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NOTE ON THE GENERAL CHAPTER

The following new general chapter deals with the qualitative use of high performance thin-layer chromatography in herbal drugs and herbal drug preparations in order to improve reproducibility of identification and test methods. In the same issue of Pharmeuropa the monographs on Birch leaf (1174), Roman Chamomile flower (0380), St. John's wort (1438), quantified St. John's wort dry extract (1874), Passion flower (1459) and Passion flower dry extract (1882) are published to illustrate the changes following the introduction of this general chapter in the Ph. Eur.

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2.8.25. HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY

High-performance thin-layer chromatography (HPTLC) is used for qualitative analysis of herbal drugs and herbal drug preparations. It is a thin-layer chromatographic technique (*2.2.27*) which, unless otherwise stated in an individual monograph, uses a glass plate coated with a uniform, typically 200 µm, porous layer (average pore size 6 nm), irregular particles of silica gel with a size between 2 µm and 10 µm and an average particle size of 5 µm, a polymeric binder and a fluorescence indicator (F_{254}). The results are qualified using a system-specific suitability test.

EQUIPMENT

The equipment used for qualitative HPTLC typically consists of:

- glass plates, as described above, usually with a size of 20 x 10 cm;
- devices suitable for the application of specified volumes of solutions as bands and allowing control of dimension and position of application;
- a device suitable for conditioning the stationary phase at prescribed relative humidity;
- a suitable chromatographic tank (for example, a twin trough chamber);
- a device suitable for reproducible drying of the developed plate;
- devices suitable for reagent application to, and heating of, the plate as part of the derivatisation procedure;
- a system suitable for electronic documentation of chromatograms under UV 254 nm, UV 366 nm and white light.

NOTE: normal thin-layer chromatographic methods using glass plates or sheets coated with particles of 5-40 µm or HPTLC aluminium backed sheets may be used, provided that the results obtained fulfil the general system suitability criteria of the bands having developed perpendicular to the lower edge of the plate and the solvent front being parallel to the upper edge of the plate and satisfy the system-specific suitability test stated in the individual monograph (where a monograph exists).

METHOD

Preparation of test solution. Unless otherwise stated in the individual monograph (where one exists), the test solution is usually prepared as follows: for dry herbal drugs or dry herbal extracts, weigh 0.5 g of the powdered herbal drug or 0.1 g of the dry herbal extract and sonicate for 15 min with 5 mL of *methanol R*. After filtration or centrifugation the filtrate or supernatant is used as the test solution.

For essential oils, dilute 50 µL in 1 mL of *toluene R* and use this solution as the test solution.

Preparation of reference solutions. Unless otherwise stated in the individual monograph (where one exists), reference solutions are usually prepared as follows: prepare a 1 mg/mL solution of the reference substance in *methanol R* or, for essential oils, in *toluene R*; prepare a 1 in 4 dilution of this reference solution using the same solvent used as the intensity reference (diluted reference solution).

Intensity marker. One of the substances used in the reference solution and in the diluted reference solution is used as an intensity marker for the evaluation of the chromatogram.

Preparation of system-specific suitability solution. Prepare the solution as stated in the individual monograph (where one exists).

Sample application and plate layout. Unless otherwise stated in the individual monograph (where one exists), samples are applied as narrow bands of 8 mm in length at a distance of 8 mm from the lower edge of the plate. The centre of the first track, which is used for the system-specific suitability solution, is positioned 20 mm from the left edge of the plate. The minimum distance between tracks (centre to centre) is 11 mm. A maximum of 15 tracks are applied onto a standard plate. If no electronic solvent front detection device is used, the developing distance is marked with a pencil close to the right or left edge of the plate.

Conditioning of the plate. Following sample application and unless otherwise stated in the individual monograph (where one exists), the plate is conditioned at a relative humidity of 33 per cent for a sufficient time to reach equilibrium (for example, by standing in a closed chamber containing a saturated magnesium chloride solution or by the use of preconditioned air).

Preparation of the tank and development of the plate. Unless otherwise stated in the individual monograph (where one exists), the chromatographic separation is performed in a saturated tank. Where a twin trough chamber is used, the rear trough is fitted with a filter paper. The tank is charged with a sufficient amount of developing solvent to wet the filter paper completely and achieve a level of 5 mm in both troughs. With the lid closed, the tank is left for 20 min for saturation. The plate is introduced in a vertical position into the front trough of the tank so that the coating layer faces the filter paper. When the mobile phase has reached 70 mm (corresponding to a development path of 6 cm), the plate is removed from the tank and dried in a vertical position in a stream of cold air. Other tank configurations and developing distances may be specified in an individual monograph.

NOTE: other tanks may be employed if the results obtained fulfil all of the system suitability criteria.

Visualisation. Chromatograms on the plate are visualised as stated in the individual monograph (where one exists). Where derivatisation reagents are used, typically 3.5 mL of reagent in solution per plate, of 20 x 10 cm, are homogenously sprayed onto the plate or the plate is immersed into the reagent solution, typically at a speed of 5 mm/s for a dwell time of usually 1 s. Observation may be performed under UV 254 nm, UV 366 nm or white light prior to and/or after derivatisation. When pictures are electronically recorded, exposure time should be adjusted on the track with the system-specific suitability solution.

System-specific suitability test. This test is based on the separation of 2 substances that have similar retardation factors (R_F values) but that are barely separable under the specified chromatographic conditions (for example, chlorogenic acid and hyperoside in chromatographic systems used for flavonoids). The results for the test and reference solutions are only valid when the system-specific suitability solution satisfies the separation requirement stated in the individual monograph.

Visual evaluation. The chromatograms obtained with the test and reference solutions are compared against the description, under *Results*, in the individual monograph (where one exists) with respect to zone position and colour, as well as intensity for the test solution. Zones of the test solution described in the result table without a descriptor have intensities similar to the zone of the intensity marker in the reference solution. Those described as ‘intense’ zones are visually more intense than the zone of the intensity marker in the reference solution; ‘faint’ zones are visually less intense than the zone of the intensity marker in the reference solution, but equal to or more intense than the zone of the intensity marker in the diluted reference solution; ‘very faint’ zones are visually less intense than the zone of the intensity marker in the diluted reference solution.