more of those given below:

a Soap
b Sodium recinoleate

a Gum tragacanth
b Gum karaya
c Sodium alginate
d Sodium carboxymethyl cellulose
e Gelatine
f Guar gum and its derivatives
i Xanthan gum
g Carraghenates
h Carboxy methyl cellulose

i Magnesium aluminium silicate
j Starch
k Hydroxy propyl cellulose
l Hydroxy ethyl cellulose

**B.1.5 Other Substances**

a Fluorides of sodium and stannous
b Monofluorophosphate of ammonium, potassium and sodium
c Sweeteners saccharine sodium, aspartame
d Petroleum jelly silicon defoaming compounds and mineral oil
e Colouring agents
f Essential Oils
g Flavouring agents
h Astringents, and
i Preservatives



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7. ISO – 11969 – Water quality – Determination of Arsenic – Atomic absorption spectrometric Method (Hydride technique).
8. ISO – 22715 – Cosmetics – Packaging and labeling.