World Health Organization

DETERMINATION OF DIETHYLENE GLYCOL AND ETHYLENE GLYCOL IN LIQUID PREPARATIONS FOR ORAL USE

Draft proposal for revision in *The International Pharmacopoeia*

(15 July 2025)

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/25.985

DETERMINATION OF DIETHYLENE GLYCOL AND ETHYLENE GLYCOL IN LIQUID PREPARATIONS FOR ORAL USE

Description	Date
Revision drafted based on information found in the scientific literature and on laboratory investigations.	May 2025
Monograph sent out for public consultation.	August – September 2025
Presentation at the 59th meeting of the Expert Committee on Specifications for Pharmaceutical Preparation	October 2025
Further follow-up action as required.	

*[**Note from the Secretariat.** It is proposed to revise the* Tests for diethylene glycol and ethylene glycol in liquid preparations for oral use*, published in the Supplementary section of* The International Pharmacopoeia. *The revision aims to:*

- *Improve resolution, sensitivity, and reproducibility by replacing the current thin-layer chromatographic (TLC) procedure with a high-performance thin-layer chromatographic (HPTLC) method,*
- *Strengthen system suitability requirements to ensure more robust and reliable analytical performance,*
- *present the HPTLC and GC procedures as complementary analytical techniques, allowing mutual confirmation of results.]*

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Introduction and scope

Diethylene glycol (DEG) and ethylene glycol (EG) are toxic substances commonly used as industrial solvents and antifreeze agents. Even small quantities can be fatal, especially for children.

Although no definitive minimum safe levels for human ingestion have been established, a detection threshold of 0.10 % for each substance is generally considered acceptable in raw materials and finished pharmaceutical products from a safety perspective.

Gas chromatography (GC) with a flame ionization detector (FID) is a reliable and widely used analytical technique for the accurate and precise detection of volatile substances. It has frequently been applied for the determination of DEG and EG in pharmaceutical excipients and formulations. However, other methods are also suitable for this purpose such as High-performance thin-layer chromatography [1], Ultrahigh-performance supercritical fluid chromatography - Mass spectrometry after precolumn derivatization [2] or Gas chromatography - Tandem mass spectrometry [3].

The document describes two procedures for the determination of DEG and EG in liquid preparations for oral use: a high-performance thin-layer chromatographic (HPTLC) and a gas chromatographic (GC-FID) procedure.

National Quality Control Laboratories should be prepared to test oral liquids from their markets for DEG and EG to safeguard patients from contaminated products.

They should implement the analytical method they want to employ based on the availability of appropriate equipment and the training and expertise of their personnel.

In line with WHO good practices for pharmaceutical quality control laboratories [4], any found contamination should be confirmed using a second method with different selectivity. In this regard, the described HPTLC and GC procedures can serve as complementary techniques, allowing mutual confirmation of results.

Laboratories that confirm the presence of DEG or EG contamination in excipients or finished products should notify the relevant regulatory authorities without delay.

References

1. Sonja Drobnjak; Detection and limit test of DEG and EG impurities in syrup products, CBS Edition: 134, 30 April 2025; https://camag.com/article/detection-and-limit-test-of-diethylene-glycol-and-ethylene-glycol-impurities-in-syrup-by-hptlc/
2. Si-Tong Zheng, Zi-Ying Wang, Zhen Liu, Yingxiang Du, Ling Cao, Sheng Tang, Hian Kee Lee, Yaozuo Yuan, Hai-Wei Shi; Rapid Determination of Trace Ethylene Glycol and Diethylene Glycol in Propylene Glycol-Contained Syrups by Ultrahigh-Performance Supercritical Fluid Chromatography-Mass Spectrometry after Precolumn Derivatization; Journal of Chromatography A; Volume 1737; 2024; https://doi.org/10.1016/j.chroma.2024.465433.
3. Altamimy, Monerah A. et al.; A Selective Gas Chromatography–Tandem Mass Spectrometry Method for Quantitation of Ethylene and Diethylene Glycol in Paediatric Syrups; Heliyon; Volume 10; Issue 7; 2024; https://doi.org/10.1016/j.heliyon.2024.e27559.
4. WHO Expert Committee on Specifications for Pharmaceutical Preparations: fifty-seventh report. Geneva: World Health Organization; 2024; Annex 1 (WHO Technical Report Series, No. 1052).

Determination by high-performance thin-layer chromatography

Note. EG and DEG exhibit similar R_F values in the described HPTLC procedure. Reference solutions containing 50:50 (m/m) mixtures of DEG and EG are used to calibrate the analyte response. As a result, analysts determine the combined concentration of DEG and EG rather than quantifying each contaminant individually.

Procedure. Carry out the test as described under *1.18 High-Performance Thin-layer chromatography*.

Prepare the following solutions:

Developing solvent system. A freshly prepared mixture of water R, concentrated ammonia solution R, toluene R and acetone R (9.5:1:5:85 *V/V/V/V*). Add the solvents in the described order and mix.

Derivatization reagent. Prepare a mixture of 0.75 g of potassium permanganate R, 5 g of sodium carbonate R and 0.625 mL of sodium hydroxide (~ 100 g/L) TS in 100 mL of water R.

Stock solutions

For solution (H1) *(EG+DEG stock solution, 20% (m/m) with reference to the sample concentration in solutions S10, 10 mg EG + 10 mg DEG per mL),* dissolve 500 mg of diethylene glycol R and 500 mg of ethylene glycol in 50.0 mL of methanol R.

For solution (H2) *(EG+DEG stock solution, 2% (m/m) with reference to the sample concentration in solutions S10, 1 mg EG + 1 mg DEG per mL),* dilute 10.0 mL of solution (H1) to 100.0 mL with methanol R.

For solution (H3) *(PG/Glycerol stock solution),* transfer 5.0 g of propylene glycol R and 5.0 g of glycerol R to a 500 mL volumetric flask, add 400 mL of methanol R, sonicate for 5 minutes and make up to volume with the same solvent.

Sample solution

For solution (S10) *(sample solution, 100 mg sample per mL)*, transfer 1.000 g of the liquid preparation for oral use under investigation to a 10.0 mL volumetric flask. Add 5 mL of methanol R, stopper the flask and shake vigorously. Make up to volume with methanol R, mix thoroughly and filtrate, if necessary.

For solution (S11) (EG spiked sample solution, 0.1% (m/m), transfer 1.000 g of the liquid preparation for oral use under investigation to a 10.0 mL volumetric flask. Add 1.0 mL of solution (R0) and 4 mL of the methanol R, stopper the flask and shake vigorously. Make up to volume with the methanol R, mix thoroughly and filtrate, if necessary.

Reference solution containing EG and DEG

For solution (R0) (EG+DEG reference solution, 10% (m/m) with reference to the sample concentration in solution (S10), 5 mg EG + 5 mg DEG per mL), transfer 10.0 mL of solution (H1) to a 20 mL volumetric flask and dilute to volume with methanol R.

For solution (R1) *(EG+DEG reference solution, 5% (m/m) with reference to the sample concentration in solution (S10), 2.5 mg EG + 2.5 mg DEG per mL)*, transfer 5.0 mL of solution (H1) to a 20 mL volumetric flask, add 5.0 mL of solution (H3) and dilute to volume with methanol R.

For solution (R2) *(EG+DEG reference solution, 1% (m/m) with reference to the sample concentration in solution (S10), 0.5 mg EG + 0.5 DEG per mL)*, transfer 5.0 mL of solution (H1) to a 100 mL volumetric flask, add 25.0 mL of solution (H3) and dilute to volume with methanol R.

For solution (R3) *(EG+DEG reference solution, 0.2% (m/m) with reference to the sample concentration in solution (S10), 0.1 mg EG + 0.1 mg DEG per mL)*, transfer

10.0 mL of solution (H2) to a 100 mL volumetric flask, add 25.0 mL of solution (H3) and dilute to volume with methanol R.

For solution (R4) *(EG+DEG reference solution, 0.1% (m/m) with reference to the sample concentration in solution (S10), 0.05 mg EG + 0.05 mg DEG per mL)*, transfer 5.0 mL of solution (H2) to a 100 mL volumetric flask, add 25.0 mL of solution (H3) and dilute to volume with methanol R.

For solution (R5) *(EG+DEG reference solution, 0.05% (m/m) with reference to the sample concentration in solution (S10), 0.025 mg EG + 0.025 mg DEG per mL)*, transfer 2.5 mL of solution (H2) to a 100 mL volumetric flask, add 25.0 mL of solution (H3) and dilute to volume with methanol R.

Analysis. Apply 5 µL of solutions (S10), (S11), (R1), (R2), (R3), (R4) and (R5) as bands and dry them in air. Precondition the plate to control its activity and develop it in a saturated chamber. Remove the plate, dry it in a stream of air at room temperature and examine it under white light, short-wave and long-wave UV light.

Use a pencil to lightly circle the spots that are visible in daylight and under UV light. The substances identified may originate from active ingredients, their impurities or degradants, excipients, or other matrix components.

To identify the spots due to EG/DEG, propylene glycol, glycerol and other components of the sample that are not visible in daylight and under UV light, spray the plate with the derivatization reagent and dry it at 100° C for 3 minutes. Let the plate rest for 15 minutes. Bright yellow spots have become visible.

The following substances are eluted with the following R_F values: sorbitol about 0.08; glycerol about 0.37; DEG and EG about 0.53; propylene glycol about 0.61.

Determine if components of the sample interfere with the EG/DEG determination by comparing the R_F values of spots circled in daylight or UV light with the R_F value of the spot due to EG or DEG after derivatization.

The test is not valid unless the chromatograms obtained with solutions (R1), (R2), and (R3) show three resolved spots due to propylene glycol, EG / DEG and glycerol und in the chromatogram obtained with solution (S11) the spot due to EG or DEG is free of interferences.

Identify a spot due to EG/DEG in the chromatogram obtained with solution (S10), if present. Estimate the approximate combined percentage content of DEG and EG (m/m) in the liquid preparation for oral use under investigation by comparing the intensity of the spot due to EG or DEG in the chromatogram obtained with solution (S10), if present, with the intensities of the spots due to EG/DEG in the chromatograms obtained with solutions (R1) to (R5).

Determination by gas chromatography

Carry out the test as described under *1.14.1 Chromatography*, Gas chromatography using the internal standard method.

For the procedure, use a capillary glass or quartz column (30 m × 0.53 mm), the inner surface of which is coated with a thick layer of macrogol 20M R (1.0 µm). Maintain the temperature of the column at 100 °C for 5 minutes. Increase the temperature at a rate of 10 °C per minute to 245 °C and maintain it at this point for 4 minutes. Maintain the temperature of the injection port and the detector at 250 °C. Use helium R or nitrogen R as the carrier gas with a linear velocity of about 38 cm per second. Use split injection ratio of 1:20 and a flame-ionization detector for detection.

The retention times and the separation of peaks are similar when using helium R or nitrogen R as the carrier gas. Although the peaks are slightly sharper when using helium R, the use of nitrogen R is preferable from a sustainability perspective.

Laboratories that can only obtain a column with a maximum temperature of 240 °C or below may change the temperature accordingly, if necessary. However, lowering the temperature may affect the ability of the method to clean the column of substances with a high boiling point thus deteriorating column performance over time.

The described procedure was validated using helium as a carrier gas. The use of nitrogen or hydrogen may also be suitable with little or no changes in peak resolution. Helium is a non-renewable gas that may be or eventually become difficult to source. Nitrogen and hydrogen can be produced on-demand using suitable generators.

For solution (IS)(10 mg IS per mL), weigh 0.500 g of the internal standard 1,3-butanediol R and dilute to 50.0 mL with water R.

During the elaboration of the analytical procedure, it was noted that the peak due to 1,3-butanediol may show peak tailing when columns with thinner layers of macrogol are used. In such cases, 1,3 propanediol R was found to be a suitable alternative to 1,3-butanediol.

For solution (A) (sample solution, 25 mg of sample per mL), weigh 0.500 g of the liquid preparation for oral use under investigation into a 20 mL volumetric flask, add 1.0 mL of solution (IS) and 1.0 mL of water R and mix thoroughly. Make up to volume with ethanol R and sonicate for 5 minutes. Place the solution in an ice bath for 15 minutes and filter.

For solution (B) (sample solution without IS), weigh 0.500 g of the liquid preparation for oral solution under investigation into a 20 mL volumetric flask, add 2.0 mL of water R and mix thoroughly. Make up to volume with ethanol R and sonicate for 5 minutes. Place the solution in an ice bath for 15 minutes and filter.

For solution (C) (EG, PG, DEG stock solution; 10 mg each per mL), weigh 1.000 g of each ethylene glycol RS, propylene glycol R and diethylene glycol RS and dilute to 100.0 mL with ethanol R.

For solution (D) (EG, PG, DEG stock solution; 1.0 mg each per mL), dilute 2.0 mL of solution (C) to 20.0 mL with ethanol R.

For solution (E) (EG, DEG calibration solution, 1.25 mg each per mL, 5% with regards to sample concentration in solution (A)), mix 25.0 mL of solution (C) with 10.0 mL of solution (IS), 15 mL of water R and dilute to 200.0 mL with ethanol R.

For solution (F) (EG, DEG calibration solution, 0.5 mg each per mL, 2% with regards to sample concentration in solution (A)), mix 1.0 mL of solution (C) with 1.0 mL of solution (IS), 1.5 mL of water R and dilute to 20.0 mL with ethanol R.

For solution (G) (EG, DEG calibration solution, 0.1 mg each per mL, 0.4% with regards to sample concentration in solution (A)), mix 2.0 mL of solution (D) with 1.0 mL of solution (IS), 1.5 mL of water R and dilute to 20.0 mL with ethanol R.

For solution (H) (EG, DEG calibration solution, 0.025 mg each per mL, 0.1% with regards to sample concentration in solution (A)), dilute 2.0 mL of solution (D) to 20.0 mL with ethanol R. Mix 5.0 mL of this solution with 1.0 mL solution (IS), 1.5 mL of water R and dilute to 20.0 mL with ethanol R.

For solution (I) (sensitivity control solution, 0.01 mg each per mL), dilute 2.0 mL of solution (D) to 20.0 mL with ethanol R. Mix 2.0 mL of this solution with 1.0 mL solution (IS), 1.5 mL of water R and dilute to 20.0 mL with ethanol R.

Inject separately 1.0 µl each of solutions (A), (B), (E), (F), (G), (H) and (I) and record the chromatograms. After each injection, wash the needle first with water R, then with methanol/water (1:1 *V*/*V*) and, finally, with 2-propanol R.

Depending on the dimensions of the liner used, the injection volume may need to be adjusted to avoid backflushing of the sample.

Measure the response of the peaks corresponding to DEG, EG, propylene glycol and 1,3-butanediol in the chromatograms obtained. The substances, if present, are eluted at

the following relative retention with reference to 1,3-butanediol (retention time about 14 minutes): propylene glycol about 0.83; EG about 0.87; and DEG about 1.20.

The test is not valid unless the resolution between the peaks corresponding to EG and propylene glycol in the chromatogram obtained with solution (G) is at least 4.0.

Also, the test is not valid unless, in the chromatogram obtained with solution (I), the signal-to-noise ratios of the peaks due to DEG and EG are both at least 10.

Furthermore, the test is not valid unless, in the chromatogram obtained with solution (B), any peak having the same retention time as 1,3-butanediol is not more than 0.01 times the area of the peak corresponding to 1,3-butanediol in the chromatogram obtained with solution (A).

The procedure was successfully applied to numerous samples from the market. However, it cannot be fully excluded that certain excipients or components of samples may interfere with the peaks due to EG, DEG or the internal standard. In such cases, the use of a mass-selective detector may enhance the selectivity of the analyte response.

Calculate the ratios between the responses of the peaks due to EG/DEG and the responses of the peak due to 1,3-butanediol in the chromatograms obtained with solutions (A), (E), (F), (G) and (H).

Determine a separate calibration function each for EG and DEG by plotting the ratios obtained for solutions (E), (F), (G) and (H) on the ordinate against the percentage content of EG/DEG reference substances with regards to the sample concentration in solution (A) on the abscissa. Consider the accurate weights of the reference substances, the accurate weight of the sample and the declared content of $C_2H_6O_2$ or $C_4H_{10}O_3$ in ethylene glycol RS and diethylene glycol RS.

Use the ratio obtained for solution (A) and the calibration functions to calculate the percentage content (m/m) of DEG and EG in the liquid preparation for oral use. If one or both results obtained are above 5%, prepare solutions (A) and (B) using a lower weight of liquid preparation for oral use under investigation and repeat the analysis to ensure that the obtained result(s) lies within the calibration range. For samples containing more than 15%, prepare an intermediate dilution in water R and use this solution to prepare solutions (A) and (B).

The percentage concentrations (m/m) of DEG and EG in the liquid preparation for oral use are each not more than 0.10%.

To be added to the *Reagent* section of The International Pharmacopoeia

Ammonia solution, concentrated R

Caution: upon exposure to air, it loses ammonia rapidly. Use care in handling concentrated ammonia solution because of the caustic nature of the solution and the irritating properties of its vapor.

Definition: content: 25.0% (m/m) to 30.0% of NH_3 (m/m).

Appearance: clear, colourless liquid, very caustic.

Solubility: miscible with water and with ethanol (96%).

Relative density: $d_{20}^{20} = 0.892$ to 0.910.

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