भारतीय मानक

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Indian Standard

अल्कोहल डिनाट्यूरेंट्स — विशिष्टि

(तीसरा पुनरीक्षण)

Alcohol Denaturants — Specification

(Third Revision)

ICS 71.100.99

भारतीय मानक ब्यूरो
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FOREWORD

This Indian Standard (Third Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Organic Chemicals, Alcohols and Allied Products Sectional Committee had been approved by the Petroleum, Coal and Related Products Division Council.

Ethyl alcohol is generally used as solvent and in the manufacture of a large number of products such as polyethylene, acetic acid, butyl alcohol, styrene, butadiene, etc. It may be used in the form of rectified spirit or ordinary denatured spirit. Besides, large quantities of specially denatured ethyl alcohol are used for specific industries. These special denaturants are added so that such specially denatured alcohol can be used in the manufacture of organic chemicals without affecting manufacturing processes.

Completely denatured alcohol (CD) and specially denatured alcohol (SD) formulations are set out in this standard with the specific composition. Both CD and SD formulations are made from spirits in accordance with international trade practices. These resulting products are not fit for human consumption; instead, the products are intended for industrial use.

CD is composed of alcohol and specified denaturants that cannot be easily removed by any simple process and can be used in a number of industrial uses. As the denaturants cannot be easily or economically removed, CD is considered to be at low risk of diversion or abuse.

SD is composed of alcohol and specified denaturants that are more readily removed by simple chemical processes. While specially denatured spirit (SDA) is not fit for human consumption by virtue of the denaturants used to denature the product, it can, through certain recovery processes, be made potable. As a result, its use is controlled through the registration of users.

This standard was first published in 1966 and simultaneously revised in 1973 and 2008. In first revision, the essential requirements as well as limit tests for the denaturants in specially denatured alcohol were incorporated. Formulae for specially denatured alcohol for specific end use were given. This standard, covering the minimum quality requirements of various alcohol denaturants along with their limit tests, was expected to introduce a measure of uniformity in the enforcement of various regulations under Excise Acts of different states in regard to procurement and usage of ethyl alcohol in industry.

It also permitted State governments to prescribe such regulations on the basis of the specified formulae as to safeguard the interests of government revenues or the policy of prohibition followed by them. This standard did not exclude such flexibility and control as was necessary in cases where there is an apprehension that none of the prescribed formulae would adequately meet the process requirements and perforce use of un-denatured alcohol is to be permitted. In such cases, the state governments have been given freedom to impose additional stringent regulations for the control over such users of un-denatured ethyl alcohol. The majority of denaturants covered had relevant detailed Indian Standard specifications for guidance. However, in the use of these chemicals for denaturing ethyl alcohol such detailed specifications were not considered essential. This standard, therefore, included only the essential requirements for each denaturant and its limit tests in the denatured alcohol

Some of the formulae covered envisaged the use of two or more denaturants in varying proportions. Obviously limit tests in these cases were difficult. In such cases, in keeping with procedures under *Excise Acts*, it was recommended that individual denaturants be first tested and then approved. Limit tests for individual denaturants can then be used on the basis of these individual denaturants.

(*Continued on third cover*)

Indian Standard

ALCOHOL DENATURANTS — SPECIFICATION

(Third Revision)

1 SCOPE

This standard prescribes the list for formulae of alcohol denaturants, essential requirements, likely/permissible end use applications, their limit tests in denatured alcohol and methods of tests for various denaturants in denatured alcohol.

2 REFERENCES

The standards listed in Annex A contain provisions which through reference in this text, constitute provisions of the standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on the standards are encouraged to investigate the possibility of applying the most recent edition of these standards.

3 TERMINOLOGY

For the purpose of this standard, definitions given in IS 6749 shall apply.

4 LIST OF PERMISSIBLE DENATURANTS AND THEIR END USES

List of denaturants along with the permissible limits is given in Annex B. Annex C covers specific end use in product/process where these formulae of denaturants can be used.

5 GRADES AND SPECIFICATIONS

5.1 The completely denatured alcohol set out in formula are mixed with specific denaturant in 100 litre of spirits

5.2 The specially denatured alcohol set out in formula are mixed with specific denaturant in 100 litre of spirits.

5.3 Where the volume of spirits to be denatured to completely denatured alcohol (CD) or specially denatured alcohol (SD) formula set out is greater or less than 100 litre, the quantity of denaturant used shall be in the same proportion to that volume of spirits that the quantity of the denaturant set out to 100 litre.

5.4 The composition of the denatured alcohol grades set out in the formula are stated in terms of ethyl alcohol of a minimum strength of 94 percent (*v/v*) ethyl alcohol by volume at 20 °C prior to denaturation in the case of all grades.

6 MANNER OF DENATURING

The denaturants should be thoroughly mixed with spirits to have a uniform effect. Table 1 covers requirement of various alcohol denaturants with reference to method of tests.

7 TEST METHODS

7.1 Tests shall be carried out as prescribed in col (5) and col (6) of Table 1.

7.2 Quality of Reagents

Unless specified otherwise, 'pure chemicals' and distilled water (*see* IS 1070) shall be employed in tests.

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

8 LIMIT TESTS FOR DENATURANTS IN DENATURED ALCOHOL

Reference may be made to Annex E for carrying out limit tests for denaturants in denatured alcohol. Gas chromatographic analysis is given in Annex F.

Table 1 Requirement of Various Alcohol Denaturants

(*Foreword*, *clauses* 6, 7.1 *and* E-6)

Sl No.	**Name of Denaturant**	**Characteristics**	**Requirement**	**Method of Test, Ref to**	
				Annex	IS/Relevant clause of IS
(1)	(2)	(3)	(4)	(5)	(6)
i)	Acetaldehyde	a) Odour	Characteristic	—	—
		b) Acetaldehyde content in aqueous solution, percent by mass, *Min*	30.0	**D-1**	—
		c) Relative density at 15 °C/15 ° C	0.920	—	—
ii)	Acetone	a) Relative density at 20 ºC/20 ºC	0.792 to 0.796		IS 170
		b) Boiling range, ºC	55.5 to 56.5 (not less than 95 percent shall distill between 55.5 ºC and 56.5 ºC)	—	
iii)	Ammonia, aqueous	a) Specific gravity at 20 °C/4 °C	0.892 0 to 0.901 0	—	IS 799
		b) Ammonia content, percent by mass, *Min*	27 to 30	**D-2**	
iv)	Benzene	a) Relative density at 20 ºC/20 ºC	0.882 to 0.885	—	IS 534
		b) Boiling range, ºC	79 to 81 (from 5 ml to 95 ml shall distill over within a range of 1 °C)	—	—
v)	A) Brucine	a) Appearance	Off white powder or crystals	—	—
		b) Melting point, ºC	105 (the anhydrous substance melts at 178 ºC)	—	—
		c) Identification:			
		i) To 10 mg of substance add a few drops of nitric acid and then a few drops of stannous chloride solution (1 part in 9 parts of hydrochloric acid, relative density 1.12)	Blood red colour changing to intense violet	—	—
				—	—

Table 1 (*Continued*)

Sl No.	**Name of Denaturant**	**Characteristics**	**Requirement**	**Method of Test, Ref to**	
				Annex	IS/Relevant clause of IS
(1)	(2)	(3)	(4)	(5)	(6)
		ii) To 4 mg of the material add a few drops of 10 percent formaldehyde and then evaporate to dryness, heat the residue with a drop of stannous chloride	Blue colour obtained changing to yellowish green on warming		
		d) Absence of strychnine	To pass the test	D-3.1	—
		e) Assay, percent by mass, *Min* (calculated as brucine $C_{23}H_{26}N_2O_4.4H_2O$)	98	D-3.2	—
	B) Brucine Sulphate	a) Appearance	White crystalline powder	—	—
		b) Solubility in water at 27 °C	1 g dissolves in 70 ml	—	—
		c) Acidity (tested by using 500 mg of brucine sulphate in 400 ml solution and titrating with sodium hydroxide solution using methyl red as indicator)	Not more than 0.5 ml of 0.02 N sodium hydroxide solution shall be required for neutralization	—	—
		d) Identification	Same as identification tests (i) and (ii) given for brucine at [Sl No.(v), (A), (c)]	—	—
		e) Absence of strychnine	To pass the test	D-3.1	—
		f) Assay, percent by mass, *Min* {calculated as brucine Sulphate $[(C_{23}H_{26}N_2O_4)2H_2SO_47H_2O]$}	98	D-3.2	—
vi)	Caustic soda liquid	Total alkali (as NaOH) content, percent by mass, *Min*	45	D-4	**A-4** of IS 252

Table 1 (*Continued*)

Sl No.	**Name of Denaturant**	**Characteristics**	**Requirement**	**Method of Test, Ref to**	
				Annex	IS/Relevant clause of IS
(1)	(2)	(3)	(4)	(5)	(6)
vii)	Chloroform	a) Relative density at 27 ºC/27 ºC	1.466 to 1.474	—	IS 5296
		b) Distillation range:			
		i) Up to 60 ºC, percent, *Max*	15	—	—
		ii) 60 ºC to 62 ºC	Remaining	—	—
viii)	Crotonaldehyde	a) Relative density at 27 ºC/27 ºC, *Max*	0.872	—	IS 2632
		b) Aldehyde content percent by mass, *Min*	85	—	
ix)	Cyclohexane	Relative density at 20 ºC/20 ºC	0.78	—	6 of IS 82
x)	Denatonium benzoate	a) Appearance	White crystalline powder	—	—
		b) Melting point, ºC	166 to 170	—	—
		c) Assay, percent by mass, *Min*	99.0	D-5	—
		d) Identification	a) Color test – Mandolin test – Violet b) UV spectrum – 263 nm and 269 nm	—	—
xi)	Denatonium saccharide (crystalline powder)	a) Appearance	White crystalline powder	—	—
		b) Melting point, ºC	175 to 178	—	—
		c) Assay, percent by mass on dry basis, *Min*	99.5	D-5	—
xii)	Denatonium saccharide (amorphous powder)	a) Appearance	White amorphous powder	—	—
		b) Melting point, ºC	173 to 176	—	—
		c) Assay, percent by mass on dry basis, *Min*	99.5	D-5	—
xiii)	Diethyl phthalate	a) Relative density at 20 ºC/20 ºC	1.194 to 1.120	D-6.1	—
		b) Purity, percent by mass, *Min*	99.0	D-6.2	—

Table 1 (*Continued*)

Sl No.	**Name of Denaturant**	**Characteristics**	**Requirement**	**Method of Test, Ref to**	
				Annex	IS/Relevant clause of IS
(1)	(2)	(3)	(4)	(5)	(6)
xiv)	Ethyl acetate	a) Relative density at 27 ºC/27 ºC	0.894 to 0.898	—	IS 229
		b) Boiling range, ºC	76 to 79 (Not less than 95 ml shall distill within the range)	—	
		c) Ethyl acetate, percent by mass, *Min*	Not less than 99	—	
xv)	Formaldehyde solution	a) Odour	Characteristic	—	—
		b) Formaldehyde content, percent by mass (*m/v*)	37 ± 0.5	D-7	IS 3321
xvi)	Gasoline (C_4–C_{12})	a) Odour	Characteristic	—	—
		b) Density at 15 ºC, kg/m^3	710 to 775	—	IS 2796
		c) Distillation recovery up to 180 ºC, percent by volume, *Min*	90	—	—
xvii)	Isopropyl alcohol	a) Appearance	Colorless liquid	—	—
		b) Specific gravity at 15.5 °C	0.810	—	IS 2631
		c) Assay, percent, *Min*	98.0	—	
xviii)	Menthol	a) Appearance	Crystalline powder	—	—
		b) Melting range, ºC	41 to 44	—	IS 3134
		c) Assay, percent, *Min*	99.0	—	
		d) Identification	Dissolve 10 mg in 1 ml of sulphuric acid and add 1 ml of a 1 percent (*w/v*) solution of vanillin in sulphuric acid, an orange yellow colour is produced. Add 1 ml of water, the color changes to violet.	—	—
xix)	Methanol (methyl alcohol)	a) Relative density at 27 °C/27 °C	0.789 to 0.798	—	IS 517
		b) Boiling range, °C	64.0 to 66.5 (not less than 95 percent by volume shall distill over between 65.5 to 66.5)	—	

Table 1 (*Concluded*)

Sl No.	**Name of Denaturant**	**Characteristics**	**Requirement**	**Method of Test, Ref to**	
				Annex	IS/Relevant clause of IS
(1)	(2)	(3)	(4)	(5)	(6)
xx)	Methyl isobutyl ketone	a) Odor	Characteristic	—	—
		b) Colour	Colorless liquid	—	IS 9850
		c) Boiling range, °C	114 to 117	—	
		d) Relative density 27 ºC/ 27 °C	0.794 to 0.799	—	
xxi)	Petroleum naphtha (C_4-C_{11})	a) Appearance	White liquid	—	—
		b) Odour	Characteristic	—	—
		c) Boiling range, °C	25 to 150	—	—
xxii)	Toluene	a) Relative density at 27 ºC/27 ºC	0.865 to 0.870	—	IS 537
		b) Boiling range, ºC	108 to 111	—	—
xxiii)	Turpentine oil (gum spirit of turpentine)	a) Odour	Characteristic	—	**4.2.2** of IS 533
		b) Relative density at 27 °C/27 °C	0.852 to 0.862	—	IS 326 (Part 3)
		c) Refractive index at 27 °C	1.468 to 1.475	—	IS 326 (Part 5)
xxiv)	Tertiary butyl alcohol	a) Appearance	Colorless liquid	**D-8**	—
		b) Boiling range, °C	78 to 85	—	—
		c) Specific gravity, 20 ºC/20 ºC	0.785 to 0.789	—	—
		d) Identification	Place five drops of a solution containing approximately 0.1 percent tertiary butyl alcohol in ethyl alcohol in a test tube. Add 2 ml of denige's reagent (dissolve 5 grams of red mercuric oxide in 20 ml of concentrated sulfuric acid; add this solution to 80 ml of distilled water, and filter when cool). Heat the mixture just to the boiling point and remove from the flame. A yellow precipitate forms within a few seconds	—	—

ANNEX A

(*Clause 2*)

LIST OF REFERRED STANDARDS

IS No.	*Title*
IS 82 : 1973	Methods of sampling and test for thinners and solvents for paints (*first revision*)
IS 170 : 2020	Acetone — Specification (*fifth revision*)
IS 229 : 2021	Ethyl acetate — Specification (*fourth revision*)
IS 252 : 2013	Caustic soda — Specification (*fourth revision*)
IS 266 : 1993	Sulphuric acid — Specification (*third revision*)
IS 323 : 2009	Rectified spirit for industrial use — Specification (*second revision*)
IS 326	Methods of sampling and test for natural and synthetic perfumery materials:
(Part 3) : 2006/ISO 279 : 1998	Determination of relative density (*third revision*)
(Part 5) : 2006/ISO 280 : 1998	Determination of refractive index (*third revision*)
IS 336 : 2021	Ether — Specification (*third revision*)
IS 517 : 2020	Specification for methanol (methyl alcohol) (*third revision*)
IS 533 : 2007	Gum spirit of turpentine (oil of turpentine) — Specification (*third revision*)
IS 534 : 2021	Benzene — Specification (*fifth revision*)

IS No.	*Title*
IS 537 : 2011	Toluene — Specification (*second revision*)
IS 799 : 2023	Ammonia, liquor — Specification (*third revision*)
IS 1070 : 2023	Reagent grade water — Specification (*fourth revision*)
IS 1448 (Part 32) : 2019/ ISO 3838 : 2004	Methods of test for petroleum and its products: Part 32 Crude petroleum and liquid or solid petroleum products — Determination of density or relative density — Capillary stoppered pyknometer and graduated bicapillary pyknometer methods (*third revision*)
IS 2631 : 2020	Iso propyl alcohol — Specification (*second revision*)
IS 2632 : 2023	Crotonaldehyde — Specification (*first revision*)
IS 2796 : 2017	Motor gasolines — Specification (*sixth revision*)
IS 3134 : 1992	Menthol — Specification (*first revision*)
IS 3321 : 1973	Specification for formaldehyde solution (*first revision*)
IS 5296 : 2024	Chloroform, pure and technical — Specification (*third revision*)
IS 6749 : 1972	Glossary of terms relating to alcohol (ethyl) industry and trade
IS 9850 : 2001	Methyl iso-butyl Ketone — Specification (*first revision*)

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ANNEX B

(*Foreword*, *Clauses* 4, C-1 *and* F-2.3)

FORMULAE FOR DENATURED ALCOHOL

Designation (1)	Denaturant (2)	Amount of Denaturant for 100 litre of Alcohol (3)	Use, Ref to Annex (4)
A - Completely Denatured Alcohol			
CD 1	Methyl alcohol + ethyl acetate	16.0 litre + 1.0 litre	C
CD 2	Iso-propyl alcohol + ethyl acetate	8.0 litre + 4.0 litre	C
CD 3	Acetone + ethyl acetate	8.0 litre + 1.0 litre	C
CD 4	Methyl alcohol + petroleum naphtha	6.5 litre + 1.0 litre	C
CD 5	Gasoline	2 litre to 5 litre	C
B - Specially Denatured Alcohol			
SD 1	Methyl alcohol	5.0 litre	C
SD 1a	Methyl alcohol	10.0 litre	C
SD 2 1)	Benzene/Toluene	5.0 litre	C
SD 2a 1)	Benzene or toluene	1.5 litre	C
2b	Cyclohexane + denatonium benzoate/ denatonium saccharide	2.0 litre + 4 g	C
SD 3	Acetone	8.0 litre	C
SD 3a	Acetone + methyl isobutyl ketone	8 litre +1.5 litre	C
SD 4 2)	Acetaldehyde	1.5 litre	C
SD 5 2)	Chloroform	5.0 litre	C
SD 6	Ethyl acetate	1.0 litre	C
SD 6 a	Ethyl acetate	5.0 litre	C
SD 7	Formaldehyde	10.0 litre	C
SD 7 a	Formaldehyde + menthol	2.5 litre + 2.5 kg	C
SD 7 b	Formaldehyde + menthol	1.25 litre + 4.5 kg	C
SD 8 2)	Caustic soda or ammonia	5.5 kg (solid) or 8.0 kg (liquid)	C
		3 litre	
SD 9	Turpentine oil	5 litre	C
SD 10	Diethyl phthalate + tertiary butyl alcohol	2.5 litre + 0.5 litre	C
SD 11	Brucine or brucine sulphate + tertiary butyl alcohol	10 g + 0.5 litre	C
SD 11a	Tertiary butyl alcohol + denatonium benzoate	0.125 litre + 0.468 g	C
SD 11b	Tertiary butyl alcohol	3.0 litre	C
SD 12	Methyl alcohol + denatonium benzoate/ denatonium saccharide	4.0 litre + 4 g	C
SD 12a	Acetone + denatonium benzoate/ denatonium saccharide	3.0 litre + 4 g	C
SD 13	Isopropyl alcohol	5.0 litre	C
SD 14	Crotonaldehyde	0.2 litre	C

1) Due to carcinogenic considerations benzene may be phased out in 5 years. Industry may make necessary alterations in their process.

2) Denaturants used in SD 4, SD 5, SD 8 formulations are separable by simple distillation. Hence customs, excise and other regulatory authorities should ascertain the actual user, specific end use quantity required for the product or process before allowing the formulas.

ANNEX C

(*Foreword*, *Clauses 4 and Clause* E-1)

PERMISSIBLE END USE APPLICATIONS

C-1 This section lists, alphabetically by product or process, formulas of denatured alcohol authorized use in those products or processes. Specially denatured alcohol formulations are for specific purpose. The specially denatured alcohol formulations given in Annex B are used for many industrial purposes. It is evident form the data given that for each industrial use there is more than one formulation is given; this gives flexibility to industry in selecting a formulation. Where ever the formula number is given col (2) it can be used for the specific end use shown in col (3).

Sl No. (1)	*Formulas Authorized* (2)	*Product or Process* (3)
	Completely Denatured Alcohol (CD)	
i)	CD 5	Alcohol denaturant for mixing in gasoline
ii)	CD 1, 2, 3, 4	General purpose
	Specially Denatured Alcohol (SD)	
i)	2a, 2b, 4 , 12, 14	Acetaldehyde
ii)	4, 6a, 14	Acetic acid
iii)	1, 1a, 2, 2b, 3, 12, 12a, 13	Adhesives and binders
iv)	1, 2a, 2b, 12, 12a	Aldehydes, miscellaneous
v)	1, 1a, 2, 2a, 2b, 3, 6a, 12, 12a, 13	Alkaloids processing
vi)	6a	Animal feed supplements
vii)	1, 1a, 2, 2a, 2b, 3, 6a, 12, 12a, 13	Antibiotics processing
viii)	12	Antifreeze, proprietary
ix)	3, 9, 12a	Antiseptic solutions, U.S.P. or N.F.
x)	1, 1a, 3, 8, 9, 10, 11, 12, 13	Bath preparations
xi)	3, 9, 10, 11, 12a	Bay rum
xii)	1, 3, 3a, 9, 10, 11, 12, 13	Biocides, miscellaneous
xiii)	1, 1a, 2, 2b, 3, 12, 12a, 13	Blood and blood products processing
xiv)	1, 12, 13	Brake fluids
xv)	3, 6a, 12a	Candy glazes
xvi)	1, 1a, 3, 12, 12a, 13	Cellulose coatings
xvii)	1, 2a, 2b, 12, 13	Cellulose compounds (dehydration)
xviii)	1, 3, 12, 12a, 13	Cellulose intermediates
xix)	1, 1a, 2, 2a, 2b, 5, 6, 8, 12, 13	Chemicals (miscellaneous) chloroform
xx)	1, 1a, 3, 3a, 8, 10, 11, 13	Cleaning solutions
xxi)	1, 3, 12, 12a, 13	Coatings, miscellaneous
xxii)	1, 3, 13	Collodions, industrial
xxiii)	13	Collodion, U.S.P.

Sl No. (1)	*Formulas Authorized* (2)	*Product or Process* (3)
xxiv)	9, 10, 11a, 11b	Colognes
xxv)	1, 1a, 2a, 2b, 3, 12, 12a, 13	Crude drugs (processing)
xxvi)	1, 12, 13	Cutting oils
xxvii)	1, 2a, 2b, 12, 13	Dehydration products, miscellaneous
xxviii)	7a, 7b, 9	Dentifrices
xxix)	3, 9, 10, 11a, 11b, 12a	Deodorants (body)
xxx)	1a, 3, 3a, 8, 12, 12a, 13	Detergents, household
xxxi)	1, 1a, 3, 11, 12, 13	Detergents, industrial
xxxii)	12	Detonators
xxxiii)	1, 3, 3a, 9, 10, 11, 12, 12a, 13	Disinfectants
xxxiv)	1, 1a, 2, 2a, 2b, 4, 6, 12, 13, 14	Drugs and medicinal chemicals
xxxv)	1, 1a, 2a, 2b, 3, 6a, 8, 12, 12a, 13	Drugs, miscellaneous (processing)
xxxvi)	1, 12, 13	Duplicating fluids
xxxvii)	1, 2, 2a, 2b, 8, 12, 13	Dyes and intermediates
xxxviii)	1, 2, 2a, 2b, 12, 13	Dyes and intermediates (processing)
xxxix)	1, 1a, 3, 12, 12a, 13	Dye solutions, miscellaneous
xl)	1, 3, 7, 12, 12a, 13	Embalming fluids, etc.
xli)	2, 2a, 2b, 4, 6, 12	Esters. Ethyl (miscellaneous)
xlii)	2a, 2b, 4, 6, 12, 14	Ether, ethyl
xliii)	2a, 2b, 4, 6, 12, 14	Ethers, miscellaneous U.S.P or N.F.
xliv)	2a, 2b, 4, 6, 12, 14	Ethyl acetate
xlv)	1, 2, 2a, 2b, 4, 6, 8, 12, 13, 14	Ethyl amines
xlvi)	2a, 2b, 4, 6, 12, 14	Ethyl chloride
xlvii)	2a, 2b, 4, 6, 12, 14	Ethylene dibromide
xlviii)	2a, 2b, 4, 6, 12, 14	Ethylene gas
xlix)	1, 2a, 2b, 13	Explosives
l)	3, 3a, 8, 9, 10, 11, 12a,	External pharmaceuticals, not U.S.P. or N.F.
li)	3, 4, 6, 9, 12a, 14	External pharmaceuticals, miscellaneous
lii)	1, 1a, 3, 12, 12a, 13	Fluid uses, miscellaneous
liii)	1, 1a, 2a, 2b, 3, 6a, 13,	Food products, miscellaneous (processing)
liv)	1, 12, 13	Fuel uses, miscellaneous.
lv)	1, 12, 13	Fuels, airplane and supplementary
lvi)	1, 12, 13	Fuels, automobile and Supplementary
lvii)	1, 12, 13	Fuels, proprietary heating
lviii)	1, 12, 13	Fuels, rocket and jet
lix)	1a, 3, 3a, 9, 10, 11, 12, 13,	Fungicides
lx)	1, 1a, 2, 2a, 2b, 3, 6a, 12, 12a, 13	Glandular products (processing)

Sl No. (1)	*Formulas Authorized* (2)	*Product or Process* (3)
lxi)	3, 3a, 9, 10, 11a, 12a	Hair and scalp preparations
lxii)	1, 2a, 2b, 3, 12, 13,	Hormones (processing)
lxiii)	1, 9, 10, 11, 13,	Incense
lxiv)	1, 1a, 3, 12, 12a, 13	Inks
lxv)	1a, 3, 3a, 9, 10, 11, 12, 13,	Insecticides, pesticide and pesticide intermediates
lxvi)	4, 6, 14	Iodine solutions (including U.S.P. and N.F. tinctures)
lxvii)	1, 1a, 13	Laboratory reagents (for sale)
lxviii)	1, 1a, 13	Laboratory uses, general (own use only
lxix)	1, 3, 12	Lacquer thinners
lxx)	9, 10	Liniments, U.S.P. or N.F.
lxxi)	3, 3a, 9, 10, 11a, 11b, 12a,	Lotions and creams (body, face and hand)
lxxii)	1, 2, 2a, 2b, 3, 6a, 12, 13	Medicinal chemicals (processing)
lxxiii)	1, 1a, 2, 2a, 2b, 3, 6a, 12, 12a, 13	Miscellaneous chemicals (processing)
lxxiv)	1, 1a, 2, 2a, 2b, 3, 6a, 12, 12a, 13	Miscellaneous products (processing)
lxxv)	7a, 7b, 9	Mouth washes
lxxvi)	1, 1a, 2a, 2b, 13	Organo-silicone products
lxxvii)	1, 1a, 2a, 2b, 3, 6a, 12, 12a, 13	Pectin (processing)
lxxviii)	1, 1a, 2, 2a, 2b, 11, 12, 13	Perfume materials (processing)
lxxix)	9, 10, 11a, 11b	Perfumes and perfume tinctures
lxxx)	1, 2a, 2b, 12, 13	Petroleum products
lxxxi)	1, 1a, 12, 13	Photoengraving dyes and solutions
lxxxii)	1, 1a, 2a, 2b, 12, 13	Photographic chemicals (processing)
lxxxiii)	1, 1a, 2a, 2b, 12, 13	Photographic film and emulsions
lxxxiv)	1, 1a, 2a, 2b, 3, 6a, 9, 12, 13	Pill and tablet manufacture
lxxxv)	1, 1a, 2, 2a, 2b, 12, 13	Plastics, cellulose
lxxxvi)	1, 1a, 2, 2a, 2b, 12, 13	Plastics, non-cellulose (including resins)
lxxxvii)	1, 1a, 12, 13	Polishes
lxxxviii)	1 , 1a, 2, 2b, 3, 9, 12, 13	Preserving solutions
lxxxix)	1, 12, 13	Proprietary solvents
xc)	1, 1a, 3, 12, 12a, 13	Refrigerating uses
xci)	3, 12, 12a	Resin coatings, natural
xcii)	1a, 3, 12, 12a , 13	Resin coatings synthetic
xciii)	1, 1a, 6, 13,	Resins, synthetic
xciv)	1, 7, 9, 10, 11, 13,	Room deodorants
xcv)	1, 2, 2b, 12, 13	Rosin (processing)
xcvi)	1, 1a, 12, 13	Rotogravure dyes and solutions
xcvii)	1, 12, 13	Rubber (latex) (processing)
xcviii)	4, 6, 14	Rubber, synthetic

Sl No. (1)	*Formulas Authorized* (2)	*Product or Process* (3)
xcix)	3a	Rubbing alcohol
c)	1, 12, 13	Scientific instruments
ci)	1, 3, 8, 9, 10, 11a, 11b, 12, 12a, 13	Shampoos
cii)	3, 12, 12a	Shellac coatings
ciii)	1, 1a, 3, 12, 12a, 13,	Soaps, industrial
civ)	1, 1a, 3, 3a, 8, 9, 10, 11b, 12, 12a, 13	Soaps, toilet
cv)	2a, 2b	Sodium ethylate, anhydrous (restricted
cvi)	1, 2a, 2b, 12, 13	Sodium hydrosulfite (dehydration)
cvii)	1, 1a, 3, 12, 12a, 13	Soldering flux
cviii)	1, 1a, 3, 12, 12a, 13	Solutions, miscellaneous
cix)	1a, 3, 12, 12a	Solvents and thinners (other than proprietary solvents of special industrial solvents)
cx)	1, 12, 13	Solvents, special (restricted sale)
cxi)	1, 1a, 3, 12, 12a, 13	Stains (wood)
cxii)	1, 1a, 2, 2b, 3, 7, 9, 12, 12a, 13	Sterilizing solutions
cxiii)	1, 7, 9, 10, 1113,	Theater sprays
cxiv)	9, 10, 11a, 11b	Toilet waters
cxv)	1, 2a, 2b, 3, 12, 12a, 13	Transparent sheeting
cxvi)	1, 13, 12,	Unclassified uses
cxvii)	1, 1a, 2a, 2b, 3, 12, 13,	Vaccine (processing)
cxviii)	4, 6, 6a, 14	Vinegar
cxix)	1, 1a, 2, 2a, 2b, 3, 6a, 12, 13,	Vitamins (processing)
cxx)	2a, 2b, 4, 6, 12, 14	Xanthates
cxxi)	1, 1a, 2, 2a, 2b, 3, 6a, 12, 13	Yeast (processing)
cxxii)	11a	Nail polish
cxxiii)	11a	Pomades and Brilliantines
cxxiv)	11a, 11b	After shave lotions
cxxv)	10, 11a, 13	Hand sanitizers
cxxvi)	11a	Mono ethylene glycol, Ethylene oxide and derivatives
cxxvii)	10	Diethyl Phthalate (DEP)

ANNEX D

(*Foreword*)

METHODS OF TESTS FOR VARIOUS DENATURANTS

D-1 ACETALDEHYDE

D-1.1 Acetaldehyde Content

Aldehydes react with hydroxylamine hydrochloride to produce free acid. The acidity of the resulting solution is determined by titrating with standard alkali.

D-1.1.1 *Reagents*

D-1.1.1.1 *Hydroxylamine hydrochloride solution*

Dissolve 35 g of hydroxylamine hydrochloride, analytical reagent grade in 650 ml of methanol (*see* IS 517) and 350 ml of water.

D-1.1.1.2 *Sodium hydroxide solution* — 0.5 N

D-1.1.1.3 *Bromophenol blue indicator* — 0.1 g in 100 ml of rectified spirit (*see* IS 323)

D-1.1.2 *Procedure*

Transfer 100 ml hydroxylamine hydrochloride solution in two 250 ml conical flasks (glass-stoppered). Add 7 to 8 drops of bromophenol blue indicator in each flask and titrate the contents of both flasks with standard sodium hydroxide solution, until the solution appears greyish-blue by transmitted light and pink by reflected light.

Take one of the flasks containing neutral hydroxylamine hydrochloride solution, weigh it in a chemical balance, add about 0.2 ml to 0.5 ml of sample by means of a graduated pipette and immediately stopper it. Weigh again and note the mass. Shake and keep it for 15 min for reaction. Reserve another flask as colour standard for the end point determination. After 15 min titrate the contents of the flask against sodium hydroxide solution until colour of the solution matches with the other flask containing only neutral hydroxylamine hydrochloride solution. Note the volume of sodium hydroxide solution used.

NOTE — Samples shall only be drawn from containers at temperature below 20 °C.

D-1.1.3 *Calculation*

$$\text{Acetaldehyde, percent by mass} = \frac{V \times N \times 4.4}{M}$$

where

V = volume, in ml, of sodium hydroxide solution consumed;

N = normality of sodium hydroxide solution; and

M = mass, in g, of the sample taken.

D-2 AMMONIA AQUEOUS

D-2.1 Ammonia Content

D-2.1.1 *Reagents*

D-2.1.1.1 *Sulphuric acid* — 1 N

D-2.1.1.2 Methyl *red indicator*

D-2.1.2 *Procedure*

Accurately weigh a glass-stoppered flask containing 25 ml of water, add about 2 ml of the sample, stopper and weigh again. Add methyl red indicator and titrate with 1 N sulphuric acid. Each ml of 1 N sulphuric acid is equivalent to 17.03 mg of NH_3.

D-2.1.3 *Calculation*

$$\text{Ammonia, percent by mass} = \frac{V \times N \times 1.7}{M}$$

where

V = volume, in ml, of sulphuric acid consumed;

N = normality of sulphuric acid, and

M = mass, in g, of the sample taken.

D-3 BRUCINE AND BRUCINE SULPHATE

D-3.1 Absence of Strychnine

When brucine sulphate (or brucine) is treated for 10 min with a solution of dilute sulphuric acid and nitric acid and the resulting solution is made alkaline with sodium hydroxide solution, only the brucine (or its sulphate) is changed to a derivative, which is soluble in alkali and insoluble in chloroform. Any strychnine, if present, is extracted with chloroform and identified by the formation of a characteristic violet colour with concentrated sulphuric acid in the presence of potassium dichromate.

D-3.1.1 *Reagents*

D-3.1.1.1 *Dilute sulphuric acid* — 10 percent (*m/v*)

D-3.1.1.2 *Dilute nitric acid* — 1 : 1

D-3.1.1.3 *Sodium hydroxide solution* — 10 percent (*m/v*)

D-3.1.1.4 *Chloroform*

D-3.1.1.5 *Concentrated sulphuric acid* — relative density 1.84 (*see* IS 266)

D-3.1.1.6 *Potassium dichromate* — analytical reagent grade

D-3.1.2 *Procedure*

Dissolve 500 mg of brucine sulphate (equivalent to 390 mg of brucine) in a mixture of 5 ml of dilute sulphuric acid and 15 ml of water and warm, if necessary. Cool to 25 °C and then add 5 ml of dilute nitric acid maintained at a temperature of 25 °C. Rotate the mixture for some time and let it stand for 10 min, shaking 3 to 4 times during this interval and keeping the temperature below 25 °C. Transfer the red solution into a separating funnel containing 40 ml of sodium hydroxide solution, extract immediately with 2 successive portions of 25 ml and the third portion of 10 ml of chloroform. Draw off the chloroform layer through a filter paper moistened with chloroform into a clean small porcelain dish. Evaporate the chloroform to a few milliliters. Wash down the sides of the dish with a few milliliter of warm chloroform and evaporate it to dryness. Cool and to the residue add 2 ml of concentrated sulphuric acid. Swirl the acid to dissolve the substance adhering on the sides of the dish and to this add a small crystal of potassium dichromate. If strychnine is present, a characteristic violet colour is produced.

D-3.2 Assay

D-3.2.1 *Reagents*

D-3.2.1.1 *Dilute sulphuric acid* — 0.1 N

D-3.2.1.2 *Sodium hydroxide solution* — 0.02 N

D-3.2.1.3 *Ammonium hydroxide solution* — 10 percent

D-3.2.1.4 *Ether* — *see* IS 336

D-3.2.1.5 *Chloroform*

D-3.2.2 *Procedure*

Weigh exactly 250 mg of brucine sulphate (or 235 mg of brucine crystals), dissolve in a mixture of 5 ml of dilute sulphuric acid and 15 ml of water and warm, if necessary. Cool to 25 °C and extract it with two 25 ml portions of ether. Discard the ether extract. The aqueous solution is then made distinctly alkaline with ammonium hydroxide solution. Extract immediately with 3 successive portions of 25 ml and the fourth portion of 10 ml of chloroform. Wash the combined chloroform extracts twice with 10 ml of water. Draw off the chloroform layer through a filter paper moistened with chloroform into a 250 ml conical flask. Wash the filter paper again, twice with 5 ml of chloroform into the conical flask. Remove the chloroform very carefully on a water-bath but not to dryness. Add 5 ml of ethyl alcohol (95 percent), evaporate to dryness, and dry for half an hour at 100 °C. Add to the dry residue 10 ml of dilute sulphuric acid, accurately measured, and 30 ml of water. Heat the mixture on a water-bath until the substance is completely dissolved. Cool to room temperature and titrate the excess acid with sodium hydroxide solution, using one drop of methyl red as indicator. Each 1 ml of 0.1 N sulphuric acid is equivalent to 0.046 65 g of brucine ($C_{23}H_{26}N_2O_4. 4H_2O$) or 0.050 67 g of brucine sulphate [$(C_{23}H_{26}N_2O_4)_2.H_2SO_4. 7H_2O$].

D-4 CAUSTIC SODA, LIQUID

D-4.1 Sodium Hydroxide Content

D-4.1.1 *Reagents*

D-4.1.1.1 *Barium chloride solution* — 0.1 percent

D-4.1.1.2 *Phenolphthalein indicator* — 1 percent

D-4.1.1.3 *Hydrochloric acid* — 0.25 N

D-4.1.2 *Procedure*

Accurately weigh 2 g of liquid caustic soda into a 100 ml volumetric flask, dissolve and dilute to the mark with distilled water at room temperature. Transfer a 25 ml aliquot of the solution to a titration flask add 10 ml of 1 percent barium chloride solution, 0.2 ml of 1 percent phenolphthalein indicator and 50 ml of distilled water. Titrate with 0.25 N hydrochloric acid to the disappearance of the pink colour.

D-4.1.3 *Calculation*

Sodium hydroxide content, percent by mass

$$= \frac{V \times N \times 4.0}{0.5}$$

where

V = volume, in ml, of hydrochloric acid solution consumed; and

N = normality of hydrochloric acid solution.

D-5 DENATONIUM BENZOATE

D-5.1 Denatonium Benzoate Content

Denatonium benzoate dissolved in glacial acetic acid. Mercuric acetate solution is added and titrated with perchloric acid in presence of crystal violet.

D-5.2 Reagents

D-5.2.1 *Glacial Acetic Acid*

D-5.2.2 *Mercuric Acetate* — 5 percent (*w/v*)

D-5.2.3 *Perchloric Acid* — 0.1 N

D-5.2.4 *Crystal Violet/Methylrosaniline chloride T.S.*

D-5.3 Procedure

D-5.3.1 *Strength of Perchloric Acid*

Weigh accurately about 0.7 g of potassium hydrogen phthalate previously powdered lightly and dried at 120 °C for 2 h. dissolve it in 50 ml of glacial acetic acid. Add two drops of crystal violet solution and titrate with perchloric acid solution**.** Until the violet color changes to emerald green. Deduct the volume of perchloric acid consumed by 50 ml of the glacial acetic acid. Each 0.020 42 g of potassium hydrogen phthalate is equivalent to 1 ml of 0.1 N perchloric acid.

D-5.3.2 *Assay*

Dissolve about 0.5 g bitrex (THS-839) accurately weighed in 60 ml glacial acetic acid, cool and then add 15 ml of 5 percent *w/v* solution of mercuric acetate in glacial acetic acid and titrate with 0.1 N perchloric acid in glacial acetic acid using 0.2 ml of a 0.5 percent *w/v* solution of crystal violet in glacial acetic acid as indicator (or methylrosaniline chloride T.S.). Repeat the titration omitting the samples. The difference between the two readings will represent the volume of perchloric acid required by the sample. Each ml of N/10 perchloric acid is equivalent to 0.044 65 g denatonium benzoate of not less than 99 percent purity $C_{28}H_{34}O_3N_2$.

D-6 DIETHYL PHTHALATE

D-6.1 Relative Density

Determine by Method A (relative density balance method) of IS 1448 (Part 32).

D-6.2 Purity

D-6.2.1 *Reagents*

D-6.2.1.1 *Standard alcoholic sodium hydroxide solution* — 0.5 N

D-6.2.1.2 *Standard hydrochloric acid* — 0.5 N

D-6.2.1.3 *Phenolphthalein indicator*

Dissolve 0.1 g of phenolphthalein in 100 ml of 60 percent rectified spirit. Make the solution faintly pink by adding dilute sodium hydroxide solution.

D-6.2.2 *Procedure*

Weigh accurately 1.0 g to 1.5 g of the material in a small tared tube and transfer the tube with its contents to a flask containing 50 ml of standard alcoholic sodium hydroxide solution. Fit a water-cooled reflux condenser to the flask and heat it for 1 h over a boiling water bath. Withdraw the flask, still carrying its condenser and immerse in cold water. When cool, rinse the inside of the condenser with distilled water. Cool, remove the flask and titrate the contents with standard hydrochloric acid using 0.5 ml of phenolphthalein indicator.

Carry out a blank determination, using all the reagents excepting the material, under similar conditions and at the same time.

D-6.2.3 *Calculation*

$$\text{Purity, percent by mass} = \frac{11.11 \times (V_1 - V_2) \times N}{M}$$

where

V_1 = volume, in ml, of standard hydrochloric acid required in the test with the material;

V_2 = volume, in ml, of standard hydrochloric acid required in the blank titration;

N = normality of standard hydrochloric acid; and

M = mass, in g, of the material taken for the test.

D-7 FORMALDEHYDE

D-7.1 Formaldehyde Content

D-7.1.1 *Reagents*

D-7.1.1.1 *Hydrogen peroxide solution* — 6 percent (20 volumes)

D-7.1.1.2 *Sodium hydroxide solution* — 1 N

D-7.1.1.3 *Dilute hydrochloric acid* — 1 N

D-7.1.1.4 *Phenolphthalein indicator solution* — prepared by dissolving 0.1 g in 100 ml of 60 percent rectified spirit.

D-7.1.2 Procedure

Take 25 ml of hydrogen peroxide solution and 50 ml of sodium hydroxide solution in a 250 ml conical flask. Add about 3 g of the material, accurately weighed; and warm on a water-bath until effervescence ceases. Cool and titrate the excess alkali with hydrochloric acid, using phenolphthalein solution as indicator. Run a blank under similar conditions. The difference between the titer values represents the sodium hydroxide solution required to neutralize the formic acid, produced by the oxidation of the formaldehyde. Each milliliter of 1 N sodium hydroxide is equivalent to 0.030 03 g of formaldehyde.

D-8 TERTIARY BUTYL ALCOHOL

D-8.1 Tertiary Butyl Alcohol (Alone)

Use gas chromatographic method for determination of tertiary butyl alcohol and compare the graph of sample with standard tertiary butyl alcohol.

D-8.2 Tertiary Butyl Alcohol in Presence of Denatonium Benzoate (Bitrex)

D-8.2.1 Ethanol is often denatured with tertiary butyl alcohol (TBA) and bitrex and may contain other volatile impurities. This method is for the quantification of tertiary butyl alcohol (typically 0.1 percent to 3 percent) in ethanol in the presence of bitrex. The method detection limit is 1 mg/l for TBA.

D-8.2.2 *Principle*

The concentration of tertiary butyl alcohol (TBA) in denatured alcohol is quantified using gas chromatography with flame ionization detector (FID).

D-8.2.3 *Apparatus*

D-8.2.3.1 *Gas Chromatograph* — equipped with an FID detector can be used with following accessories and typical operating conditions:

Column	:	Fused silica capillary column
Dimensions	:	30 m × 0.25 mm (internal diameter) × 0.25 µm
Oven program	:	40 °C for 7 min Ramp 35 °C/min to 250 °C
Carrier gas	:	Helium
Carrier flow rate	:	Constant flow 1.0 ml
Split flow	:	50 ml
Injection volume	:	1.0 µl
Injector temperature	:	250 °C
Detector	:	Flame ionization detector
Detector temperature	:	300 °C
Run time	:	14 min

D-8.2.3.2 *Disposable pasteur pipettes* — 3 ml

D-8.2.3.3 *Analytical balance* — capable of accurately weighing to the nearest 0.000 1 g

D-8.2.3.4 *Volumetric flasks* — of capacity 25 ml and 10 ml

D-8.2.3.5 *GC auto sampler vials* (2 ml) *and caps*

D-8.2.4 *Reagents*

D-8.2.4.1 *Ethanol, absolute* (*greater than* 99 *percent, pure*) — highly flammable

D-8.2.4.2 *Tertiary butyl alcohol* (*greater than* 99 *percent, pure*) — flammable harmful

D-8.2.4.3 *Bitrex, denatonium benzoate* (*greater than* 99 *percent, pure*) — irritant

D-8.2.4.4 *Standard tertiary butyl alcohol* (*TBA*) *solution*

Dissolve 0.197 3 g of tertiary butyl alcohol (TBA) into 25 ml volumetric flask and make it up to the volume with absolute ethanol. This corresponds to 1 percent (*w/v*) TBA.

NOTE — Density of absolute alcohol at 20 °C is 0.789 g/ml.

D-8.2.5 *Procedure*

D-8.2.5.1 *Standard preparation*

Aliquot 0.5 ml, 1.0 ml, 2.0 ml and 5.0 ml of standard solutions (*see* **D-8.2.4.4**) to 10 ml volumetric flasks and make it up the volume with absolute alcohol to get 0.05 percent, 0.1 percent, 0.25 percent and 5.0 percent TBA standards respectively.

D-8.2.5.2 *Procedure for sample preparation*

Inject 1.0 µl of the sample (if needed, make suitable dilutions to fit the sample peak area of TBA within the calibration plot) for GC analysis. Conduct all analysis in duplicates.

D-8.2.6 *Calculation and Expression of Results*

D-8.2.6.1 Make a calibration curve by plotting concentration of standard (in percent) versus peak area obtained for the solutions, and obtain the linear regression equation for the curve.

D-8.2.6.2 Calculate the concentration of TBA in the sample

$$\text{Concentration of TBA, percent} = \frac{\text{Peak area of the TBA peak in sample} \times \text{Dilution factor}}{\text{Slope (obtained from linear regression equation)}}$$

D-8.2.6.3 Express the results in percent, *w/v*

D-8.2.7 *Quality Assurance and Control*

D-8.2.7.1 The method detection limit is 1 mg/l (based on S/N ratio 3 : 1).

D-8.2.7.2 Retention time of TBA is getting detected at 2.47 min ± 0.02 min.

D-8.2.7.3 *Precision*

Instrument repeatability — Standard TBA solution (0.25 percent) was injected five times to check the repeatability. The relative standard deviation (RSD) was found to be 0.02 percent and 0.69 percent for retention time and peak area respectively.

Sl No.	*Sample*	*Area* (µV)	*Retention Time* (min)	*Average*		*Standard Deviation*		*RSD*	
				Area	*RT*	*Area*	*RT*	*Area*	*RT*
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
i)	Inj 1	261948	2.472	264 374	2.47	1 830.26	0.00	0.69	0.02
ii)	Inj 2	265366	2.472						
iii)	Inj 3	263378	2.472						
iv)	Inj 4	266726	2.472						
v)	Inj 5	264451	2.473						

D-8.2.7.4 *Accuracy*

Standard TBA (1 percent solution) is spiked to two samples and the recovery was found to be 99.2 percent and 105 percent respectively.

Sl No.	*Sample*	*Area* µV	*Concentration* (percent)	*Average Concentration* (percent)	*Expected Concentration* (percent)	*Percent Recovery*
(1)	(2)	(3)	(4)	(5)	(6)	(7)
i)	Sample spike 1	369 818	0.352	0.361	0.365	99.2
ii)	Repeat	388 858	0.370			
iii)	Sample spike 2	851 450	0.810	0.818	0.800	105
iv)	Repeat	868 254	0.826			

D-8.2.7.5 *Interferences*

A standard bitrex solution in ethanol (0.1 percent, *w/v*) is prepared and injected into the GC and there is no interfering component at the retention time of TBA. A mixed standard solution of TBA (0.25 percent, *w/v*) and bitrex (0.1 percent, *w/v*) in ethanol is injected into the instrument and the concentration of TBA is calculated thereafter. The calculated result was in agreement with theexpected value (*see* Fig. 3).

D-8.2.8 *Annexures*

D-8.2.8.1 Standard calibration plot and chromatograms of standards and samples

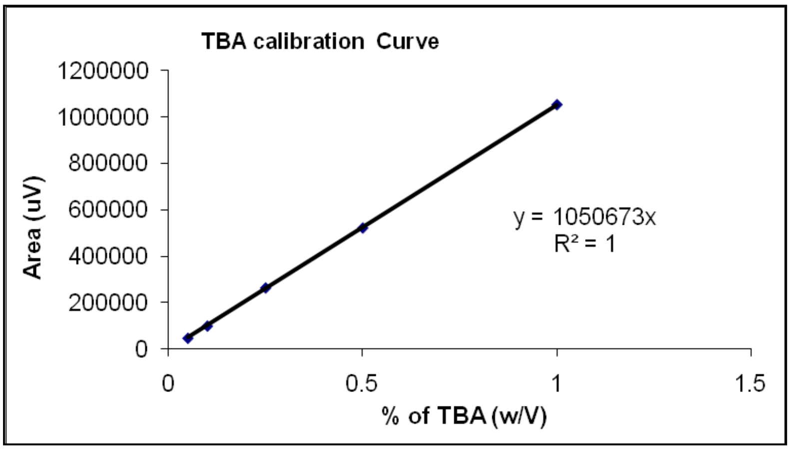

FIG. 1 TBA CALIBRATION CURVE

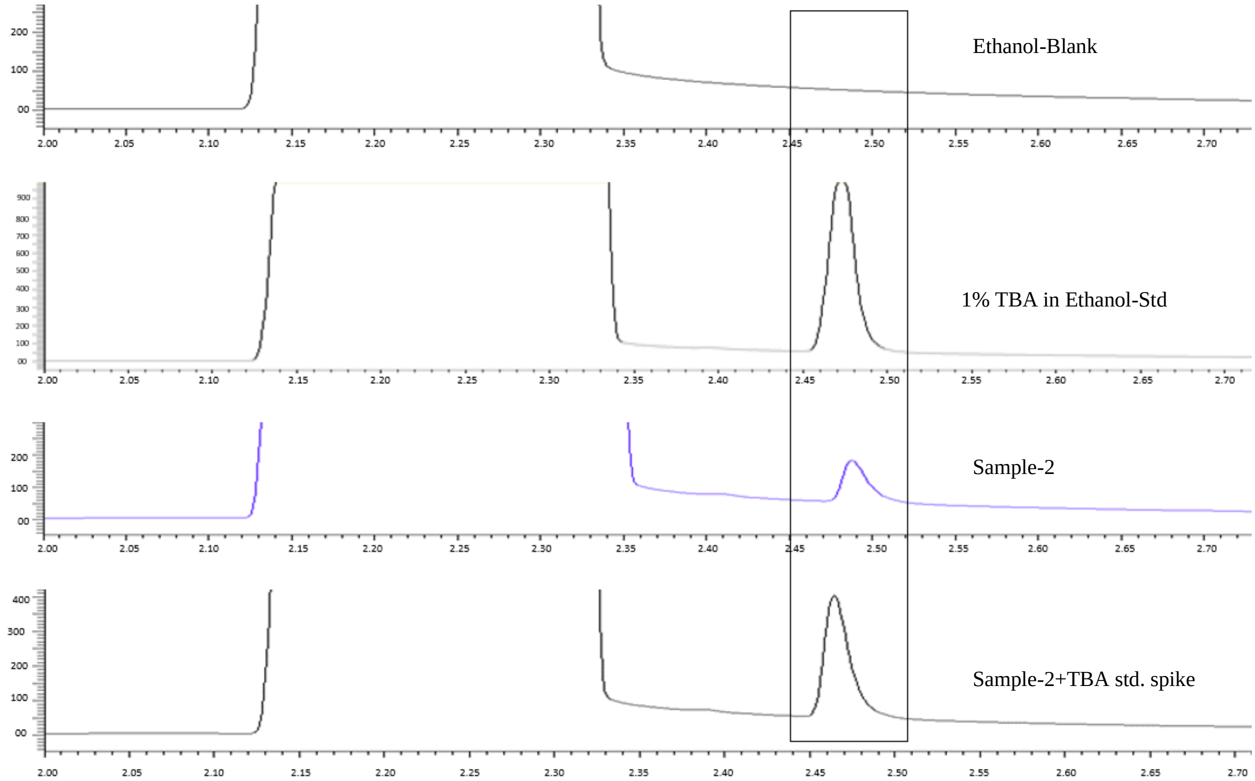

FIG. 2 CHROMATOGRAMS (ZOOMED) OF BLANK, STANDARD, SAMPLE AND STANDARD-SPIKE SAMPLE (ZOOMED)

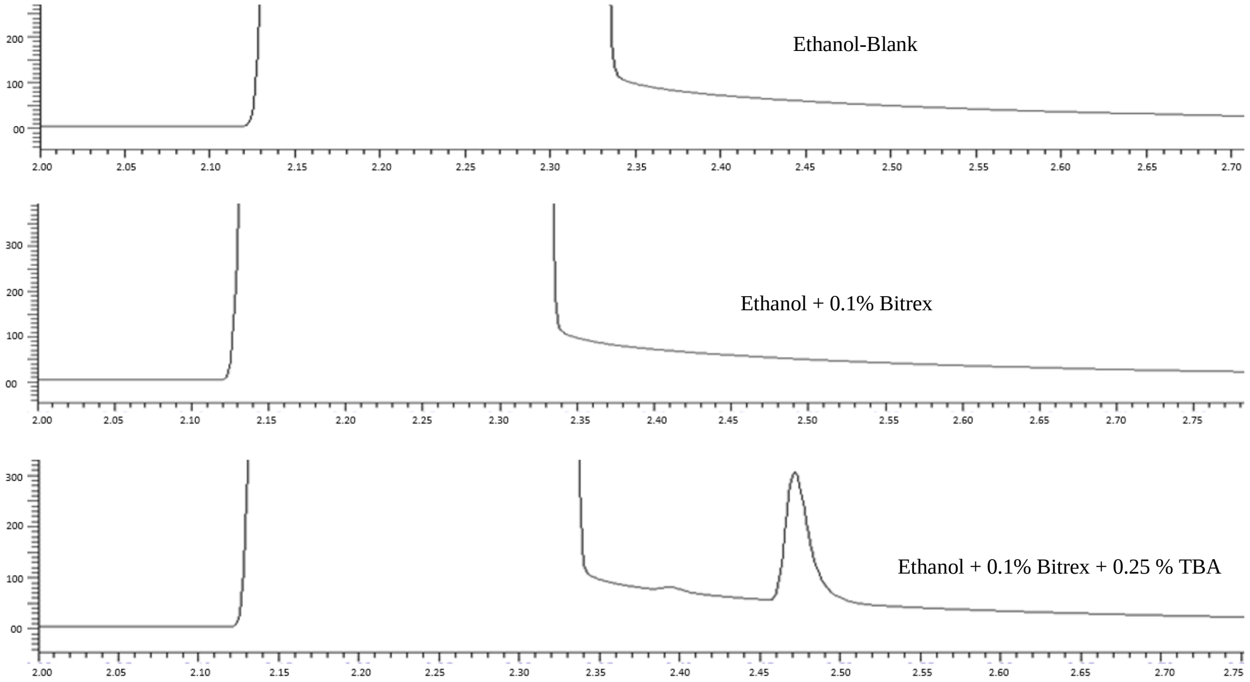

FIG. 3 CHROMATOGRAMS (ZOOMED) SHOWS NO INFERENCE FROM BITREX (ZOOMED)

ANNEX E

(*Foreword* and *Clause* 8)

LIMIT TESTS FOR DENATURANTS IN DENATURED ALCOHOL — GENERAL METHODS

E-1 Limit tests for various denaturants as prescribed here in are applicable to concentrations of the denaturants in ethyl alcohol as covered by corresponding formula (*see* Annex C).

E-2 ACETALDEHYDE

E-2.1 Outline of the Method

Colour developed with schiff's reagent is matched with that produced with a standard solution of pure acetaldehyde.

E-2.2 Apparatus

E-2.2.1 *Nessler Cylinders* — two, 50 ml capacity

E-2.3 Reagents

E-2.3.1 *Schiff's Reagent*

Dissolve 0.25 g of rosaniline hydrochloride in 150 ml of water. To this add 25 ml of 10 percent sodium bisulphite solution followed by 2.5 ml of concentrated hydrochloric acid (relative density: 1.14). Make it up volume to 250 ml.

E-2.3.2 *Acetaldehyde* — 0.30 percent

E-2.4 Procedure

Dilute one volume of denatured alcohol 10 times with rectified spirit. Take 1 ml of diluted, denatured alcohol in a Nessler cylinder and add 5 ml of schiff's reagent. Make up volume with rectified spirit and allow to stand. In another Nessler cylinder add 0.9 ml of 0.30 percent acetaldehyde, 5 ml of schiff's reagent and make up volume to 50 ml. Match colour developed in the two cylinders.

E-2.5 The denatured alcohol shall be taken as conforming to the limit test, if the violet colour developed by the sample is not lighter than that of the standard.

E-3 ACETONE

E-3.1 Outline of the Method

Hydroxylamine hydrochloride yields an oxime on reacting with the carbonyl group. The hydrochloric acid, which is set free, is then titrated with alcoholic potassium hydroxide solution; alternatively, an accurately measured amount of alkali is added, and the excess alkali back titrated with a standard acid solution.

E-3.2 Reagents

E-3.2.1 *Hydroxylamine Hydrochloride Solution*

Dissolve 1.5 g of hydroxylamine hydrochloride in 100 ml of water.

E-3.2.2 *Methyl Orange Indicator* — prepared by dissolving 0.01 g in 100 ml of water

E-3.2.3 *Sodium Hydroxide Solution* — 0.1 N

E-3.3 Procedure

Add 5 ml of the denatured alcohol to a glass-stoppered conical flask containing 30 ml of hydroxylamine hydrochloride solution (accurately neutralized to methyl orange) and allow to stand for 45 min. Titrate the liberated acid with sodium hydroxide solution.

E-3.4 The denatured alcohol shall be taken as conforming to the limit test, if 5 ml of denatured alcohol consumes not less than;

a) 18.0 ml of 0.1 N sodium hydroxide solution for acetone in SD 12a; and

b) 48.0 ml of 0.1 N sodium hydroxide solution for acetone in SD 3, 3a.

E-4 AMMONIA SOLUTION

The denatured alcohol is tested for the presence of ammonia by assay methods given in **D-2**.

E-5 BENZENE

Benzene is nitrated and the resulting dinitrobenzene condensed with butanone to obtain pink colour. The colour is then compared with a standard obtained by treating known quantities of benzene in an analogous manner.

E-5.1 Reagents

E-5.1.1 *n-Hexane* — technical grade, having a refractive index of 1.375 4 to 1.387 0 at 20 °C.

E-5.1.2 *Ether* (*see* IS 336)

The ether shall be freed from peroxides and aldehydes by shaking with water, and then with saturated ferrous sulphate solution and 10 percent sulphuric acid, until a sample of ether gives no reaction with potassium iodide solution.

E-5.1.3 *Butanone*

Commercial samples of this material are liable to be impure. The substance shall be fractionated with a good column and only the first four-fifths of the fraction boiling between 72 °C and 81 °C shall be used.

E-5.1.4 *Nitration Mixture*

Equal volumes of concentrated sulphuric acid relative density 1.84 and fuming nitric acid relative density 1.50.

E-5.1.5 *Sodium Chloride Solution*, saturated.

E-5.1.6 *Potassium Hydroxide Solution* — 40 percent (*m/v*)

E-5.2 Procedure

Measure 100 ml of the denatured alcohol to be tested in a 500 ml distillation flask and add 200 ml of water along with a few pieces of carborandum. Connect the flask to a condenser and distill, collecting the distillate in a 25 ml cylinder immersed in ice-cold water. Distil 20 ml at the rate of 1.0 ml/min to 1.5 ml/min. This distillate consists of 80 percent alcohol (*m/m*) and contains all the benzene. Transfer this distillate to a 125 ml separating funnel; add 25 ml of saturated sodium chloride solution and about 20 ml of hexane. Stopper the funnel and shake vigorously for 30 s. After the 2 layers have been separated, discard the aqueous layer; wash the hexane solution with two successive portions of water and finally with 10 ml of sodium chloride solution. The hexane extract is then drawn into 25 ml measuring flask and exactly made up to 25 ml with hexane.

Place 10 ml of the nitration mixture into a 150 ml glass stoppered flask, immersed in an ice bath. After the nitration mixture has become cold, introduce very slowly 2 ml of the hexane extract measured exactly with the aid of a pipette. Stopper and allow it to stand for at least 1 h for the nitration to be complete. Dilute the contents of the flask with 100 ml of ice-cold water cautiously, keeping the flask at the same time in ice-cold water. Extract the nitrated material with 50 ml of ether in a separating funnel, run the aqueous layer into the second separating funnel and extract it with another 25 ml of ether. Reject this aqueous layer and mix this 25 ml ether with the original 50 ml ether extract. Wash the combined ether extract with 25 ml water and run down the ether extract into a 100 ml volumetric flask. Make to volume with 85 percent alcohol. Transfer a 10 ml aliquot to suitable Nessler cylinders, add 5 ml of butanone and 5 drops of potassium hydroxide solution, shake the mixture, and allow to stand for 10 min. If benzene is present in the original denatured alcohol, a pink colour appears and if toluene is present a blue colour will appear.

Compare the colour thus produced with a standard prepared with known concentrations (5 percent solution in SD 2, 1.5 percent solution in SD 2a) of benzene treated similarly.

After 10 min, add 5 drops of glacial acetic acid, agitate the Nessler cylinders and allow to stand for another 10 min. Again compare the colour with the standard, treated similarly. The blue colour developed due to toluene disappears whereas colour developed due to benzene remains unaffected by this treatment. The method is sensitive to 0.000 88 mg quantities of benzene.

E-6 BRUCINE AND BRUCINE SULPHATE

The denatured alcohol is tested for the presence of brucine or brucine sulphate by corresponding identification tests [Table 1, Sl No. (v)] after evaporating off the alcohol. Apart from this, the denatured alcohol shall comply with the test for absence of strychnine as prescribed under **D-3.1** and shall contain not less than 98 percent of the declared contents of brucine or brucine sulphate by following the method of assay given under **D-3.2** after evaporating off the alcohol.

E-7 CAUSTIC SODA

This is determined by titrating the denatured alcohol with sulphuric acid, using methyl orange as indicator. The standard should match with the denatured alcohol by the procedure given in **D-4**.

E-8 CHLOROFORM

The colour developed by the denatured alcohol when treated with sodium hydroxide and pyridine is matched against the colour developed similarly by standard solution of chloroform and rectified spirit.

E-8.1 Apparatus

E-8.1.1 *Nessler cylinders* — two 50 ml capacity

E-8.2 Reagents

E-8.2.1 *Pyridine* — technical grade

E-8.2.2 *Sodium Hydroxide Solution* — 10 percent (*m/v*)

E-8.2.3 *Standard Chloroform Solution* — 5 percent (*v/v*) in rectified spirit

E-8.3 Procedure

Dilute 1 ml of the denatured alcohol with 100 ml of water. Transfer 50 ml of the diluted solution to a nessler cylinder. Add 4 ml of water, 5 drops of sodium hydroxide solution and 15 ml of pyridine. Mix well and heat in a boiling water-bath for 5 min. Cool by dipping in a cold water-bath for 7 min. Mix well and compare the colour after 10 min with that developed in another Nessler cylinder to which 0.95 ml of standard chloroform solution together with the same reagents have been added.

The denatured alcohol shall be taken as conforming to the limit test, if the colour developed in the Nessler cylinder containing the denatured alcohol under test is not lighter than that developed in other Nessler cylinder containing standard chloroform solution.

E-9 CROTONALDEHYDE

The colour developed by the material with schiff's reagent is matched with that produced by a standard solution of crotonaldehyde and rectified spirit with schiff's reagent.

E-9.1 Apparatus

E-9.1.1 *Nessler Cylinders*

E-9.2 Reagents

E-9.2.1 *Schiff's Reagent* — same as E-2.3.1

E-9.2.2 *Standard Solution of Crotonaldehyde*

Dissolve 0.2 ml percent pure crotonaldehyde (or equivalent) in 100 ml of rectified spirit.

E-9.3 Procedure

Transfer 1 ml of the denatured alcohol under test to a nessler cylinder. Add 5 ml of Schiff's reagent and make up to the volume. Mix the contents of the Nessler cylinder and allow to stand for 1 h. To another Nessler cylinder similarly add 1 ml of the standard crotonaldehyde solution and 5 ml of schiff's reagent. Mix the contents and match the color.

The denatured alcohol shall be taken as confirming to the limit tests if the violet color produced in the Nessler cylinder containing the denatured alcohol under test conditions not lighter than that produced in the Nessler's cylinder containing standard solution of crotonaldehyde.

E-10 DENATONIUM BENZOATE AND DENATONIUM SACCHARIDE

Two methods have been given, Method A ultra violet absorption (routine method) and Method B is based on high performance liquid chromatography using UV detector for detection of denatonium benzoate and denatonium saccharide in alcohol. In case of dispute, Method B is the referee method.

E-10.1 Method A

Ethanolic solutions of denatonium benzoate/denatonium saccharide in the concentration ranges 20 ppm to 50 ppm yield a measurable ultra violet absorption at the characteristic wave length 263 nm.

E-10.1.1 *Procedure*

Make standard concentration of denatonium benzoate/denatonium saccharide 50 ppm, 75 ppm, 100 ppm, 125 ppm and 150 ppm in redistilled rectified spirit. Measure absorbance on an ultraviolet spectrophotometer at 263 nm. Plot a calibration curve. Measure absorbance at 263 nm for individual denaturant solutions. Read the concentration by interpolation from the calibration plot.

E-10.2 Method B

E-10.2.1 *Apparatus*

E-10.2.1.1 *HPLC* — any suitable instrument, with suitable configuration to test the sample.

E-10.2.1.2 *Glassware* — calibrated

E-10.2.1.3 *Balance* — analytical balance capable of weighing to within ± 0.01 mg.

E-10.2.2 *Reagents*

E-10.2.2.1 *HPLC grade water*

E-10.2.2.2 *HPLC grade acetonitrile*

E-10.2.2.3 *AR grade disodium hydrogen phosphate*

E-10.2.2.4 *Reference material of denatonium benzoate*

E-10.2.2.5 *Reference material of denatonium saccharide*

E-10.2.2.6 Chromatographic conditions given below are for guidance only:

a) Mobile phase

1) Acetonitrile

2) 20 *m/m*, disodium hydrogen phosphate buffer

Weigh 3.56 g disodium hydrogen phosphate buffer (Na_2HPO_4) dissolve in 1 000 ml of water. Adjust to *p*H 3.0 with phosphoric acid (H_3PO_4). Filter through 0.45 µm membrane filter or equivalent.

b) Conditions

Temperature : Ambient
Wavelength : 220 nm
Flow rate : 1.0 ml/min
Injection volume : 20 µl
Run time : 20 min
Diluent : I : H_2O :: 50 : 50

E-10.2.2.7 *Standard preparation*

Take 25 mg reference standard of denatoniumbenzoate and denatonium saccharide in 50 ml volumetric flask separately, dissolve and dilute with diluents. Further take 1.0 ml solution to 100 ml volumetric flask and make up with diluents. Following solution contains 5.0 ppm of reference standards. Inject 20 µl of standard sample and record the standard chromatogram.

E-10.2.2.8 *Test preparation*

Take 6 g to 7 g sample of ethanol in 50 ml volumetric flask. Dissolve and dilute with diluents. Inject 20 µl of test sample and record the chromatogram.

E-10.2.3 *Analytical Procedure*

Inject a mobile phase as blank until a suitable baseline is achieved and no interferences are observed at the retention time(s) of interest. Inject the reference standard, followed by injections of each test sample solution

E-10.2.4 *Calculations* (*Based on External Standard Method*)

$$\text{Analyte (w/w), ppm} = \frac{\begin{array}{c}\text{Peak area of interest}^{1)}\\ \text{component in test sample}\end{array} \times \text{weight of standard} \times \text{purity of standard}}{\text{Peak area of interest component in standard solution} \times \text{sample taken}} \times \text{DF} \times 10\ 000$$

where

DF = dilution factor.

Sl No. (1)	*Name* (2)	*Approximate RT* (*Min*) (3)	*Approximate* (*RRT*) (4)	*Classification of Components* (5)
i)	Denatonium benzoate	4.75	1.000	Denaturants
ii)	Denatonium saccharide	3.60	1.000	Denaturants

NOTES

1 Ensure that peaks of denatonium benzoate and denatonium benzoate in alcohol and all related impurities are resolved properly, that is, base to base separation should take place, if required make necessary adjustment.

2 Perform the analysis of denatonium benzoate and denatonium saccharide separately. Change the standard concentration as per the interest components presents in the sample.

3 Typical retention time (RT) and relative retention time (RRT) of interest components.

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1) Interest components means denatonium benzoate or denatonium saccharide.

E-11 DIETHYL PHTHALATE

The ester is saponified with alcoholic potassium hydroxide solution and the resulting hydrolyzate titrated with standard sulphuric acid.

E-11.1 Reagents

E-11.1.1 *Alcoholic Potassium Hydroxide Solution* — 0.5 N. Dissolve 28 g of potassium hydroxide in minimum quantity of water and make up the volume to 1 litre with rectified spirit. Allow the solution to stand overnight and filter.

E-11.1.2 *Phenolphthalein Indicator*

E-11.1.3 *Standard Sulphuric Acid* — 0.5 N

E-11.2 Procedure

Transfer 25 ml of the denatured alcohol to a 250 ml conical flask fitted with a reflux condenser. To this add 25 ml of alcoholic potassium hydroxide solution and reflux for 45 min on a boiling water bath. Cool the contents and titrate with sulphuric acid, using phenolphthalein as indicator. Record the titer value as (V_1).

Carry out the blank titration using 25 ml of the denatured alcohol to which 25 ml of alcoholic potassium hydroxide solution has been added in an ice-cold bath. Record the blank titer value as V_2. The difference of two values (V_2 - V_1) gives the volume of 0.5 N potassium hydroxide consumed by diethyl phthalate.

The denatured alcohol shall be taken as conforming to the limit test if 25 ml of the denatured alcohol do not consume less than 12.5 ml of 0.5 N alcoholic potassium hydroxide solution for SD 10.

E-12 ETHYL ACETATE

E-12.1 Reagents

same as D-7

E-12.2 Procedure

same as D-7

E-12.3 The denatured alcohol shall be taken as conforming to the limit test if 25 ml of the denatured alcohol do not consume less than:

a) 4.5 ml of 0.5 N alcoholic potassium hydroxide solution for SD 6, and
b) 22.5 ml of 0.5 N alcoholic potassium hydroxide solution for SD 6a.

E-13 FORMALDEHYDE

Formaldehyde is oxidized to formic acid, which reacts with excess of standard sodium hydroxide solution. The un-reacted excess of sodium hydroxide is determined by titrating it with sulphuric acid from which the volume of formic acid generated is determined.

E-13.1 Reagents

E-13.1.1 *Hydrogen Peroxide Solution* — 6 percent (20 volumes)

E-13.1.2 *Sodium Hydroxide Solution* — approximately 1 N

E-13.1.3 *Sulphuric Acid* — 1 N

E-13.1.4 *Phenolphthalein Indicator*

E-13.2 Procedure

Transfer 10 ml of the denatured alcohol to a 250 ml conical flask. To this add 15 ml of hydrogen peroxide and 25 ml of sodium hydroxide solution. Warm the contents of conical flask on a hot water bath, using reflux condenser, until effervescence ceases. Cool the contents and titrate with sulphuric acid, using phenolphthalein as indicator. Carry out a blank without the addition of the denatured alcohol. The difference in the 2 litre values gives the volume of 1 N sulphuric acid used equivalent to the formic acid generated.

The denatured alcohol shall be taken as conforming to the limit test if 10 ml of the denatured alcohol do not consume;

a) less than 10.0 ml of 1 N sulphuric acid in SD 7;
b) less than 2.5 ml of 1 N sulphuric acid in SD 7a; and
c) less than 1.25 ml of 1 N sulphuric acid in SD 7b.

E-14 ISOPROPYL ALCOHOL

Colour developed with furfural reagent is matched with that produced with a standards solution of isopropanol.

E-14.1 Reagents

E-14.1.1 *Sodium Phosphate Saturated Solution*

E-14.1.2 *Saturated Solution of Potassium Permanganate*

E-14.1.3 *Sodium Hydroxide* — 0.25 N

E-14.1.4 *Furfural Solution* (1 : 100)

E-14.1.5 *Hydrochloric Acid*

E-14.2 Procedure

To 1 ml of denatured alcohol add 1.0 ml of water, 1 ml of solution saturated sodium phosphate and add 3 ml of saturated solution of potassium permanganate. Warm the solution to 45 °C to 50 °C and allow standing till potassium permanganate color is discharged. Add 3 ml of 2.5 N sodium hydroxide and filter through a sintered glass filter. Add 1 ml of furfural solution and allow to stand for 10 min. Take 1 ml of the solution and add 3 ml of hydrochloric acid. Any pink colour produced in the solution is not more intense than that in control.

Control sample: 1 ml of saturated solution of sodium phosphate, 3 ml of 2.5 N sodium hydroxide and required quantity of isopropyl alcohol. Then add 1 ml of furfural solution and allow standing for 10 min. Take 1 ml and add 3 ml of hydrochloric acid.

E-15 METHANOL (METHYL ALCOHOL)

Methanol is oxidized with potassium permanganate solution. The excess permanganate is removed with oxalic acid and the colour developed by the solution with 5 ml of schiff's reagent is matched with the colour developed by a standard solution of methyl alcohol, which has been similarly treated.

E-15.1 Apparatus

E-15.1.1 *Nessler Cylinders* — two, 50 ml capacity

E-15.2 Reagents

E-15.2.1 *Potassium Permanganate Solution*

Dissolve 3 g of the pure crystals of the substance in 100 ml of water containing 15 ml of orthophosphoric acid.

E-15.2.2 *Oxalic Acid Solution*

Dissolve 5 g of pure oxalic acid in 100 ml of dilute hydrochloric acid.

E-15.2.3 *Schiff's Reagent* — same as in E-13.1.

E-15.3 Procedure

Dilute 1 ml of denatured alcohol to 100 ml with distilled water. Transfer 1 ml of this diluted solution to a Nessler cylinder containing 4 ml of water and 2 ml of potassium permanganate solution. Allow it to stand for 10 min with occasional stirring. Then add 2.5 ml of oxalic acid solution to decolourize the permanganate followed by 6 ml of Schiff's reagent. Mix the contents and allow to stand for 1 h.

A control for comparison is prepared by taking 5 ml of methanol (96 percent) in 100 ml of rectified spirit. Take 1 ml of the dilute solution and make it to 100 ml with distilled water. 1 ml of this aqueous solution is then treated in another Nessler cylinder similarly as in the test (*see* E-15.3).

E-15.4 The denatured alcohol shall be taken as conforming to the limit test, if the violet colour developed in the Nessler cylinder containing the sample is not lighter than that produced in the blank (equivalent to 0.048 percent of methanol).

NOTE — Samples denatured with SD 1 – 5 litre, SD 1a – 10 litre, SD 12 – 4 litre when tested similarly should conform to the standards prepared from corresponding equivalent quantities of methanol.

E-16 MENTHOL

Menthol in acid medium is treated with aniline in acid medium to give orange yellow colour.

E-16.1 Apparatus

E-16.1.1 *Nessler Cylinders* — two, 50 ml capacity

E-16.2 Reagents

E-16.2.1 *Concentrated Sulphuric Acid*

E-16.2.2 *Vanillin in Sulphuric Acid* — 1 percent (*w/v*)

E-16.2.3 *Standard Solutions:*

a) 2.5percent solution of menthol in Ethanol for SD 7a; and
b) 4.5percent solution of menthol in Ethanol for SD 7b.

E-16.3 Procedure

Take 10 ml of the standard solutions in 5 ml of concenterated sulphuric acid and add 5 ml of 1 percent (*w/v*) solution of vanillin in sulphuric acid, an orange yellow color is produced. Compare the colour with 10 ml of denatured alcohol with standards.

E-17 METHYL ISOBUTYL KETONE

To identify methyl isobutyl ketone in presence of acetone gas chromatographic methods should be used.

E-18 TERTIARY BUTYL ALCOHOL

Yellow precipitate formed by reaction with denige's reagent is identified.

E-18.1 Reagents

E-18.1.1 *Denige's Reagent*

Dissolve 5 g of the red mercuric oxide in 20 ml of concentrated sulphuric acid. Add this solution to 80 ml of distilled water and filter when cool.

E-18.2 Procedure

Place five drops of a solution containing approximately 0.1 percent tertiary butyl alcohol in ethyl alcohol in a test tube. Add 2 ml of denige's reagent and heat the mixture just to the boiling point and remove from the flame. A yellow precipitate forms within a few seconds.

E-19 TOLUENE

Toluene is nitrated and the resulting dinitrotoluene condensed with butanone, a blue colour turning to violet develops, the intensity of which is proportional to the quantity of toluene nitrated. The above colour completely fades out on addition of acetic acid. A series of standards containing known quantities of toluene that have been nitrated in the same manner are prepared simultaneously for colour comparison.

E-19.1 Reagents

All the reagents given under E-5.1 are required.

E-19.2 Procedure

Measure 100 ml of the denatured alcohol to be tested in a 500 ml distillation flask and add 200 ml of water along with a few pieces of carborandum. Connect the flask to a condenser and distil, collecting the distillate in a 25 ml cylinder immersed in ice cold water. Distil 20 ml at the rate of 1.0 ml/min to 1.5 ml/min. This distillate consists of 80 percent alcohol (*m/m*) and contains all the toluene. Transfer this distillate to a 125 ml separating funnel and add 25 ml of saturated sodium chloride solution and about 20 ml of hexane. Stopper the funnel and shake vigorously for 30 s. After the 2 layers have been separated, discard the aqueous layer, wash the hexane solution with 2 successive portions of water and finally with 10 ml of sodium chloride solution. The hexane extract is then drawn into a 25 ml measuring flask and exactly made up to 25 ml with hexane.

Place 10 ml of the nitration mixture into a 150 ml glass stoppered flask immersed in an ice-bath. After the nitration mixture has become cold, introduce very slowly 2 ml of the hexane extract measured exactly with the aid of the pipette. Stopper and allow it to stand for at least 1 h for the nitration to be complete. Dilute the contents of the flask with 100 ml of ice cold water cautiously keeping the flask at the same time in ice cold water. Extract the nitrated material with 50 ml of ether in a separating funnel, run the aqueous layer into a second separating funnel and extract it with another 25 ml of ether. Reject this aqueous layer and mix this 25 ml ether with the original 50 ml ether extract. Wash the combined ether extract with 25 ml water, and run down the ether extract into a 100 ml volumetric flask. Make to volume with 95 percent alcohol. Transfer a 10 ml aliquot to suitable Nessler cylinder, add 5 ml of butanone and 5 drops of potassium hydroxide solution, shake the mixture, and allow to stand for 10 min. If toluene is present in the original denatured alcohol, a blue colour turning into violet will appear. The colour is then compared with a standard obtained from a known concentration of toluene treated similarly. The above colour completely fades out on addition of acetic acid.

E-20 TURPENTINE OIL

The turpentine oil is extracted with xylole and then increase in the volume of xylole layer noted.

E-20.1 Apparatus

E-20.1.1 *Cassia Flask* — 100 ml capacity, graduated to 0.1 ml

E-20.2 Reagents

E-20.2.1 *Sodium Chloride Solution* — saturated

E-20.2.2 *Xylole*

E-20.3 Procedure

Shake 25 ml of the denatured alcohol with 5 ml of xylole and 70 ml of sodium chloride solution in a cassia flask. Read the xylole layer, which separates on standing.

E-20.4 The denatured alcohol shall be taken as conforming to the limit test if the volume of the upper xylole layer is not less than 11.0 ml.

ANNEX F

(*Foreword* and *Clause* 8)

LIMIT TESTS USING GAS CHROMATOGRAPHIC METHODS

F-1 These test methods covers the determination of the denaturants in SD 1, SD 1a, SD 2, SD 2a, SD 2b, SD 3, SD 3a, SD 4, SD 5, SD 6, SD 6a, SD 13, SD 14, CD 1, CD 2, CD 3, CD 4, CD 5. Denatured alcohol formulations by gas chromatography.

F-2 These methods are for comparative purposes. Accurate determination of denaturants is possible. These methods are for volatile denaturants only.

F-2.1 Summary of Test Method

A denatured alcohol sample is introduced into a gas chromatograph with a capillary column. At given oven and gas conditions the components are vaporized and eluted from the column in order. The flame ionization detector signal is processed by an electronic data acquisition system.

F-2.2 Apparatus

F-2.2.1 *Gas Chromatograph* — with flame ionization detector capable of operating at the conditions given below.

F-2.2.1.1 *Sample introduction* — manual/automatic

F-2.2.1.2 *Column*

This method utilizes polar, slightly polar and non-polar columns. As different companies produce different columns with their brand name a comparative columns list is given as Table 2. One can use any one of these columns or any other equivalent column, if they get proper separation of the denaturant.

F-2.2.1.3 *Operating conditions*

The operating conditions given below are for semi - polar columns. For polar and non-polar columns, the conditions may vary slightly. These conditions are applied when acetaldehyde, methanol, acetone, ethyl acetate, chloroform, benzene,crotonaldehyde, cyclohexane, toluene, methyl isobutyl ketone, isopropyl alcohol and tertiary butyl alcohol denaturant formulae are tested.

Oven temperature:

Initial temperature	:	40 °C
Hold time	:	5 min
Heating rate	:	2 °C/min
Hold temperature	:	90 °C
Heating rate	:	30 °C/min
Final temperature	:	230 °C
Hold time	:	2 min
Run time	:	36.4 min and vary in given conditions
Injector temperature	:	140 °C
Injection mode	:	Split injection (split ratio 2 : 1)
Detector temperature	:	Flame ionization detector at 240 °C
Carrier gas and flow rate	:	Nitrogen/Hydrogen 3ml/min
Injection volume	:	0.2 ml

Table 2 Guidelines for Selection of Suitable Columns

(*Clause* F-2.2.1.2)

Sl No.	Nature	Denaturant Formula	Phase Composition of the Column	Examples
(1)	(2)	(3)	(4)	(5)
i)	Polar	SD 1, SD 1a, SD 3, SD 3a SD 3b, SD 4 SD 5, SD 6 SD 6a, SD 7 SD 13	Poly ethylene glycol or Poly ethylene glycol TPA	BP 20, BP 21 HP – FFAP HP – 20M Carbo wax 20 M DB Wax DB FFAP Supelcowax-10 SPB 1000 CP-WAX 52 CB CP WAX 58 CB, etc
ii)	Semi-polar	SD 1, SD 1a, SD 2, SD 2a SD 2b, SD 3 SD 3a, SD 3b SD 4, SD 5 SD 6, SD 6a SD 7, SD 13 SD 14, SD 15	50 percent phenyl 50 percent methyl polysiloxane or 6 percent cyano propylphenyl 94 percent dimethyl polysiloxane	BP 624, BPX-50 HP 50+, HP-VOC DB 17, DB 624 SPB 50, SPB 624-4 CP SIL 24 CB CP 624 CB, etc
iii)	Non-polar	SD 2 SD 2a SD 2b CD 4 CD 5	Dimethyl poly siloxane oil or 1 percent vinyl – 5 percent phenyl methyl polysiloxane or 5 percent phenyl polysiloxane	BP-1, BPX1 BPX5, BP-5 HP 5, HP5-Trace HP 1, Ultra 2, Ultra 1 DB 2887, SPB 5 SPB 1, SPB HAP CP-SIL5CB CP SIL 8 CB, etc

F-2.3 Procedure

Prepare synthetic mixture containing the exact amount of denaturant mentioned in the Annex B. Inject 0.2 µl of standard and note down the area of denaturant (a). Inject 0.2 µl of the sample and note down the area of denaturant (b). Find out the factor as follows:

$$\text{Factor} = \frac{\text{Amount of denaturant in the sample}}{\text{Area of denaturant (a)}}$$

Calculate the percent denaturants as follows:

$$\text{Percent denaturant} = \text{Factor} \times \text{Area (b)}$$

NOTE — Area normalization is simple method of calculation and results are accurate as the components in the sample are compared with the standard.

F-3 TESTS FOR CD 4 AND CD 5 FORMULATIONS

Gas chromatograms can easily identify petroleum naphtha and gasoline. This method differentiates the individual denaturants and any combination. Petroleum naphtha is having carbon atoms in the range C_4 to C_{11}. Gasoline is having carbon atoms in the range C_4 to C_{12}.

These denaturants when analyzed by gas chromatograph gives specific chromatogram. The specific chromatograms for CD 4 and CD 5 with the chromatographic conditions are given below for guidance. The chromatograms may differ in appearance depending on the instrument parameters.

Sample and respective standards has to be analyzed in similar conditions.

Ethanol peak is removed from calculation using software or calculate the area of other than ethanol for total area comparison. For these denaturants 10 percent total area from the standard has to be taken as confirms to the limit tests for these denaturants.

F-3.1 Operating Conditions

The following operating conditions are for non-polar columns.

Oven temperature:

Initial temperature : 50 °C
Hold time : 0 min
Heating rate : 5°C/min
Hold temperature : 250°C
Hold time : 10 min
Run time : 20 min
Injector temperature : 260°C
Injection mode : Split injection (split ratio 1 : 60)
Detector temperature : Flame ionization detector at 260 °C
Carrier gas and flow rate : Nitrogen/Hydrogen 3 ml/min
Injection volume : 0.2 ml

The sample chromatograms for CD 4 (naphtha), CD 5 (gasoline) are given below for guidance (*see* Fig. 4 and Fig. 5). To determine the carbon atom numbers calibrate the instrument with stock hydrocarbon calibration standard and compare the sample.

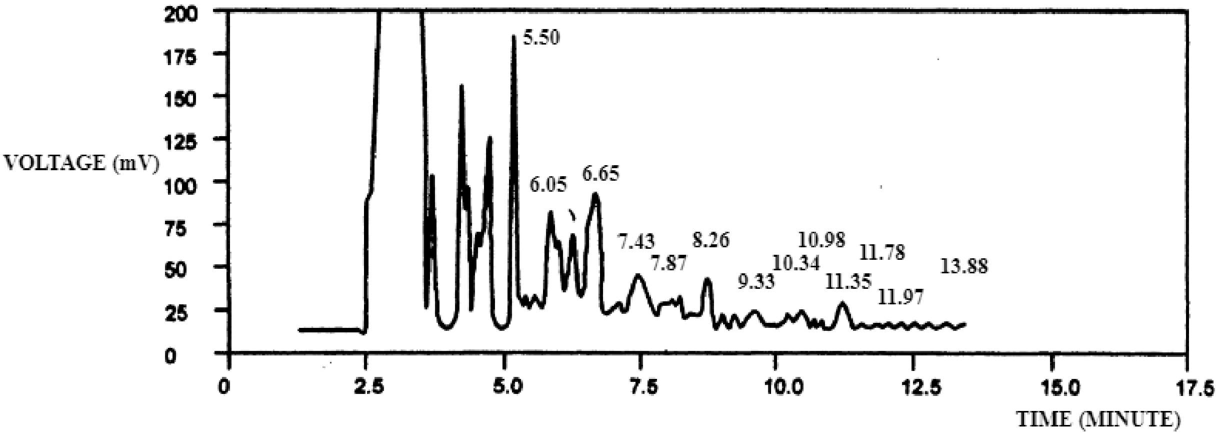

FIG. 4 SAMPLE CHROMATOGRAM OF PETROLEUM NAPHTHA (CD 4) (1 LITRE IN 100 LITRE ETHANOL)

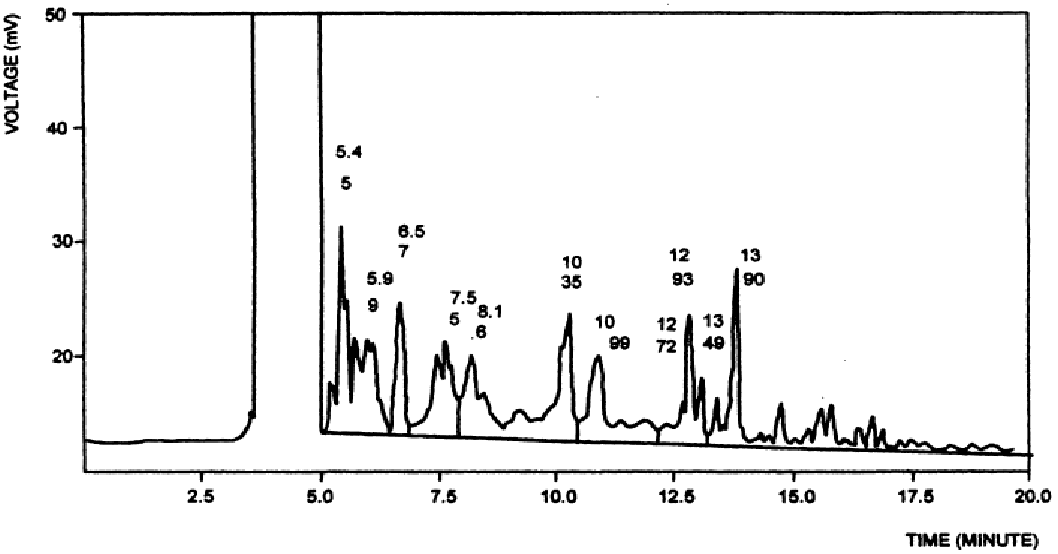

FIG. 5 SAMPLE CHROMATOGRAM OF GASOLINE (CD 5) (2 LITRE IN 100 LITRE ETHANOL)

ANNEX G

(*Foreword*)

COMMITTEE COMPOSITION

Organic Chemicals, Alcohols and Allied Products Sectional Committee, PCD 09

Organization	*Representative*(*s*)
National Chemical Laboratory (NCL), Pune	Dr C. V. Rode **(*Chairperson*)**
Alkyl Amines Chemicals Limited, Mumbai	Shri S. V. Nikumbhe Shri Sameer Katdare (*Alternate*)
All India Alcohol-Based Industries Development Association (AABIDA), Mumbai	Shri K. L. Raphael Shri Kirti Gajjar (*Alternate*)
All India Distillers Association (AIDA), New Delhi	Shri V. N. Raina
BASF India Limited, Mumbai	Shri Kiran Bhat Shri Hemal (*Alternate*)
Chemical and Petrochemicals Manufacturers Association (CPMA), New Delhi	Shri Uday Chand
CSIR - Central Drug Research Institute (CDRI), Lucknow	Dr Sanjeev Kanojiya
Deepak Fertilizer, New Delhi	Dr L. B. Yadawa Shri Suresh Amle (*Alternate*)
Deepak Phenolics Limited, Vadodara	Shri Dharmesh Siddhapuria Shri Sandip Kumar Pandya (*Alternate*)
Department of Chemicals and Petrochemicals, Ministry of Chemicals and Fertilizers, New Delhi	Shri O. P. Sharma Shri Varun Singh Poonia (*Alternate*)
Dow Chemical International Private Limited, Mumbai	Shri V. Mohandoss Shri Govind Gupta (*Alternate*)
Godavari Biorefineries, Mumbai	Shri Shanul Laxmanrao Pagar Shri Appasaheb J. Wani (*Alternate*)
Gujarat Narmada Valley Fertilizers Company Limited, Ahmedabad	Dr R. M. Patel Shri C. S. Patel (*Alternate*)
Hindustan Organic Chemicals Limited (HOCL), Mumbai	Shri Deleep Kumar K. Dr B. Rajeev (*Alternate*)
India Glycols Limited, Uttarakhand	Dr R. K. Sharma Shri Alok Singhal (*Alternate*)
Indian Chemical Council (ICC), Mumbai	Dr Mritunjay Chaubey Shri J. Sevak (*Alternate*)

Organization	*Representative(s)*
Indian Oil Corporation Limited, Panipat	DR Y. S. JHALA
Jubilant Agri and Consumer Products Limited, Gurugram	DR KANAK BARAN DASS
Jubilant Life Sciences Limited, Noida	SHRI MANSUKH D. KANZARIYA
Laxmi Organic Industries, Mumbai	SHRI KRISHNA A. RAO SHRI KAMLESH FULCHAND SHINDE (*Alternate*)
National Chemical Laboratory (NCL), Pune	DR RAVINDAR KONTHAM DR UDAYA KIRAN MARELLI (*Alternate*)
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United Phosphorus Limited (UPL), Mumbai	SHRI M. D. VACHHANI
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Member Secretary
MS ADITI CHOUDHARY
SCIENTIST 'B'/ASSISTANT DIRECTOR
(PETROLEUM, COAL AND RELATED PRODUCTS), BIS

(*Continued from second cover*)

Apart from ensuring uniformity in the minimum quality requirements and methods of test for alcohol denaturants as such and in the specially denatured alcohol, this standard assisted consumers of specially denatured alcohol in complying with Excise Regulations and the Excise Authorities in effecting implementation of the regulations current in different states in the country. Since wood naphtha was no longer used as denaturant, therefore, in the first revision the requirements of wood naphtha were deleted.

In the light of development in the technologies and also experience gained over a period of more than three decades, the Committee responsible for formulation of this standard felt the need to revise the standard. Information on revision had been gathered from various customs and excise departments in the country and effort had been made to align the standard with international trade practices.

In second revision, completely denatured formulations with mineral and synthetic pyridine bases and light caoutchoucine were deleted. New completely denatured formulations according to modern trade practices were introduced. Diethyl ether, castor oil, chloral hydrate and ethyl benzoate, which were not in use, were substituted by new denaturants.

In this revision, requirement of acetaldehyde and crotonaldehyde have been modified. Further, the requirement of denatonium saccharide has been bifurcated as crystalline powder and amorphous powder. The three amendments issued to 2008 version are also incorporated in this revision.

The detailed specifications for individual denaturants have been given in Table 1. List of denaturants (formulae), along with their permissible limits has been given in Annex B. The permissible end use applications are given in Annex C. Methods of tests for various denaturants are given in Annex D, while limit tests for denaturants in denatured alcohol are given in Annex E. Gas chromatographic methods for denaturants (volatile) in ethanol are also given for quick identification/compliance to the denatured formulations in Annex F.

The composition of the Committee responsible for the formulation of this standard is given in Annex G.

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.

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