World Health Organization

ETHANOL 96% (*V/V*)

(ETHANOLUM 96% (*V/V*))

Draft proposal for inclusion for *The International Pharmacopoeia*

(January 2021)

DRAFT FOR COMMENTS

Please send any comments you may have on this draft working document to **Dr Herbert Schmidt**, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (schmidth@who.int), with a copy to Ms Claire Vogel (vogelc@who.int) by **31 March 2021**.

Our working documents are sent out electronically and they will also be placed on the WHO Medicines website (https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/pharmaceuticals/current-projects) for comments under the "*Working documents in public consultation*" link. If you wish to receive our draft guidelines, please send your e-mail address to jonessi@who.int and your name will be added to our electronic mailing list.

SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/21.872:

ETHANOL 96% (*V/V*)

(**ETHANOLUM 96% (*V/V*)**)

Description	**Date**
Monograph drafted based on the corresponding, internationally-harmonized text developed by the Pharmacopoeial Discussion Group.	December 2020
Draft monograph sent out for public consultation.	February – March 2021
Discussion at the Consultation on Screening Technologies, Laboratory Tools and Pharmacopoeial Specifications for Medicines	May 2021
Presentation to the 56th WHO Expert Committee on Specifications for Pharmaceutical Preparations.	October 2021
Further follow-up action as required.	

*[**Note from the Secretariat**. It is proposed to include the monograph on Ethanol 96% (V/V) in* The International Pharmacopoeia*.*

The monograph is based on the corresponding, internationally-harmonized text developed by the Pharmacopoeial Discussion Group (PDG). Editorial modifications have been made in order to be in line with the style used in The International Pharmacopoeia*.]*

ETHANOL 96% (*V/V*) (ETHANOLUM 96% (*V/V*))

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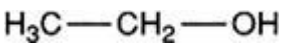
Graphic formula.



Molecular formula. C_2H_6O

Relative molecular mass. 46.07

Chemical name. Ethyl alcohol; ethanol; CAS Reg. No. 64-17-5.

Other name. Alcohol 96% (*V/V*).

Description. A colourless, clear and mobile liquid.

Miscibility. Miscible with water R.

Category. Solvent; antiseptic.

Storage. Ethanol 96% (*V/V*) should be kept in a well-closed container and stored, whenever possible, at a temperature between 8 and 15 °C.

Additional information. Ethanol 96% (*V/V*) contains approximately 4% (*V/V*) of water. It is flammable, burning with a blue smokeless flame, and hygroscopic. Boiling point, about 79 °C.

Requirements

Definition. Ethanol 96% (*V/V*) contains not less than 95.1% (*V/V*) and not more than 96.9% (*V/V*) of C_2H_6O, corresponding to not less than 92.6% (*m/m*) and not more than 95.2% (*m/m*) of C_2H_6O, at 20 °C.

Identity tests

A. Determine the relative density d_{20}^{20} of the test substance as described under *1.3 Determination of mass density, relative density and weight per millilitre*. The relative density d_{20}^{20} is 0.805 to 0.812.

B. Carry out the test as described under *1.7 Spectrophotometry in the infrared region*. The infrared absorption spectrum of the test substance is concordant with the reference spectrum of ethanol 96% (*V/V*)

Clarity and colour of solution. The test substance is clear when tested as described under *1.18 Clarity and degree of opalescence of liquids [**Note from the Secretariat**. Chapter 1.18 is currently under elaboration]* and colourless when compared with water R as described under *1.11.2 Degree of coloration of liquids*, Method II. Dilute 1.0 mL to 20 mL with water R. After standing for 5 minutes, the dilution remains clear when compared with water R.

Non-volatile residue. Place 100 mL of the test substance in a porcelain dish and heat on a water-bath until volatilized, dry the residue at 105 °C for 1 hour, and weigh; not more than 2.5 mg (25 ppm *m/V*).

Acidity or alkalinity. Add 20 mL of carbon-dioxide-free water R and 0.1 mL of phenolphthalein solution TS to 20 mL of the test substance; the solution is colourless. Add 0.1 mL of carbonate-free sodium hydroxide (0.01 mol/L) VS; the solution is pink (30 ppm, expressed as acetic acid).

Absorbance. Record the absorption spectrum (*1.6*) of the test substance in a cuvette or cell with an optical pathlength of 50 mm against water R between 235 nm and 340 nm. The

absorbance at 240 nm is not more than 0.40, not more than 0.30 between 250 nm and 260 nm, and not more than 0.10 between 270 nm and 340 nm. The spectrum shows a steadily descending curve with no observable peaks or shoulders.

Volatile impurities. Carry out the test as described under *1.14.5 Gas chromatography*.

Use a fused-silica column (30 m × 0.32 mm) coated with a stationary phase of cyanopropyl(3)phenyl(3)methyl(94)polysiloxane R (1.8 µm).

As a detector, use a flame ionization detector.

Use helium R as the carrier gas with a linear velocity of 35 cm/s. Use a split ratio of 1:20.

Maintain the temperature of the column at 40 °C for 12 minutes. Increase the temperature at a rate of 10 °C per minute to 240 °C, and then maintain it at this temperature for 10 minutes. Maintain the temperatures of the injection port and that of the detector at 200 °C and 280 °C, respectively.

Prepare the following six solutions: For solution (1), use the test substance. For solution (2), add 150 µL of 4-methylpentan-2-ol R to 500.0 mL of the test substance. For solution (3), dilute 100 µL of anhydrous methanol R to 50.0 mL with the test substance. Dilute 5.0 mL of the solution to 50.0 mL with the test substance. For solution (4), dilute 50 µL of anhydrous methanol R and 50 µL of acetaldehyde R to 50.0 mL with the test substance. Dilute 100 µL of the solution to 10.0 mL with the test substance. For solution (5), dilute 150 µL of acetal R to 50.0 mL with the test substance. Dilute 100 µL of the solution to 10.0 mL with the test substance. For solution (6), dilute 100 µL of benzene R to 100.0 mL with the test substance. Dilute 100 µL of the solution to 50.0 mL with the test substance.

Inject alternately 1 µL each of solutions (1), (2), (3), (4), (5) and (6) and record the chromatograms.

The test is not valid unless the resolution between the peaks corresponding to acetaldehyde (the first peak) and methanol (the second peak) in the chromatogram obtained with solution (4) is at least 1.5.

Methanol (impurity F)

In the chromatogram obtained with solution (1), the area of any peak corresponding to methanol is not greater than 0.5 times the area of the peak due to methanol in the chromatogram obtained with solution (3) (200 ppm (*V/V*)).

Acetaldehyde (impurity B) and acetal (impurity A)

Calculate the sum of the contents of acetaldehyde and acetal in parts per million (*V/V*) using the following expression:

$$\frac{10 \times A_E}{A_T - A_E} + \frac{30 \times C_E}{C_T - C_E} \times \frac{44.05}{118.2}$$

where

A_E = area of any peak corresponding to acetaldehyde in the chromatogram obtained with solution (1);

A_T = area of the peak due to acetaldehyde in the chromatogram obtained with solution (4);

C_E = area of any peak corresponding to acetal in the chromatogram obtained with solution (1);

C_T = area of the peak due to acetal in the chromatogram obtained with reference solution (5);

44.05 = molecular mass of acetaldehyde;

118.2 = molecular mass of acetal.

The sum of the contents is not greater than 10 ppm (*V/V*), expressed as acetaldehyde.

Benzene (impurity D)

Calculate the content of benzene in parts per million (*V/V*) using the following expression:

$$\frac{2B_E}{B_T - B_E}$$

where

B_E = area of any peak corresponding to benzene in the chromatogram obtained with solution (1);

B_T = area of the peak due to benzene in the chromatogram obtained with solution (6).

The content of benzene is not greater than 2 ppm (*V/V*).

If necessary, the identity of benzene can be confirmed using another suitable chromatographic system (stationary phase with a different polarity).

Other volatile impurities

In the chromatogram obtained with solution (2), the sum of the areas of the peaks for any other impurities is not greater than the area of the peak due to 4-methylpentan-2-ol (300 ppm). Disregard any peak with an area less than 0.03 times than the area of the peak due to 4-methylpentan-2-ol (9 ppm).

Assay. Calculate the mass density at 20 °C (ρ_{20}) of the test substance using the value for the relative density d_{20}^{20} obtained in identity test A. Determine the % (*V/V*) of C_2H_6O using the alcoholimetric table given in 1.3.2. *[**Note from the Secretariat**. Chapter 1.3.2 is currently under revision. The revised version will include the mentioned alcoholimetric table]*

Impurities

***[Note from the Secretariat.** The chemical structures will be added at a later stage.]*

A. 1,1-Diethoxyethane (acetal)

B. Acetaldehyde
C. Propan-2-one (acetone)
D. Benzene
E. Cyclohexane
F. Methanol
G. Butan-2-one (methyl ethyl ketone)
H. 4-Methylpentan-2-one (methyl isobutyl ketone)
I. Propan-1-ol (propanol)
J. Propan-2-ol (isopropyl alcohol)
K. Butan-1-ol (butanol)
L. Butan-2-ol
M. 2-Methylpropan-1-ol (isobutanol)
N. Furan-2-carbaldehyde (furfural)
O. 2-Methylpropan-2-ol (1,1-dimethylethyl alcohol)
P. 2-Methylbutan-2-ol
Q. Pentan-2-ol
R. Pentan-1-ol (pentanol)
S. Hexan-1-ol (hexanol)
T. Heptan-2-ol
U. Hexan-2-ol
V. Hexan-3-ol

New reagents for Anhydrous Ethanol and Ethanol 96% (*V/V*) monographs

Phenolphthalein solution TS

Procedure. Dissolve 0.1 g of phenolphthalein R in 80 mL of ethanol (~750 g/L) TS and dilute to 100 mL with water R.

Test for sensitivity. Add 0.1 mL of the phenolphthalein solution to 100 mL of carbon dioxide-free water R; the solution is colourless. Not more than 0.2 mL of sodium hydroxide (0.02 mol/L) VS is required to change the colour to pink.

Colour change. pH 8.2 (colourless) to pH 10.0 (red).

Cyanopropyl(3)phenyl(3)methyl(94)polysiloxane R

Polysiloxane substituted with 3% cyanopropyl groups, 3% of phenyl groups and 94% of methyl groups.

4-Methylpentan-2-ol R

4-Methyl-2-pentanol; $C_6H_{14}O$

Description. Clear, colourless, volatile liquid.

Refractive index. n_D^{20} = about 1.411

Relative density. d_4^{20} = about 0.802

Boiling point. About 132 °C.

Methanol, anhydrous R

Procedure. Treat 1000 mL of methanol R with 5 g of magnesium R. If necessary initiate the reaction by adding 0.1 mL of mercuric chloride (54 g/L) TS. When the evolution of gas has ceased, distil the liquid and collect the distillate in a dry container protected from moisture.

Water (2.8). Not more than 0.3 g/L.

Magnesium R

Mg

Description. Silver-white ribbon, turnings or wire, or a grey powder.

Mercuric chloride (54 g/L) TS

A solution of mercuric chloride R containing 54 g of $HgCl_2$ per litre.

Acetal R

Acetaldehyde diethyl acetal; 1,1-Diethoxyethane; $C_6H_{14}O_2$

Description. Clear, colourless, volatile liquid.

Miscibility. Miscible with water and with ethanol (~750 g/L) TS.

Refractive index. n_D^{20} = about 1.382

Relative density. d_{20}^{20} = about 0.824

Boiling point. About 103°C

Amendment to existing reagent entry

The existing entry for Benzene will be replaced with the following:

Benzene

C_6H_6 (SRIP, 1963, p.48)

Where benzene is used to prepare a reference solution, for safety reasons, the pure reagent may be replaced by a commercially available reference material containing a certified amount of benzene.

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