

# <203> HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY PROCEDURE FOR IDENTIFICATION OF ARTICLES OF BOTANICAL ORIGIN

## INTRODUCTION

This chapter describes a procedure for use in a USP *Identification* test that relies on the technique of high-performance thin-layer chromatography (HPTLC). It is applicable to the identification of articles of botanical origin in USP's compendia that serve as a drug substance or drug product, or as an ingredient or a dietary supplement. Careful control of the variables for the HPTLC technique is briefly described with references to more detailed information provided in equipment manuals. Reproducibility of the results allows comparison of closely related botanical materials that are not official ingredients. The analytical technique uses high-performance plates, appropriate equipment to control variables, and a system suitability test for purposes of performance qualification. [NOTE—See *Identification of Articles of Botanical Origin Using High-Performance Thin-Layer Chromatography Procedure* (1064) for additional information.]

## REQUIRED EQUIPMENT

The equipment used for HPTLC technique consists of the following:

- Plates: Unless otherwise specified in the individual monograph, use plates coated with a uniform 200- $\mu\text{m}$  layer of porous (60- $\text{\AA}$  pore size) silica gel having irregular particles of 2–10  $\mu\text{m}$  and an average particle size of 5  $\mu\text{m}$ , a polymeric binder, and a fluorescence indicator ( $F_{254}$ ) of 20  $\times$  10 cm. [NOTE—Chromatographic methods using high-performance thin-layer chromatographic glass plates are preferred over aluminum-backed sheets because of greater mechanical stability.]
- A device suitable for the application of specified volumes of samples as bands with specified length at the specified positions
- A suitable chromatographic chamber (for example, a twin trough chamber) allowing for control of saturation and developing distance
- A device suitable for controlling the activity of the stationary phase via relative humidity
- 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 heating as part of the derivatization procedure, if required
- A system suitable for documentation of chromatograms under UV 254 nm, UV 366 nm, and white light

Each of these devices as well as the system as a whole should pass installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ) in order to assure that the instruments are working according to their specifications to control the variables within their intended ranges. [NOTE—See *Analytical Instrument Qualification* (1058) for additional information.] Installation qualification and operational qualification are typically performed by the instrument manufacturer. The system suitability test is performed by the analyst as proof of performance qualification.

## PROCEDURE

### Preparation of Test Solution

Unless otherwise stated in an individual monograph, 100 mg of a powdered botanical ingredient, 10 mg of a dry extract or fraction, 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 centrifugation the filtrate or supernatant is used as the *Sample solution*. Unless otherwise stated in an individual monograph, 50  $\mu\text{L}$  of an essential oil is dissolved in 1 mL of toluene and used as the *Sample solution*.

### Preparation of the Standard Solutions

Unless otherwise stated in an individual monograph, USP Reference Standards of individual marker compounds are dissolved at a concentration of 1 mg/mL in methanol. The USP Reference Standard extracts are shaken and sonicated in methanol at a concentration of 10 mg/mL or, for essential oils, the USP Reference Materials are dissolved in toluene at a concentration of 50  $\mu\text{L}/\text{mL}$ .

## Sample Application and Plate Layout

Samples are applied as narrow bands of  $8.0 \pm 0.5$  mm length at a distance of  $8.0 \pm 0.5$  mm from the lower edge of the plate. The system suitability standards are applied on the lane nearest to the edge at NLT 20 mm from the edge of the plate. The distance between tracks (center to center) is NLT  $11.0 \pm 0.5$  mm. All application volumes are specified in the individual monograph. Application volumes usually range from 2.0 to 10.0  $\mu\text{L}$ . The developing distance is marked with a pencil close to one of the edges of the plate before the development, although an electronic solvent front detection device may be substituted.

## 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% for a minimum of 10 min (for example, by standing in a closed chamber containing a saturated solution of magnesium chloride).

## 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 left 20 min for saturation. The plate is introduced in a vertical position into the front trough of the chamber so that the coating layer faces the filter paper. When the mobile phase has reached a distance corresponding to a development path of 6 cm, the plate is removed from the chamber and dried in a vertical position in a stream of cold air that does not affect the integrity of the separated zones. Other 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, defined volumes of reagents in solution (typically 1–2 mL) are homogeneously 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 dwell time of 1 s works for most nonaqueous reagents.]

## Visualization

Chromatograms on the plate are visualized as stated in an individual monograph. Observation and evaluation may be performed under UV 254 nm, UV 366 nm, or white light prior to and after derivatization.

## System Suitability

To check the suitability of the system for resolution, position, and color of the bands, unless otherwise stated in an individual monograph, 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) in chromatographic systems used for flavonoids. USP Reference Standard mixtures for system suitability may be provided, or the substances designated to check the system suitability for resolution, position, and colors of the bands may be included in the USP Reference Standard extracts. Description of the resolution, position, and colors for the key bands of the reference material fingerprint should match the description in the monograph within a specified tolerance range. The system suitability requirements in an individual monograph are satisfied when the results obtained comply with those specified in the monograph.

## Evaluation and Acceptance Criteria

Chromatograms of the *Sample solution* and *Standard solution* are compared against the description in the *Acceptance criteria* section of the monograph with respect to zone position, zone separation, color, and relative intensity.

## Documentation

Documentation is necessary to record the results in an auditable manner to comply with current good manufacturing practices. Proper documentation tools should be employed; for example, a camera suitable for taking digital pictures under UV and white light and an imaging software suitable for archiving, retrieving, and analyzing the results makes it easy to maintain electronic records.