

## CombiFlash NextGen 300+

For a High Precision Purification  
of Organic Molecules and Natural  
Products Workshop

Speakers

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## Outline :

- Introduction to the steps of organic molecule synthesis
- History of development of chromatography from traditional column to automated flash chromatography
- Similarities and differences between flash and HPLC
- Demonstration of CombiFlash NextGen 300+ parts
- Some features of CombiFlash NextGen 300+

# Introduction

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There are  
three  
steps of  
organic  
synthesis:

**Reaction Setup**

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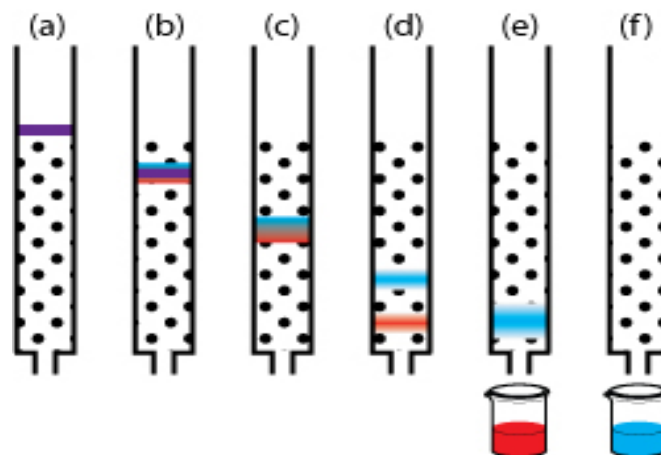
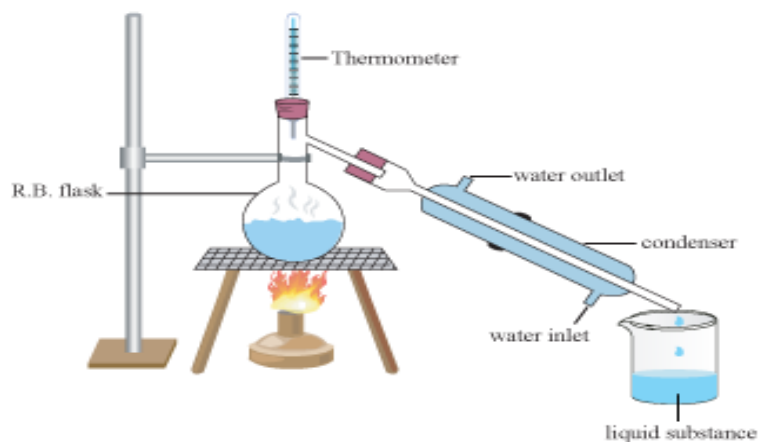
**Workup , PURIFICATION**

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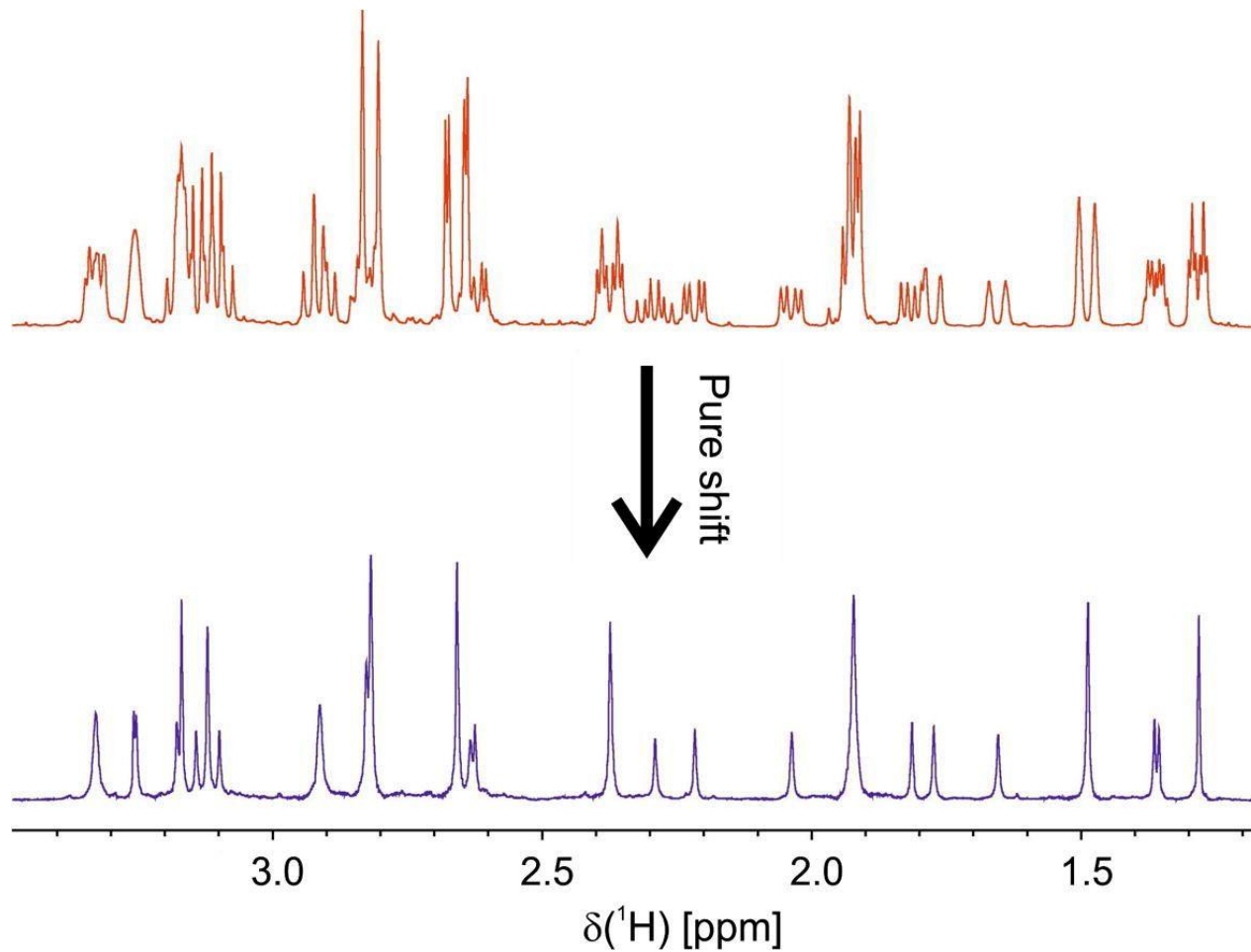
**Final Product Analysis**

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# Compound Purification



# Effect of Purification on NMR

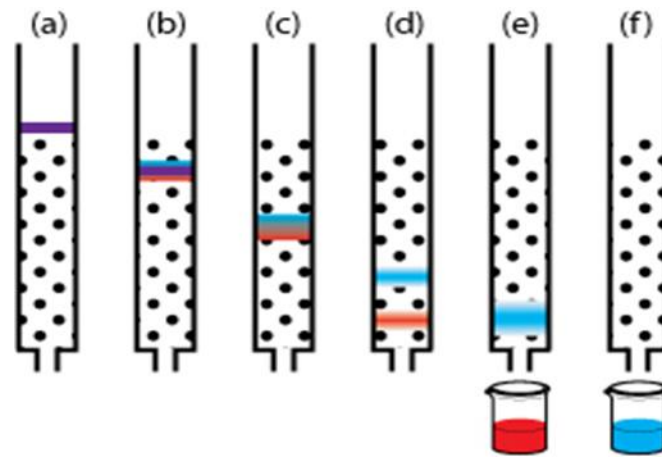


# Traditional Column Chromatography

- Traditional column chromatography applies a crude reaction mixture on top of a bed of silica gel loaded in a glass column.

A gravity-fed solvent mixture (mobile phase)

passes through the vertical column of silica gel (stationary phase), separating the individual products of the crude reaction mixture.



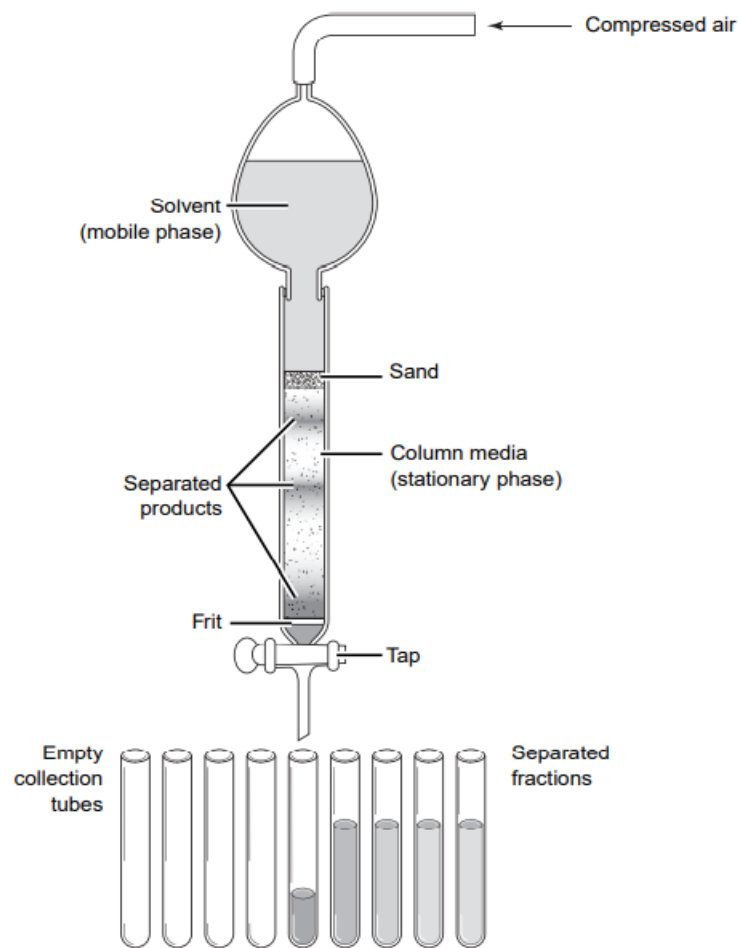
# Flash Chromatography

- This term was coined in **1978** by W. Clark Still and coworkers at Columbia University



# Flash Chromatography

- separations in **which a gas pressurized solvent reservoir is used to accelerate solvent flow**
- chemical separation is done in **less time** than traditional **gravity-based column chromatography**



*Illustration of basic elements in a traditional Flash column chromatography apparatus*



# Automated Flash Chromatography

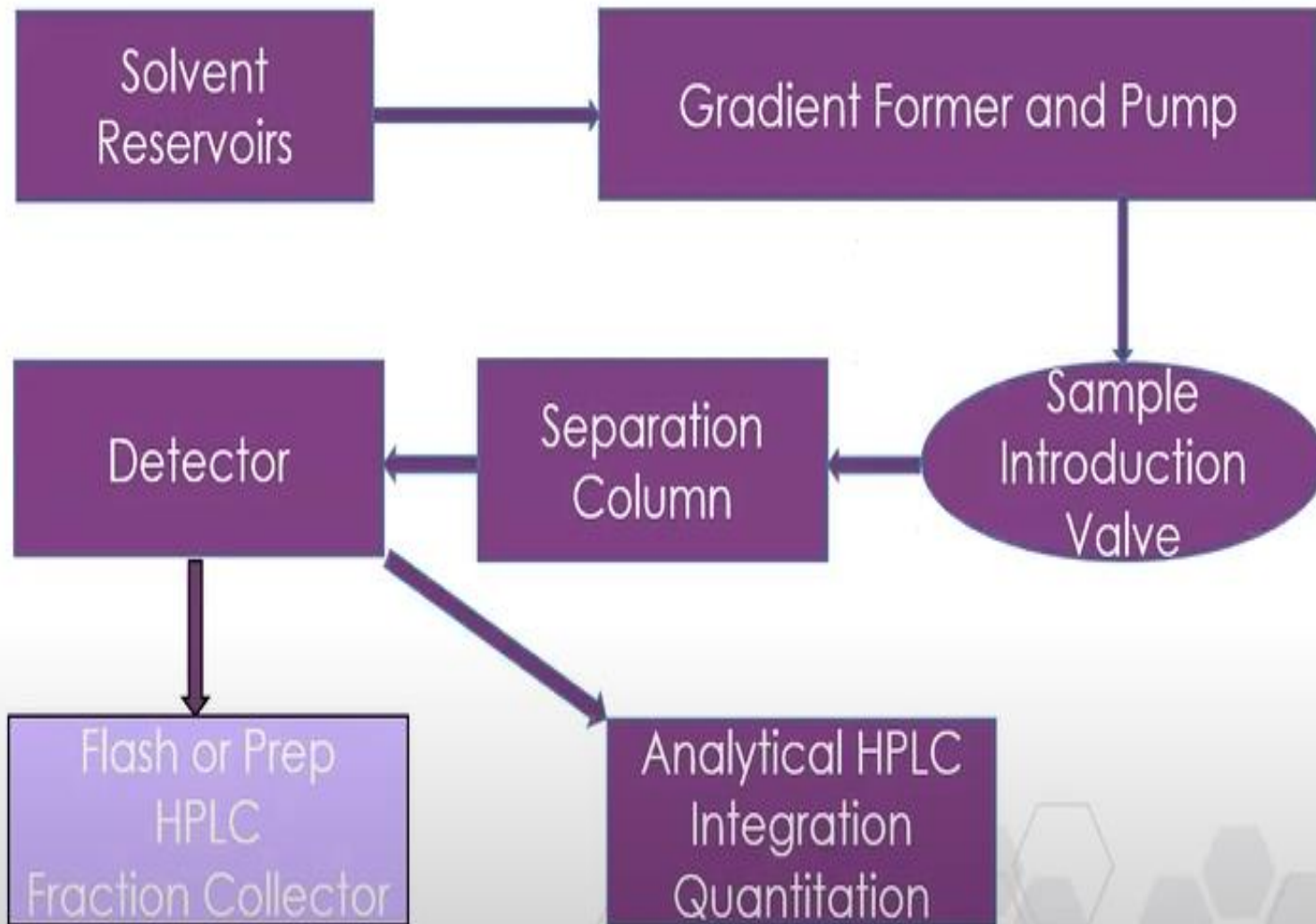
- CombiFlash equipment designed by Teledyne ISCO.
- The advantages of using automated Flash chromatography are
  - It's easy, fast, inexpensive,  
requires minimal development time,  
and has high resolution ,  
more efficient separation,  
required less solvent.



# Automated Flash Chromatography

- Flash chromatography is currently one of the most popular techniques for purifying.
- Pharmaceutical intermediates
- Final organic products.
- It is also widely used in natural products research

# Similarities Between Flash and HPLC



# Differences Between Flash and HPLC

<b>Flash chromatography</b>	<b>HPLC</b>
Lower pressure (<300 psi)	High pressure (>300 psi)
Columns larger particle size -less expensive , disposable	Columns -smaller particle size -Increased resolution -More expensive , reusable
Focus on purification and speed	Focus on analysis and quantification Late stage purification
High flow and high sample loading	Low flow and low sample loading
Typically silica base Normal Phase	Typically modified silica (C18) Reversed Phase

# Advantages of Flash Chromatography

- Speed in purifying **MILLIGRAMS to GRAMS** of material
- Typical purity of greater than 90% on single pass
- Operation cost lower than HPLC
  - Acquisition cost
  - Higher sample loading
  - Column costs

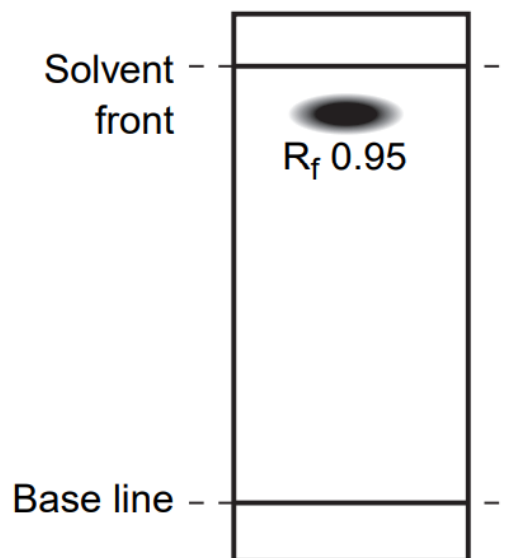
# Flash Chromatography Essentials

## ➤ Some definition :

The Retention Distance  $R_f$  : on a TLC plate represents the distance of a given compound migrates from the origin with respect to the solvent front on the plate.

**Ex:**

$$R_f = 4.75/5$$



## ➤ Some definition :

### column volume (CV):

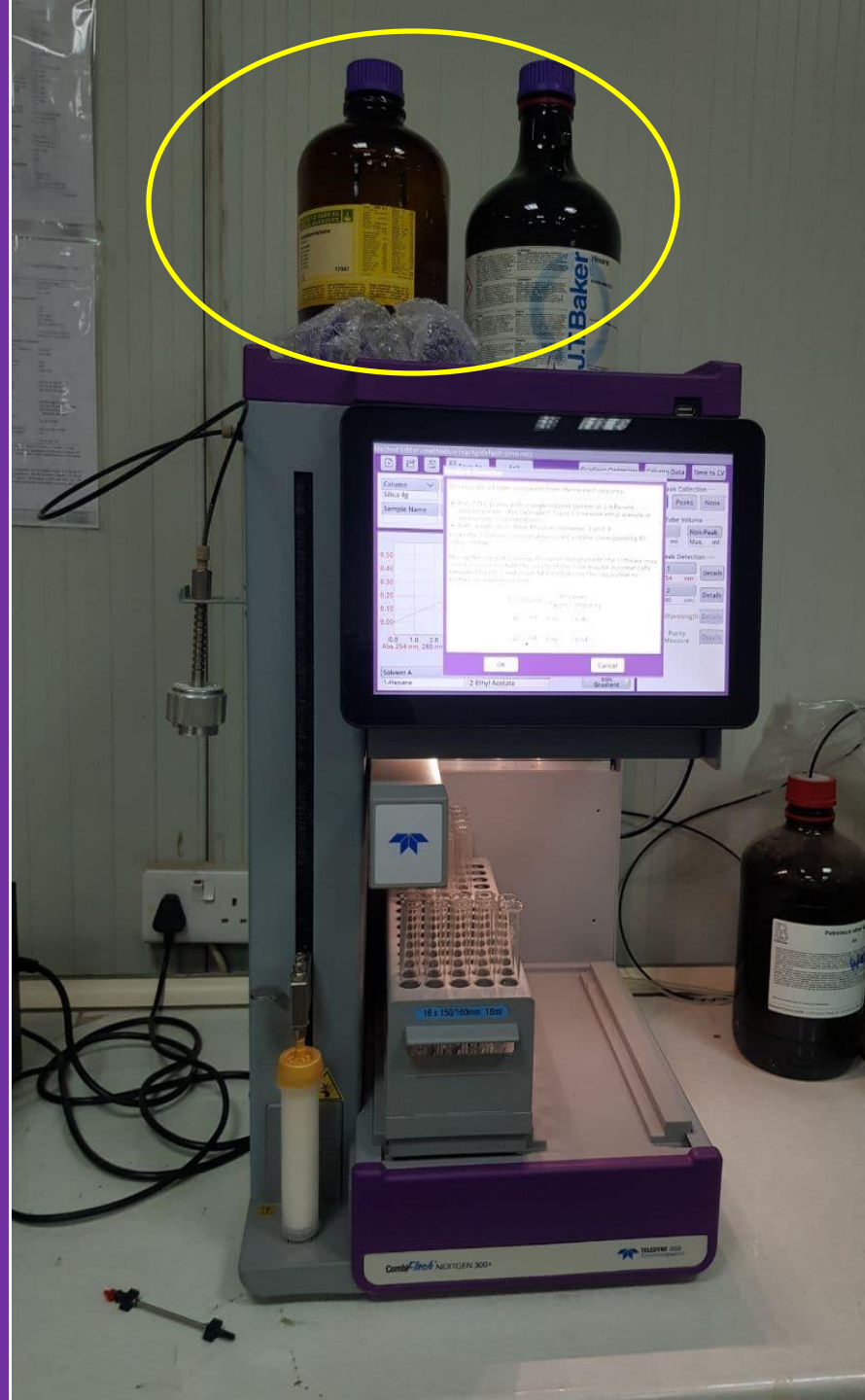
- In Flash chromatography, the solvent is pumped through the stationary phase instead of relative distances,

- **Retention in Flash Chromatography**

is defined in terms of the volume of solvent necessary to move the components through the column. This volume, expressed *in column volume (CV)*

$$R_f = \frac{1}{CV}$$

# MOBILE PHASE

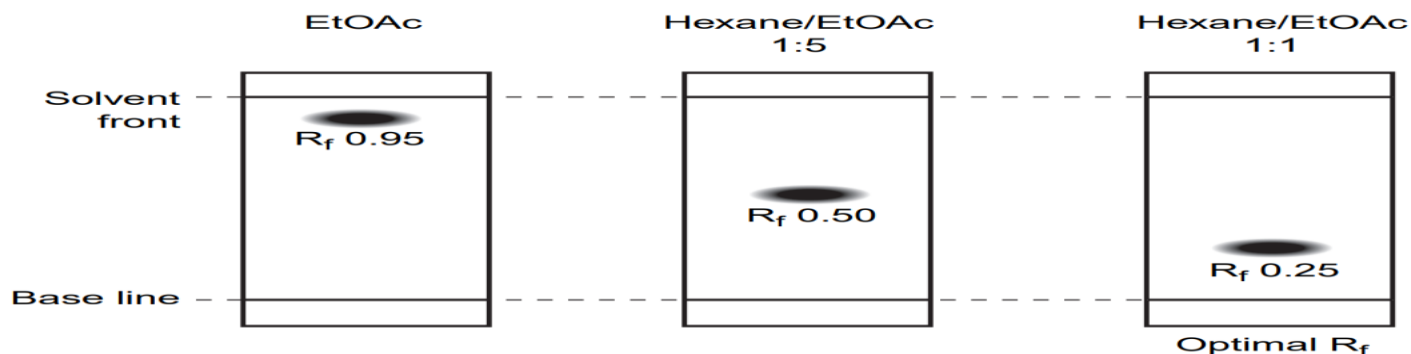




# Mobile Phase:

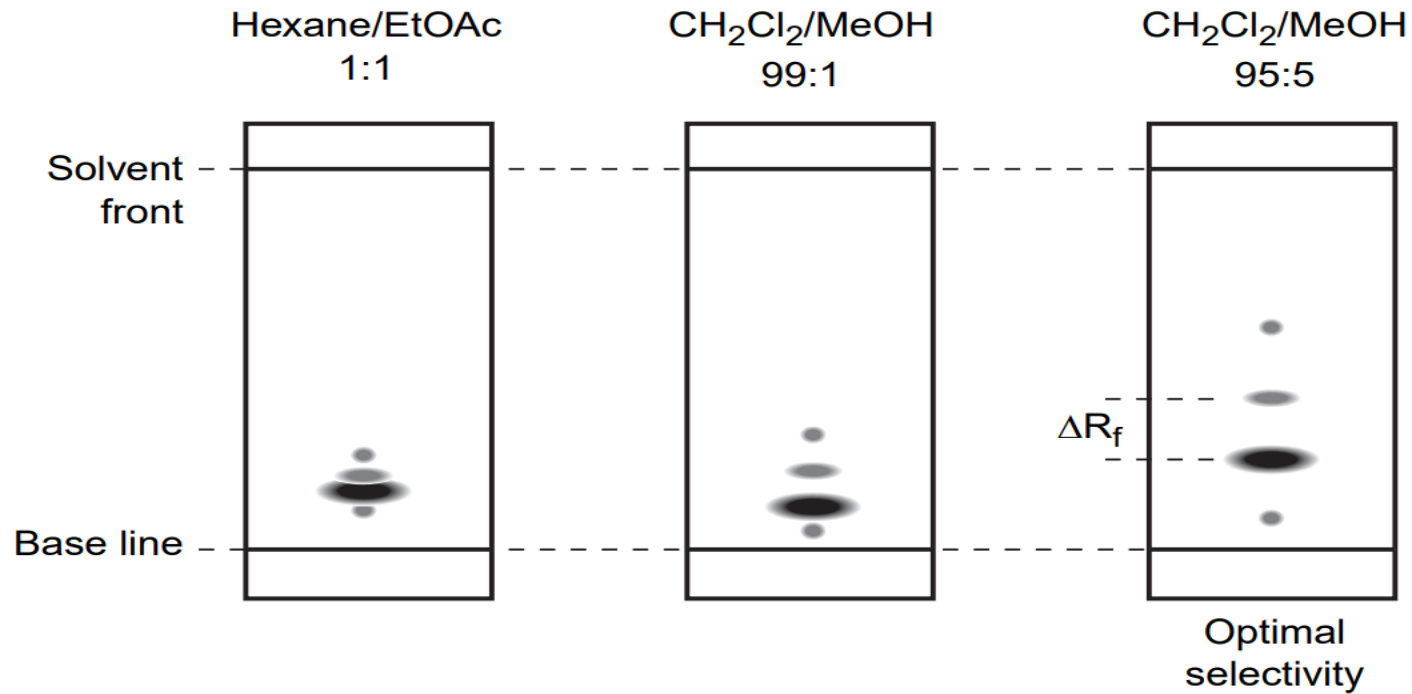
- Dependent on the polarity of the product(s) to be isolated and the type of stationary phase to be used.
- During the TLC analytical trials, the medicinal chemist will seek the:

**1. solvent system** that moves the desired product to  
 $R_f=0.25\pm0.05$

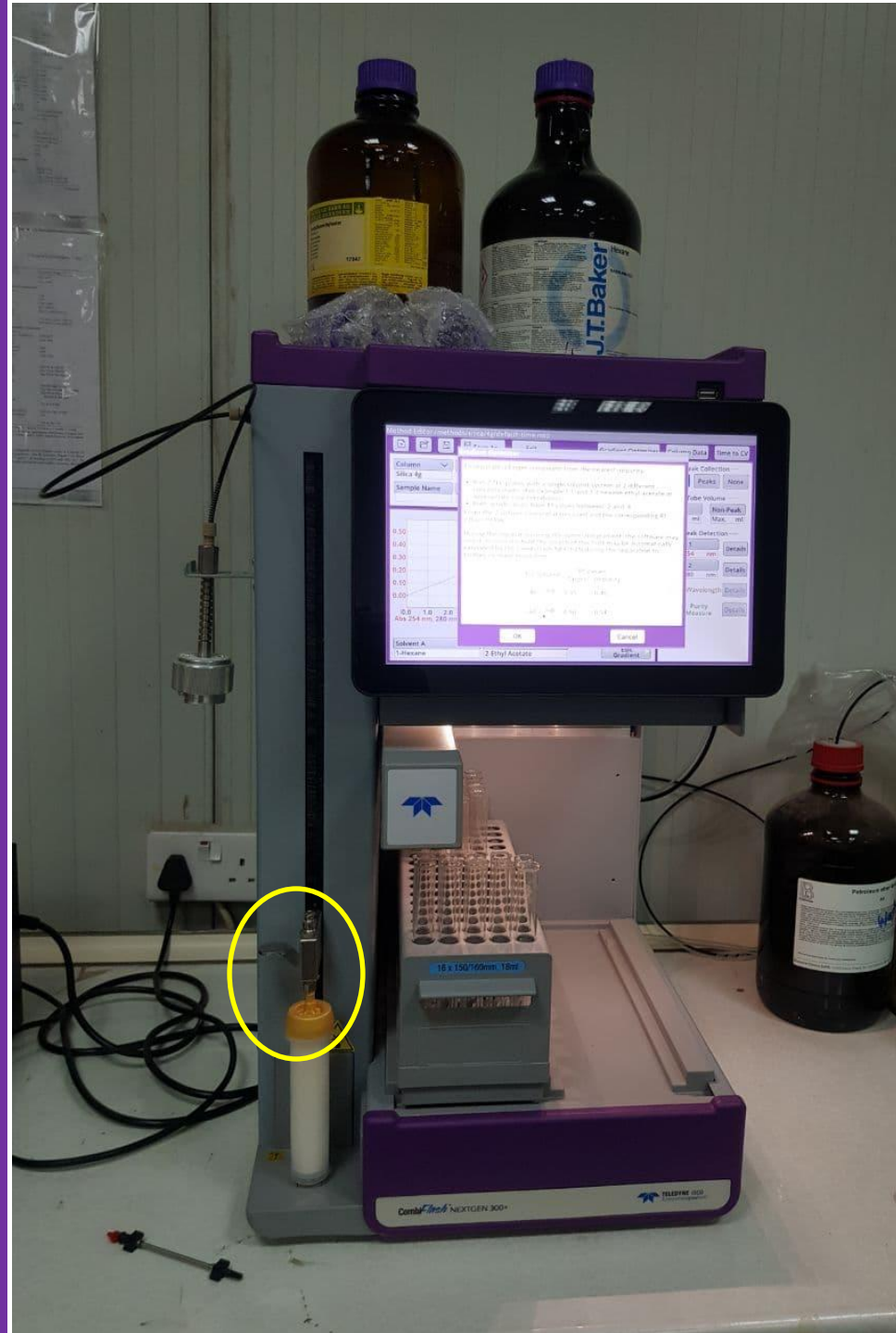


# Mobile Phase:

2. and keeps other undesired products to a distance of at least  $\Delta R_f=0.2$ .



# LOADING CAPACITY



# Loading Capacity

## 1. COLUMNS SIZE :

- **1-20%** of the column size depending upon resolution of impurities and column stationary phase (**for bare silica**)
- **0.1-2%** of the column size for functionalized media like **C18**

# Loading Capacity

## 2. selectivity :

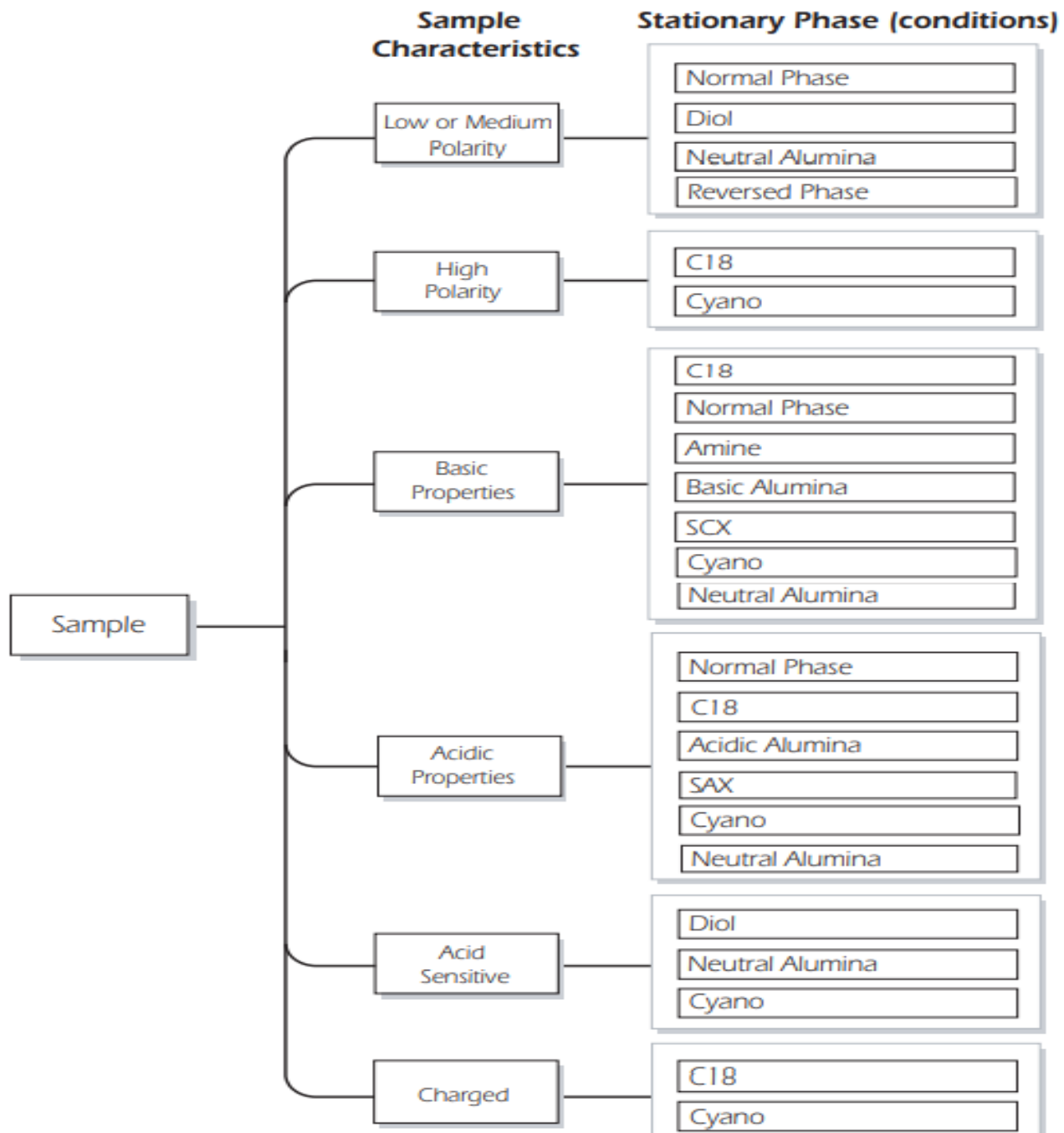
- The selectivity obtained will determine the sample loading capacity on the column.
- The lower the retention time ( $R_f$ ) and the higher the selectivity ( $\Delta R_f$ ) between product spots on the TLC plate, the higher the amount of sample can be loaded.

	<i>Loading</i>			
	<i>Light</i>	<i>Moderate</i>	<i>Significant</i>	<i>Heavy</i>
<i>Column size (g silica)</i>	$\Delta R_f < 0.2$	$0.2 - 0.4$	$0.4 - 0.6$	$> 0.6$
4 g (69-2203-304)	0.0004 - 0.004	0.004 - 0.16	0.16 - 0.28	0.28 - 0.4
12 g (69-2203-312)	0.0012 - 0.012	0.012 - 0.48	0.48 - 0.84	0.84 - 1.2
24 g (69-2203-324)	0.0024 - 0.024	0.024 - 0.96	0.96 - 1.68	1.68 - 2.4
40 g (69-2203-340)	0.004 - 0.04	0.04 - 1.6	1.6 - 2.8	2.8 - 4
80 g (60-2203-380)	0.008 - 0.08	0.08 - 3.2	3.2 - 5.6	5.6 - 8
120 g (69-2203-320)	0.012 - 0.12	0.12 - 4.8	4.8 - 8.4	8.4 - 12
125 g (69-2203-314)	—	—	5 - 8.75	8.75 - 12.5
220 g (69-2203-422)	0.022 - 0.22	0.22 - 8.8	8.8 - 15.4	15.4 - 22
330 g (69-2203-330)	0.033 - 0.33	0.33 - 13.2	13.2 - 23.1	23.1 - 33
750 g (69-2203-275)	0.075 - 0.75	0.75 - 30	30 - 52.5	52.5 - 75
1500 g (69-2203-277)	0.15 - 1.5	1.5 - 60	60 - 105	105 - 150

# STATIONARY PHASE

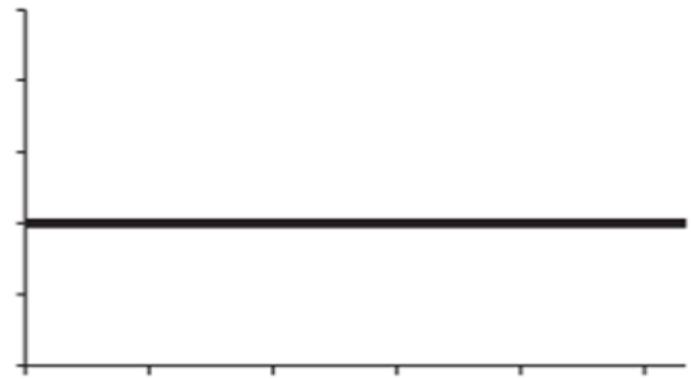


RediSep Rf  
Media

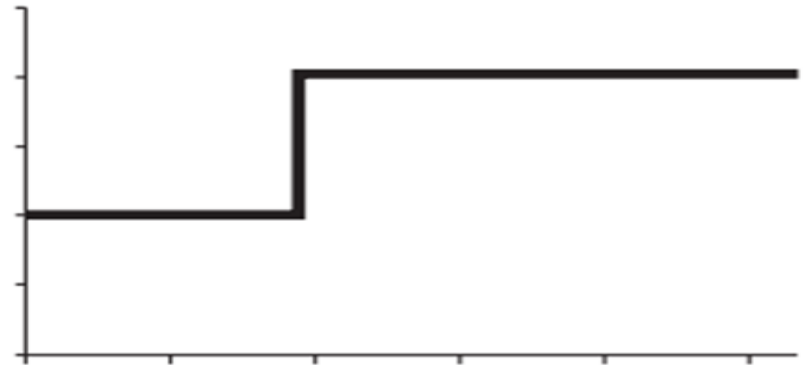




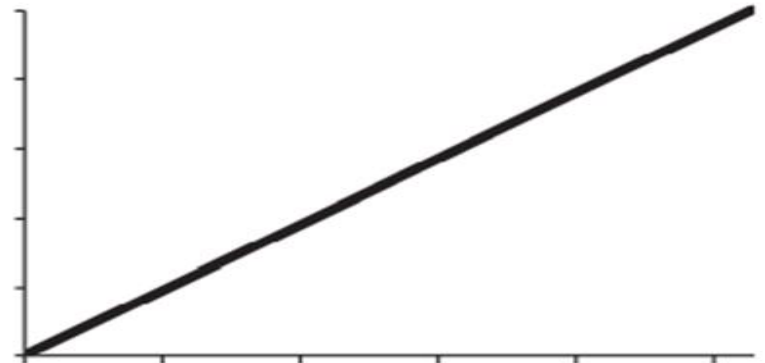
# GRADIENT FORMER AND PUMP



Isocratic



Stepped



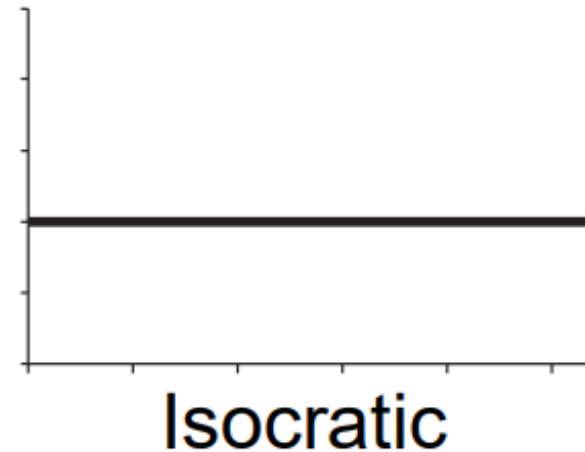
Linear

# Mobile Phase Techniques

## 1. ISOCRATIC ELUTION

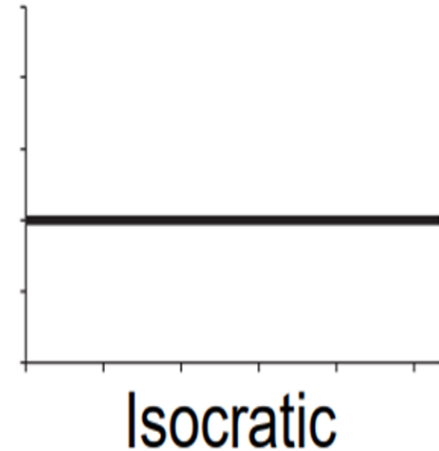
### ➤ Classical Method

➤ the mobile phase may be a single solvent or a mixture, but the mobile phase composition is the same throughout the separation.

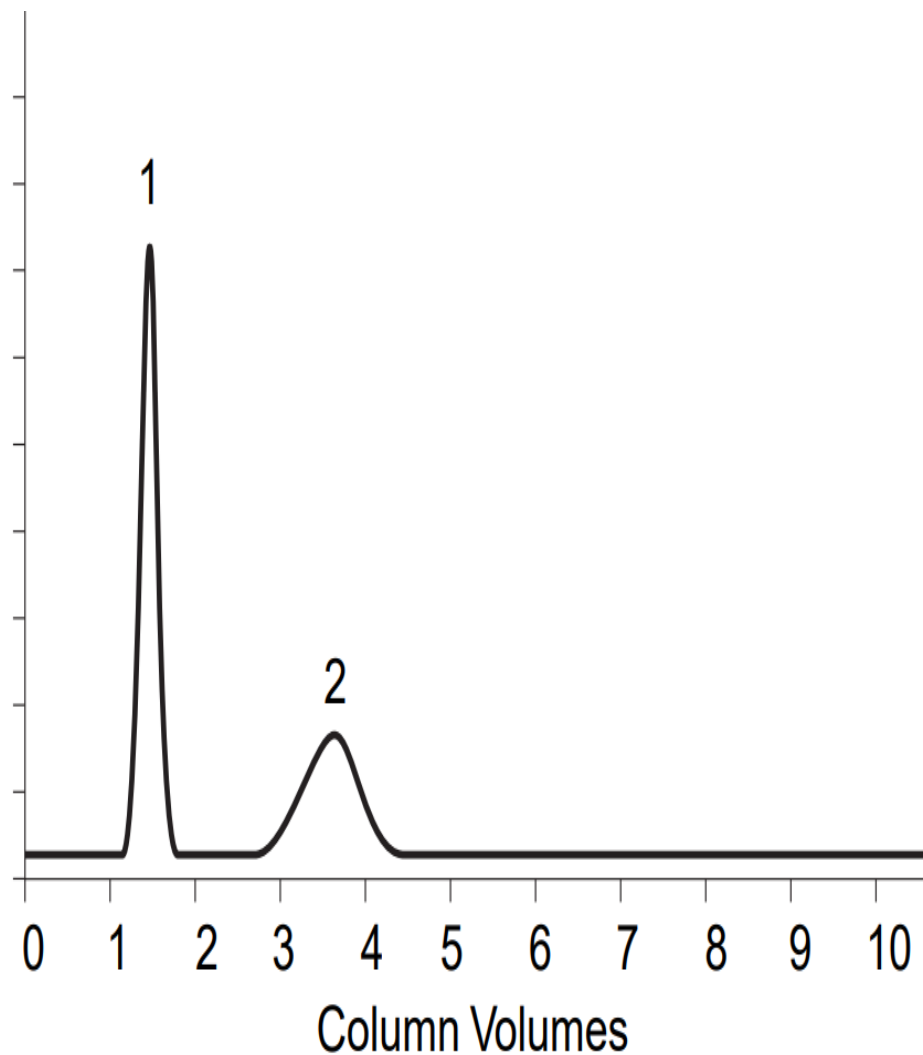
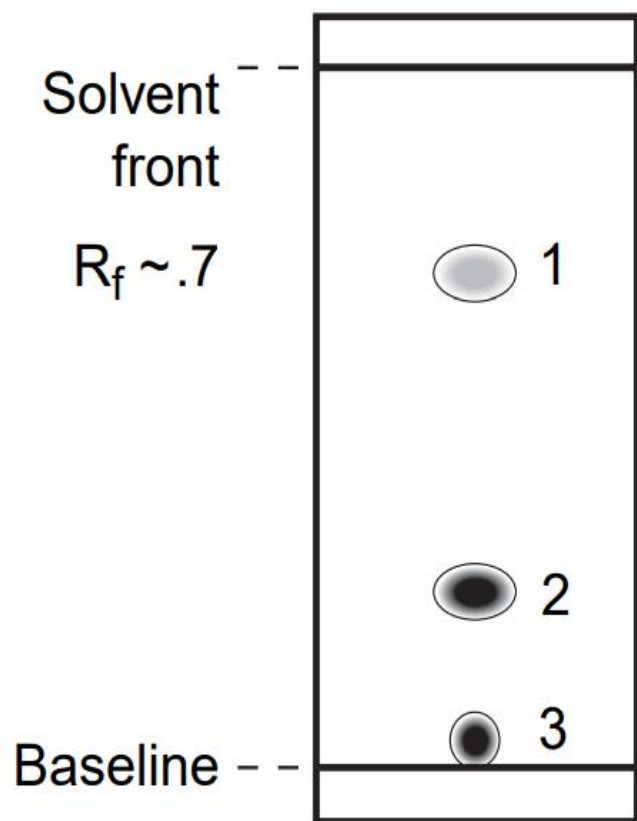


# Isocratic Mobile Phase :

- The separation is selective.
- That will not separate a wide variety of compounds.
- Column capacity is typically limited when using isocratic mobile phases. **If the sample size is increased too much, the mixture's compounds will contaminate each other.**

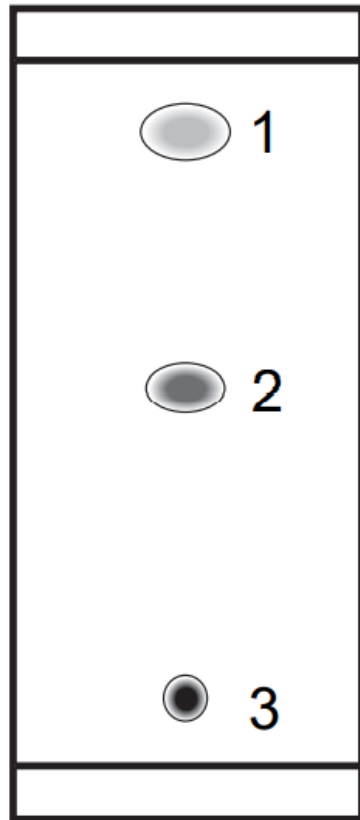


# Illustration of isocratic 20% EtOAc in hexane

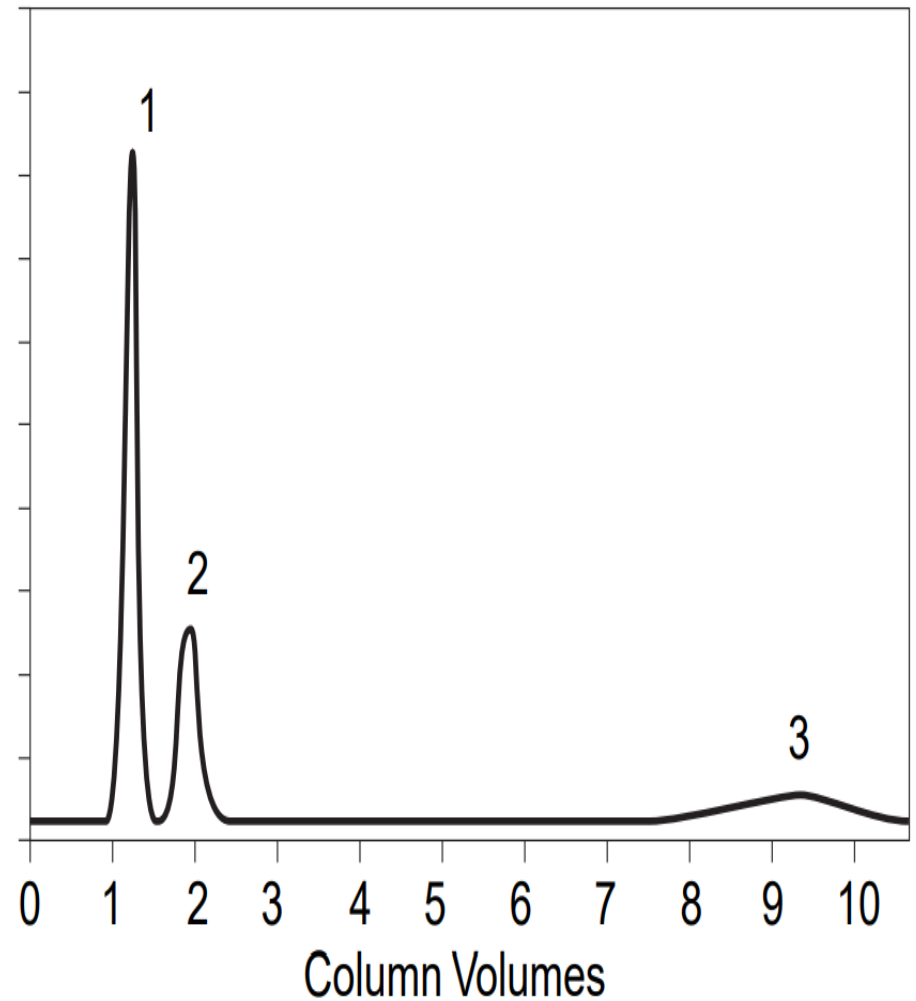


# Illustration of isocratic 30% EtOAc in hexane

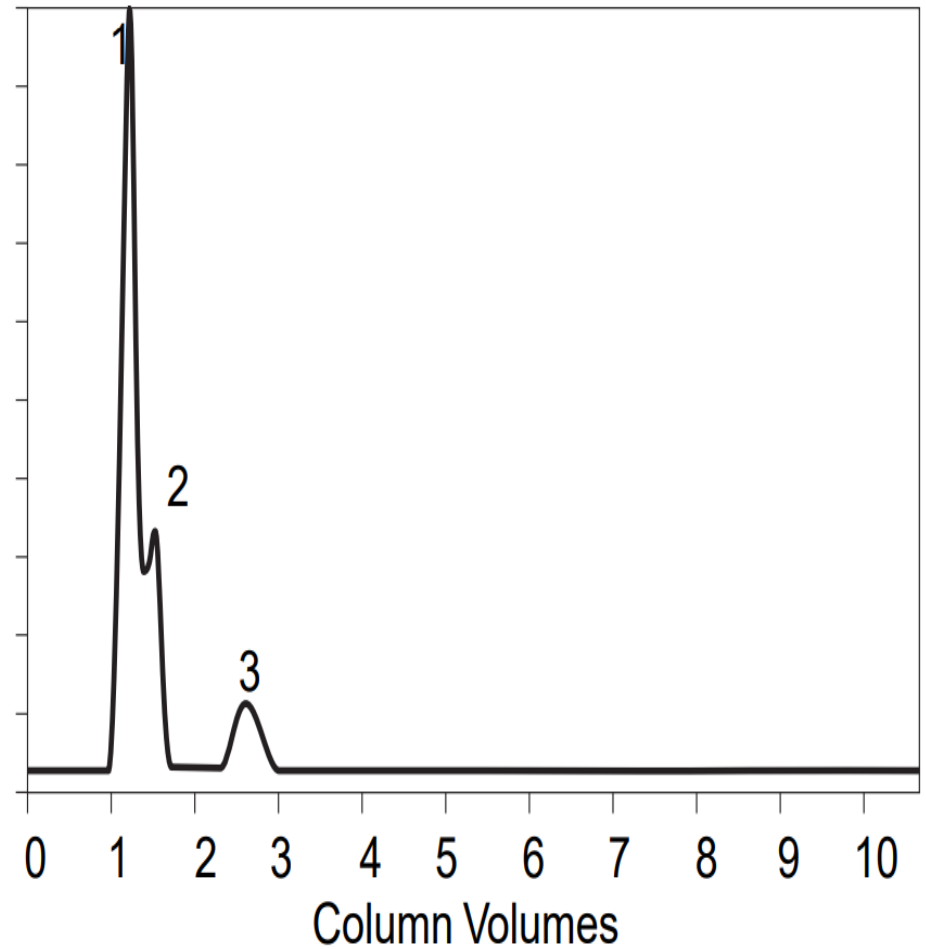
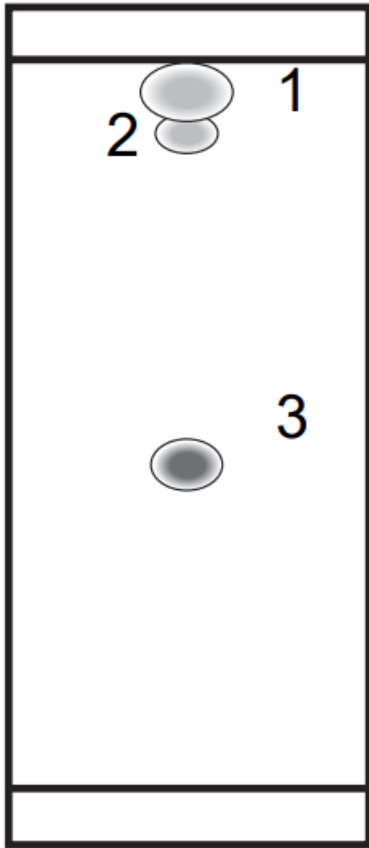
$R_f \sim .9$



$R_f \sim .1$

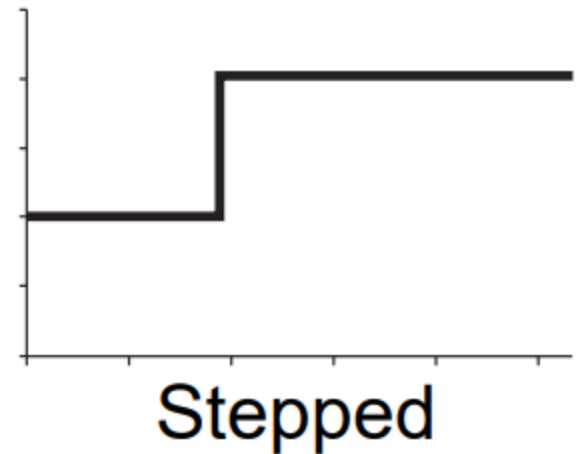


# Illustration of isocratic 40% EtOAc in hexane

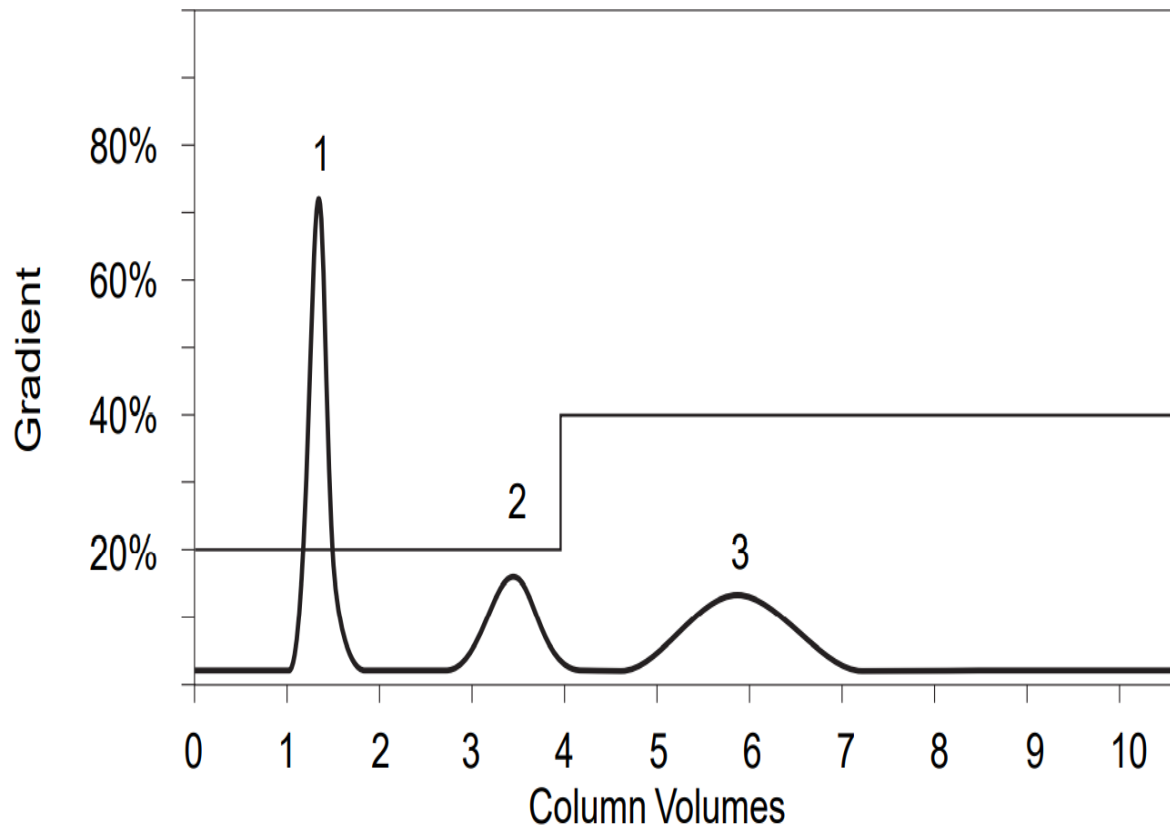
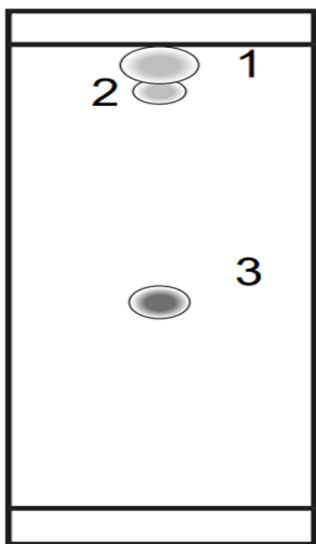
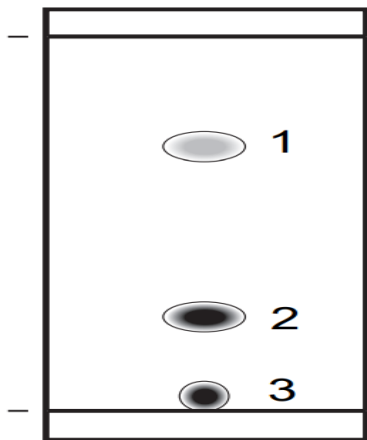


# Stepped Gradient

The solvent strength is **increased only after the previous compound has separated**, greatly improving **selectivity**. As a result, **column capacity can be increased**.



# Stepped Gradient

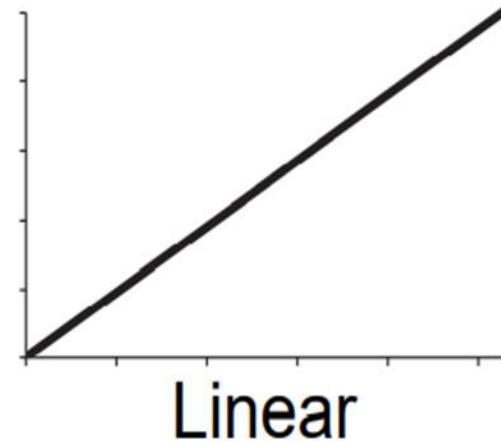


*Illustration of a stepped gradient and chromatogram*

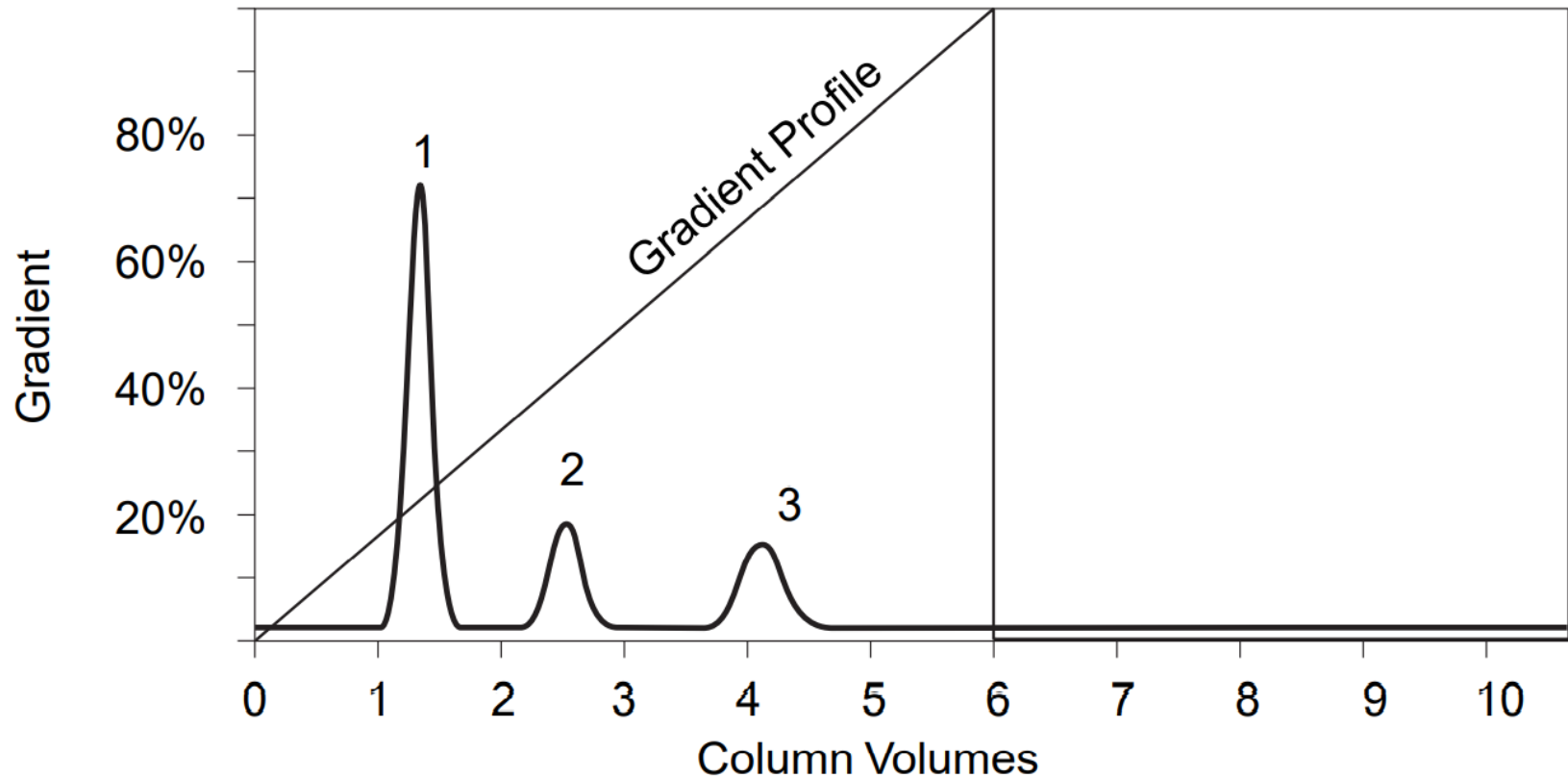


# Linear Gradient

- Linear gradients **BEGIN** with a low-strength solvent blend. until the separation **ENDS** at a high-strength solvent blend.
- It is not necessary to perform as many TLCs
- TLC work to determine that the solvent system and stationary phase



# Linear Gradient



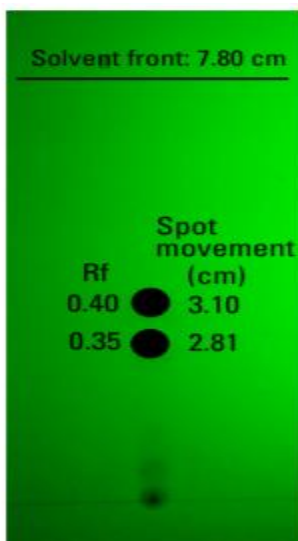
*Illustration of a linear gradient and chromatogram*

# Gradient Optimizer

- The Gradient Optimizer is a feature in PeakTrak that **generates a gradient containing an isocratic hold.**
- This method is useful when purifying compounds that **elute closely.**

# Gradient Optimizer

- Run two TLC plates with a single solvent system at two different concentrations.
- For example 40% EA in Hexane and 60% EA in Hexane



- Need **Rf values for target** compound and nearest impurity
- **Rf values between 0.2-0.8**

# Gradient Optimizer

itor /methods/silica/4g/default-time.mtd

Save As... Exit... Gradient Optimizer... Column Data... Time to

Name

To separate a target compound from the nearest impurity.

- Run 2 TLC plates with a single solvent system at 2 different concentrations. (For example 1:1 and 1:3 hexane:ethyl acetate or appropriate concentrations).
- Both results must have Rf values between .2 and .8.

Enter the 2 solvent concentrations used and the corresponding Rf values below.

During the separation using the optimized gradient, the software may insert an isocratic hold. The length of this hold may be automatically extended by the CombiFlash NEXTGEN during the separation to further increase resolution.

TLC Solvent	Rf Values	
	Target	Impurity
40 %B	0.35	0.40
60 %B	0.50	0.54

OK Cancel

Exit Gradient

Peak Collection  
Peaks Non-Peaks

Tube Volume  
ml Non-Peak Max.

Peak Detection  
1 54 nm Detail  
2 80 nm Detail

Wavelength Detail

Purity Measure Detail

nt A  
ane

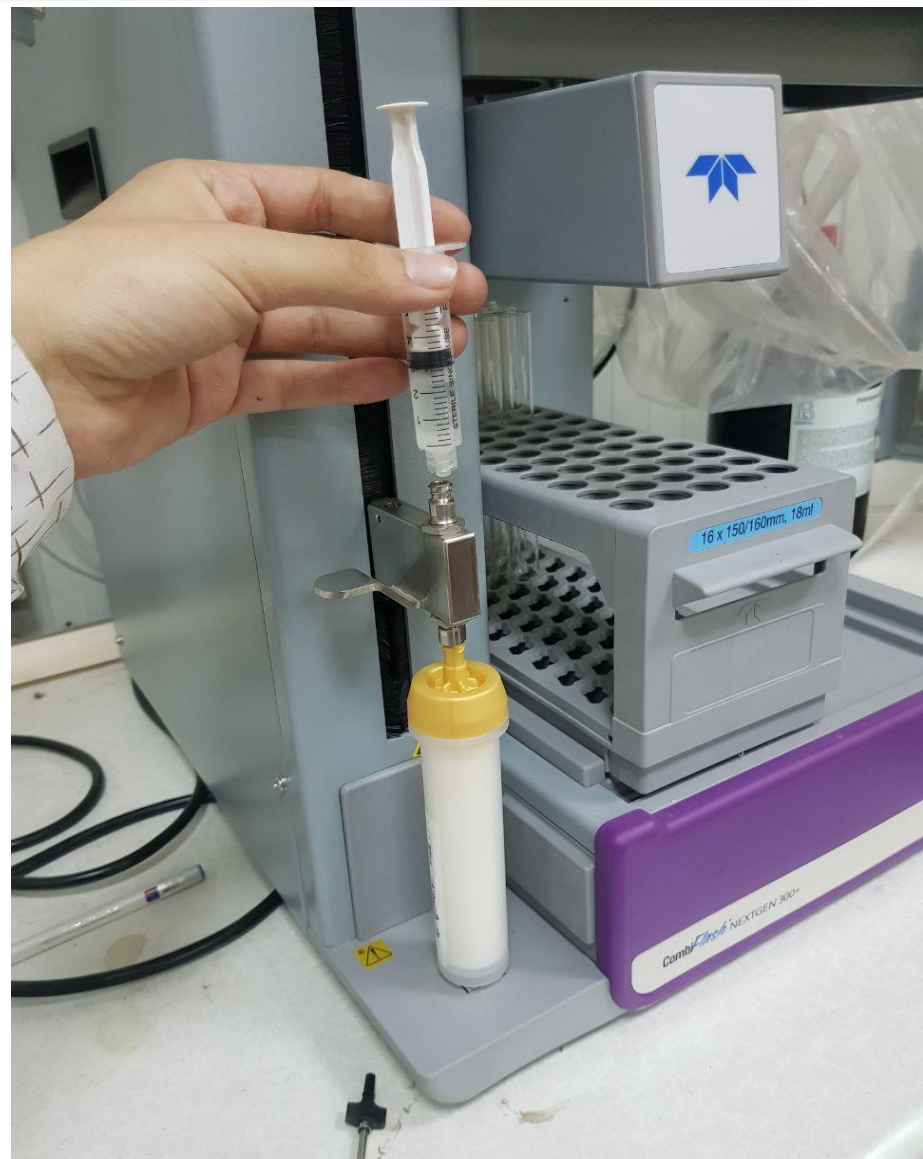
2-Ethyl Acetate



# **SAMPLE LOADING TECHNIQUE**

# Liquid injection :

- Great for neat liquid samples
- If not liquid .then dissolve sample and add directly onto column after equilibration
- Same rules as traditional open column chromatography
  - - minimize the amount of solvent
  - -the weaker the dissolution solvent the better



# SOLID LOADING

- Great for **hard to dissolve** sample that require **large amount** of strong solvent to do liquid injection .

## Solid load cartridge :

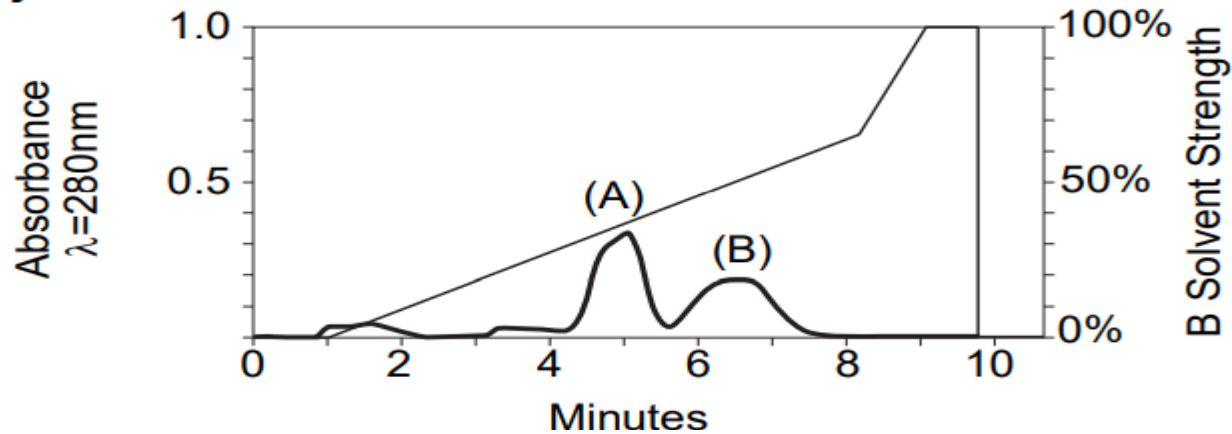
- **Dissolve** compound and then absorb onto **silica gel**
- **Evaporate** dissolution solvent from silica mixture, and **load** silica mixture onto an empty **solid load cartridge** and add cap .



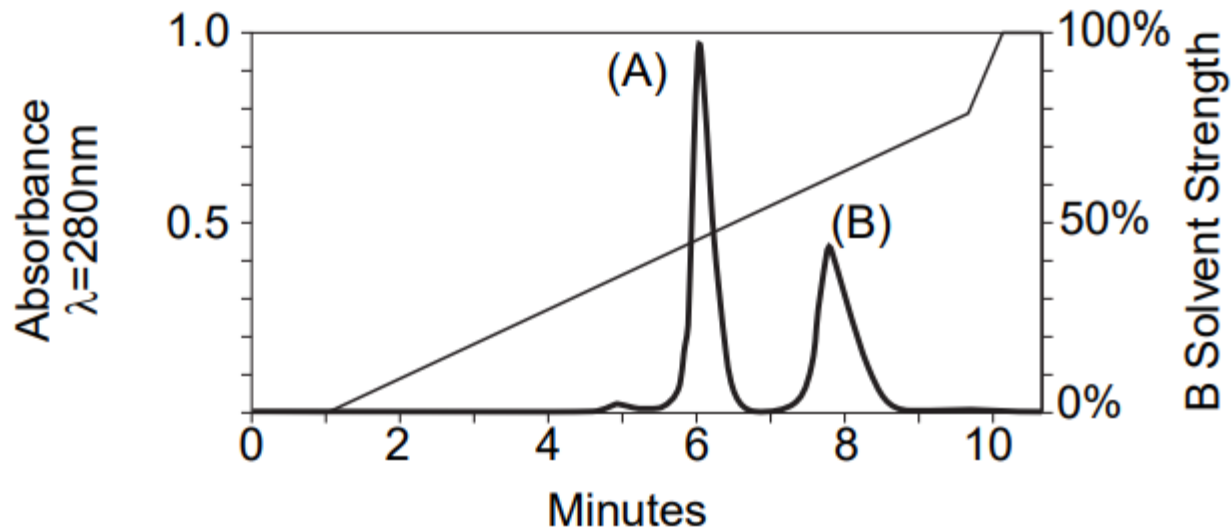


- The dry **SOLID LOAD** cartridge shows **BETTER RESOLUTION** than the **LIQUID INJECTION**.

**Syringe Injection**



**Solid Load Cartridge  
(dried)**



**What Detection  
Options are Available  
and Useful for  
Purification**

# Available Methods of Detection

- UV (200-400 nm) or UV-Vis (200-800 nm)

- **Integrated ELSD**



- **Purlon Mass Spectrometer**



# Evaporative Light Scattering Detector ELSD

- **Considered a universal detector**, as it can detect compounds without chromophores.
- **Limitations:**
  - **Destructive detection technique**

# MS Detection

- Enable this option **to monitor or detect compounds with a Purlon mass spectrometer system (Purlon systems only).**

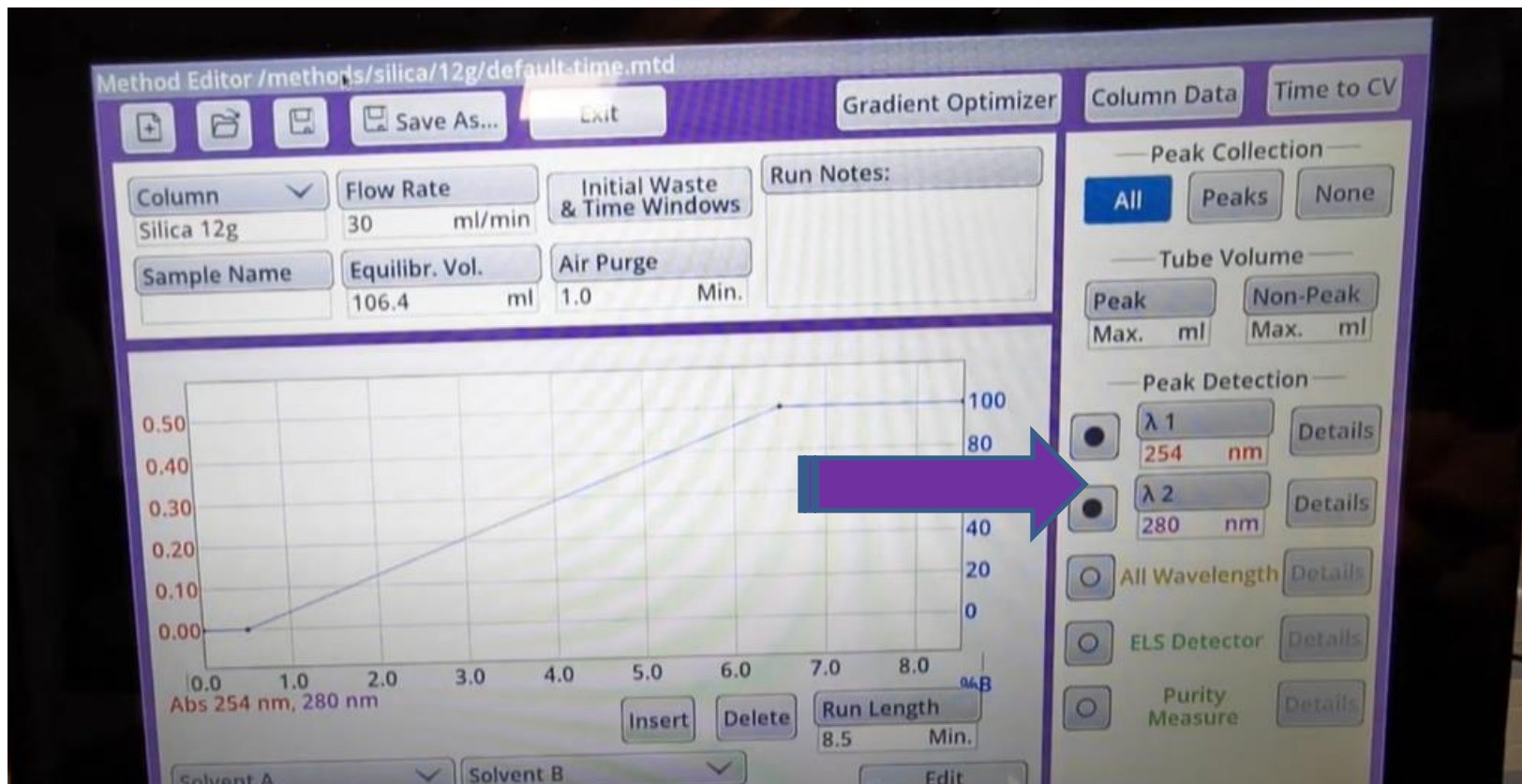


# UV and UV-Vis Detection

- **Non-destructive technique**
- **UV (200-400 nm) or UV-Vis (200-800 nm) configuration**
  - Requires a chromophore for detection
  - Entire UV spectrum is saved throughout the chromatogram

# **Getting the most from your UV and UV-Vis Methods of Detection**

# Can choose to trigger collection or monitor up to 2 single wavelengths





# All-wavelength detection

The image shows a software interface for method editing. The main window is titled "Method Editor / methods/silica/12g/default-time.mtd". A "Detection Options" dialog box is open, showing the following settings:

- Signal Gain: 1x
- Peak Width: 1 min
- Threshold: 0.20 AU
- Minimum  $\lambda$ : 200 nm
- Maximum  $\lambda$ : 300 nm
- Detection Method:  All Wavelength

Below the dialog box, a purple arrow points to the text "Detection Options for All Wavelength Detection".

In the background, the "Peak Detection" section of the software is visible, with a blue arrow pointing to the "All Wavelength" option:

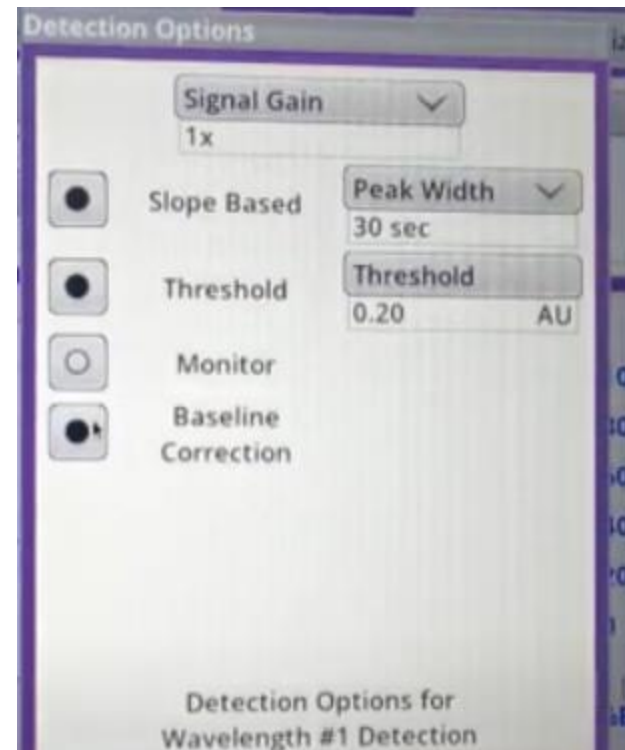
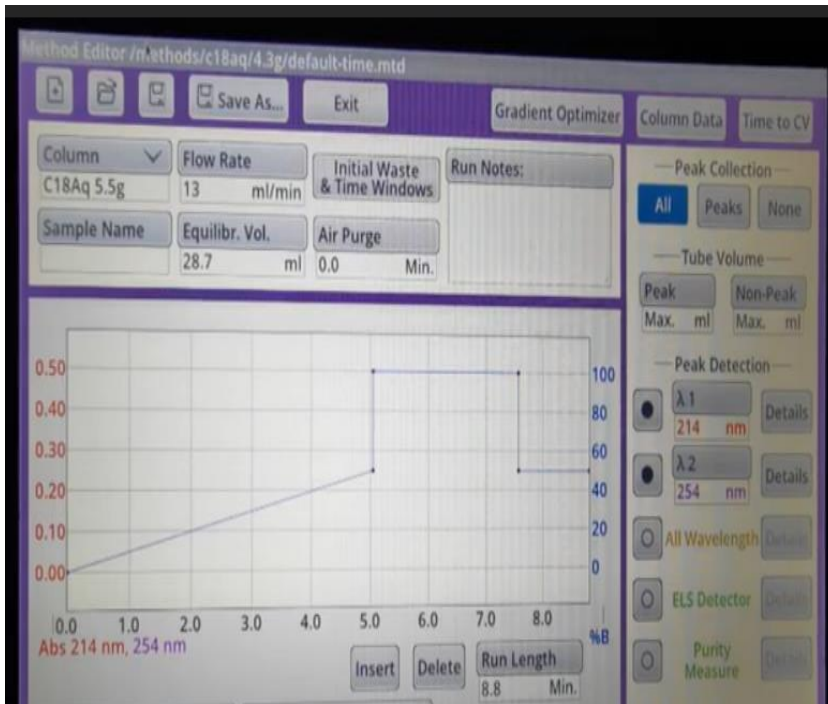
- $\lambda$  1 254 nm Details
- $\lambda$  2 280 nm Details
- All Wavelength Details
- ELS Detector Details
- Purity Measure Details

The "Peak Collection" section shows "All" selected, and the "Tube Volume" section shows "Peak Max. ml" and "Non-Peak Max. ml" fields.

At the bottom left, a graph shows absorbance vs. time (0.0 to 3.0 minutes) with a linear increase. The x-axis is labeled "Abs 254 nm, 280 nm, 200-300 nm".

# Baseline Correction :

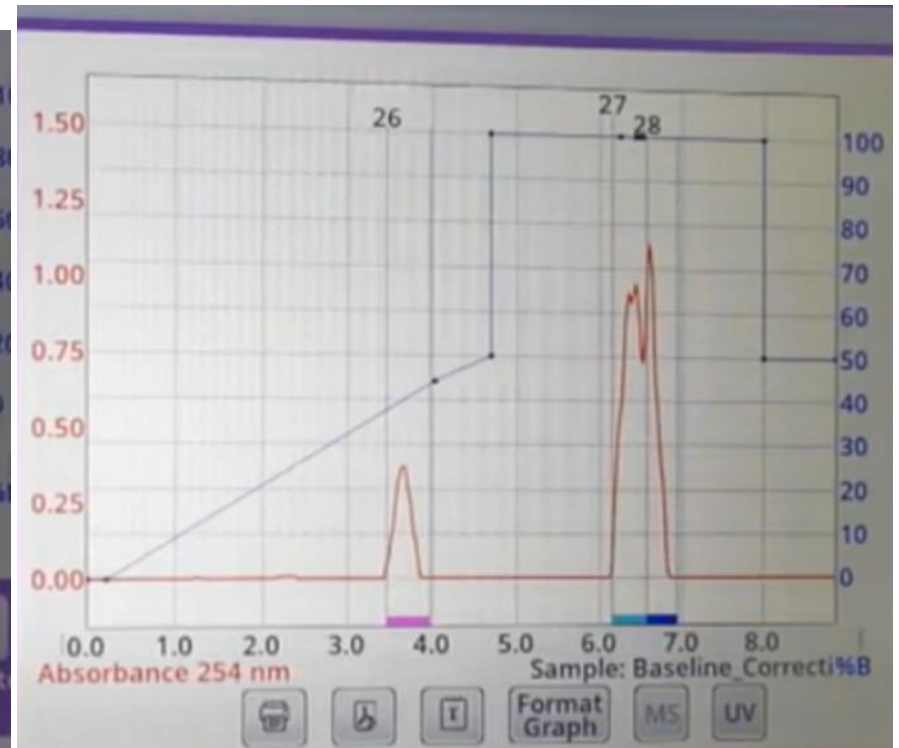
- Its important when the solvent and the compound that absorb in **the same UV region**



# Baseline Correction

- Enables a short pre-run gradient to measure baseline absorbance
- Allow the system to subtract baseline from run
- Expand detection abilities across all wavelengths, not limited by solvent UV cut-off

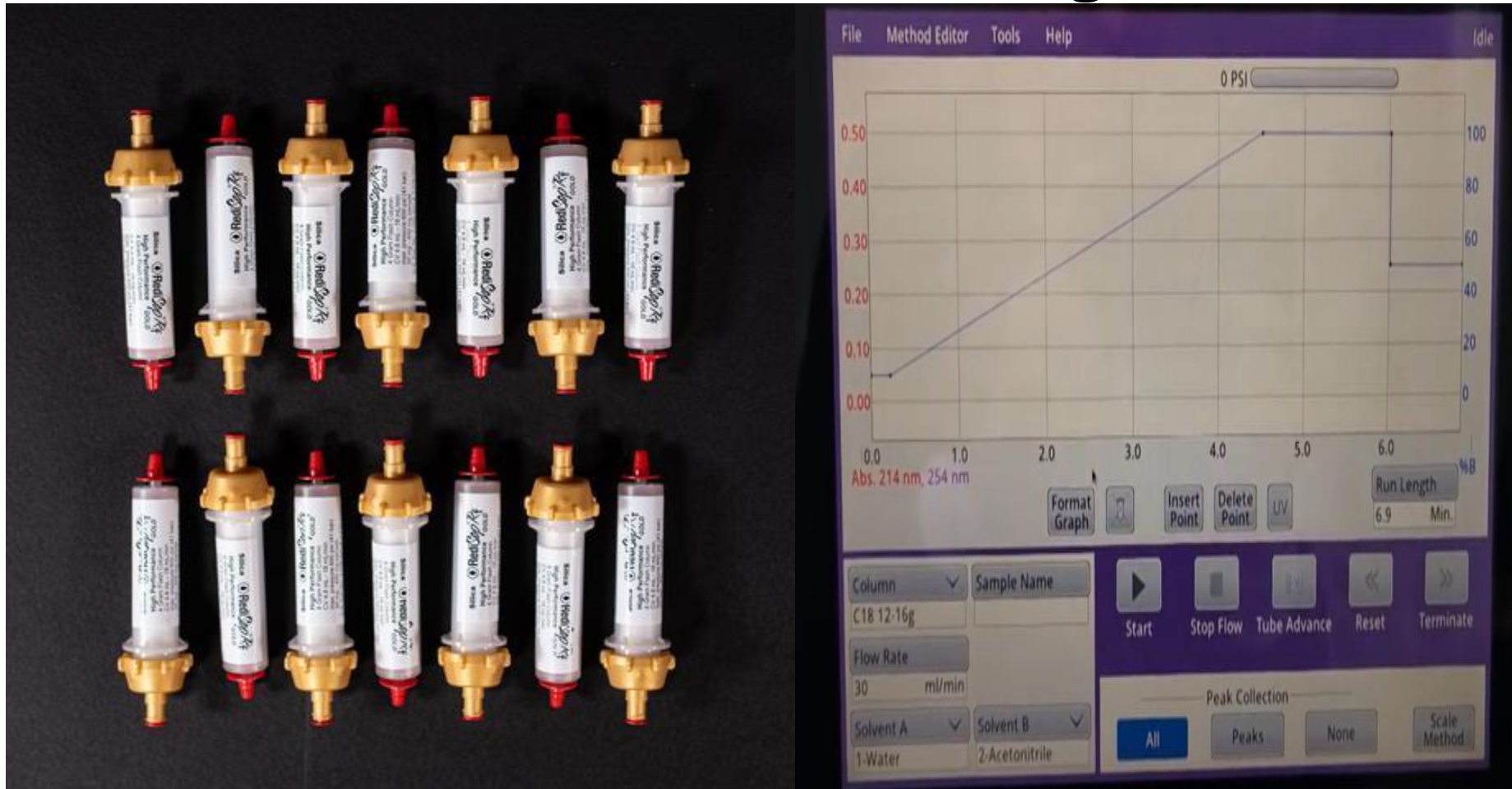
# Baseline Correction



# **SOME FEATURES OF COMBIFLASH NEXETGEN300+**

# The Radio-frequency Identification RFID Tags:

- All columns contain RFID Tag



# The RFID Tags

The screenshot displays a chromatography software interface with a 'Column Data' pop-up window. The background interface includes a 'Method Editor' window with various parameters and a 'Peak Collection' section on the right. The 'Column Data' window provides the following information:

- RediSep Rf Column: C18 12-16g
- Lot Number: 281637189W
- Rated Pressure: 300
- Rated Flow Rate: 30 ml/min
- Number of Times Used: 3
- Last Fluid Used: 50% Water, 50% Acetonitrile
- First Used on: 2/11/2020
- Last Used on: 2/11/2020

The background interface shows a 'Method Editor' window with the following parameters:

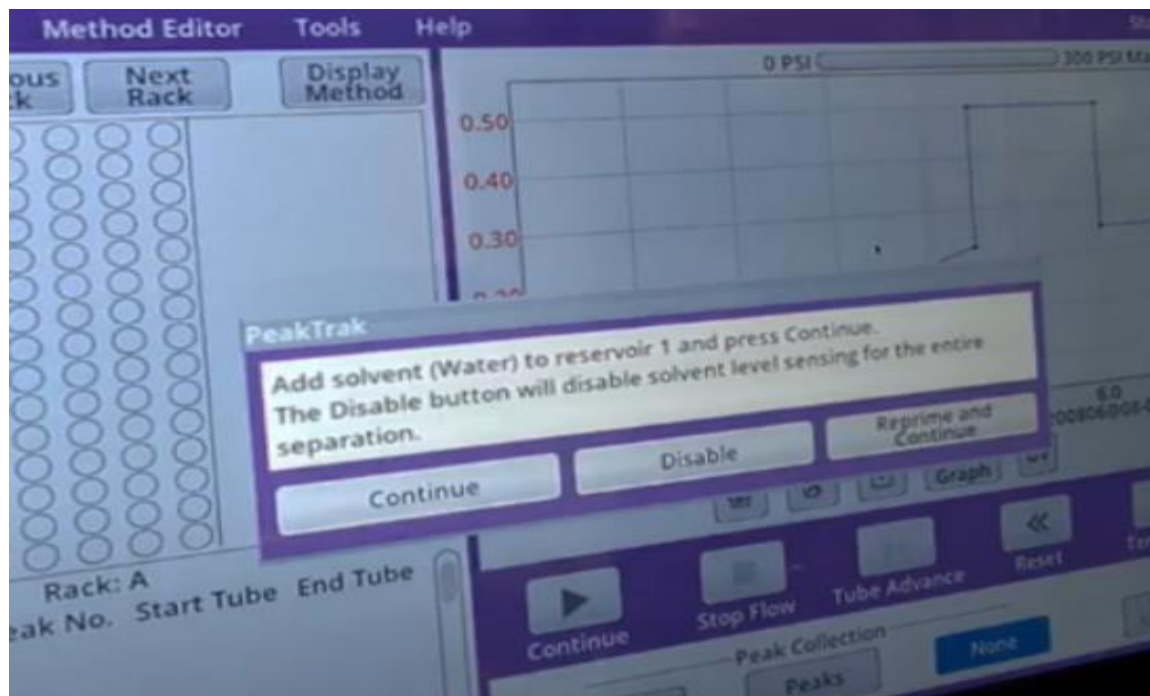
- Column: C18 12-16g
- Flow Rate: 30 ml/min
- Sample Name: [Empty]
- Equilibr. Vol.: 77.4
- Initial Waste & Time Windows: [Empty]
- Run Notes: [Empty]
- Gradient Optimizer: [Active]
- Column Data: [Active]
- Time to CV: [Active]
- Peak Collection: All, Peaks, None
- Tube Volume: Peak Max. ml, Non-Peak Max. ml
- Peak Detection:   $\lambda$  1 214 nm,   $\lambda$  2 254 nm,  All Wavelength,  Purity Measure
- Solvent A: 1-Water, Solvent B: 2-Acetonitrile
- Run Length: 6.9 Min.

## SOLVENT LEVEL SENSING:

Its actively measure the depth of your inlet  
**solvent and depth of your waste solvent.**

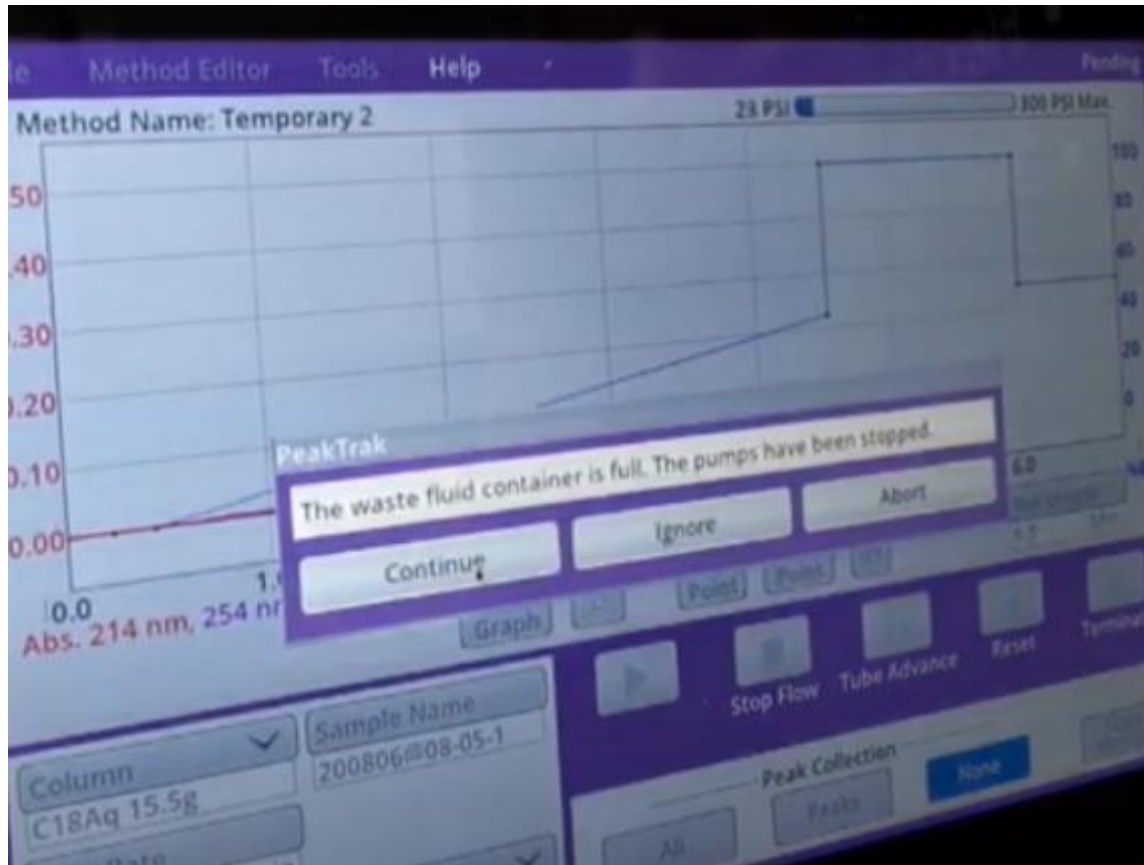


# SOLVENT LEVEL SENSING:



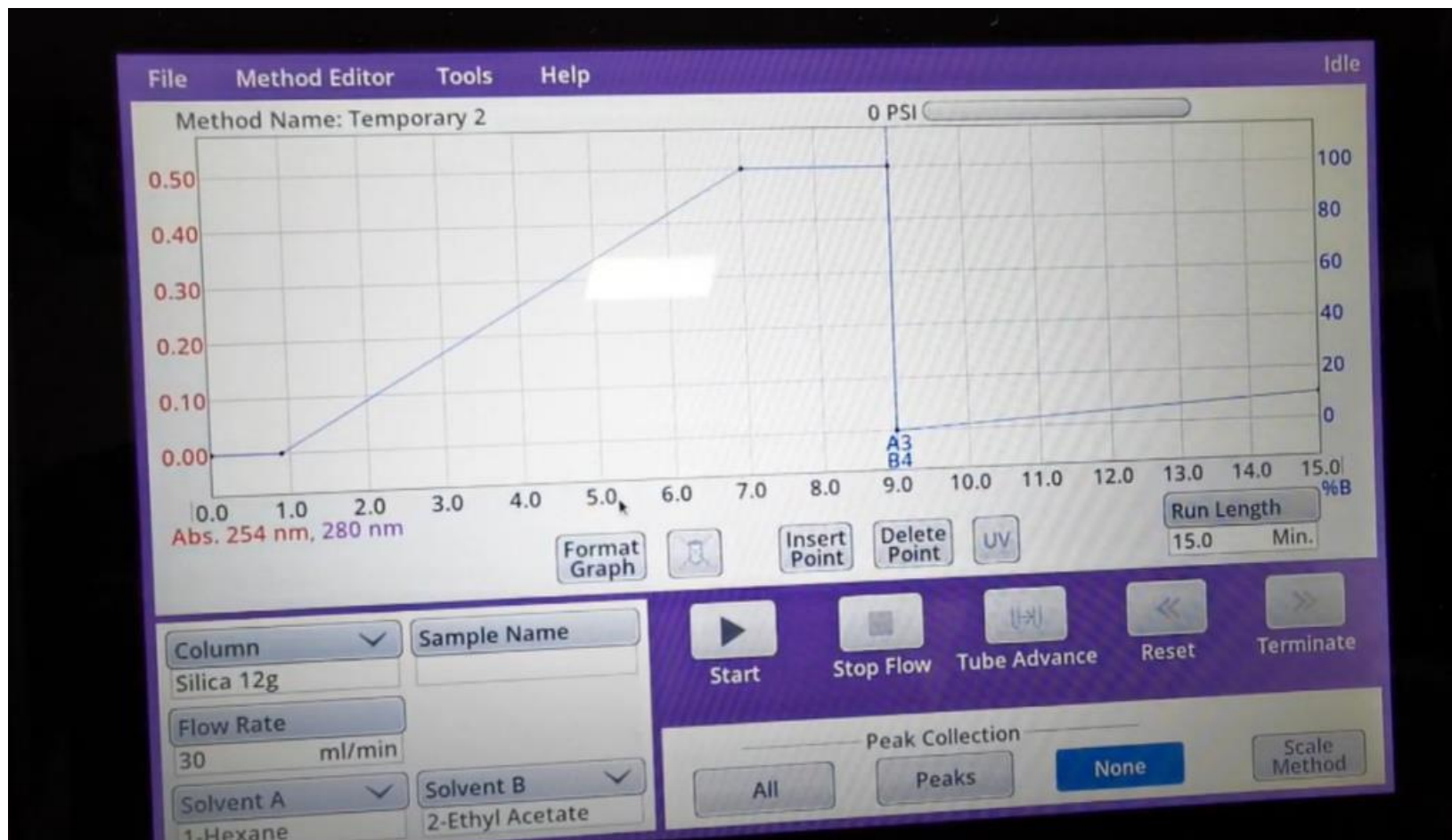
You can not run your columns dry

# SOLVENT LEVEL SENSING:



Can not overflow your waste

# Use the 4 Solvent Lines



THANK YOU