World Health Organization

1.18 HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY

Draft proposal for inclusion in *The International Pharmacopoeia*

(15 July 2025)

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For any technical questions, you may 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 **09 September 2025**. Please note that only comments received by this deadline will be considered for the preparation of this document.
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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/25.984

1.18 HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY

Description	Date
Revision drafted by the Secretariat based on information found in the scientific publications and in other pharmacopoeias.	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**. The new chapter* 1.18 High performance thin-layer chromatography *is proposed for inclusion in* The International Pharmacopoeia.*]*

1.18 HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY

Introduction. High-performance thin-layer chromatography (HPTLC) is a chromatographic technique (1.14.1), in which variables are controlled within narrow ranges, using a standardized methodology and appropriate equipment, in order to achieve more reproducible results compared to traditional thin-layer chromatography (TLC).

Equipment. The stationary phase consists of a uniform, typically 200 µm layer of porous (average pore size 6 nm or 60 Å), irregular particles of silica gel with an average particle size of 5 um size (ranging from 2 to 10 µm) and a polymeric binder with or without a fluorescence indicator (F_{254}), coated onto a support, which is typically a glass plate or aluminium foil. Other stationary phases, such as chemically bonded phases (C8, C18, CN, NH2, DIOL) or microcrystalline cellulose, are also available. Besides the stationary phase, the following devices are used:

- devices suitable for the application of specified volumes of solutions as bands of specified length at the designated positions on the plate;
- a sealed chamber for preconditioning the plate at the prescribed relative humidity to control the activity of the stationary phase (for example, an empty desiccator);
- a chromatographic tank to maintain reproducible vapor phase composition (a twin trough chamber with a lid is recommended);
- a device to treat the plate with derivatization reagent, if required.
- a device to control the heating of the plate as part of the derivatization procedure, if required;

- a device to illuminate the plate under the prescribed observation conditions, typically, at short-wave UV (nominally 254 nm), long-wave UV (nominally 365 or 366 nm) and white light;

- a photographic system to document the chromatograms under the prescribed visualization conditions.

Procedure. Follow the procedure described below, unless otherwise described in individual monographs.

Prepare the test and reference solutions as described and apply them as narrow bands of 8 mm in length at a distance of 8 mm from the lower edge of the plate. Position the first band so that its centre is 20 mm from the left edge of the plate. Allow a minimum distance of 11 mm between tracks (centre to centre). Apply the specified volume (usually 2 to 10 µL) of a maximum of 15 solutions onto a 20 x 10 cm plate. Mark the developing distance on the right or left edge of the plate (usually 70 mm from the lower edge of the plate).

Expose the plate for a minimum of 10 minutes to air with a relative humidity of 33%, obtained over a saturated magnesium chloride solution TS in a sealed chamber (for example, a desiccator).

Line the rear trough of the twin-trough chamber with filter paper. Add sufficient developing solvent to both troughs to achieve a solvent level of approximately 5 mm. Pour a greater volume into the rear trough to ensure complete wetting of the filter paper. Close the lid and allow the chamber to stand for 20 minutes to reach vapor equilibrium.

Introduce the plate vertically into the front trough of the chamber with the chromatographic layer facing the filter paper. When the mobile phase has reached the prescribed distance, remove the plate from the tank and dry it in a vertical position in a stream of air at room temperature.

Where derivatization is required, spray an appropriate volume of the described reagent (typically 1 to 4 mL) uniformly onto the plate or immerse the plate into the reagent solution at a defined speed and for a defined dwell time. An immersion speed of 50 mm/s and a dwell time of 1 second are recommended.

Observe the plate typically under short-wave UV, long-wave UV or with light prior to and after derivatization. Assess the described system suitability criteria every time that analysis is performed. A general requirement is that the solvent front and the chromatographic bands are strictly parallel to the horizontal sides of the plate. A curved solvent front may be attributed to a gas leak at the edges of the chamber. This can be prevented by placing a weight on the cover to ensure that the chamber is sealed.

Failure to meet the system suitability requirements invalidates the analysis. In the event of failure, its cause needs to be identified and corrected and the analysis repeated until the suitability requirements have been met.

Document the results in an auditable manner employing proper documentation tools, such as a camera capable of capturing images under the prescribed illumination, or a scanning densitometer along with a suitable software.

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

Magnesium chloride solution TS, saturated

Fill a bottle with 200 mL of water R and mark the water level. Pour out the water and transfer 225 g of magnesium chloride R into the bottle. Ad 50 mL of water R and heat at 40 °C to 50 °C for 10 minutes under stirring. Add more water R to reach the 200 mL mark, heat again at 40 °C to 50 °C for 10 minutes under stirring and cool to room

118 temperature. The final solution should contain a layer of about 2.5 cm of $MgCl_2$ crystals
119 at the bottom. The solution can be used for 1 year.