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Draft Indian Standard

ORAL RINSES — SPECIFICATION

(ICS No. 71.100.70)

Cosmetic Sectional Committee	Last date for receipt of comment is
PCD 19	26-06-2022

FOREWORD
(Formal clauses will be added later)

In this standard cognizance has been taken of new and emerging technologies in the field of oral care and dentistry. In formulation of this standard, assistance has been derived from International Standard, ISO 16408 : 2015 'Dentistry — Oral care products — Oral rinses' in developing this standard. An attempt has been made to incorporate relevant parts of this standard while keeping in mind specific needs in the Indian context.

The main theme of safety of the consumer while using the product is maintained as central in this standard. The oral rinses, when used in a normal manner, shall not cause injury to the teeth, gums, and mucous membrane of the mouth or the body in general. The role of the oral rinses is to clean the oral cavity and also to prevent/reduce the incidence of oral dental diseases like caries, gingivitis or periodontal diseases. The use of oral rinses improves the oral hygiene. Hence oral rinses with active ingredients that help in improving oral hygiene are part of this specification. Fluoride has been unequivocally proven to be effective in caries control but under certain conditions. Excessive ingestion of fluoride may contribute to fluorosis. Keeping both aspects in mind, the Ministry of Health and Family Welfare has imposed a restriction on the limit of fluoride ion in toothpaste. Since oral rinses fall under the same category of oral care products, the limit of 1000 ppm of maximum available fluoride level has been retained for oral rinses as well.

Specific qualitative and quantitative requirements for freedom from biological hazards are not included in this standard, however it is recommended that reference be made to ISO 7405 : 2018 Dentistry — Evaluation of biocompatibility of medical devices used in dentistry' and ISO 10993-1 : 2018 'Biological evaluation of medical devices: Part 1 Evaluation and testing within a risk management process' when assessing possible biological or toxicological hazards.

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 IS 2 :2022 'Rules for rounding off numerical values (*second revision*).' 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 draft standard prescribes the requirements and methods for sampling and test for the following types of oral rinses:

a) Ready-for-use solutions;
b) Concentrated solutions for use after dilution with water; and
c) Solutions for use after mixing.

1.2 This draft standard does not cover other product forms, for example, mouth sprays, foams, powders, tablets etc. Therapeutic and medicated oral rinses are also not covered in the standard.

1.3 This draft standard does not specify biological safety aspects of oral rinses.

2 REFERENCE

The standards which are necessary adjuncts to this draft standard are listed below. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standard:

Indian/ International Standard No.	*Title*
IS 2088 : 1983	Methods for determination of arsenic (*second revision*)
IS 3958 : 1984	Methods of sampling cosmetics (*first revision*)
IS 4011 : 2018	Methods of test for safety evaluation of cosmetics (*third revision*)
IS 4707	Classification of cosmetic raw materials and adjuncts
(Part 1) : 2020	Colourants (*fourth revision*)
(Part 2) : 2017	List of raw materials generally not recognized as safe for use in cosmetics (*fourth revision*)
IS 4117 : 2008	Alcohol denaturants - Specification (*second revision*)
IS 14648 : 2011	Microbiological examination of cosmetics and cosmetic raw materials — Methods of test (*second Revision*)
IS 16913 : 2018	Methods of test for cosmetics — Determination of heavy metals (Arsenic, Cadmium, Lead and Mercury) by Atomic Absorption Spectrometry (AAS)
ISO 28888 : 2013	Dentistry — Screening method for erosion potential of oral rinses on dental hard tissues

3 TERMINOLOGY

For the purpose of this standard, the following definitions shall apply.

3.1 Oral Rinse — Also referred to as mouth rinse or mouth wash, is a liquid formulation used for oral care purpose.

4 TYPES

Oral rinses shall be classified into following two types based on their application by the user:

a) Type 1 — Non-Fluoridated
b) Type 2 — Fluoridated

5 REQUIREMENTS

5.1 General

Oral rinses generally consist of water, ethanol, humectant, surfactant, sweetener, *p*H adjuster, flavour, colour and active agents. The product may be opaque, transparent or combination thereof, coloured or white, packed in suitable container, from which it can be poured.

5.2 Ingredients

5.2.1 Oral rinses shall not contain readily fermentable carbohydrates.

5.2.2 Unless specified otherwise, all the raw materials used in the manufacture of oral rinses shall conform to the requirements prescribed in the relevant Indian Standards where such standards exist.

5.2.3 All ingredients of oral rinses shall comply with the provisions of IS 4707 (Part 1) and IS 4707 (Part 2) subject to the provisions of *the Drugs and Cosmetics Act*, 1940 and Rules framed there under.

5.2.4 For safety evaluation of novel ingredients used in formulation of oral rinse; it shall comply to IS 4011.

5.2.5 For oral rinses containing alcohol, the alcohol used shall conform to IS 323 and it shall be free from methanol when tested as per the test method prescribed in Annex A. Alcohol content (percent by volume) in such oral rinses shall be determined as per the method prescribed in Annex A.

5.2.6 The denaturant used in the manufacture of oral rinses containing alcohol shall comply with the provisions of IS 4117.

5.3 Stability

The product shall show no sign of deterioration, such as agglomeration or change in clarity, after being subjected to the determination of stability to ageing procedure specified in Annex B.

5.4 Compatibility with Oral Tissues

Oral rinses shall not cause irritation or damage to the oral hard and/or soft tissue, when used in accordance with the manufacturer's recommendation for frequency and duration of use and experience with known side effects.

5.5 The oral rinses shall also comply with the requirements given in Table 1 when tested according to the methods referenced in col 5 of Table 1.

Table 1 Requirements for Oral Rinses

(*Clause* 5.5)

Sl, No.	Characteristic	Requirement for		Method of Test Ref to Annex/ IS
		Type 1 (Non-Fluoridated)	Type 2 (Fluoridated)	
(1)	(2)	(3)	(4)	(5)
i)	Alcohol, *Max (%v/v)*	30	30	A (A-1)
ii)	*p*H at (27 ± 2)ºC[1]	3.0 – 10.5	3.0 – 10.5	C
iii)	Heavy metals (as lead)[2], parts per million, *Max*	20	20	D/ IS 16913
iv)	Arsenic (as As_2O_3)[2], parts per million, Max	2	2	E/ IS 16913
v)	Mercury, parts per million, *Max*	1	1	IS 16913
vi)	Available Fluoride ion[3], parts per million, *Max*	50 (Type Test) [4]	1000	F or G
vii)	Microbial limit [5]			
	a) Total microbial count, CFU/g, *Max*	100	100	IS 14648
	b) Yeast and mould count, CFU/g, *Max*	100	100	IS 14648
	c) Escherichia coli, per gram	Absent	Absent	IS 14648
	d) Pseudomonas aeruginosa, per gram	Absent	Absent	IS 14648
	e) Staphylococcus aureus, per gram	Absent	Absent	IS 14648
	f) Candida albicans, per gram	Absent	Absent	IS 14648

NOTES :

[1)] In the *p*H value of an oral rinse is below 5.5, it shall pass a screening test as specified in ISO 28888.

[2)] In case of any dispute with respect to heavy metal and arsenic content, methods of test prescribed at Annex D and E, respectively shall be the reference method.

[3)] In case of any dispute, methods of test prescribed at Annex F shall be the reference method.

[4)] Type test is recommended to be done on the formulation only once to pass the above criteria.

[5)] As per IS 14648 for product containing >20% v/v alcohol, microbial test can be exempted.

6 PACKAGING AND MARKING

6.1 Packing

6.1.1 The oral rinses shall be packed in suitable dispensing systems. When packed in containers, the containers shall be properly sealed and have a leak-proof cap or closure.

6.1.2 The container and/or dispensing system shall neither contaminate nor permit contamination of the oral rinse.

6.1.3 The liquid formulation should be pourable from container in which it is packed.

6.2 Marking

The labelling and marking of oral rinses shall comply with the provisions of the Drugs and Cosmetic Rules, the Legal Metrology Rules and any other relevant statutory requirement. In addition, the packaging shall be legibly marked with the following information:

a) The wording "oral rinse" or "Mouth wash" or equivalent
b) Type of oral rinse (Fluoridated or Non-Fluoridated)
c) Fluoride ion content in ppm for fluoridated oral rinse;
d) If the oral rinse contains alcohol, the declaration of alcohol content, as volume fraction;
e) Instructions and warning for proper use with children;
f) Caution: "Not suitable for children under 6 years of age unless medically recommended";
g) Warning: "Not to be swallowed" ; and
h) Any special storage conditions (if applicable) (for example, need for refrigeration).

6.3 BIS Certification Marking

The product may also be marked with the Standard Mark.

6.3.1 The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act*, 2016 and the Rules and Regulations framed thereunder, and the products may be marked with the standard mark.

7 SAMPLING

7.1 Representative samples of the product shall be drawn as prescribed in IS 3958.

7.2 Test for all characteristics shall be carried out on the composite sample.

7.3 The product shall be taken to have conformed to the specification if the composite sample passes all the tests.

8 QUALITY OF REAGENTS

8.1 Unless specified otherwise, pure chemicals and distilled water [*see* IS 1070 : 1992 'Reagent grade water (*third revision*)'] shall be employed in tests.

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

ANNEX A
(*Clause* 5.2.5)
GAS CHROMATOGRAPHIC METHOD OF TEST FOR ALCOHOL CONTENT AND ABSENCE OF METHANOL FOR ORAL RINSES

A-1 DETERMINATION OF ALCOHOL CONTENT

A -1.1 Apparatus

A-1.1.1 *Gas Chromatograph* — Equipped with Flame Ionization Detector (FID) and split injection port.

Chromatographic condition :

A-1.1.1.1 GC Conditions for β – DEX 225 Chiral Column

Column	Fused silica capillary column packed with 6 percent cyanopropylphenyl and 94 percent dimethyl polysiloxane
Film Thickness	1.8 µm
Column Dimension	30 m × 0.32 mm ID
Injector Temperature	200°C
Split Ratio	1 : 40
Sample Size	0.5 µl (2 percent solution in suitable solvent)
Carrier Gas and Flow	Nitrogen or Helium, at the flow rate of about 1.2 ml/min
Hydrogen gas flow	300 ml/min
Column oven Temperature	60°C for 5 min, then raised to 150°C at a rate of 10°C per min
Detector type	FID
Detector Temperature	250°C

NOTE — Optimum operating conditions may vary with column and instrument used and must be determined by using standard solutions. Adjust the parameters for maximum peak sharpness and optimum separation. With high level standard, 1-propanol should give almost complete baseline separation from ethanol.

A-1.2 Reagents and Solutions

A-1.2.1 *Ethanol* — 99.9 percent (*v/v*), *Min*

A-1.2.2 *1-Propanol* — 99.9 percent (*v/v*), *Min*

A-1.2.3 *Methanol* — 99.9 percent (*v/v*), *Min*

A-1.2.4 *Internal Standard Stock Solution* — Dilute 5.0 ml of 1-propanol (**A-1.2.2**) to 100 ml.

A-1.2.5 *Ethanol Stock Solution* — Dilute 5.0 ml of ethanol (**A-1.2.1**) to 100 ml.

A-1.2.6 *Ethanol Standard Solution* — Take 10 ml of ethanol stock solution (**A-1.2.5**) in a 100 ml volumetric flask, add 10 ml of internal standard stock solution (**A-1.2.4**) and make up volume to 100 ml.

A-1.2.7 *Internal Standard Solution* — Dilute 5 ml of internal standard stock solution (**A-1.2.4**) to 50 ml.

A-1.2.8 *Test Solution* — Take sample equivalent to 0.5 ml of ethanol (**A-1.2.1**) in a 100 ml volumetric flask, add 10 ml of internal standard stock solution (**A-1.2.4**) and make up volume to 100 ml.

A-1.2.9 *Methanol Stock Solution* — Dilute 5.0 ml of methanol (**A-1.2.3**) to 100 ml.

A-1.2.10 *Methanol Standard Solution* — Take 5.0 ml of methanol stock solution (**A-1.2.9**) in a 100 ml volumetric flask , add 10 ml of internal standard stock solution (**A-1.2.4**) and make up volume to 100 ml.

A-1.3 Procedure

A-1.3.1 Set the instrument as per chromatographic condition as given in **A-1.1.1** above and allow the instrument till stable base line is achieved.

A-1.3.2 Inject separately 2µl of each, ethanol standard solution, internal standard solution and methanol standard solution and determine the retention time of ethanol, 1-propanol and methanol.

A-1.3.3 Inject 5 injections of ethanol standard solutions and calculate Relative Standard Deviation (RSD) of internal standard response ratio. RSD should be less than 2. Use average peak area of five injections for calculation.

A-1.3.4 Inject 2 µl of test solution in duplicate. Use average peak area of two injections for calculation.

A-1.4 Calculation

Calculate ethanol content in sample as follows:

Ethanol content, percent (*v/v*) = $\frac{R_2 \times W_s \times D \times 100}{R_1}$

where

R_2 = peak ratio of ethanol to 1-propanol for sample solution;
W_s = concentration of ethanol in standard solution in percent (*v/v*);

D = dilution factor for sample solution; and
R_1 = peak ratio of ethanol to 1-propanol for standard solution.

NOTE — Other chromatographic parameters and combinations including (but not limited to) usage of FID with validated procedures and similar sensitivity may also be used.

A-2 DETERMINATION OF ABSENCE OF METHANOL

Observe the chromatograms obtained with test solution and methanol standard solution. Test complies if no peak observes, in the chromatogram obtained with test solution at the retention time of methanol.

ANNEX B
(*Clause* 5.3)
DETERMINATION OF STABILITY AGAINST AGEING

B-1 One of the following two tests shall be performed.

B-1.1 Accelerated Test

Store the oral rinse at (40 ± 2) °C for 3 months at (75 ± 5) percent relative humidity or under such conditions of time and temperature as will stimulate storage at room temperature for 36 months.

B-1.2 Real Time Test

Store the oral rinse at (27 ± 2)°C at (65 ± 5) percent relative humidity for 36 months or for the period indicated by the expiry date listed on the product label.

ANNEX C
[*Table* 1, *Sl No.* (i)]
DETERMINATION OF *p*H

C-1 APPARATUS

C-1.1 *p*H meter — preferably equipped with glass electrode.

C-2 PROCEDURE

Determine the *p*H of oral rinse in its intended concentration for use at a temperature of 27 ± 2° C, using a *p*H meter.

ANNEX D
[*Table* 1, *S1 No.* (ii)]
TEST FOR HEAVY METALS

D-1 OUTLINE OF THE METHOD

The color produced with hydrogen sulphide solution is matched against that obtained with standard lead solution.

D-2 APPARATUS

D-2.1 Nessler Cylinders — 50-ml capacity.

D-3 REAGENTS

D-3.1 Dilute Hydrochloric Acid — Approximately 5 N.

D-3.2 Dilute Acetic Acid — Approximately 1 N.

D-3.3 Hydrogen Sulphide Solution — Standard.

D-3.4 Standard Lead Solution — Dissolve 1.600 g of lead nitrate in water and make up the solution to 1 000 ml. Pipette out 10 ml of the solution and dilute again to 1 000 ml with water. One milliliter of this solution contain 0.01 mg of lead (as Pb).

D-4 PROCEDURE

D-4.1 Weigh about 2.000 g of material in a crucible and heat on a hot plate and then in a muffle furnace to ignite it at 600°C to constant mass. Add 3 ml of dilute hydrochloric acid, warm (wait till no more dissolution occurs) and make up the volume to 100 ml. Filter the solution. Transfer 25 ml of the filtrate into a Nessler's cylinder. In the second Nessler's cylinder, add 2 ml of dilute acetic acid, 1.0 ml of standard lead solution and make up the volume with water to 25 ml.

D-4.2 Add 10 ml of hydrogen sulphide solution to each Nessler cylinder and make up the volume with water to 50 ml. Mix and allow to stand for 10 min. Compare the colour produced in the two Nessler's cylinders. Blank determination without samples are recommended to avoid errors arising out of reagents.

D-5 RESULTS

The sample may be taken to have passed the test, if the colour developed in the sample solution is less than that of standard solution.

ANNEX E
[*Table* 1, *S1 No.* (iii)]
DETERMINATION OF ARSENIC

E-1 OUTLINE OF THE METHOD

Arsenic present in a solution of the material is reduced to arsine, which is made to react with mercuric bromide paper. The stain produced is compared with a standard stain.

E-2 REAGENTS

E-2.1 Mixed Acid — Dilute one volume of concentrated sulphuric acid with four volumes of water. Add 10 g of sodium chloride for each 100 ml of the solution.

E-2.2 Ferric Ammonium Sulphate Solution

Dissolve 64 g of ferric ammonium sulphate in water containing 10 ml of mixed acid and make up to one liter.

E-2.3 Concentrated Hydrochloric Acid [*see* IS 265 : 1993 'Hydrochloric acid — Specification (*fourth revision*)']

E-2.4 Stannous Chloride Solution — Dissolve 80 g of stannous chloride ($SnCl_2.2H_2O$) in 100 ml of water containing 5 ml of concentrated hydrochloric acid.

E-3 PROCEDURE

Carry out the test as prescribed in IS 2088, adding into the Gutzeit bottle, 2 ml of ferric ammonium sulphate solution, 0.5 ml of stannous chloride solution and 25 ml of sample solution as prepared in **D-4.1**.

For comparison, prepare a stain using 0.001 mg of arsenic trioxide.

ANNEX F
[*Table* 1, *Sl No.* (v)]
DETERMINATION OF FLUORIDE ION BY POTENTIOMETRIC METHOD

F-1 GENERAL

F-1.1 This method is suitable for the determination of water soluble fluoride species in oral rinses, including free fluoride and hydrolyzable complexes, for example, sodium mono fluorophosphates.

F-1.2 Principle

Water soluble species are converted to fluoride ion by acid hydrolysis. The fluoride ion activity is then determined potentiometrically with the help of fluoride ion sensitive electrode.

F-2 APPARATUS

F-2.1 *p*H Meter (Potentiometer) — Scale readable to ± 0.5 mV or better.

F-2.2 Fluoride Ion Sensitive Electrode — Orion 94-09 or similar.

F-2.3 Single Junction Reference Electrode — Orion 90-01, or similar, with filling solution.

F-2.4 Magnetic Stirrer

F-2.5 Polythene/Polypropylene Beakers and Volumetric Flasks — 100, 250 ml and pipettes.

F-2.6 Semi-log Graph Papers – 2/3 cycles.

F-3 REAGENTS

F-3.1 Sodium Fluoride, analytical grade

F-3.2 Trisodium Citrate, analytical grade

F-3.3 Sodium Chloride, analytical grade

F-3.4 Hydrochloric Acid, analytical grade — l M.

F-3.5 Sodium Hydroxide — l M.

F-3.6 Sodium Acetate Trihydrate, analytical grade

F-3.7 Glacial Acetic Acid

F-3.8 TISAB L (Total Ionic Strength Adjusting Buffer) Solution — Dissolve 294 g trisodium citrate, 29 g sodium chloride and 68 g sodium acetate trihydrate in 600 ml of hot water. Cool, adjust to *p*H 6.4 with glacial acetic acid. Dilute to 1 litre with distilled water.

F-3.9 TISAB LF (TISAB Containing Fluoride) Solution — Prepare 100 ml of l mg F^-/100 ml solution as described in **F-3.11**. Dissolve 294 g trisodium citrate, 29 g sodium chloride and 68 g sodium acetate trihydrate in 600 ml of hot water. Cool, pipette in 10 ml of 1 mg F^- /100 ml solution and adjust to *p*H 6.4 with glacial acetic acid. Dilute to 1 litre with distilled water. Store in a polythene or polypropylene bottle.

F-3.10 Fluoride Blank Solution — Take 100 ml hydrochloric acid solution (l M) in 1 litre flask and then add 200 ml sodium hydroxide (l M), by measuring cylinder. Dilute to 1 litre with distilled water and mix well.

F-3.11 Standard Sodium Fluoride Solution (0.01 mg F^- per ml)

Dry the sodium fluoride at 110ºC for 4 h 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 X). Each ml of Solution X contains 1 mg fluoride ion (F^-). Take 10 ml of this Solution X in 1000 ml volumetric flask and make up this volume to the mark (Solution Y). Each ml of Solution Y contains 0.01 mg fluoride (F^-) ion.

Transfer Solution X and Solution Y to polythene bottles for storing.

F-3.12 Preparation of standard solutions of Sodium Fluoride Take 1, 2, 5, 10, 20 and 25 ml of Solution Y (*see* **F-3.11**) in 100 ml volumetric flask marked A, B, C, D, E and F, respectively. To

each add 50 ml of TISAB L buffer solution and 10 ml of fluoride blank solution. Check that the *p*H is in the range of 6.4 ± 0.1, and if necessary correct with 1 M NaOH or 1 M HCl. Transfer quantitatively to a 100 ml polypropylene volumetric flask 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 mV measurement.

F-4 mV MEASUREMENT OF STANDARD SOLUTIONS OF SODIUM FLUORIDE

F-4.1 Preparation of Electrodes — Remove protective cap and soak the fluoride electrode in TISAB LF solution for 15 min.

F-4.2 Fill the reference electrode with filling solution.

F-4.3 Rinse the electrodes with de-ionized water and keep the tips immersed in TISAB LF solution until immediately before use.

F-4.4 Check that the electrodes are correctly connected to the *p*H meter.

F-4.5 Rinse the electrodes with deionized water before use and carefully blot dry with a paper tissue.

F-5 mV MEASUREMENT

F-5.1 Transfer the contents of solution A from 100 ml flask into a clean, dry 150 ml polypropylene beaker.

F-5.2 Immerse the tips of the electrodes in the solution while stirring the solution with a magnetic stirrer. Ensure that no air bubbles adhere to the electrode surfaces.

F-5.3 Leave until the potential reading is constant. This should take approximately 2 or 3 min.

F-5.4 Record the potential reading in mV and check the temperature of the solution.

F-5.5 Rinse the electrodes with de-ionized water and blot dry with a paper tissue.

F-5.6 Repeat the procedure prescribed in **F-5.1** to **F-5.5** for solutions B, C, D, E and F to record mV of these solutions.

F-5.7 Plot the calibration graph on semi log graph paper with the mini volt reading on-the linear ordinate and the final concentration of fluoride in the standard F solution on the logarithmic abscissa. The graph should be a straight line with a gradient of ~ 57-59 mV per decade change in concentration.

F-6 TEST SOLUTION

F-6.1 Weigh about 5 g of oral rinses to the nearest mg. Dilute with deionized water to 100 ml with deionized water in a polypropylene volumetric flask and mix well.

NOTE — In case of non-fluorinated product, 20 g of sample shall be taken for preparation of test solution.

F-6.2 Ensure that the dispersion is homogeneous and then centrifuge about 60 ml of the dispersion in a polypropylene centrifuge tube, closed with a cap to prevent evaporation, until clear. This will take about 20 min at 4000 rpm.

F-6.3 Pipette 20 ml of the clear supernatant into a 250 ml round-bottomed flask.

F-6.4 Add a few anti-bumping granules then add 10 ml hydrochloric acid solution (1 M approximately) by measuring cylinder. Attach a reflux condenser and boil gently for 5 min.

F-6.5 Add, almost immediately, 20 ml of 1 M sodium hydroxide via the condenser, rinsing down with approximate 20 ml of distilled water. Then transfer quantitatively to a 100 ml polypropylene volumetric flask and dilute to volume with distilled water.

NOTE - For samples containing Free Fluoride Ions, steps F-6.2 to F-6.5 can be avoided

F-6.6 Pipette 25 ml of the clear solution prepared above into a 100 ml polypropylene beaker, add 25 ml TISAB L (Solution L) and check *p*H. If necessary adjust to *p*H 6.4 by addition of approximately 1 M hydrochloric acid or 1 M sodium hydroxide. Transfer quantitatively into a 100 ml volumetric flask and dilute to volume.

F-6.7 Transfer the contents of 100 ml flask to a clean, dry 100 ml polythene beaker, immerse the tips of the electrode in the solution while stirring the solution with a magnetic stirrer. Ensure that no air bubbles adhere to the electrode surfaces.

F-6.8 Leave until the potential reading is constant (this should take two or three minutes). Record the potential reading in mV for the test solution.

NOTE — The reading for standard fluoride (F^-) and test solutions should be taken simultaneously.

F-7 CALCULATION

A graph is plotted for concentration of fluoride (F^-) against potential mV on a semi-logarithmic paper for standard F^- solutions, the potential mV is plotted on X-axis and mg of F^- on Y-axis (on logarithmic scale). Read the F^- concentration in test solution for measured mV from this graph.

Concentration of fluoride (F^-) in oral rinses, parts per million = $\frac{2a \times 10000}{M}$

where

a = fluoride, in mg, from calibration graph for test solution, and
M = Mass, in g, of sample.

ANNEX G

[*Table* 1, *Sl No.* (v)]

DETERMINATION OF FLUORIDE ION BY ION CHROMATOGRAPHY (IC)

G-1 APPARATUS

G-1.1 Ion Chromatographic System with Conductivity detector (IC-CD)

G-1.2 Mobile Phase Filtering Apparatus

G-1.2.1 *0.45 µm Nylon Filters (47 mm)* or equivalent.

G-1.2.2 *0.45 µm PTFE Syringe Filters (25 mm)* or equivalent.

G-1.2.3 *A Grade Pipettes*

G-1.3 Polymethyl Pentane (PMP)/ Polypropylene Volumetric Flasks

G-1.4 Polymethyl Pentane (PMP)/ Polypropylene Autosampler Vials

G-1.5 Polypropylene Disposable Syringes

G-2 REAGENTS

G-2.1 Sodium Carbonate, anhydrous powder.

G-2.2 Sodium Hydroxide (1 N solution)

G-2.3 Sodium Fluoride, working standard.

G-3 IC CONDITIONS

Parameter	Description
Column	250 × 4.6 mm or any suitable anion exchange column
Guard column	50 × 4.6 mm or any suitable anion exchange guard column
Mobile phase	Solution of 0.15 g/l Sodium carbonate and 0.1 percent 1 N sodium hydroxide
Flow rate	1.0 ml/min
Injection volume	20 µl
Fluoride standard	~50ppm

NOTE — Other chromatographic parameters and mobile phase(s) combinations including (but not limited to) usage of sodium bicarbonate with validated procedures and similar sensitivity may also be used.

G-4 SAMPLE AND STANDARD PREPARATION

G-4.1 Pipette 5.0 ml of sample into a 100 ml volumetric flask.

NOTE — In case of non-fluorinated product, 20 g of sample shall be taken for preparation of test solution.

G-4.2 Add approximately 35 ml of deionised water, stopper and tap the flask until no air bubbles are visible.

G-4.3 Dilute to volume with deionised water and mix thoroughly.

NOTE — Repeat the process of tapping the flask in between the addition of the remaining water to remove any air bubbles.

G-4.4 Pipette approximate 15.0 ml solution (**G-4.3**) into a suitable volumetric flask, to match the concentration of the sample fluoride to ~50 ppm. Dilute to volume with deionised water and mix thoroughly.

G-4.5 Condition the syringe and PTFE 0.45 µm filter (or equivalent) by passing at least 3 ml of each sample solution through the filter.

G-4.6 Filter the sample solution into an IC autosampler vial and chromatograph as per the sections on equipment settings and conducting the analysis.

G-4.7 Prepare the standard solution in similar steps to achieve the concentration of the standard fluoride ~50 ppm.

G-5 CALCULATIONS

The following calculations are included as examples and are only suitable for single point calibration. The use of a single point calibration curve linear through zero is preferable.

$$\text{Concentration of fluoride } (\text{F}-) \text{ in oral rinses, parts per million}$$

$$= \frac{R\text{u} \times W \times \text{sample dilution} \times P}{R\text{s} \times \text{standard dilution} \times \text{sample volume} \times 100} \times 1000$$

where

R_u = Peak Area of Sample;
R_s = Peak Area of Standard;
W = mass, in mg, of sodium fluoride standard; and
P = Potency of standard.