A Concise Quality Control Guide on Essential Drugs and other Medicines

Manual

Accompanying the GPHF-Minilab™

Special Edition 2024
Detection of Hazardous Anti-freeze in Liquids for Oral Use
incl. Video Tutorial

Physical Testing & Thin-Layer Chromatography

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GPHF
GLOBAL PHARMA HEALTH FUND E.V.

A charity voluntarily supported by
Merck KGaA, Darmstadt, Germany

Health & Safety

Important Notice

The chemicals travelling alongside the GPHF-Minilab™ as well as pharmaceuticals to be tested may contain hazardous substances. Hence, users of the Minilab and bystanders should closely follow all instructions given in this and the main manual in order to avoid potential health risks resulting from accidental contact with these chemical and pharmaceutical substances.

Care must be exercised in the handling of chemicals and pharmaceuticals in order to avoid generating excessive dust or vapours in the atmosphere. Extraction should be used at points of activity that, in more austere circumstances, might be replaced by simple but sufficient air ventilation.

Symptoms such as drowsiness, respiratory problems, nausea or skin rash must be reported to the supervisor especially after accidental spillage of large amounts of organic solvents.

In the event of accidental spillage or splashing of liquids affecting skin or eyes, wash with copious amounts of water, report to the supervisor and if necessary, to the local surgery for further attention. Use protective clothes and safety spectacles when handling aggressive test solutions, for example strong acids and caustic solutions.

Use protective clothing, for example an apron and safety spectacles, prior to starting any work on medicines quality testing. Wash hands and face thoroughly after work.

Diethylene glycol and ethylene glycol as impurities in liquids for oral use

Introduction and Scope

I. SCREENING FOR NON-COMPLIANCE BY THIN-LAYER CHROMATOGRAPHY *

Diethylene glycol (DEG) and ethylene glycol (EG) are toxic substances used as industrial solvents and antifreeze agents, potentially fatal even in small amounts, especially for children. While the exact safe ingestion levels for DEG and EG are unknown, a detection level of 0.1% for each substance is considered adequate for screening raw materials and finished products. Gas chromatography (GC) is a precise and accurate technique widely used for testing DEG and EG in pharmaceutical products. For National Quality Control Laboratories (NQCLs) without GC access, thin-layer chromatography (TLC) can screen for DEG and EG at concentrations down to about 0.2% (m/m). However, the detection limits may vary between laboratories, and analysts should verify these limits. TLC cannot distinguish between DEG and EG, so the combined concentration of both contaminants is determined using a 50:50 (m/m) mixture for calibration. Given the semi-quantitative nature of TLC and its potential detection limit above the 0.1% safety threshold, there is a risk of missing products with individual concentrations of DEG or EG above 0.1% but below the detection limit. For instance, with a detection limit of 0.5%, a sample with 0.3% DEG and no EG would yield a false negative result. To mitigate this risk, NQCLs using TLC should consider confirming results at collaborating laboratories or regional centres using GC. Pending the inclusion in *The International Pharmacopoeia*, working groups involved in DEG/EG impurities testing have expressed a desire to transfer the existing DEG/EG TLC screening method to the mobile Minilab TLC kit.

II. RESULTS & ACTIONS TO BE TAKEN

National Drug Quality Control Laboratories with access to gas chromatography (GC) should use it directly for confirmatory testing instead of the TLC screening method. GC is at least twice as sensitive as TLC, detecting DEG or EG concentrations as low as 0.1% (m/m). GC can also analyse excipients used in pharmaceutical manufacturing. Laboratories that confirm DEG or EG contamination in excipients or finished products must promptly inform the regulatory authorities.

Limit and Safe Level Testing by Thin-Layer Chromatography (TLC)

I. PRINCIPLE

A detection level of 0.1% for DEG and EG is considered adequate for screening finished pharmaceutical products. A combined concentration of DEG and EG is measured, using a 50:50 (m/m) mixture for calibration. For the limit test, the levels of DEG and EG in oral liquids should be below the lowest combined calibration solution of 0.2% DEG/EG. As this lower calibration solution is hardly detectable, no corresponding spots should be visible in the sample solutions under investigation. Or, if a corresponding spot is detectable, the sample solutions contain DEG/EG above the safety level.

II. EQUIPMENT AND REAGENTS

1) Aluminium foil
2) Funnel
3) Spatula
4) Label tape
5) Marker pen
6) Pencil and ruler
7) 10-ml vials
8) Set of graduated pipettes (1 to 25 ml)
9) Set of laboratory glass bottles (25 to 100 ml)
10) Merck TLC aluminium plates pre-coated with silica gel 60 F_{254}, size 5x10 cm
11) Glass microcapillaries (2-µl filling capacity)
12) TLC developing chamber (500-ml jar)
13) Hot plate
14) Filter paper
15) Pair of scissors
16) Pair of tweezers
17) Plastic beaker (250 ml)
18) Potassium permanganate
19) Sodium carbonate (anhydrous)
20) Sodium hydroxide (granulated)
21) Toluene
22) Acetone
23) Methanol
24) Ammonia solution 25%
25) Distilled/tap/bottled water
26) Electronic pocket balance
27) Reference agents, for example diethylene glycol (DEG) and ethylene glycol (EG) as pure substances from commercial sources

III. PREPARATION OF THE STOCK CALIBRATION SOLUTION

A DEG/EG mixture 50/50 (m/m) is required for the preparation of the calibration stock solution, whereby DEG and EG themselves are obtained as a pure substance with a purity of almost 100 % from commercial sources. Using the electronic pocket balance and a 50-ml laboratory glass bottle provided, weigh in correctly about 0.3 g of neat diethylene glycol and 0.3 g of neat ethylene glycol. In order to overcome the balance's in-build dynamic inertia and to ensure correct readings, lift the lab bottle or tap the weighing pan with a pen or spatula each time after a few more milligrams have been added. Dilute with 29.4 ml of methanol using a suitable graduated straight pipette. Adjust the amount of methanol when the weighing result differs from the target weight. Close the lab bottle and shake until all of the DEG/EG mix is dissolved. The solution obtained should contain 10 mg of total diethylene glycol and 10 mg of total ethylene glycol per ml and be labelled as *'DEG/EG Stock Calibration Solution'*. Ideally, this solution should be freshly prepared for each test, although it appears to be stable over several days if used repeatedly. The final solution obtained should be clear and colourless.

IV. PREPARATION OF THE WORKING CALIBRATION SOLUTION 1%

Pipette 0.5 ml of the stock solution into a 10-ml vial and add 9.5 ml of methanol. Close and shake the vial. The solution obtained should contain 1.0 mg of total DEG/EG per ml and be labelled as *'DEG/EG Working Calibration Solution 1%'*.

V. PREPARATION OF THE WORKING CALIBRATION SOLUTION 0.5%

Pipette 0.2 ml of the stock solution into a 10-ml vial and add 7.8 ml of methanol. Close and shake the vial. The solution obtained should contain 0.5 mg of total DEG/EG per ml and be labelled as *'DEG/EG Working Calibration Solution 0.5%'*.

VI. PREPARATION OF THE WORKING CALIBRATION SOLUTION 0.2%

Pipette 0.2 ml of the stock solution into a 25-ml vial and add 19.8 ml of methanol using suitable graduated pipettes. Close and shake the vial. The solution obtained should contain 0.2 mg of total DEG/EG per ml and be labelled as *'DEG/EG Working Calibration Solution 0.2%'*.

VII. PREPARATION OF THE WORKING SAMPLE SOLUTION

Using the electronic pocket balance and a 10-ml laboratory glass bottle provided, weigh in correctly about 1 g of sample solution. In order to overcome the balance's in-build dynamic inertia and to ensure correct readings, lift the lab bottle or tap the weighing pan with a pen or spatula each time after a few more milligrams have been added. Dilute with 9 ml of methanol using a suitable graduated straight pipette. Adjust the amount of methanol when the weighing result differs from the target weight. Close the lab bottle and shake until all of the sample is dissolved. The solution obtained should contain 100 mg of total sample per ml and be labelled as *'Sample Working Solution'*. This solution should be freshly prepared for each test. Precipitation or phase separation may occur. Continue working with the clear or hazy methanolic supernatant liquid.

If information is available on the excipients and active substances in the oral liquid preparation to be analysed, prepare additional solutions by dissolving an appropriate amount of each of the compounds in an appropriate volume of methanol and filtering if necessary.

VIII. SPOTTING

Draw an origin line parallel to and about 1.5 cm from the bottom edge of the chromatoplate. Using microcapillary pipettes, apply 2 µl of each test and calibration solution as shown in the picture on the next page. Place up to five spots on the plate, ensuring they are circular, uniform, and evenly spaced. Varying spot intensities are acceptable, but diameters should be consistent. If spot sizes differ, repeat the step.

Gently dry the spots by holding the chromatoplate with tweezers in a hot air stream above the heating plate for about 10 seconds. Shake the TLC plate constantly and allow his underside to touch the heating plate's surface for fractions of a second when moving downward.

IX. DEVELOPMENT

Using suitable graduated pipettes and a transfer pipette, add 17 ml of acetone, 1 ml of toluene, 1.9 ml of water and 2 drops of ammonia solution 25% to the jar serving as TLC developing chamber. Close the chamber and mix thoroughly. Line the chamber's wall with filter paper and wait for about 15 minutes thus ensuring saturation of the chamber with solvent vapour. Carefully place the loaded TLC plate into the jar. Close the jar and develop the chromatoplate until the solvent front has moved about three-quarters of the length of the plate, the developing time being about 12 minutes. Remove the TLC plate from the chamber, mark the solvent front and allow excess solvent to evaporate by gentle drying. To do this, hold the chromatoplate with the supplied tweezers in the hot air stream directly above the heating plate for about one minute. Shake the TLC plate constantly and each time the chromatoplate moves downwards, its underside is allowed to touch the surface of the heating plate for fractions of a second.

X. DETECTION

Stain the developed chromatography plate with a potassium permanganate solution. Prepare the solution by dissolving 125 mg of sodium hydroxide, 3.7 g of anhydrous sodium carbonate, and 1.5 g of potassium permanganate in 200 ml of water. A slight turbidity after sodium carbonate dissolution is acceptable. Use the provided 250 ml plastic beaker to hold the solution, which can later be stored in a closed lab container at room temperature for at least one month. Immerse the TLC plate in the solution using tweezers, remove it immediately, let excess solution to run down the left long side of the plate onto a paper towel, and dry the back with a tissue. By using the long left side, any streaks will move in the least disruptive direction. At maximum heating level, dry the entire chromatoplate on a hot plate for about two to three minutes, placing three TLC plates as spacers to avoid direct heat. Observe the spots for diethylene and ethylene glycol from the calibration solutions and other spots from the test solutions becoming visible as the permanganate solution discolors due to the presence of oxidisable compounds to reveal yellowish to white spots. Read the TLC plate a second time after a rest period of about 10 minutes. The process can also be done by spraying the solution and drying with a hairdryer, but both procedures require repeated practice to become perfect. The TLC assay is valid if DEG/EG spots from the 0.5% calibration solution are visible. Immersion staining is illustrated on page 36 of the main manual, and a corresponding video can be viewed using the provided QR code.

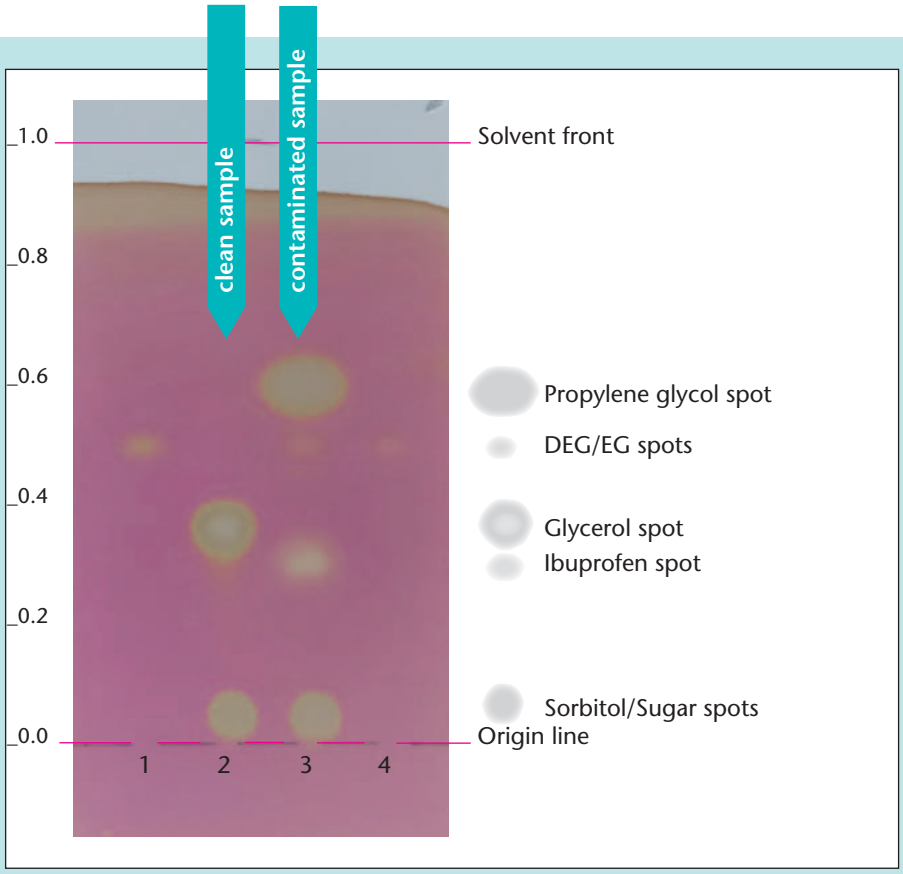
CHROMATOPLATE OBSERVED AT DAYLIGHT AFTER PERMANGANATE STAINING

Run No.1:
Working calibration solution 0.5% (Validity limit of the method/spot has to be visible)

Run No.2:
Clean non-medicinal syrup based on glycerol and sugar

Run No.3:
Contaminated ibuprofen syrup based on propylene glycol and sugar

Run No.4:
Working calibration solution 0.2% (DEG/EG limit value)



XI. OBSERVATIONS MADE AT DAYLIGHT AFTER PERMANGANATE STAINING

After staining with permanganate solution, a dark purple background forms. Compounds sensitive to the visualisation solution turn light yellow to white, making the TLC plate appear as a negative. Small yellowish spots at a travel distance of about 0.51 indicate the presence of diethylene or ethylene glycol or both exceeding the limit. Strong spots at various travel distances suggest other ingredients: a white spot at about 0.62 indicates propylene glycol, at about 0.37 indicates glycerol, at about 0.30 indicates ibuprofen, and at about 0.06 near the origin line indicates sorbitol or sucrose. Further strong spots may occur depending on the ingredients in the oral liquid. Spots may overlap due to their proximity or size. Other excipients contained in various finished products, like artificial sweeteners, preservatives, and colouring agents, may cause no or weak spots, migrating to the solvent front or staying near the origin.

XII. RESULTS & ACTIONS TO BE TAKEN

The assay is valid only if the chromatogram from the 0.5% calibration solution shows a spot for diethylene/ethylene glycol (DEG/EG). Determine the detection limit by evaluating the DEG/EG spots in chromatograms from 1%, 0.5%, and 0.2% calibration solutions performing a second run. The sample clearly passes the test if there is no spot or a DEG/EG spot below the spot size of the 0.2% calibration solution. While this TLC method helps less-resourced National Drug Quality Control Laboratories detect adulterated products, they should consider ways to reduce false negatives, such as confirming results at collaborating laboratories or regional centres using gas chromatography (GC). Laboratories with GC equipment should confirm results directly with GC, bypassing TLC screening for non-compliance. If DEG or EG contamination is confirmed in excipients or finished products, notify regulatory authorities immediately, quarantine the affected batches, and keep samples for reference. Document all TLC readings with digital photos.

Additional Minilab Inventory Items Needed

Next to the existing GPHF-Minilab™ kit the following items must be added to the Minilab inventory in order to perform the new test protocol on impurity testing for diethylene glycol (DG) and ethylene glycol (EG) contaminations.

The contact details for the procurement of GPHF-Minilab™ kits and additional items for DEG/EG impurity testing are as follows:

Technologie Transfer Marburg (TTM)
Industriestrasse 10
35091 Cölbe, Germany
ttm@ttm-germany.de
phone +49-6421-8737-30
fax +49-6421-8737-37

GPHF-Minilab Reference Agents

Reference Agents on Impurity Testing

Order No.	*Item*	*Qty*
AG030206	Diethylene glycol, 100 g	1
AG030207	Ethylene glycol, 25 g	1

GPHF-Minilab Solvents and Chemicals

Chemicals of analytical reagent grade of commerce

Order No.	*Item*	*Qty*
AG010042	Potassium permanganate, 25 g	1
AG010043	Sodium carbonate (anhydrous), 300 g	1
AG010044	Sodium hydroxide (granulated), 100 g	1

* This new GPHF-Minilab™ test protocol is based on the procedure *'Tests for Diethylene Glycol and Ethylene Glycol in Liquid Preparations for Oral Use'*, which was proposed as a new chapter for inclusion in *'The International Pharmacopoeia'* on 31 October 2023. The full version of the proposed procedure can be accessed via the link as follows: https://cdn.who.int/media/docs/default-source/medicines/pharmacopoeia/2023-11-16-deg-eg-inliquidoraldosageforms-qas22-922rev3.pdf?sfvrsn=284f645d_1 This link to the working document QAS/23.922/rev3 was last time accessed in July 2024.

- Detecting falsified and substandard medicines in low and middle-income countries
- Protecting consumers and medicines supply chains
- Boosting medicines testing capacities for priority medicines
- Assisting in post-marketing medicines quality monitoring
- Complementing the work of existing medicines control laboratories

The GPHF-Minilab™
is a unique miniature laboratory which comes with
affordable test methods for a rapid and easy detection of
falsified and substandard medicines as entry-level technology for
resource limited health settings in low- and middle-income countries.

In more than twenty-five years of project work, the GPHF-Minilab™ has
proven its suitability in more than a 100 countries.

This special edition for the Minilab manual extends the testing of active pharmaceutical ingredients to the testing of pharmaceutical excipients. More specifically, the testing of liquid preparations for oral use is extended to include the presence of antifreeze agents, e.g. diethylene and ethylene glycol impurities.

The method inventory of the GPHF-Minilab™ manual now includes a collection of test methods for 119 active pharmaceutical ingredients as well as one impurity test for rapid quality verification of a wide range of finished pharmaceutical products.

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