

TLC Introduction

Thin Layer Chromatography to Preparative Chromatography

One of the first steps in scale-up of preparative liquid chromatography separations is selection of an appropriate mobile phase. Two methods are commonly used to determine the proper mobile phase composition: Thin Layer Chromatography (TLC) or High Performance Liquid Chromatography (HPLC). The use of TLC will be discussed here to deal with the successful correlation between the TLC separation to the preparative silica column.

TLC is a liquid-solid adsorption technique where the mobile phase ascends the thin layer of stationary phase coated onto a backing support such as glass by capillary action. There is a similar relationship to column chromatography where the solvent travels down through the column's adsorbent. The similar relationship allows TLC to be a rapid method for determining solvent composition for preparative separations.

Steps for Method Development

Choose Stationary Phase

Choose a scalable TLC plate, preferably that has an identical media as the preparative column. Choose between normal and reverse phase based on sample polarity and solubility.

Choose a Mobile Phase

Criteria for Choosing a Preparative Solvent

- Solubility
- Affinity
- Resolution

1. Solubility

Many solvent systems provide the minimal solubility for the sample, but to elute a sample from a column the mobile phase must have a greater solubility for the sample, as the sample concentration is usually very high. When possible, it is best to dissolve the sample in the mobile phase. The first step in solvent selection is determination of the solubility of the sample. The desired mobile phase would provide the greatest solubility, while providing affinity for the sample on the stationary phase.

Solvent Solubility Screening Table

Water	↑ Increasing Polarity
Methanol	
Ethanol	
Acetone	
Diethyl Ether	
Ethyl Acetate	
Dichloromethane	
Toluene	
Chloroform	
Cyclohexane	
Petroleum Ether	
Hexane	

2. Affinity

To achieve a separation, the sample must have a relatively equal affinity for the solvent and the packing material. If the sample has a higher affinity for the stationary phase than the solvent, the sample will remain at the origin (R_f value will be too low).

3. Resolution

Resolution is improved by optimizing the affinity between sample, solvent, and support. The optimum solvent for separating two or more compounds will maximize the difference in the compounds. Most TLC and preparative mobile phase systems contain a polar solvent and a chromatographically dissimilar less-polar solvent. As a guide for method development, a substitution in the polar solvent often results in a change in resolution, while a change in the less-polar solvent results primarily in a change in R_f of the sample components. The table below shows some common tendencies of various functional groups to adsorb onto the silica.

Affinity of Functional Groups for Silica Gel

-NH ₂	Amine	↑ Increasing Affinity
-COOH	Carboxylic Acid	
-COH	Alcohol	
-CONH ₂	Amide	
-C=O	Carbonyl	
-C-CO ₂ R	Ester	
-C-O-C	Ether	
-C1	Halocarbons	
-CC-	Hydrocarbons	

Select Visualization Technique

Once a mobile phase is selected, visualization techniques will need to be determined. Common techniques include SWUV, I₂/SWUV, I₂/KI for Nitrogen containing compounds, H₂SO₄/LWUV, H₂SO₄/PMA for non-nitrogen containing compounds.

Perform TLC Analysis

Look up the affinity for the type of compound as well as the solvent strengths to find a starting point for method development or look up a reference from a similar structure, then adjust the mobile phase composition to adjust the R_f . It is common to try 3–6 solvent systems for the first round of method development. Review the results after visualization and adjust the R_f if necessary, increase the separation and evaluate visualization techniques to make sure you are seeing all necessary compounds.

Optimizing TLC Separations for Preparative Separations

The optimum separation of compounds by TLC is usually achieved when R_f values are between 0.3–0.5.

$$R_f = \frac{\text{Distance from origin to center of spot}}{\text{Distance from origin to solvent front}}$$

Generally, adjusting the compound's R_f between 0.3–0.5 is done first for a TLC separation. For scale-up to preparative separations, the TLC solvent system's polarity must be decreased to lower the R_f between 0.15–0.35. This R_f range is optimal for a preparative separation, in terms of sample load, resolution, residence time, and solvent usage.

Determination of Column Volumes (C.V.)

The equation $C.V. = 1/R_f$ relates the TLC values and the preparative LC column volumes to elute each component. This equation is only a guideline and the relationship between the R_f values and the column volume will vary in use. Generally, the LC column volumes will be equal to or less than the calculated values. The elution volume will also be dependent upon the sample load and solvent used to solubilize the sample.

TLC Plates

Davisil® Silica TLC Plates

- Made with the same Davisil® silica as sold in bulk for easy method development
- Scored to customize to your plate size preference



Davisil® Silica TLC Plates

Description	Layer Thickness	Qty.	Part No.
<i>Hard Layer, Organic Binder, Fluorescent Indicator, 254nm</i>			
<i>Scored, 4, 5 x 20cm Sections</i>			
20 x 20cm	250µm	25	8617580
<i>Scored, 8, 2.5 x 10cm Sections</i>			
10 x 20cm	250µm	25	8617610

GraceResolv™ Silica TLC Plates

- Made with the same high-purity Davisil® silica as used in the GraceResolv™ flash cartridges for easy method development
- Scored to customize to your plate size preference

GraceResolv™ Silica TLC Plates

Description	Layer Thickness	Qty.	Part No.
<i>Hard Layer, Organic Binder, Fluorescent Indicator, 254nm</i>			
<i>Scored, 4, 5 x 20cm Sections</i>			
20 x 20cm	250µm	25	8618900



3179

Grace has a large selection of TLC plates to suit your separation needs.

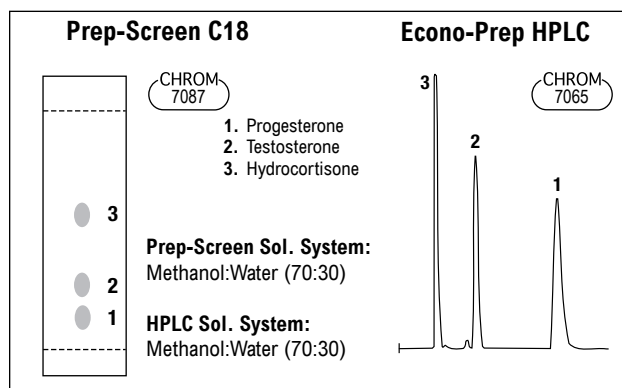


5369

Alltech® Prep-Screen TLC Plates

- Made with the 10–12µm spherical silica gel used for Alltech® prep-HPLC cartridge columns
- Available in both Econosphere™ silica gel and C18 (reversed-phase) bonded versions

Prep-Screen HPTLC Plates help to optimize sample separation parameters for use in preparative HPLC. These plates allow a quick, inexpensive preview of sample traits in various mobile phase systems prior to HPLC analysis. The adsorbent is the same Econosphere™ silica or C18 used in Alltech packed HPLC columns.



Prep Screen HPTLC Plates

Description	Qty.	Part No.
<i>Prep-Screen HPTLC Plates, 10 x 10cm, 200µm Layer</i>		
Silica Gel, 10µm Spherical Adsorbent	10	16328
C18, 10µm Spherical Adsorbent	10	16332

technical assistance

Contact Tech Support: Phone: 1.800.255.8324 (North America)
Email: contact.alltech@grace.com
Online: www.discoverysciences.com

related products

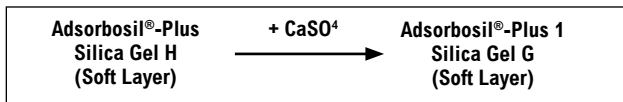
See pages 194–198 for tanks, sprayers, applicators, and other TLC accessories.

tlc

TLC Plates

Alltech® Prekotes

- High purity silica gel for increased sensitivity
- Controlled particle size for faster separations and improved resolution (>80% in 6–15µm range)
- Precoated plates provide convenience and superior layer quality



Soft Layer

- Contains no organic binders
- Sample zones are easy to remove for further analysis

Soft Layer Adsorbosil® Prekotes

Description	Qty.	Part No.
<i>Soft Layer Prekotes, Glass-Backed</i>		
Conventional, 250µm Layer Thickness, 20 x 20cm		
Adsorbosil®-Plus	25	16384
Adsorbosil®-Plus P*	25	16376
Adsorbosil®-Plus 1	25	16330
Adsorbosil®-Plus 1 P*	25	16322

*P = With Fluorescent Indicator, 254 wavelength.

Hard Layer

- Contains a proprietary inorganic binder to add abrasion resistance to layer
- Use when strong charring is required for visualization
- Write on layer with pencil or felt pen
- Sample zones can be removed for further analysis

Hard Layer Adsorbosil® Prekotes

Description	Qty.	Part No.
<i>Hard Layer Prekotes, Glass-Backed</i>		
Conventional, 250µm Layer Thickness, 20 x 20cm		
Adsorbosil®-Plus 1	25	16324
Adsorbosil®-Plus 1 P*	25	16326

*P = With Fluorescent Indicator, 254 wavelength.

Adsorbosil® HPTLC Plates

- 150µm layer thickness for fast, high resolution separations
- Smooth surface for noise-free densitometry

HPTLC with Inorganic Binder

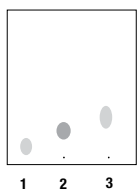
- Allows use of 100% water in the solvent system
- Use when strong charring is required

High-Performance TLC Plates

Description	Qty.	Part No.
<i>Adsorbosil®-Plus 1 Plates with Inorganic Binder, 10 x 10cm</i>		
Adsorbosil®-Plus 1P HPTLC, with F254 UV Indicator	25	16401

Reversed-Phase HPTLC Plates

- Coated with non-polar, C18 chemically bonded support
- Cross-linked organic polymer binder makes an abrasion resistant layer and a smooth surface
- Use to separate mixtures of lipophilic components, hydrocarbons, fats and waxes, fat soluble vitamins or steroids



Fatty Acid Separation

1. Methyl Oleate
2. Methyl Linoleate
3. Methyl Linolenate

CHROM
7088

TLC Plate: Adsorbosil® RP HPTLC (Part No. **16314**)

Sol. System: Acetonitrile:Acetic Acid:Water (70:10:1)

Development Time: 25min

Visualization: Iodine Vapor

High-Performance TLC Plates

Description	Qty.	Part No.
<i>Reversed-Phase C18 HPTLC, 150µm Layer Thickness</i>		
Adsorbosil® RP HPTLC, 10 x 10cm	25	16314
Adsorbosil® RP HPTLC Plates with F254, 10 x 10cm	25	16315
Adsorbosil® RP HPTLC Plates, Prescored, 10 x 20cm	25	16318
Adsorbosil® RP HPTLC Plates, Prescored, with F254, 10 x 20cm	25	16319

TLC Plates

Macherey-Nagel

Silica Gel Plates

- 60Å pore size
- Particle size, 5 to 17µm
- Binder is organic, stable in most organic solvents and aggressive detection reagents

Glass-Backed Macherey-Nagel TLC Plates

Description	Size	Layer	Qty.	Part No.
<i>Silica Gel Plates, Glass-Backed</i>				
Conventional Layers, Silica Gel 60				
SIL G-25	5 x 10cm	250µm	50	809017
SIL G-25	5 x 20cm	250µm	100	809011
SIL G-25	20 x 20cm	250µm	25	809013
SIL G-25 UV254	5 x 20cm	250µm	100	809021
SIL G-25 UV254	20 x 20cm	250µm	25	809023
SIL G-25 UV254+366	20 x 20cm	250µm	25	809123
Preparative Layers				
SIL G-100 UV254	20 x 20cm	1000µm	15	809063
SIL G-200 UV254	20 x 20cm	2000µm	12	809083

Specialty Plates

- **Silica Gel and Cellulose**—Specific for separation of food preservatives
- **Silica Gel**—Specific for aflatoxin analysis

Specialty Plates

Description	Size	Layer	Qty.	Part No.
<i>Specialty Plates, Glass-Backed</i>				
Silica Gel and Cellulose — Mixed Layer				
SILCEL-Mix-25 UV254	20 x 20cm	250µm	25	810043
Silica Gel Highly Purified with Gypsum				
SIL G-25 HR	20 x 20cm	250µm	25	809033
SIL G-25 HR UV254	20 x 20cm	250µm	25	809043

related products

Performing flash separations?

See GraceResolv™ flash consumable product line on pages 184–187.

Nano-Series HPTLC Plates

- Particle size, 2–10µm
- Higher speed
- Better resolution and sensitivity than regular TLC plates

Nano-SIL HPTLC Plates

Description	Size	Layer	Qty.	Part No.
<i>Nano-Series HPTLC Plates, Glass-Backed</i>				
Silica Gel 60				
Nano-SIL-20	10 x 20cm	200µm	50	811013
Nano-SIL-20/UV254	10 x 20cm	200µm	50	811023
<i>Nano-Series HPTLC Plates, Aluminum-Backed*</i>				
Silica Gel 60				
Nano-SIL G	20 x 20cm	200µm	25	818141
Nano-SIL G/UV254	20 x 20cm	200µm	25	818143

*Do not use with mineral acids or concentrated ammonia.

technical assistance

Contact Tech Support: Phone: 1.800.255.8324 (North America)
 Email: contact.alltech@grace.com
 Online: www.discoverysciences.com

TLC Plates

Macherey-Nagel Polygram® Series

Silica Gel 60

- SIL G and SIL N layers have different binders and show different separation characteristics

Cellulose, Avicel-Microcrystalline

Cellulose MN 300—Native Fibrous Cellulose

- Fibers are 2–20µm in length

Cellulose, Ion-Exchanger

- DEAE carries positive charges at neutral and acidic pH
- Use DEAE to separate proteins, enzymes, and hormones
- Use PEI to analyze nucleic acids

Polyamide 6

- Specific for separation of dansyl and DNP amino acids

Polygram® Flexible-Backed TLC Plates

Description	Size	Layer	Qty.	Part No.
<i>Polyester-Backed TLC Plates</i>				
<i>Silica Gel 60</i>				
SIL G	20 x 20cm	250µm	25	805013
SIL G/UV254	4 x 8cm	250µm	50	805021
SIL G/UV 254	20 x 20cm	250µm	25	805023
SIL N-HR/UV254	20 x 20cm	200µm	25	804023
<i>Cellulose Plates</i>				
<i>Cellulose MN 400, Avicel-Microcrystalline Cellulose</i>				
CEL 400	20 x 20cm	100µm	25	801113
CEL 400 UV254	20 x 20cm	100µm	25	801123
<i>Cellulose MN 300</i>				
CEL 300	20 x 20cm	100µm	25	801013
CEL 300 UV254	20 x 20cm	100µm	25	801023
<i>Cellulose, Ion-Exchanger</i>				
CEL 300 DEAE	20 x 20cm	100µm	25	801073
CEL 300 PEI*	20 x 20cm	100µm	25	801053
CEL 300 PEI/UV254	20 x 20cm	100µm	25	801063
<i>Specialty Polyester-Backed Plates</i>				
<i>Polyamide 6</i>				
POLYAMIDE 6	20 x 20cm	100µm	25	803013
POLYAMIDE 6 UV254	20 x 20cm	100µm	25	803023

*PEI plates must be refrigerated.

Hybrid Plates

- “Wettable” RP plate
- For both reversed- and normal-phase chromatography
- Determine polarity by eluent selection
- Activate layer at 110–115°C prior to use
- Particle size of 2–10µm for improved separations

Hybrid TLC Plates

Description	Layer	Qty.	Part No.
<i>Silica Gel RP18/UV254s* Plates, Aluminum-Backed</i>			
4 x 8cm	150µm	50	818144
5 x 20cm	150µm	50	818145
20 x 20cm	150µm	25	818146

*Acid-resistant fluorescent indicator.

technical assistance

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 Online: www.discoverysciences.com

Analtech TLC Plates

Silica Gel H and HF Uniplates™

- Soft Layer
 - Contains no organic binder
 - Contains no calcium sulfate binder
- Hard Layer
 - Contains an organic binder
 - 80% water resistant
 - Visualization by charring up to 150°C

Silica Gel H and HF Uniplates™

Description	Layer Thickness	Qty.	H Part No.	HF* Part No.
<i>Soft Layer, No Binders</i>				
Conventional				
20 x 20cm	250µm	25	710011	720011
10 x 20cm	250µm	25	710021	7200210
<i>Hard Layer, Organic Binder</i>				
Conventional				
20 x 20cm	250µm	25	746011	747011
10 x 20cm	250µm	25	746021	747021
Scored, Four 5 x 20cm Sections or Eight, 2.5 x 10cm Sections				
20 x 20cm	250µm	25	—	747511
10 x 20cm	250µm	25	—	747521

*F-Fluorescent Indicator, 254nm.

HPTLC Uniplates™

- Smooth surface gives a high signal-to-noise ratio for increased sensitivity and precision
- High resolution separations in five minutes
- Ideal for densitometric scanning

HPTLC Uniplates™

Description	Layer Thickness	Qty.	Part No.
<i>HPTLC Silica Gel with Inorganic Binder</i>			
10 x 10cm, HP-GHL	150µm	25	756077
10 x 10cm, HP-GHLF*	150µm	25	757077
<i>Reversed-Phase HPTLC Unibond™**</i>			
10 x 10cm, HP-RP18F	150µm	25	763077

*F-Fluorescent Indicator, 254nm.

**Due to the extreme hydrophobic nature of the RP18 adsorbent, aqueous wettability is limited to approximately 60% water in the mobile phase. (see www.analtech.com)

Silica Gel G and GF Uniplates™

- Calcium Sulfate Binder
- Soft Layer
 - Contains no organic binder
- Hard Layer
 - Contains an inorganic binder
 - 100% water resistant
 - Visualization with strong charring reagents
 - Abrasion resistant

Silica Gel G Uniplates™

Description	Layer Thickness	Qty.	G Part No.	GF* Part No.
<i>Soft Layer, No Binders</i>				
Conventional				
20 x 20cm	250µm	25	701011	702011
10 x 20cm	250µm	25	701021	702021
Preadsorbent				
20 x 20cm	250µm	25	731011	732011
<i>Preparative Uniplates™, Soft Layer</i>				
Conventional Prep				
20 x 20cm	500µm	25	—	702012
20 x 20cm	1000µm	25	—	702013
20 x 20cm	1500µm	25	—	702014
20 x 20cm	2000µm	25	—	702015
<i>Hard Layer, Inorganic Binder</i>				
Conventional				
20 x 20cm	250µm	25	711011	721011
10 x 20cm	250µm	25	711021	721021

*F-Fluorescent Indicator, 254nm.

related products

Looking for a fast and easy way to score your own TLC plates?
See our TLC plate cutter on page 196.



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