⟨203⟩ HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY PROCEDURE FOR IDENTIFICATION OF ARTICLES OF BOTANICAL ORIGIN

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INTRODUCTION

This chapter describes a procedure for use in a USP Identification test that ▲employs▲ (USP 1-May-2020) the technique of high-performance thin-layer chromatography (HPTLC). ▲Precise▲ (USP 1-May-2020) control of the ▲experimental▲ (USP 1-May-2020) variables ▲is essential for reproducible analysis. [NOTE—For additional information,▲ (USP 1-May-2020) see *Identification of Articles of Botanical Origin by High-Performance Thin-Layer Chromatography Procedure* ⟨1064⟩.]

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▲▲ (USP 1-MAY-2020)EQUIPMENT

The equipment used for HPTLC ▲analysis typically includes:▲ (USP 1-May-2020)

- Plates: Unless otherwise specified in the individual monograph, use 20 × 10 cm plates coated with a uniform 200-µm layer of porous (60-Å pore size) silica gel having irregular particles of 2–10 µm and an average particle size of 5 µm, a polymeric binder, and a fluorescence indicator (F_{254}). [NOTE—▲▲ (USP 1-May-2020)Glass plates are preferred over aluminum-backed sheets.▲▲ (USP 1-May-2020)]
- A device suitable for ▲application of indicated volumes as bands of▲ (USP 1-May-2020) specified length at the ▲designated▲ (USP 1-May-2020) positions.
- A device suitable for controlling the activity of the stationary phase ▲by preconditioning the plate to a prescribed▲ (USP 1-May-2020) relative humidity.
- A suitable chromatographic chamber ▲to maintain reproducible vapor phase composition and to allow for control of developing distance▲ (USP 1-May-2020) (for example, a twin-trough chamber).
- A device suitable for reproducible drying of the developed plate.
- A device suitable for treatment of the plate with derivatization reagent, if required.
- A device suitable for ▲controlled plate▲ (USP 1-May-2020) heating as part of the derivatization procedure, if required.
- A ▲device▲ (USP 1-May-2020) suitable for ▲illumination under prescribed observation conditions, typically, short-wave UV (nominally 254 nm), long-wave UV (nominally 365 or 366 nm),▲ (USP 1-May-2020) and white light. ▲ [NOTE—Light sources used for chromatogram visualization typically produce broad-band emission; therefore, the maxima quoted above carry only nominal significance.]
- A photographic system for documentation of chromatograms under the prescribed visualization conditions.▲ (USP 1-May-2020)

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PROCEDURE

Preparation of the Test Solution

Unless otherwise stated in an individual monograph, 100 mg of a powdered botanical ingredient, 10 mg of a dry extract, ▲▲ (USP 1-May-2020) or the amount of a dosage form containing the equivalent of the aforementioned quantities of the botanical ingredient is sonicated for 15 min with 1 mL of methanol. After ▲filtration or▲ (USP 1-May-2020) centrifugation, the filtrate or supernatant is used as the *Sample solution*. ▲For essential oils, 50 µL/mL solution in toluene is▲ (USP 1-May-2020) used as the *Sample solution*.

Preparation of the Standard Solutions

Unless otherwise stated in an individual monograph, Reference Standards of individual marker compounds are dissolved ▲in methanol▲ (USP 1-May-2020) at a concentration of 1 mg/mL. The Reference Standard extracts are shaken and sonicated in methanol at a concentration of 10 mg/mL or, for essential oils, ▲in toluene at 50 µL/mL.▲ (USP 1-May-2020)

Sample Application and Plate Layout

Samples are applied as narrow bands of ▲8-mm▲ (USP 1-May-2020) length at a distance of ▲8 mm▲ (USP 1-May-2020) from the lower edge of the plate ▲ [NOTE—Refer to ⟨1064⟩ for additional information.]▲ (USP 1-May-2020) The system suitability standards are applied to the ▲track▲ (USP 1-May-2020) nearest to the ▲lateral▲ (USP 1-May-2020) edge at NLT 20 mm from the ▲lateral▲ (USP 1-May-2020) edge of the plate. The distance between tracks, center-to-center, is NLT ▲11 mm.▲ (USP 1-May-2020) All application volumes are specified

in the individual monographs, ▲and▲ (USP 1-May-2020) usually range from 2–10 µL. The developing distance is marked with a pencil close to one of the ▲lateral▲ (USP 1-May-2020) edges of the plate before the development; ▲alternatively,▲ (USP 1-May-2020) an electronic solvent front detection device ▲is used.▲ (USP 1-May-2020)

Preconditioning of the Plate

Following sample application and unless otherwise stated in an individual monograph, the plate is conditioned at a relative humidity of 33% ▲by exposure to the atmosphere generated by vapors in equilibrium with saturated magnesium chloride solution in a sealed chamber▲ (USP 1-May-2020) for a minimum of 10 min. ▲Other frequently used saturated salt solutions of potassium thiocyanate and sodium chloride establish equilibrium relative humidity of 47% and 75%, respectively, at 25°.▲ (USP 1-May-2020)

Preparation of the Developing Chamber and Development of the Plate

Where a twin-trough chamber is used, the rear trough is fitted with filter paper. The chamber is charged with a sufficient volume of developing solvent to wet the filter paper completely and achieve a level of developing solvent of exactly 5 mm in both troughs. With the lid closed, the chamber is ▲allowed to achieve saturation for▲ (USP 1-May-2020) 20 min. ▲▲ (USP 1-May-2020) The plate is introduced ▲vertically▲ (USP 1-May-2020) into the front trough of the chamber ▲with the chromatographic layer facing▲ (USP 1-May-2020) the filter paper. ▲Once the solvent front▲ (USP 1-May-2020) has ▲ascended 6 cm from the application line,▲ (USP 1-May-2020) the plate is removed from the chamber and dried in a vertical position in a current of cold air that does not affect the integrity of the separated zones. ▲Alternative▲ (USP 1-May-2020) chamber configurations and developing distances may be specified in an individual monograph. [NOTE—Other development chambers may be employed if the results obtained fulfill all of the system suitability criteria.]

Derivatization Procedure

Where derivatization reagents are used, ▲appropriate▲ (USP 1-May-2020) volumes of reagents ▲▲ (USP 1-May-2020) (typically 1–2 mL) are ▲uniformly▲ (USP 1-May-2020) sprayed onto the plate or the plate is immersed into the reagent solution at a defined speed and for a defined dwell time. [NOTE—Immersion speed of 50 mm/s and the dwell time of 1 s ▲are recommended▲ (USP 1-May-2020) for most nonaqueous reagents.]

Visualization

Chromatograms on the plate are visualized as directed in an individual monograph, ▲typically under short-wave UV, long-wave UV,▲ (USP 1-May-2020) or white light prior to and after derivatization.

System Suitability

▲A general system suitability requirement is that the solvent front and the chromatographic bands are strictly parallel to the horizontal sides of the chromatographic plate. Specific suitability parameters are detailed in the monographs. Typically, to assess▲ (USP 1-May-2020) suitability of the chromatographic system for resolution, position, and color of the bands, ▲▲ (USP 1-May-2020) two or more reference substances are selected that have similar but just separable R_F values under the chromatographic conditions to be used; for example, chlorogenic acid (blue) and hyperoside (yellow-orange) ▲are commonly▲ (USP 1-May-2020) used for flavonoids ▲methods. System suitability is required every time the analysis is performed; it is conducted on the same plate alongside the test articles. Failure to meet the system suitability requirements invalidates the analysis. In the event of failure, its cause needs to be identified and corrected (see ⟨1064⟩ for potential sources of failure), and the analysis repeated until the suitability requirements have been met. Individual▲ (USP 1-May-2020) Reference Standards, ▲or their▲ (USP 1-May-2020) mixtures, ▲are typically employed▲ (USP 1-May-2020) for system suitability ▲monitoring, and the use of▲ (USP 1-May-2020) the USP Reference Standard extracts ▲is common. The▲ (USP 1-May-2020) resolution, position, and colors of the key bands of the ▲Reference Standard chromatogram▲ (USP 1-May-2020) should match the description in the monograph within a specified tolerance. ▲▲ (USP 1-May-2020)

Evaluation and Acceptance Criteria

Chromatograms of the *Sample solution* and *Standard solution* are compared against the descriptions in the *Acceptance criteria* section of the monograph with respect to ▲band▲ (USP 1-May-2020) position, ▲▲ (USP 1-May-2020)separation, color, and relative intensity.

Documentation

▲Test results are required to be documented▲ (USP 1-May-2020) in an auditable manner to comply with the current good manufacturing practices. Proper documentation tools should be employed; for example, a ▲digital▲ (USP 1-May-2020) camera ▲capable of capturing images▲ (USP 1-May-2020) under ▲prescribed illumination, or a scanning densitometer along with the▲ (USP 1-May-2020) imaging software suitable for archival, retrieval, and analysis of the results.

▲Standard Parameters

Unless otherwise indicated in an individual monograph, the following standard parameters, as specified in *Table 1*, are to be employed in every procedure referencing this chapter.

Table 1

Parameter	Standard
Chromatographic plate, glass, 20 × 10 cm	200-µm silica layer, 5-µm average particle size, with fluorescence indicator F_{254}
Application position, from plate lower edge	8 mm
Band application length	8 mm
Band spacing, center-to-center	NLT 11 mm
Relative humidity	33%
Plate conditioning time	10 min
Chamber saturation time	20 min
Developing distance	6 cm
Temperature	22 ± 5°▲ (USP 1-May-2020)