World Health Organization

TEST FOR DIETHYLENE GLYCOL AND ETHYLENE GLYCOL IN LIQUID PREPARATIONS FOR ORAL USE

Draft proposal for inclusion in *The International Pharmacopoeia*

(27 July 2023)

DRAFT FOR COMMENTS

Please submit your comments through the online platform, PleaseReview™ (https://who.pleasereview.net/Main/Default.aspx?action=loaddocument&reviewid=196) If not registered or included in our mailing list, kindly submit your request with your full name, email address and organization/affiliation to jonessi@who.int, nsp@who.int.

For any technical queries, please contact **Dr Herbert Schmidt**, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (schmidth@who.int), with a copy to Ms Sinéad Jones (jonessi@who.int, nsp@who.int).

Comments should be submitted through the online platform on or by **27 September 2023**. Please note that only comments received by this deadline will be considered for the preparation of this document.

Our working documents are sent out electronically and uploaded into PleaseReview™. The working documents are also placed on the WHO Medicines website (https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/pharmaceuticals/working-documents-public-consultation) under the "Working documents in public consultation". If you wish to receive all our draft guidelines during the course of the year, please send your full name, organization/ affiliation, and email address to jonessi@who.int, nsp@who.intand your name will be added to our electronic mailing list and review platform.

SCHEDULE FOR DRAFT WORKING DOCUMENT QAS/23.922

Test for diethylene glycol and ethylene glycol in liquid preparations for oral use

Description	Date
Text drafted by the Secretariat.	February 2023
Discussion at the Consultation on Quality Control and Pharmacopoeial Specifications.	April 2023
Draft text sent out for public consultation.	July – August 2023
Submission to the Expert Committee on Specifications for Pharmaceutical Preparations.	October 2023
Further follow-up action as required.	

*[**Note from the Secretariat.** Unacceptable amounts of ethylene glycol and diethylene glycol have, once again, recently been found in medicines and jeopardized the safety of patients (for more information see https://www.who.int/teams/rethylene glycolulation-prequalification/incidents-and-SF/full-list-of-who-medical-product-alerts and https://www.who.int/news/item/23-01-2023-who-urges-action-to-protect-children-from-contaminated-medicines).*

The International Pharmacopoeia *(Ph.Int.) already prescribes a gas-chromatographic method (GC) for the test for diethylene glycol and ethylene glycol in paracetamol oral solutions. Following up on requests from WHO Member States to provide alternative procedures for National Quality Control Laboratories without access to gas chromatographs, the Secretariat proposes a tiered approach where samples are first screened for non-compliance using a semi-quantitative TLC method and suspected contaminations are subsequently confirmed by GC at regional centres.*

The test is proposed for inclusion in the Supplementary information section of Ph.Int. Reference to this chapter will be made in the general monograph Liquid preparations for oral use. The mentioned reagents, test and volumetric solutions are described in the Reagent Section of the Ph.Int.

Users of The International Pharmacopoeia *are kindly requested to provide their feedback on the proposed tiered approach.*

National Quality Control Laboratories, WHO Collaborating Centres or other laboratories wishing to assess, demonstrate, or acquire the competency to perform the described testing, are kindly requested to analyse a sample with a known ethylene glycol or diethylene glycol concentration and to answer the questions listed under Feedback on the described procedures – Guidance for laboratories in the Annex.

These responses will help the World Health Organization evaluate the suitability of the described test procedures, and support regions in identifying training needs and setting up networks/competency centres for the testing of samples for ethylene glycol and diethylene glycol contamination.]

Test for diethylene glycol and ethylene glycol in liquid preparations for oral use

Diethylene glycol and ethylene glycol are toxic substances used as industrial solvents and antifreeze agents that can be fatal even taken in small amounts, in particular for children.

A suitable and widely used analytical technique to test pharmaceutical products precisely and accurately for diethylene glycol and ethylene glycol is gas chromatography. For a National Quality Control Laboratory without access to a gas chromatograph, a tiered approach is described, where suspicious samples are first identified using a semi-quantitative thin-layer chromatography method (see *Screening for non-compliance by thin-layer chromatography*) and suspected contaminations subsequently confirmed at regional centres using gas chromatography (see *Confirmatory testing by gas chromatography*).

Using the thin-layer chromatography method, diethylene glycol or ethylene glycol concentrations down to 1% (m/m) are detectable. A discrimination between the two contaminants is not possible as both co-elute in the described system.

The sensitivity of the gas chromatographic method is increased by a factor of at least 10 compared to the thin-layer chromatographic procedure. Diethylene glycol or ethylene glycol concentrations down to 0.1% (m/m) for each contaminant can be determined.

Laboratories whose investigations have confirmed diethylene glycol or ethylene glycol contaminations in excipients or finished products shall inform the responsible regulatory authorities without delay.

Screening for non-compliance by thin-layer chromatography

Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography, using a plate coated with silica gel R5 and of a suitable size (e.g. 10 cm x 10 cm, 10 cm x 20 cm or 20 cm x 20 cm).

Saturate a chromatographic tank of a suitable size by lining its walls with filter paper. Pour a sufficient quantity of a freshly prepared mixture of toluene R, acetone R and ammonia (~100 g/L) TS (5:85:10 V/V/V) into the chromatographic tank. After impregnation of the filter paper, the mobile phase shall form a layer of appropriate depth, usually 1 cm. Replace the lid and allow the tank to stand for 1 hour at 20-25 °C to complete the saturation of the tank.

Prepare the following solutions, using methanol R as a solvent.

For solution (A) *(sample solution)*, transfer 1.000 g of the oral solution under investigation to a 5.0 mL volumetric flask and fill up to volume.

For solution (B) *(diethylene glycol reference solution, 20% (m/m), 40 mg diethylene glycol/mL)*, dissolve 1.000 g of diethylene glycol R in 25.0 mL.

For solution (C) *(diethylene glycol reference solution, 10% (m/m), 20 mg diethylene glycol /mL)*, dilute 10.0 mL of solution (B) to 20.0 mL.

For solution (D) *(diethylene glycol reference solution, 1% (m/m), 2 mg diethylene glycol /mL)*, dilute 5.0 mL of solution (B) to 100.0 mL.

For solution (E) *(ethylene glycol reference solution, 20% (m/m), 40 mg ethylene glycol/mL)*, dissolve 1.000 g of ethylene glycol R in 25.0 mL.

For solution (F) *(ethylene glycol reference solution), 10% (m/m), 20 mg ethylene glycol /mL)*, dilute 10.0 mL of solution (E) to 20.0 mL.

For solution (G) *(ethylene glycol reference solution, 1% (m/m), 2 mg ethylene glycol /mL)*, dilute 5.0 mL of solution (E) to 100.0 mL.

Apply separately to the plate, 2 µL of each of solutions (A), (B), (C), (D), (E), (F) and (G) at a distance of about 2 cm from the lower edge and from the sides of the plate and on a line parallel to the lower edge. Allowing the standard solutions to bracket the sample solution. Use a device that allows the reproducible dispensing of the prescribed volume onto the plate (e.g. micro capillary pipettes).

Apply the solutions in sufficiently small portions to obtain circular spots of 2-5 mm in diameter. Allow an interval of at least 10 mm between the centres of circular spots.

When the solvent of the applied solutions has evaporated, place the plate in the chromatographic tank, ensuring that the plate is as vertical as possible and that the spots are above the surface of the mobile phase. Close the chromatographic tank and maintain it at 20-25 °C, protected from sunlight and placed in a draught-free area. Remove the plate when the mobile phase has moved over a distance of about 7 cm (for 10 cm x 10 cm or 10 cm x 20 cm plates) or 15 cm (for 20 cm x 20 cm plates) measured between the points of application and the solvent front.

Dry the plate and visualise the spots by spraying the plate with a solution containing 0.632 g of potassium permanganate R in 100 mL of acetone R. Heat the plate at 105 °C for 15 minutes. Ethylene glycol and diethylene glycol appear as yellow spots on a reddish background.

In the chromatograms obtained, the following substances are eluted with the following Rf values: sorbitol about 0.1; glycerol about 0.45; diethylene glycol and ethylene glycol about 0.55; propylene glycol about 0.65.

The test is not valid unless the chromatograms, obtained with solutions (C) and (F), show spots due to diethylene glycol or ethylene glycol with a similar position and appearance (diethylene glycol and ethylene glycol co-elute).

Identify the spots due to diethylene glycol or ethylene glycol in the chromatograms obtained and estimate their percentage content (m/m) in the liquid preparation of oral use under investigation by comparing the intensity of the spot due to diethylene glycol

or ethylene glycol in the chromatogram obtained with solution (A), if present, with the intensity of the spots due to diethylene glycol or ethylene glycol in the chromatograms obtained with solutions (B) to (G).

If a contamination of the oral solution under investigation is determined, confirm the estimated diethylene glycol or ethylene glycol concentration by gas chromatography.

Confirmatory testing 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 230 °C and maintain it at this point for 4 minutes. Maintain the temperature of the injection port and the detector at 230 °C. Use helium R as the carrier gas with a linear velocity of about 38 cm per second. For application of the solutions, use splitless injection followed by a split ratio of 1:20 after 30 seconds. Use a flame-ionization detector for detection.

Prepare the following solutions in a 1:1 mixture of acetonitrile R and water R.

For solution (IS), weigh 0.175 g of the internal standard 1,3-butanediol R and dilute to 100.0 mL.

For solution (A), weigh 10.000 g of the liquid preparation for oral solution under investigation, add 4.0 mL of solution (IS) and dilute to 100.0 mL.

For solution (B), weigh 10.000 g of oral solution under investigation and dilute to 100.0 mL.

For solution (C), weigh 0.500 g of each ethylene glycol RS, propylene glycol R and diethylene glycol RS and dilute to 50.0 mL.

For solution (D) mix 25.0 mL of solution (C) with 2.0 mL of solution (IS) and dilute to 50.0 mL.

For solution (E) mix 10.0 mL of solution (C) with 4.0 mL of solution (IS) and dilute to 100.0 mL.

For solution (F) mix 1.0 mL of solution (C) with 4.0 mL of solution (IS) and dilute to 100.0 mL.

For solution (G) dilute 2.0 mL of solution (C) to 20.0 mL. Mix 2.0 mL of this solution with 4.0 mL solution (IS) and dilute to 100 mL.

Inject separately 1.0 µl each of solutions (A), (B), (C), (F) and (G) and record the chromatograms.

Measure the response of the peaks corresponding to diethylene glycol, ethylene glycol, propylene glycol and 1,3-butanediol in the chromatograms obtained.

In the chromatogram obtained with solution (E), the analyte peaks are eluted at the following relative retention with reference to 1,3-butanediol (retention time about 14 minutes): propylene glycol about 0.73; ethylene glycol about 0.76; diethylene glycol about 1.04; and glycerol about 1.27.

The test is not valid unless the resolution, between the peaks corresponding to ethylene glycol and propylene glycol in the chromatogram obtained with solution (E), is at least 4.0 and no peak having the same retention time as 1,3-butanediol can be detected in the chromatogram obtained with solution (B). Also, the test is not valid unless, in the chromatogram obtained with solution (G), the signal-to-noise ratios of the peaks due to diethylene glycol and ethylene glycol are at least 10.

Calculate the ratios between the responses of the peaks due to diethylene glycol/ ethylene glycol and the responses of the peak due to 1,3 butanediol in the chromatograms obtained with the solutions (A), (D), (E) and (F).

Prepare a calibration function each for ethylene glycol and diethylene glycol by plotting the ratios obtained for solutions (D), (E) and (F) on the ordinate against the amount of the reference standard on the abscissa. Consider 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 diethylene glycol and ethylene glycol in the liquid preparation for oral use. The percentage concentration (m/m) of diethylene glycol or ethylene glycol in the liquid preparation for oral use should be below 0.1%.

If the result obtained is above 5.0%, prepare solution (1) using a lower weight of liquid preparation for oral use under investigation and repeat the analysis.

International Chemical Reference Substances to be established

Ethylene glycol ICRS

Diethylene glycol ICRS

Reagents to be added to The International Pharmacopoeia:

1,3-Butanediol R

$C_4H_{10}O_2$; CAS Reg. No. 107-88-0.

Contains not less than 99.0% of $C_4H_{10}O_2$.

Ethylene glycol R

Ethane-1,2-diol; $C_2H_6O_2$; CAS Reg. No. 107-21-1.

Contains not less than 99.0% of $C_2H_6O_2$.

Description. Colourless, slightly viscous liquid, hygroscopic, miscible with water R and dehydrated ethanol R.

Relative density. d_{20}^{20} = 1.113 to 1.115.

Refraction index. n_D^{20} about 1.432.

Boiling point. About 198 °C.

Melting point. About -12 °C.

Acidity. To 10 mL, add 20 mL of water R and 1 mL of phenolphthalein/ethanol TS. Not more than 0.15 mL of sodium hydroxide (0.02 mol/L) VS is required to change the colour of the indicator to pink.

Water (2.8): Not more than 2 mg/g.

Diethylene glycol R

2,2′-Oxydiethanol; $C_4H_{10}O_3$; CAS Reg. No. 111-46-6.

Contains not less than 99.5% of $C_4H_{10}O_3$.

Description. Clear, colourless liquid, hygroscopic, miscible with water R, acetone R and dehydrated ethanol R.

Relative density. d_{20}^{20} about 1.118.

Refraction index. n_D^{20} about 1.447.

Boiling point. 244 °C to 246 °C.

Storage. In an airtight container.

Annex:

Feedback on described analytical procedures – Guidance for laboratories

National Quality Control Laboratories, WHO Collaborating Centres or other laboratories wishing to assess, demonstrate or acquire the competency to perform the described testing are kindly requested to:

I. analyse a sample with a **known** ethylene glycol or diethylene glycol concentration according to the provision *Test for diethylene glycol and ethylene glycol in liquid preparations for oral use*; and
II. answer the questions below and send the responses, together with the requested documents, to *schmidth@who.int*.

These responses will help the World Health Organization evaluate the suitability of the described test procedure and support regions in identifying training needs and setting up networks/competency centres for the testing of samples for ethylene glycol and diethylene glycol contamination.

To I.

To be able to evaluate the performance of the test procedure, the laboratory is requested to test a sample with a **known** ethylene glycol or diethylene glycol concentration. Such a sample can be prepared by adding a known ethylene glycol or diethylene glycol concentration to a sample free of these substances from the market.

Please perform the following steps:

Take a liquid preparation for oral use from your market and ensure that it is not contaminated with diethylene glycol or ethylene glycol by analysing it according to

the provision: *Test for diethylene glycol and ethylene glycol in liquid preparations for oral use, Confirmation by gas chromatography*.

Spike the preparation with either ethylene glycol or diethylene glycol, accurately weighed, so that the sample contains between 1.0% to 15.0% (m/m) of one of these contaminants.

Test the spiked sample firstly according to the analytical procedure *Screening for non-compliance by thin-layer chromatography* and secondly, if a gas chromatograph is available, according to *Confirmatory testing by gas chromatography*. As the International Chemical Reference Substances on diethylene glycol and ethylene glycol are currently under establishment, use other suitable reference substances for the latter procedure.

To II.

Please summarize the results of your investigations in a comprehensive laboratory report. Respond, in particular, to the following questions/points:

1. Name and address of your laboratory.
2. Name, title and email address of a contact person.
3. Which sample (name, manufacturer, lot number) has been spiked with diethylene glycol or ethylene glycol? Please provide details of the composition of the sample (active ingredient(s), strength(s) and excipients).
4. Which of the tests were performed: *Screening for non-compliance by thin-layer chromatography*, *Confirmatory testing by gas chromatography*, or both? If Confirmatory testing by gas chromatography was not performed, please provide an explanation as to why.
5. While performing the *Screening for non-compliance by TLC*, did the analyst deviate from the prescribed procedure? If yes, please describe the deviations in detail and provide a rational as to why changes were made.

6. While performing the *Confirmatory testing by gas chromatography*, did the analyst deviate from the prescribed procedure? If yes, please describe the deviations in detail and provide a rational as to why changes were made.
7. What was the diethylene glycol or ethylene glycol target concentration in the spiked sample (calculated based on the amount with which the sample was spiked)?
8. Which diethylene glycol or ethylene glycol concentration/percentage content was estimated based on the performance of the *Screening of non-compliance by TLC* on the spiked sample?
9. Which diethylene glycol or ethylene glycol concentration/percentage content was determined based on the performance of the *Confirmatory testing by gas chromatography* on the spiked sample?
10. Please include (photocopies of) the chromatogram(s) obtained when performing the TLC and GC methods. Indicate on the print-outs which solutions were analysed.
11. Did the analyst encounter problems/challenges when performing the analysis? If so, please describe them in detail.
12. Please evaluate your laboratory´s competency to perform the *Screening of non-compliance by TLC* on liquid preparations for oral use. If your laboratory does not yet have this competency, please identify/describe areas/topics for which your laboratory would need further training/guidance in order to obtain this competency.
13. Please evaluate your laboratory´s capacity to perform the *Confirmatory testing by GC*. If your laboratory does not yet have this competency, please identify/describe areas/topics for which your laboratory would need further training/guidance in order to obtain this competency.

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