

## General Methodology for HPTLC

Version 04: Effective Date: 25.06.2009

### Revision History

Version 01: replaces all earlier versions  
Version 02: minor revision and update  
Version 03: D) Development: details on ADC2 parameters added; E) Derivatization: plate drying modified; G) Labeling: saving of images modified  
Version 04: B) sample application parameters changed (20 mm, 15 Tracks) D) appropriate volume for development increased, tilting of chamber removed; minor revisions

### Related documents

GDL 50.000.01 Use of CAMAG equipment and software  
GDL 50.002.01 Record keeping  
GDL 60.000.01 Housekeeping rules and schedule  
SOP 50.001.02 Labeling

### I. PURPOSE

This SOP provides general guidance for analysis by HPTLC.

### II. APPLICATION

This SOP applies to all work regarding HPTLC analysis unless the resulting chromatograms or the applied method require adjustments.

### III. PROCEDURE

#### General

1. Prior to using an instrument, log-in the corresponding instrument log according to GDL 50.000.01 Use of CAMAG equipment and software.
2. Observe safety and housekeeping rules according to GDL 60.000.01 Housekeeping rules and schedule and SOP 60.000.02 Chemical Hygiene Plan.
3. For use of instrument refer to GDL 50.000.01 Use of CAMAG equipment and software.
4. Record any performed work in appropriate worksheets as specified in GDL 50.002.01 Record keeping. Include temperature and humidity at least once a day in any active worksheets.

#### A) Plate material

Handle plates with extreme caution to avoid any damage to the layer. Store plates in the original package with the lid closed. Remove plate from storage only immediately prior to use.

Plates are generally handled only at the upper edge to avoid contamination.

Unless otherwise necessary Merck HPTLC plates silica gel 60 F 254 in the format 10x10 cm or 20x10 cm are used. For most work plates are used without pre-treatment unless chromatography produces impurity fronts due to contamination of the plate. For reproducibility studies and quantitative analyses plates are pre-washed as follows.

1. Mark the direction of development with a pencil at the upper edge of the plate.
2. Develop plate with 20 mL methanol per trough in a 20 x 10 cm Twin Trough Chamber (TTC) to the upper edge. Up to two 20 x 10 cm or four 10 x 10 cm plates can be

- developed back to back in each trough of the TTC.
- Remove the plate and dry for 20 minutes in a clean drying oven at 120°C.
  - Equilibrate plate with lab atmosphere (temperature, relative humidity) in a suitable container providing protection from dust and fumes.

### B) Sample application

Unless otherwise necessary apply samples using the spray-on technique with ATS 4 or Linomat 5. In case of spot application by contact use the Nanomat or the ATS 4 and dissolve the sample in the solvent of lowest suitable solvent strength.

Use the following application parameters:

Parameter	HPTLC
Distance from lower edge of plate for use in TTC	8 mm
Distance from lower edge of plate for use in HDC	5 mm
x-position of first track	20 mm
Minimum space between bands/spots	2 mm
Maximum diameter of application spot	5 mm
Band length	8 mm
Maximum number of tracks on a 10 x 10 cm plate	7
Maximum number of tracks on a 20 x 10 cm plate	15

### C) Preparation and storage of developing solvents

Developing solvents consisting of more than 1 component are prepared by measuring the required volume (respectively weight) of each component separately and transferring them into a solvent bottle of appropriate size. The bottle is closed with a lid and shaken to ensure proper mixing of the content.

Volumes smaller than 1 mL are measured with a suitable micropipette. Volumes up to 20 mL are measured with a graduated volumetric pipette of suitable size. Volumes larger than 20 mL are measured with a graduated cylinder of appropriate size.

To minimize volume errors developing solvents are prepared in a volume that is sufficient for one working day.

### D) Development

Plates are generally developed in a saturated Twin Trough Chamber according to the following procedure:

- Prepare the appropriate volume (15 mL for 10x10 cm, 30 mL for 20x10 cm TTC) of developing solvent.
- Open chamber and place a correctly sized (10x10 cm; 20x10 cm) piece of filter paper in the rear trough.
- Pour appropriate volume (10 mL for 10x10 cm, 20 mL for 20x10 cm TTC) of solvent into rear trough of the chamber so that the filter paper is thoroughly wetted and adheres to rear wall of TTC.
- Pour appropriate volume (5 mL for 10x10 cm, 10 mL for 20x10 cm TTC) of solvent into front trough of the chamber.
- Set chamber on bench, replace the lid and let chamber equilibrate for 20 minutes.
- Mark the desired developing distance (70 mm from lower edge of plate) with a pencil on the right edge of the plate.
- Slide off the lid to the side.
- Insert the plate into the front trough. The layer should face the filter paper and the back of the plate is resting against front wall of TTC.
- Replace lid.
- Develop plate to the mark.
- Open lid, remove plate.

12. Dry the plate (vertically in direction of chromatography) 5 minutes in a stream of cold air.
13. After each development remaining mobile phase and filter paper are discarded. Prior to being prepared for the next run the chamber is dried and, if necessary, also cleaned.

Alternatively the automatic developing chamber ADC 2 can be used for development.

- To adjust the activity of the HPTLC plate the humidity control option of the ADC 2 is used. Consult the ADC2 manual for handling.
  - If the instrument is operated in stand alone mode, and unless otherwise specified, the standard method S2 is applied using  $MgCl_2$  for humidity control.
  - If the instrument is operated under software control, and unless otherwise specified, developing distance is set to 70 mm from lower edge of plate, chamber saturation is set to 20 min, and the activity of the plate is adjusted to 33% relative humidity using saturated magnesium chloride solution for 10 min.
  - The following manual steps are performed:
1. If a developing solvent is used for the first time the ADC2 is rinsed using 7 mL of developing solvent for each inlet.
  2. Insert plate in ADC2.
  3. Place an ADC2-filter paper in the rear trough of the twin-trough chamber.
  4. Position the twin-trough chamber in the ADC2.
  5. Pour 10 mL of solvent into the left inlet of the ADC2 (for development) and 25 mL into the right inlet (for saturation).
  6. For humidity control make sure the appropriate bottle is attached.
  7. Start development using winCATS software, or in stand alone mode by pressing the "run" button.
  8. After each development remaining mobile phase and filter paper are discarded. Prior to being prepared for the next run the chamber is dried and, if necessary, also cleaned.

### E) Derivatization

Transfer of reagent for derivatization of samples on a HPTLC plate may be accomplished by spraying or dipping. Dipping is the preferred method and should be used whenever possible. Spraying is done in the TLC spray cabinet. If derivatization includes heating the plate heater is used. Always refer to the HPTLC method for details of the derivatization procedure.

#### Dipping (Chromatogram Immersion Device)

1. Charge tank of immersion device with 200 mL of reagent.
2. Place plate in holder of immersion device, set parameters according to method and press start.
3. Let excess reagent drip off plate, wipe off back of the plate with paper towel.
4. Wait until no liquid film is seen on the plate surface.
5. Remove plate from plate holder.

#### Spraying (Glass sprayer)

1. Charge the bottle of the sprayer with up to 50 mL of reagent.
2. Place plate in spray cabinet against a filter paper or a paper towel.
3. Spray plate with horizontal and vertical motion until it is homogeneously covered with reagent avoiding any local over-saturation of layer.

#### Heating (Plate Heater)

1. Turn on plate heater and select temperature.
2. Wait until the temperature is stable.
3. Place plate onto plate heater.
4. After the time specified by the method remove hot plate from heater.

## F) Documentation of plates (Visualizer)

Each developed plate is documented with the digital documentation system under UV 254 nm, UV 366 nm and white light.

If a type of light does not produce usable information that fact must be documented. If a plate is derivatized images are taken prior and after derivatization.

All images are labeled and listed in the project work sheet.

## G) Labeling

### Plates

Each plate is given an individual identification number (ID), which will be written in pencil in the top right corner. The ID includes project number, dash, year (YY), month (MM), day (DD), dash, and a consecutive number each day.

Example: The first plate developed for Project P91 on January 10, 2002 is labeled P91-020110-01. The sixth plate developed for this project on the same day will have the ID P91-020110-06.

### Images

Electronic images are saved as part of the winCats file corresponding to each plate. When exporting images as cpf-file or in other formats they are labeled individually with a file name corresponding to the plate ID followed by the description of light used for capturing. Example: The image of the first plate of January 10, 2002 for Project P91 taken under UV 254 nm is saved as P91-020110-01-254. The image of a derivatized plate will additionally be labeled with a letter abbreviating the reagent name. Example: P91-020110-01-A366.cpf is the image of the first plate on January 10, 2002 for Project P91 after derivatization with anisaldehyde under UV 366 nm.

The following abbreviations are used:

254	short-wave UV light, reflection
366	long-waver UV light, reflection
W	white light (reflectance)
WT:	white light (transmittance)
WRT:	white light (reflectance / transmittance)
A:	anisaldehyde reagent
S:	Sulfuric acid reagent
NP:	Natural products reagent
NPP:	Natural products reagent followed by polyethylene glycol

Other letters to indicate other specific reagents as specified on the work sheet

## Image Comparison Viewer

Image comparison Viewer file (.cx6) are saved with the project number and if necessary with a descriptor (e.g. P194 different solvents.cx6) in the electronic folder of the corresponding project. The content of the .cx6 file is briefly described in the worksheet.

## H) Quantitative Evaluation

Quantitative evaluation is performed either with the TLC Scanner 3 using winCATS software or based on digital images with VideoScan.

The winCATS analysis files are labeled to reflect the plate ID and any additional descriptive information if multiple evaluation under different conditions is performed.

Example: P91-020110-01-A-520.cna for the analysis file containing scan data at 520 nm obtained after derivatization with anisaldehyde reagent as opposed to P91-020110-01-210.cna containing scan data at 210 nm obtained prior to derivatization

The Video scan file is saved with the name of the corresponding image file and the addition "scan". Example P91-020110-01-A366-scan.cpf

## I) Documentation of work

All work performed is documented in a project worksheet.