Standard operating procedure for HPTLC

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Purpose
This standard operating procedure (SOP) provides general guidance for analysis by high-performance thin-layer chromatography HPTLC.

Definitions
HPTLC is performed on 20x10 cm HPTLC glass plates coated with silica gel 60 F254. Suitable (preferably software-controlled) instruments must be employed for sample application, chromatogram development, derivatization, and documentation. NOTE: if no automatic developing chamber is available a 20x10 cm twin trough chamber may be used.*
NOTE: Record temperature and relative humidity in the laboratory

1. Preparation of plates

1.1 Obtain HPTLC plate silica gel 60 F 254 (20x10 cm). Record the batch number.
1.2 Inspect plate under UV 254 nm for any damage of the layer. If damage is detected discard plate.
1.3 With a soft pencil label the plate in the upper right corner with: your initials – date (dd/mm/yy) – consecutive number for the day. Example ER-23/02/10-001.
1.4 On the right side of the plate mark developing distance at 70 mm from lower edge of plate. NOTE: left handed persons may label /mark the plate on left side.

2. Preparation of chamber (manual development only)

2.1 Obtain a twin trough chamber for 20x10 cm plates.
2.2 Fit the rear trough of chamber with a filter paper of corresponding size.
2.3 Pour 20 ml of developing solvent over the filter paper into the rear trough ensuring complete wetting. Pour a sufficient amount of developing solvent into the front trough to have a level of 5 mm.
2.4 Close the lid of the chamber and allow 20 min for saturation.

3. Sample application

3.1 Select the following application parameters on the application device:
band length 8 mm
number of tracks 15
first application position X: 20 mm
application position Y: 8 mm
distance between tracks: automatic (minimum 11 mm)
sample solvent type: methanol
3.2 Disable any unused tracks.

* NOTE: 10x10 cm HPTLC plates may be used for manual development only. Use a twin trough chamber for 10x10 cm plates and the following application parameters:
band length 8 mm
number of tracks: 7
first application position X: 15 mm
application position Y: 8 mm
distance between tracks: automatic (min. 11 mm)
sample solvent type for application: methanol
developing solvent in rear trough (with filter paper): 10 ml
developing solvent in front trough: a sufficient amount to have a level of 5 mm

3.3 Apply the application volumes as according to the standardized procedure for selected herbal drugs.

4. Plate conditioning (manual development only)

After sample application place the plate for 45 min in a suitable desiccator containing a saturated solution of $MgCl_2$.

5a. Manual development

5a.1 Slowly open the lid of the saturated chamber and insert the conditioned plate into the front trough so that the back of the plate rests against the front wall of the chamber and the layer faces the inside of the chamber. Close the lid.
5a.2 Let the mobile phase ascend until it reaches the mark.
5a.3 Open the lid and remove the plate. Place it upright in a rack under a fume hood.
5a.4 Dry plate with cold air from a hair dryer for 5 min.

5b. Automatic development

Use the following settings of the automatic chamber:

Enable pre-drying
Saturation with filter paper 20 min
Humidity control 10 min with $MgCl_2$
Migration distance 70 mm
Drying time 5 min
10 ml of developing solvent
25 ml of saturation solvent

NOTE: if no humidity control is available follow step 4.

6. Derivatization

Use the reagent specified in the individual monograph.
Derivatization is either performed by using an automatic dipping device (typical immersion time 5 cm/s and no dwell time) or an automatic spraying device (typically 2-4 mL of reagent).
Examples:

6a. Flavonoids – Derivatization by dipping

6a.1 Heat the dry plate for 5 min at 100°C.
6a.2 While hot dip plate for 1 sec into a solution of 0.5% NP reagent in ethyl acetate. Then, after two min of waiting, dip the plate for 1 sec into a solution of 5% macrogol 400 in dichloromethane.

6b. Flavonoids – Derivatization by automatic spraying

6b.1 Heat the dry plate for 5 min at 100°C.
6b.2.1 While hot spray the plate with 3.5 ml of a solution of 1% NP reagent in methanol then with a solution of 5% macrogol 400 in methanol.
OR 6b.2.2 While hot spray the plate with 3.5 ml of a solution of 0.5% NP reagent in ethyl acetate then with 3.5 ml solution of 5% macrogol 400 in dichloromethane.

7. Documentation

30 min after the second derivatization step, take an image of the derivatized plate under UV 366 nm.

8. Reporting

Create a copy of software based report or use own reporting documents.