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

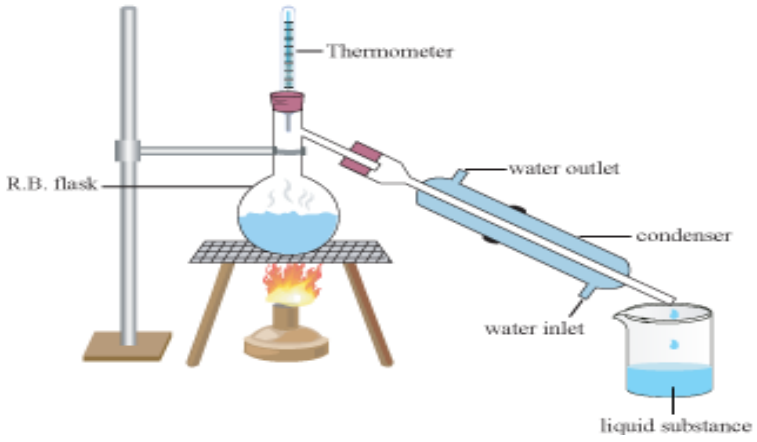
There are three steps of organic synthesis:

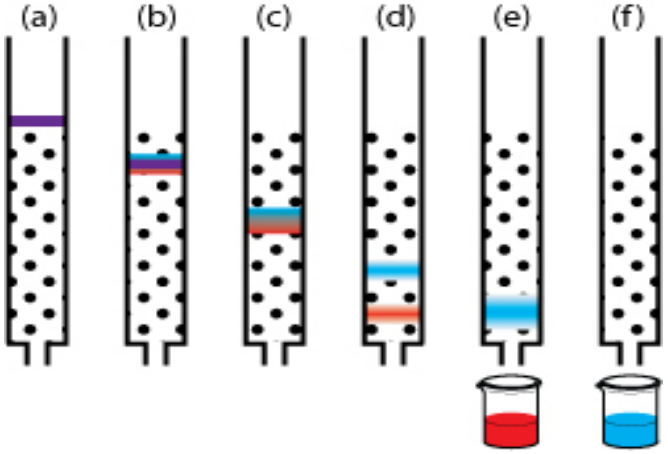
Reaction Setup

Workup , PURIFICATION

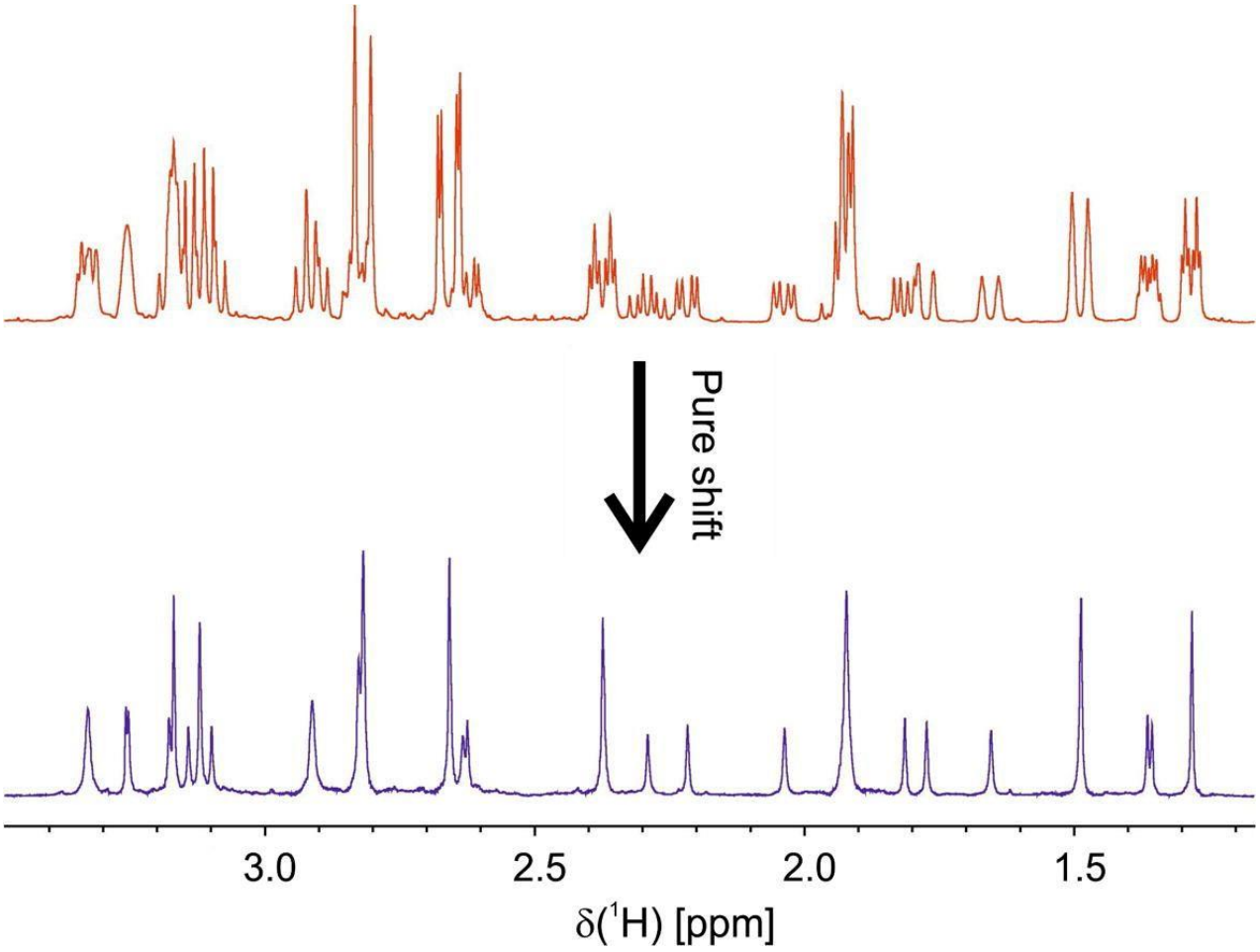
Final Product Analysis

Compound Purification





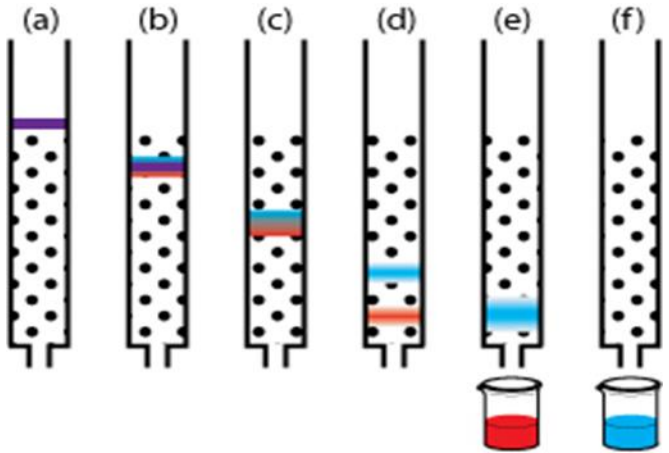
Effect of Purification on NMR



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**

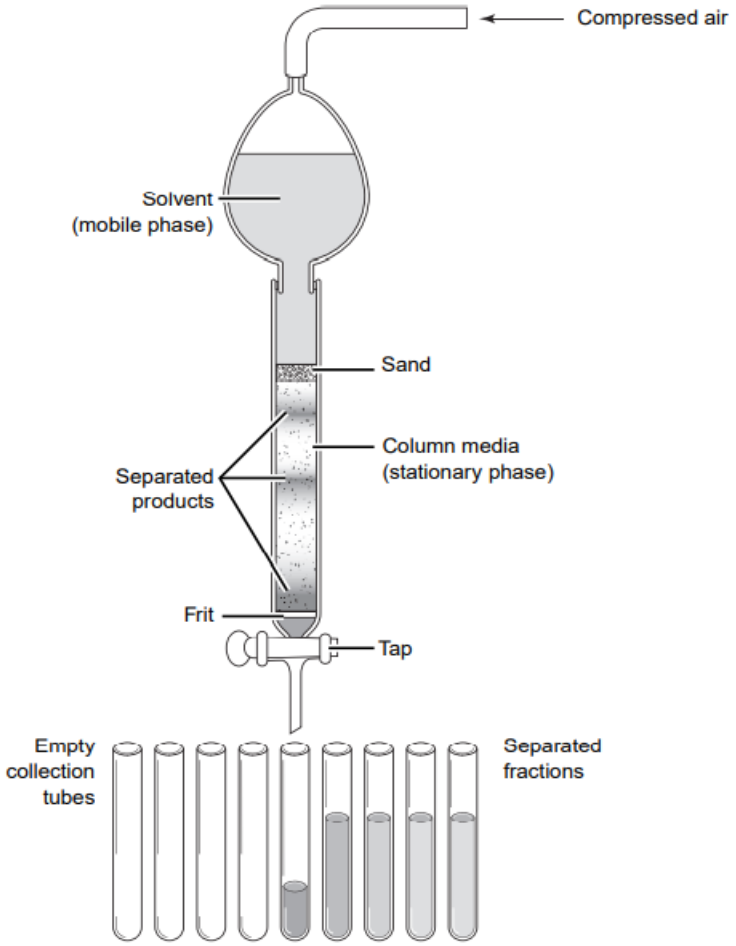


Illustration of basic elements in a traditional Flash column chromatography apparatus

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 ,

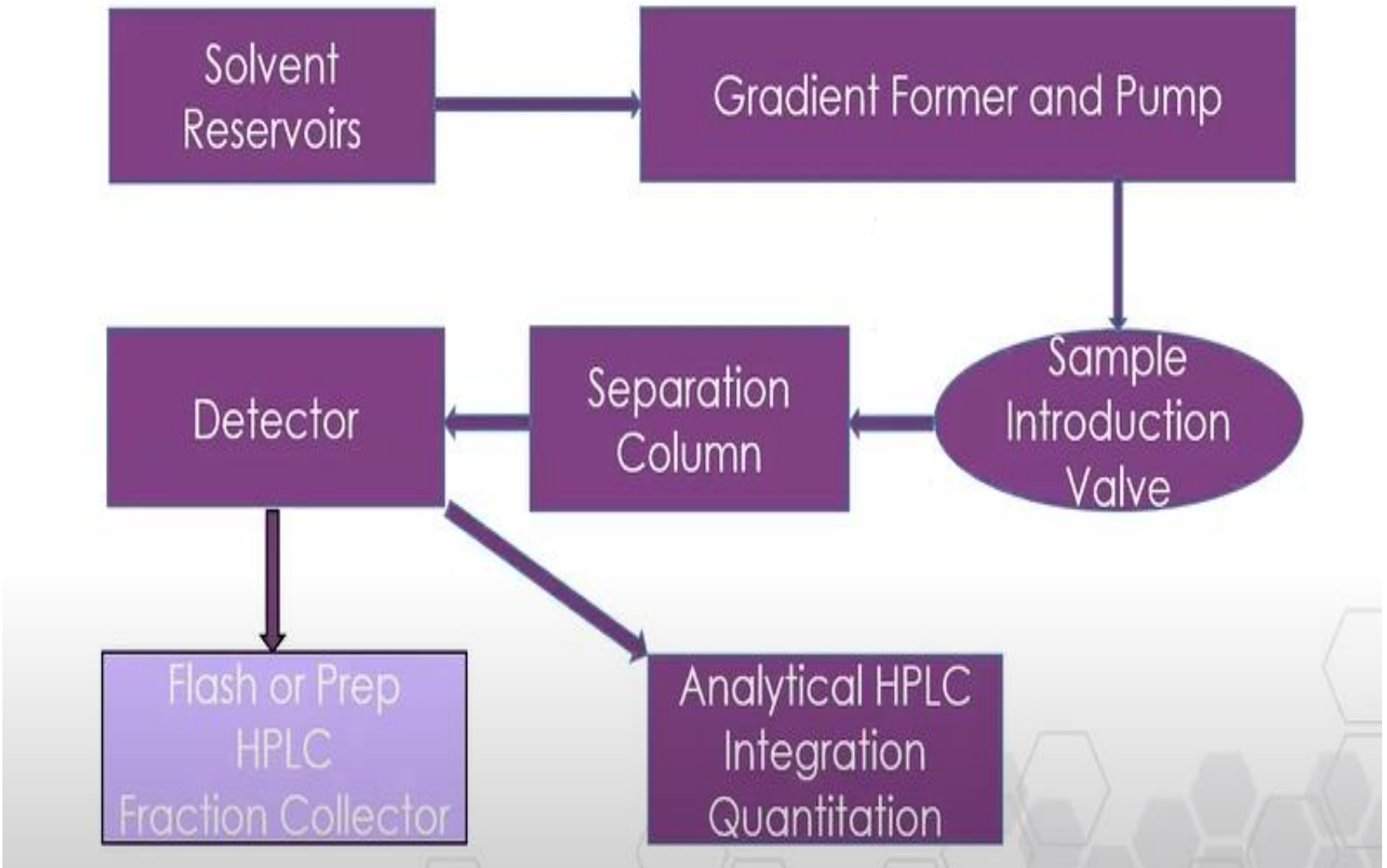
more efficient separation,

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

Flash chromatography	**HPLC**
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

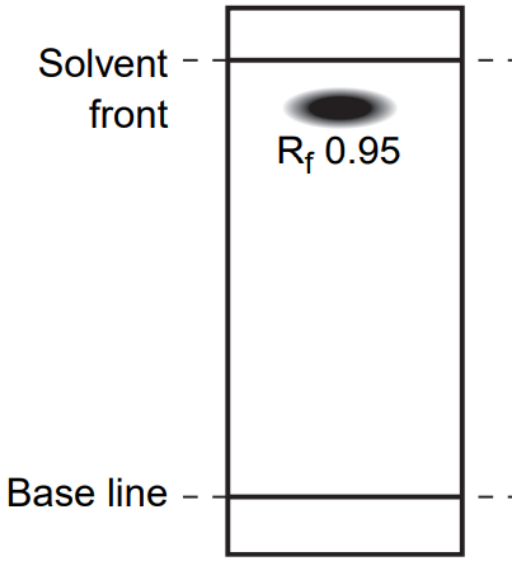
Flash Chromatography Essentials

➢Some definition :

The Retention Distance Rf : 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:

Rf = 4.75/5



➢Some definition :

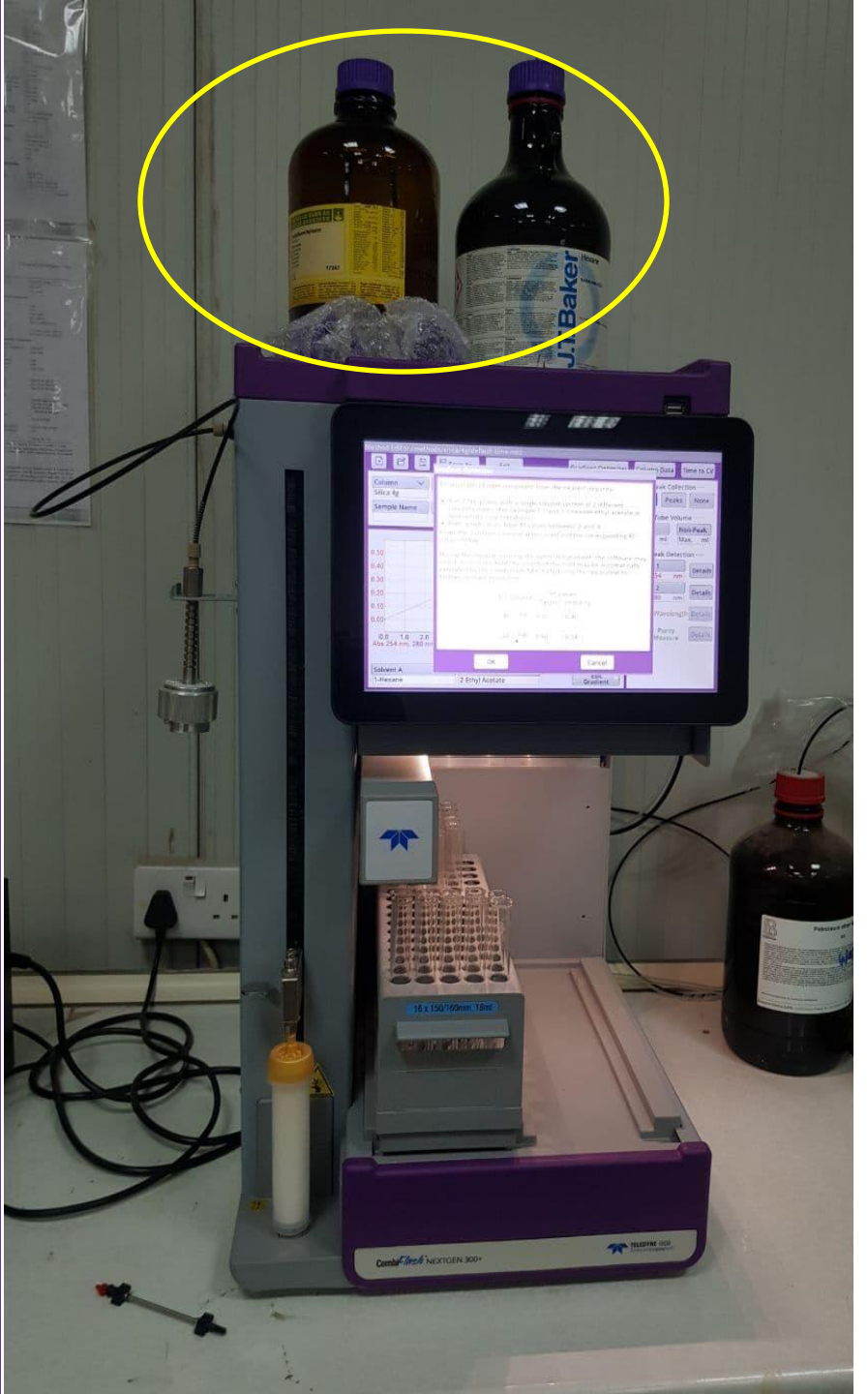
column volume (CV):

- 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)***

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

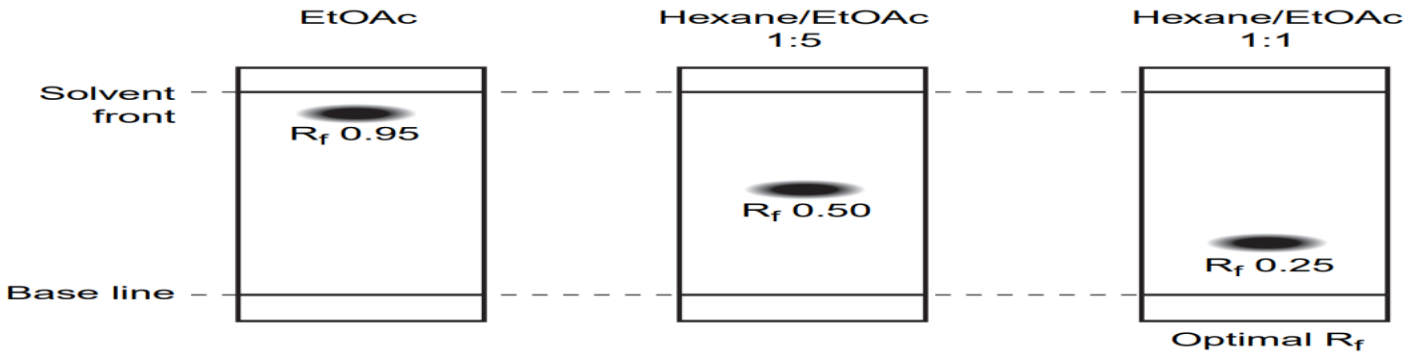
MOBILE PHASE



Mobile Phase:

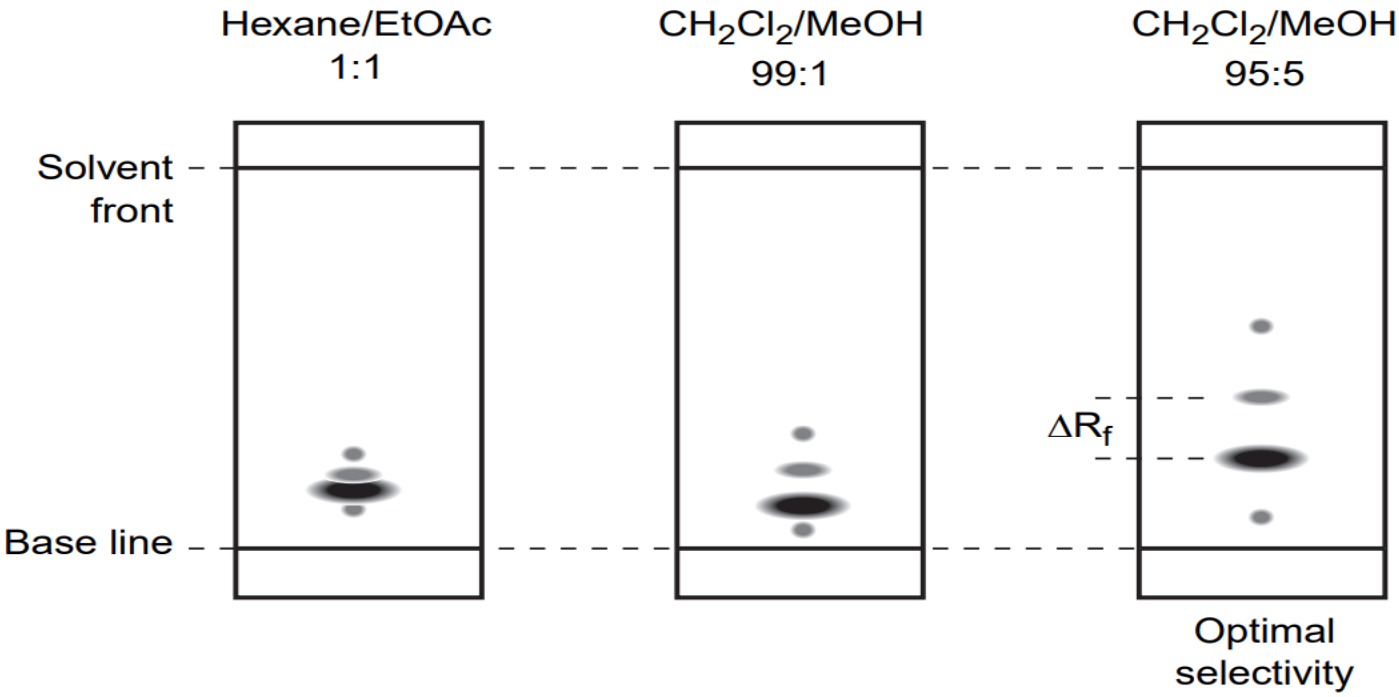
- Dependent on **the polarity of the product(s)** to be isolated and **the type of stationary phase to be used.**
- During the TLC analytical trials, the medicinal chemist will **seek the:**

1. solvent system that moves the desired product **to Rf=0.25±0.05**

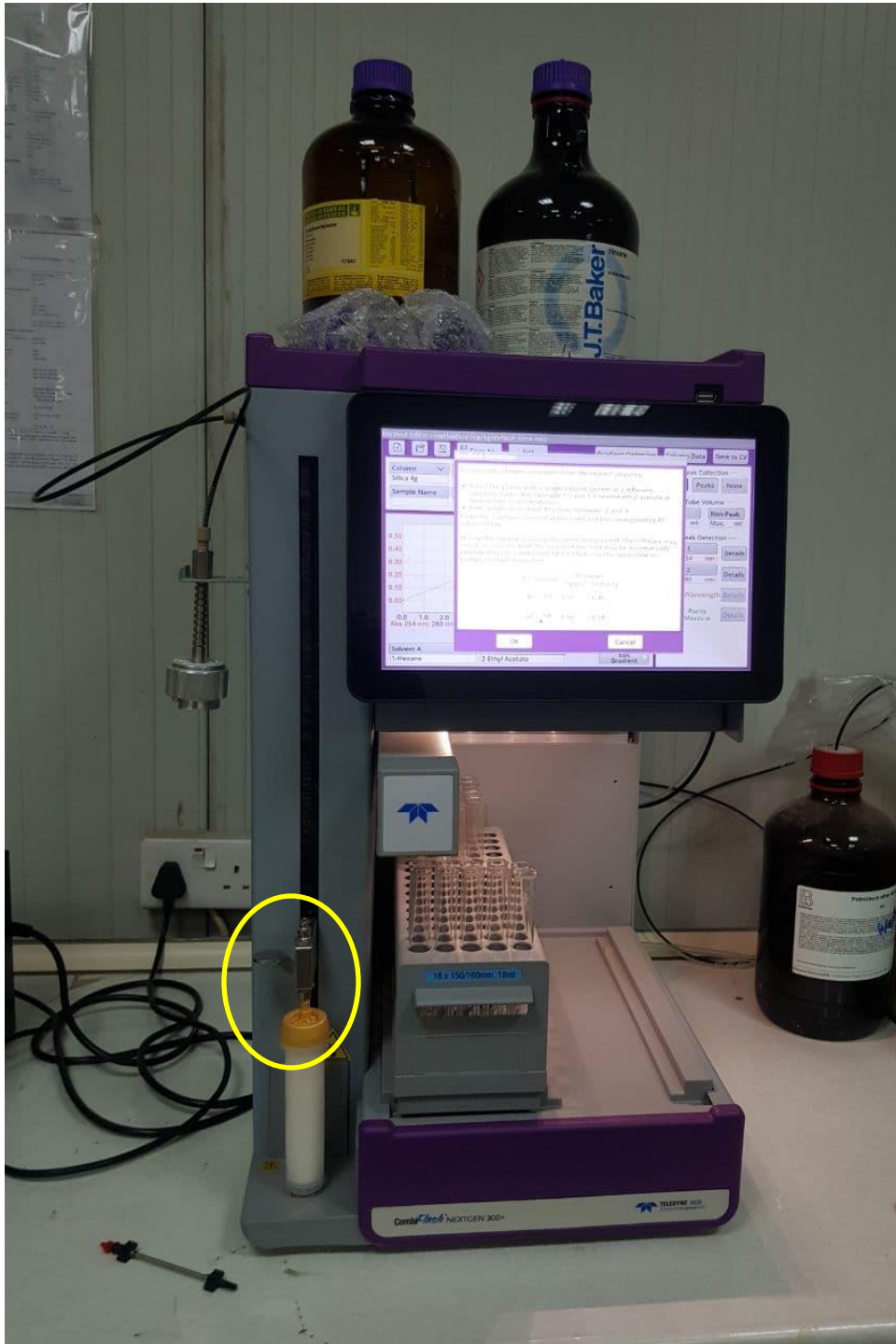


Mobile Phase:

2.and keeps **other undesired products to a distance of at least ΔRf=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**

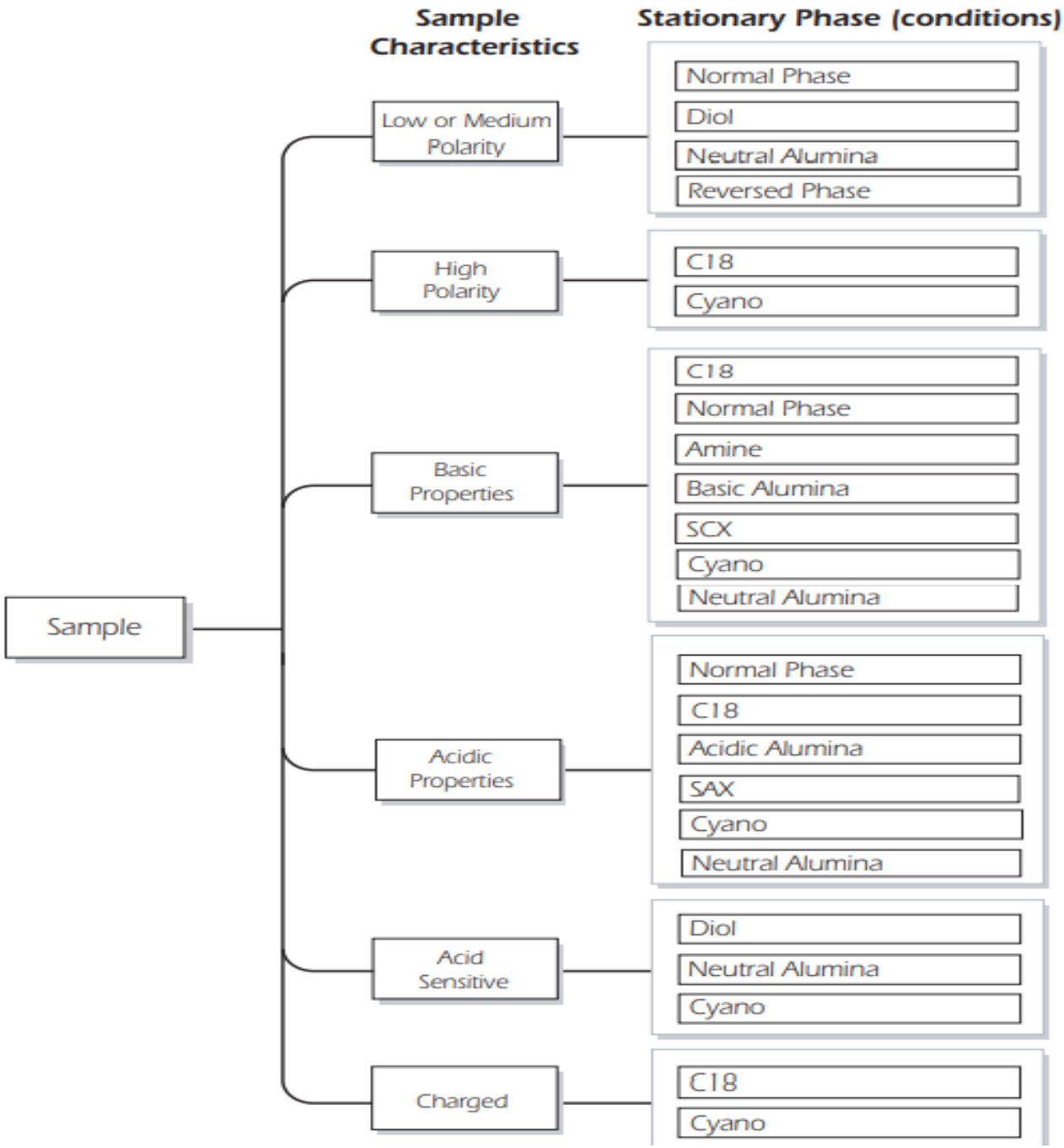
Loading Capacity

2. selectivity :

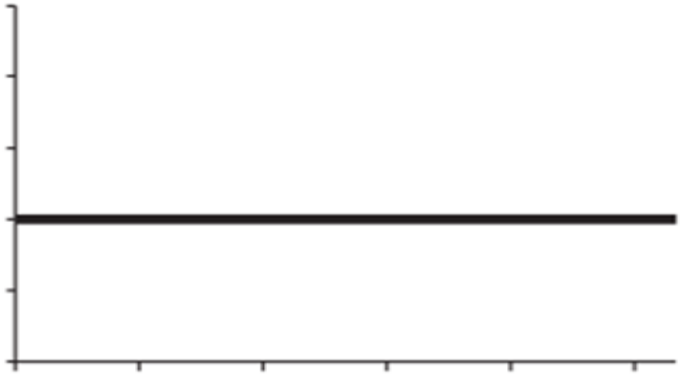
- The selectivity obtained will determine the sample loading capacity on the column.
- **The lower the retention time (Rf)** and the **higher the selectivity (ΔRf)** between product spots on the TLC plate, **the higher the amount of sample can be loaded.**

	Loading			
	Light	*Moderate*	*Significant*	*Heavy*
Column size (g silica)	*ΔRf < 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

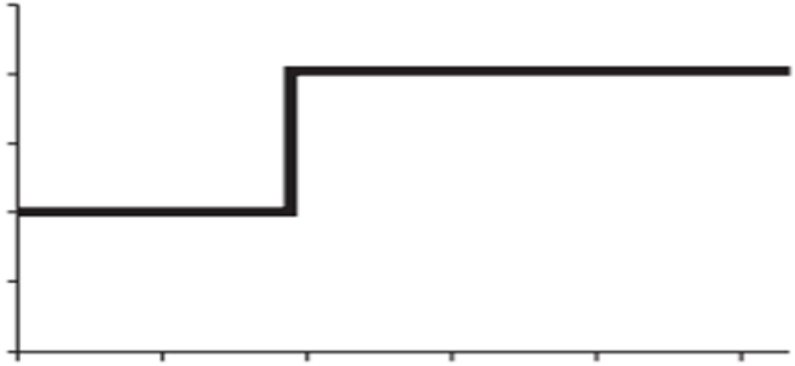
STATIONARY PHASE


Sample Characteristics
Stationary Phase (conditions)
Sample
Low or Medium Polarity
Normal Phase
Diol
Neutral Alumina
Reversed Phase
High Polarity
C18
Cyano
Basic Properties
C18
Normal Phase
Amine
Basic Alumina
SCX
Cyano
Neutral Alumina
Acidic Properties
Normal Phase
C18
Acidic Alumina
SAX
Cyano
Neutral Alumina
Acid Sensitive
Diol
Neutral Alumina
Cyano
Charged
C18
Cyano

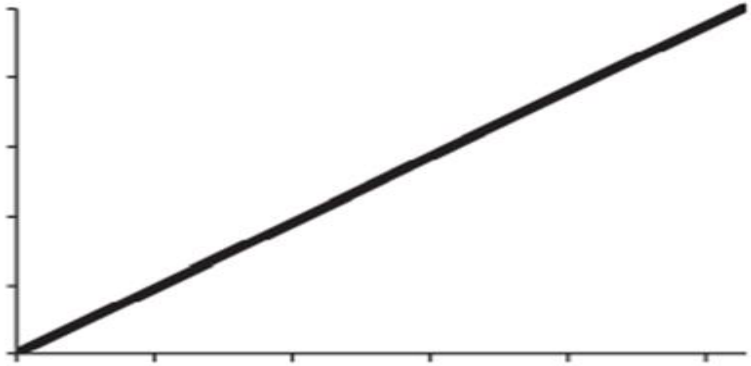
GRADIENT FORMER AND PUMP



Isocratic



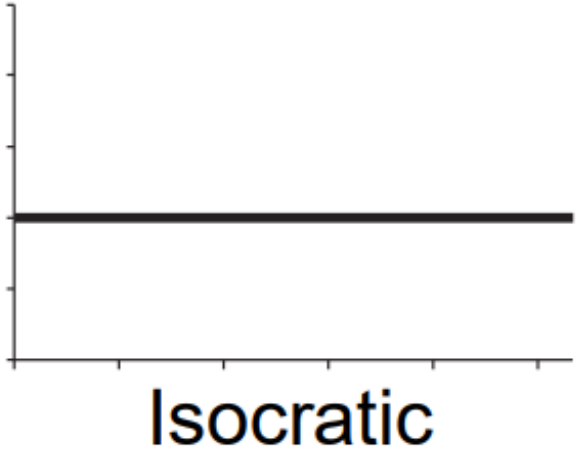
Stepped



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**.

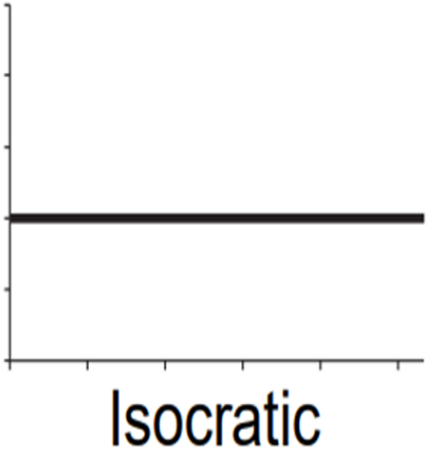
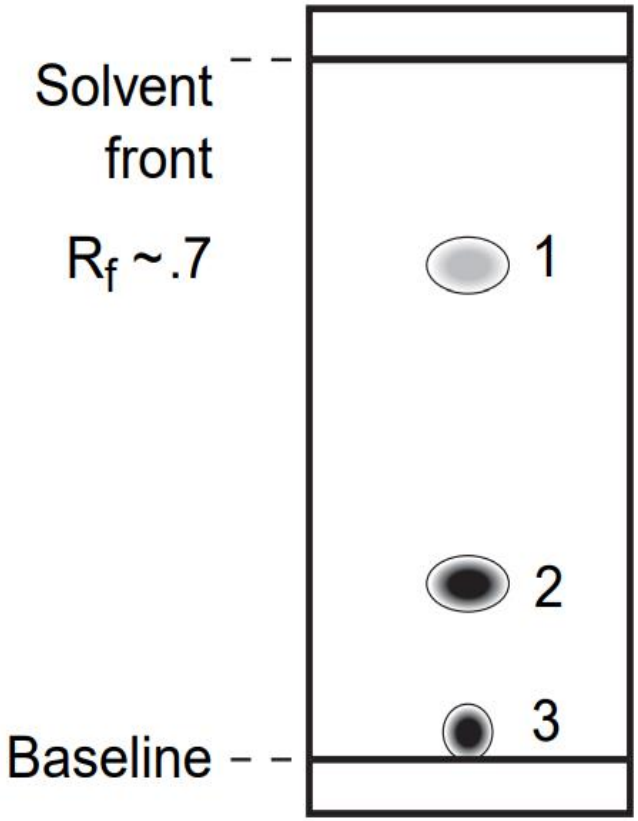


Illustration of isocratic 20% EtOAc in hexane



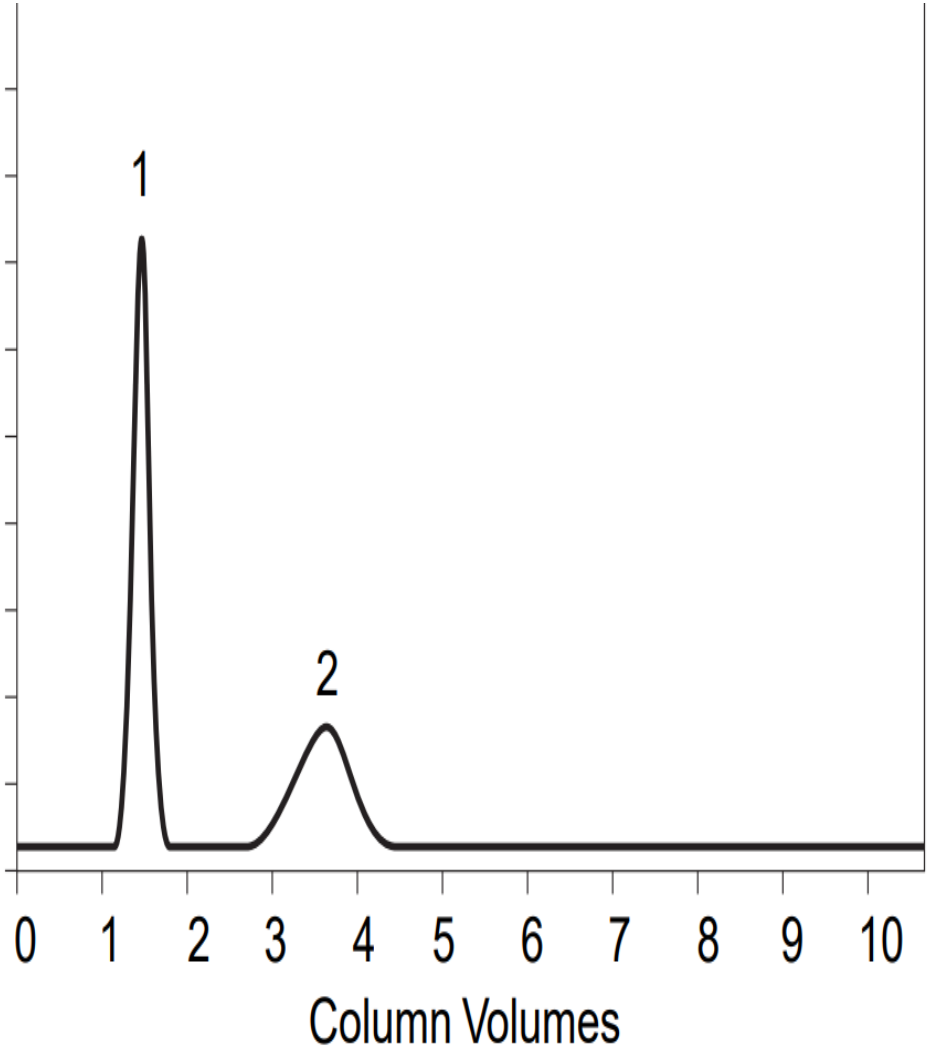
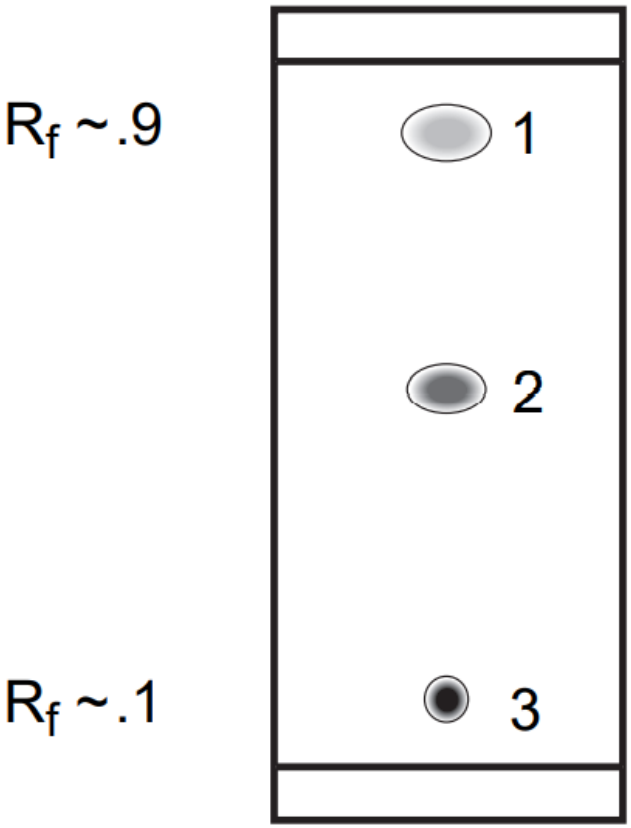


Illustration of isocratic 30% EtOAc in hexane



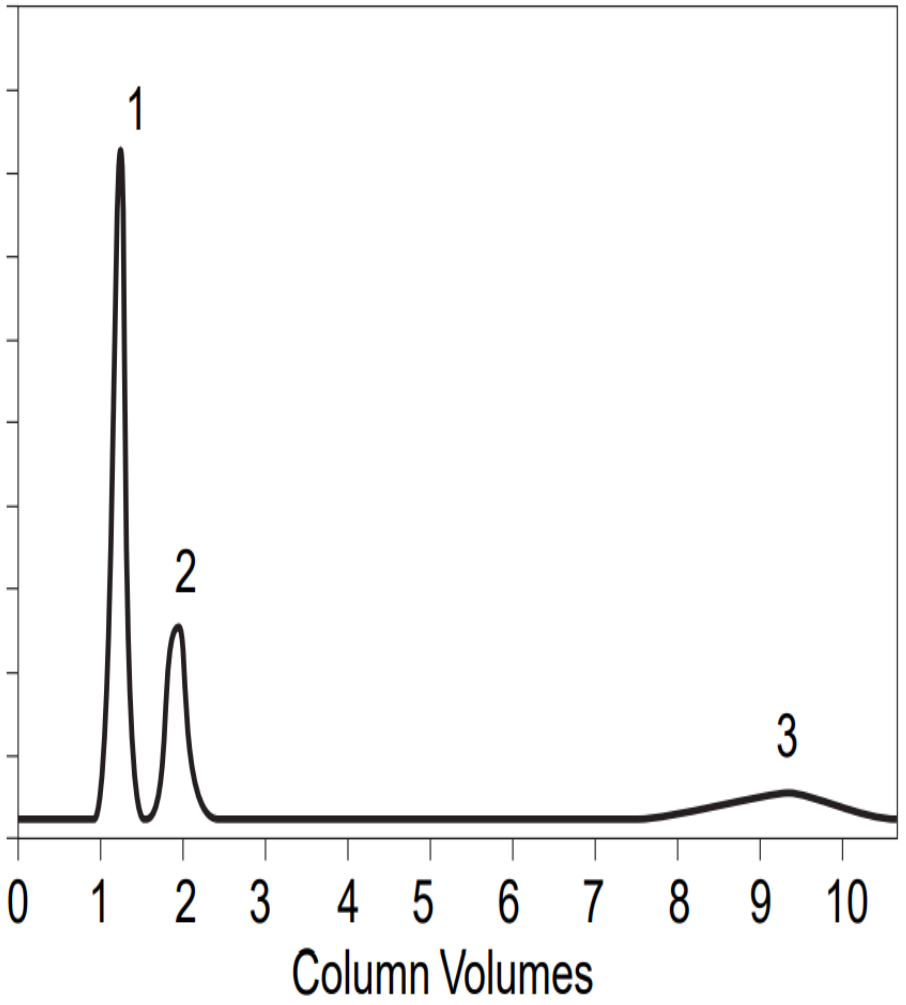
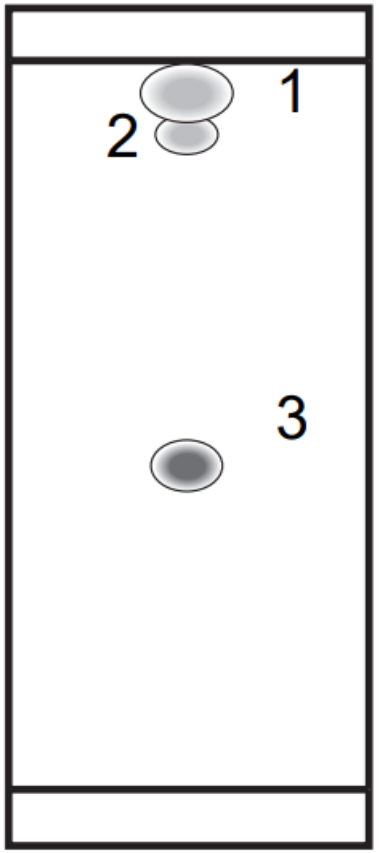
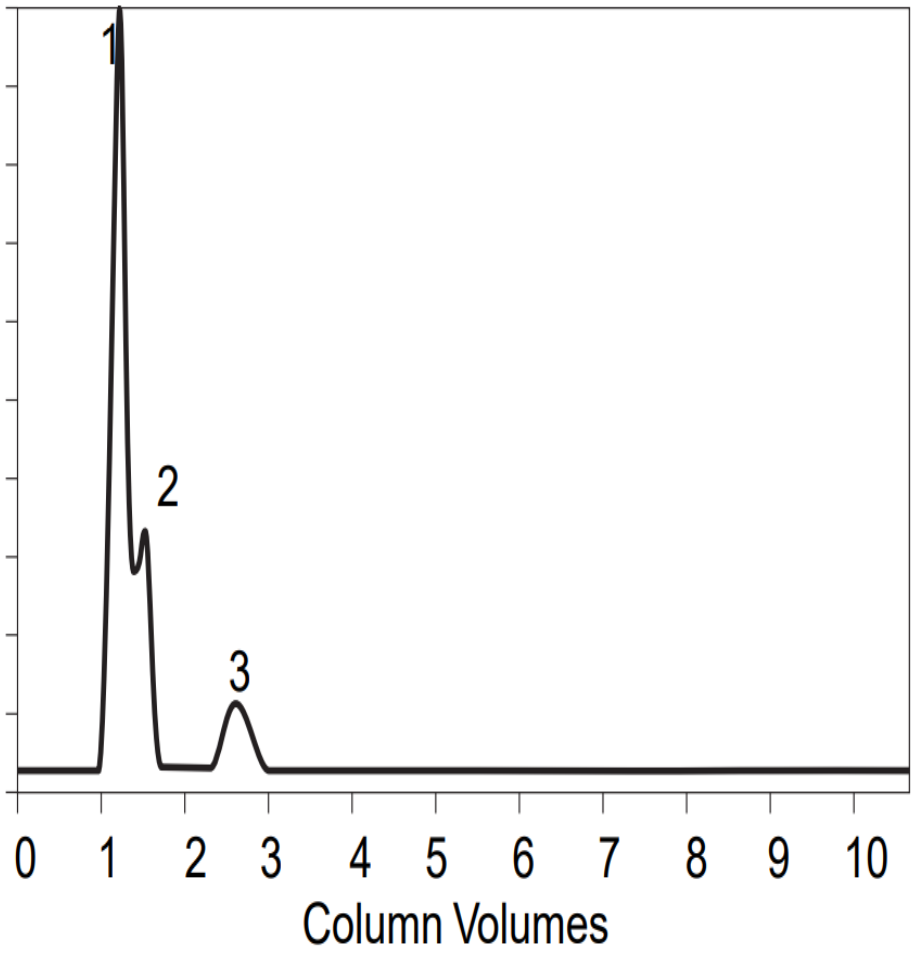


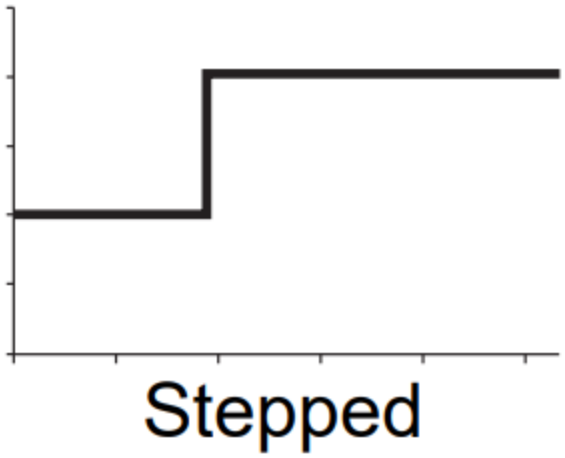
Illustration of isocratic 40% EtOAc in hexane



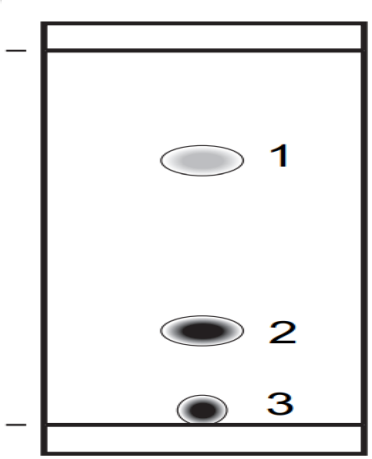


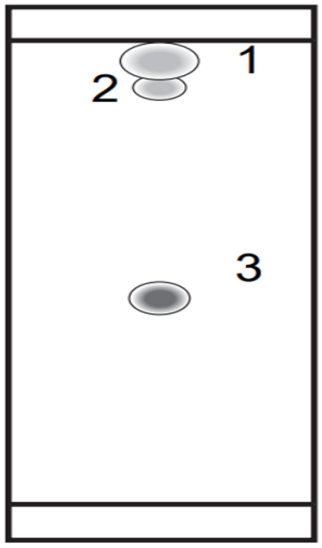
Stepped Gradient

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





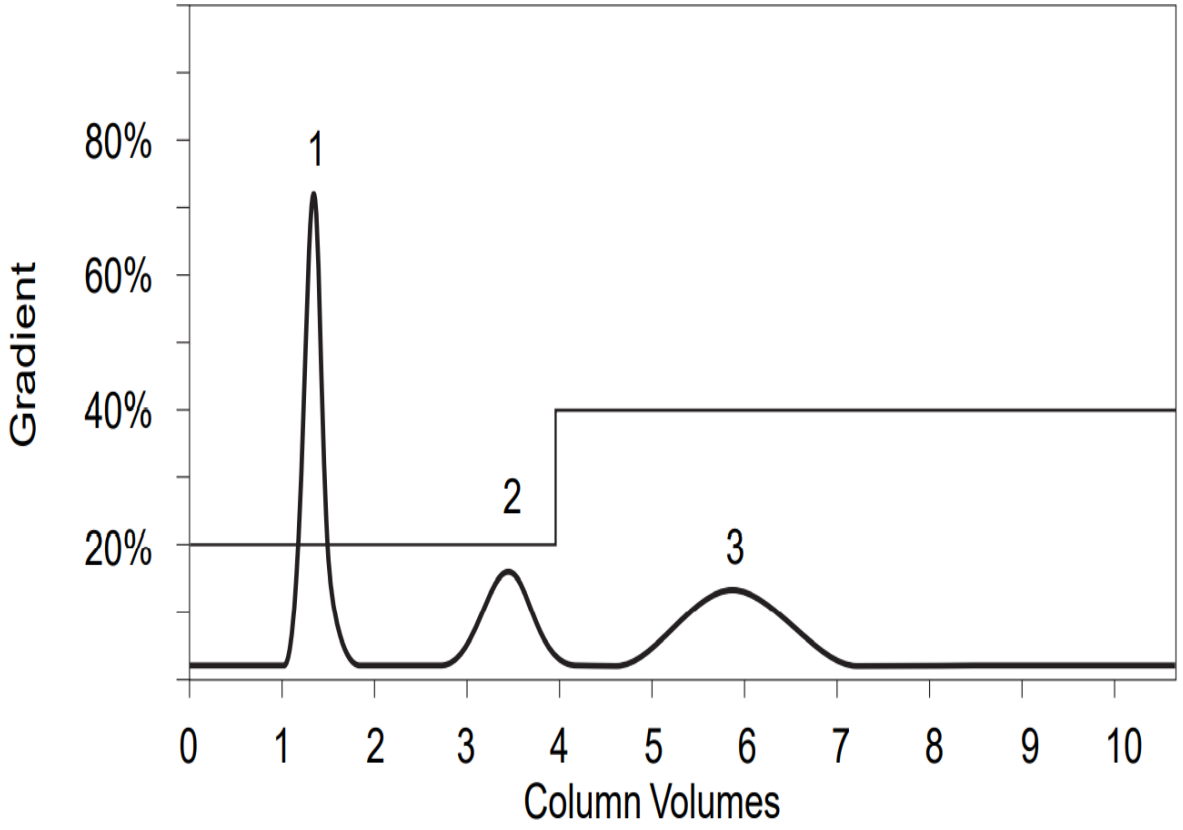


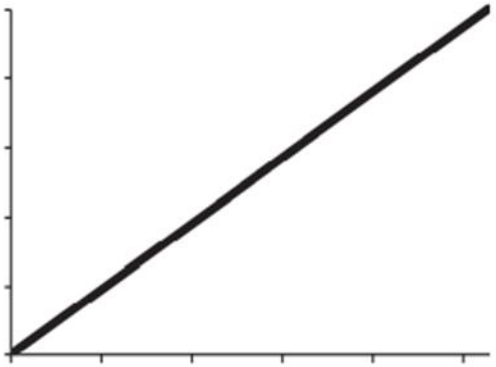
Illustration of a stepped gradient and chromatogram

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

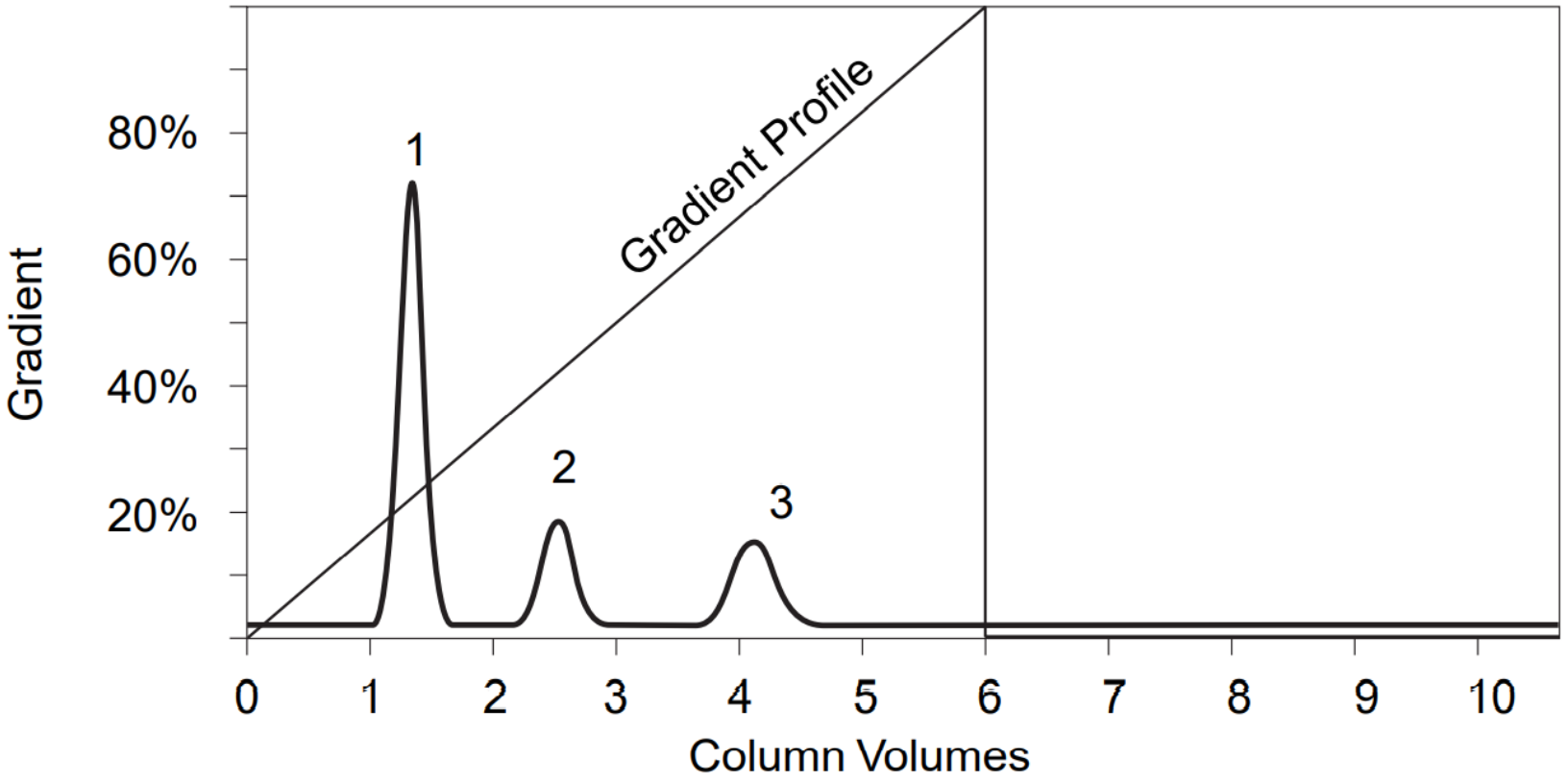


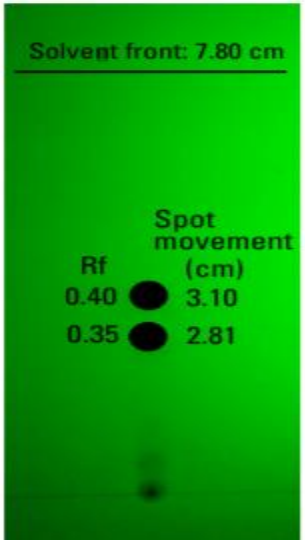
Illustration of a linear gradient and chromatogram

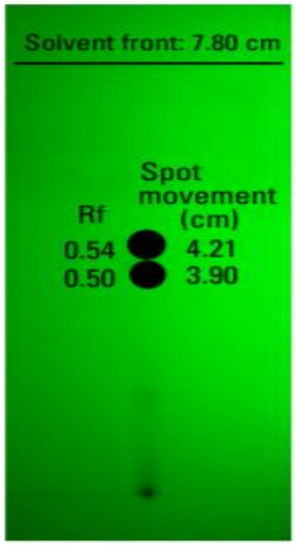
Gradient Optimizer

- 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

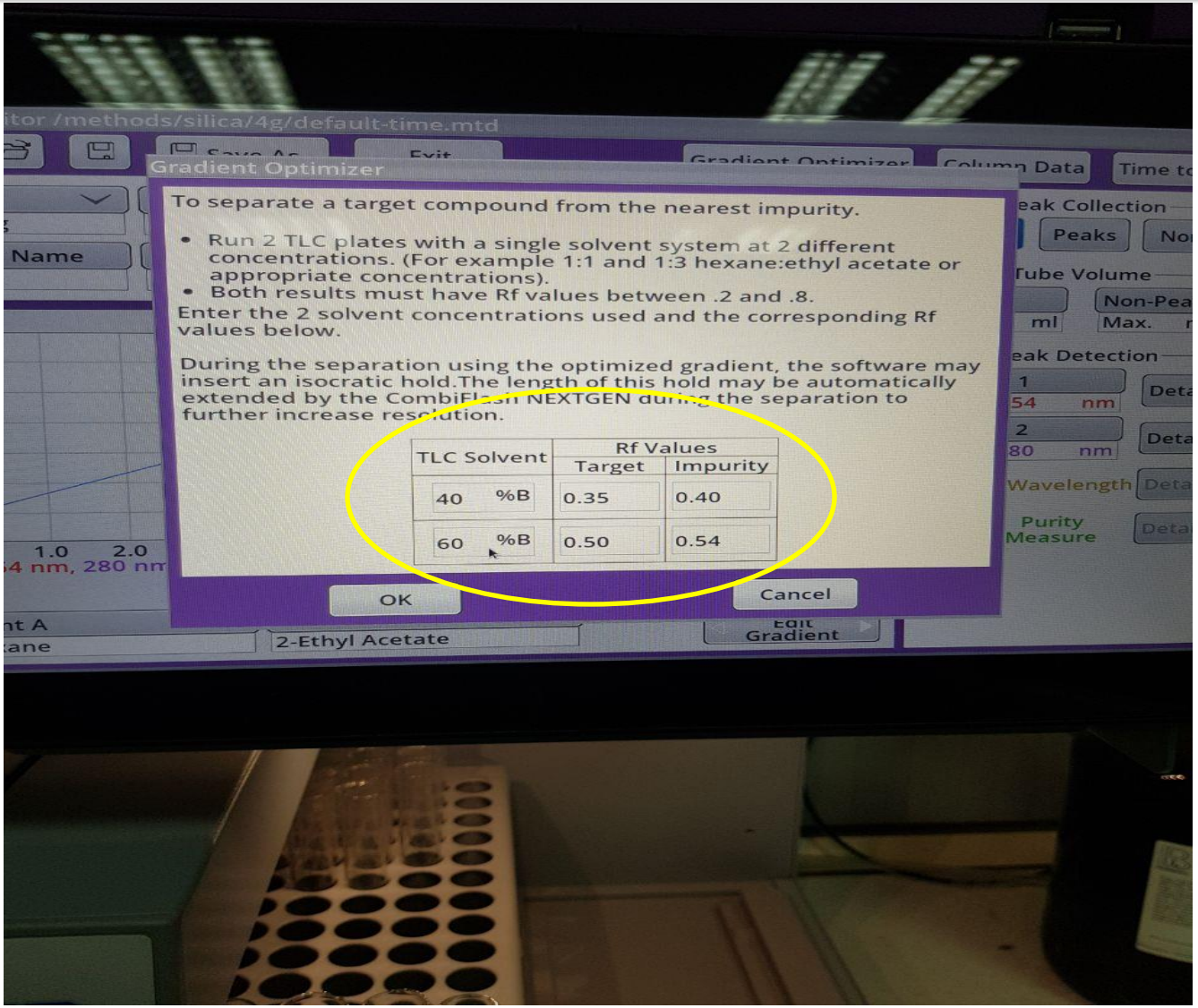
- Run two TLC plates with a single solvent system at two different concentrations.
- For example 40% EA in Hexane and 60% EA in Hexane





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

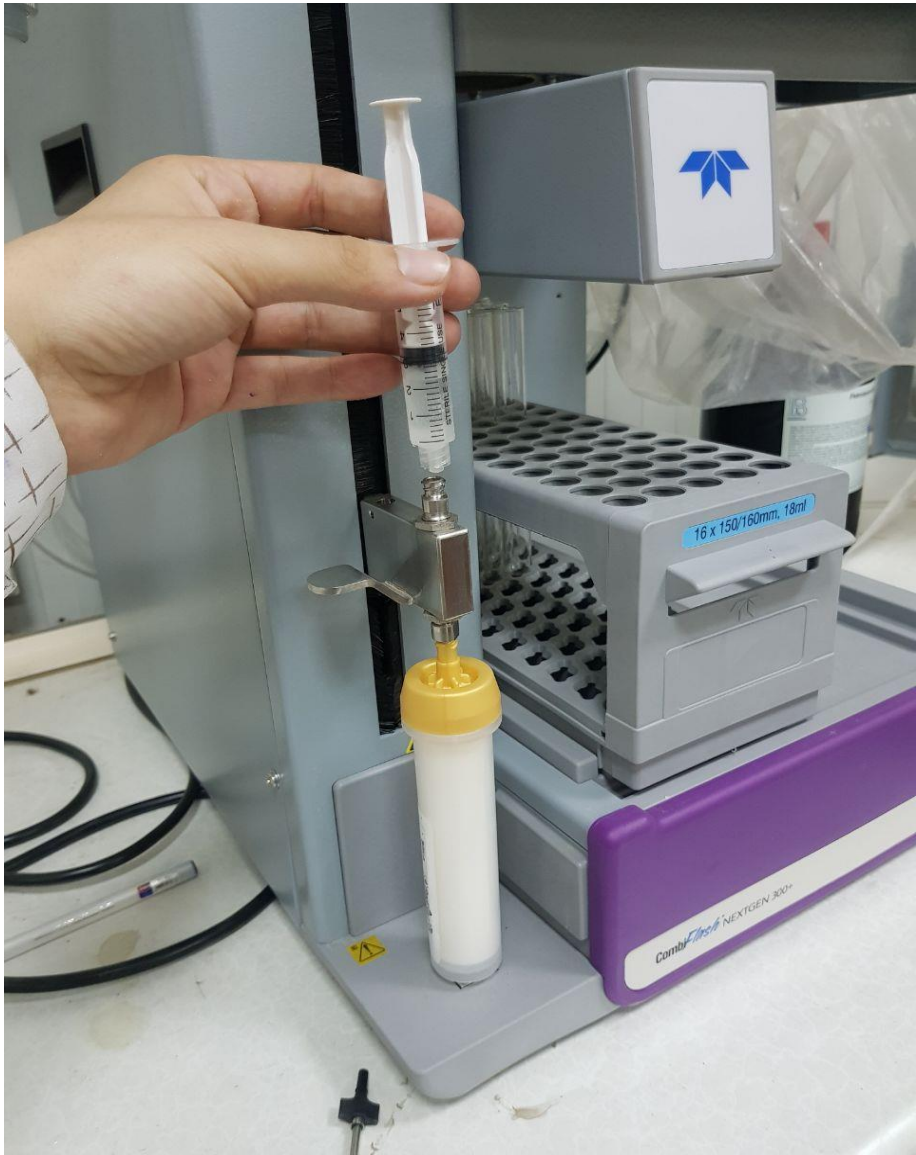
Gradient Optimizer



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**

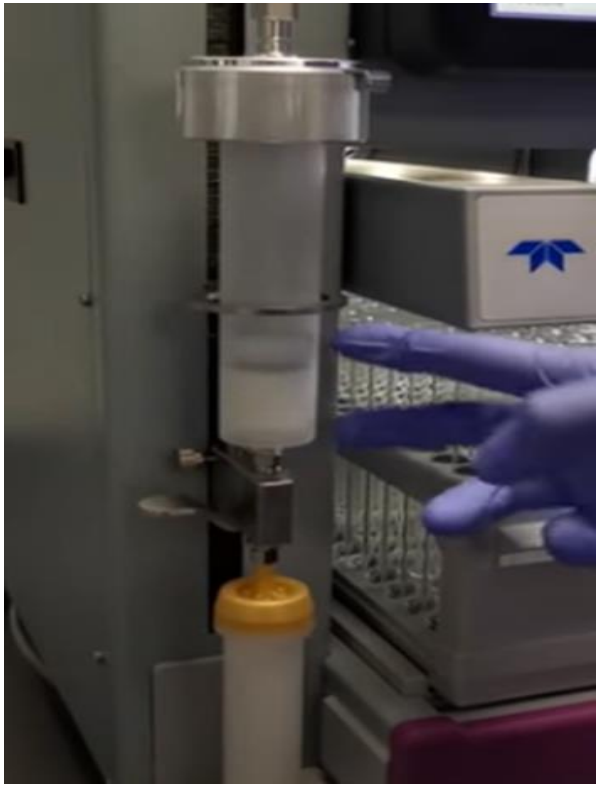


SOLID LOADING

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

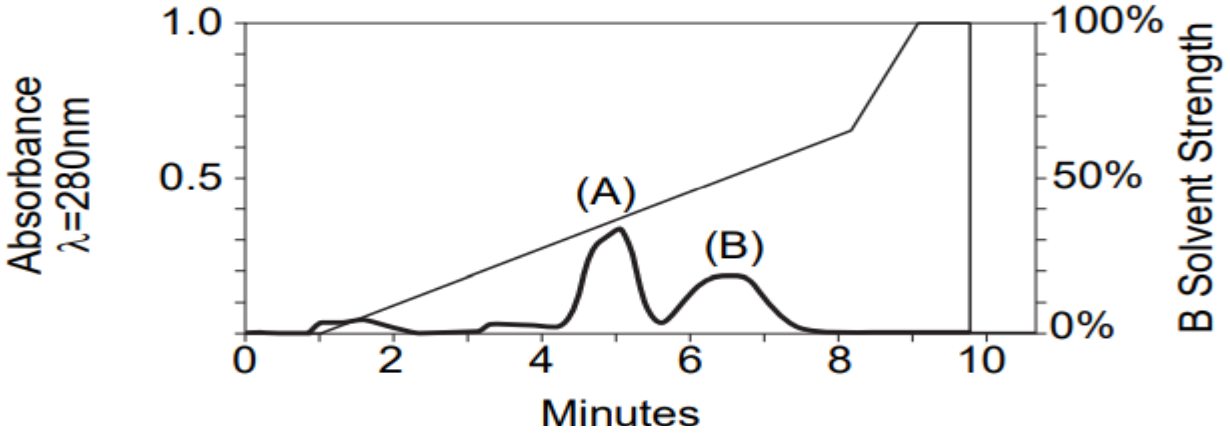
Solid load cartridge :

- **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 .

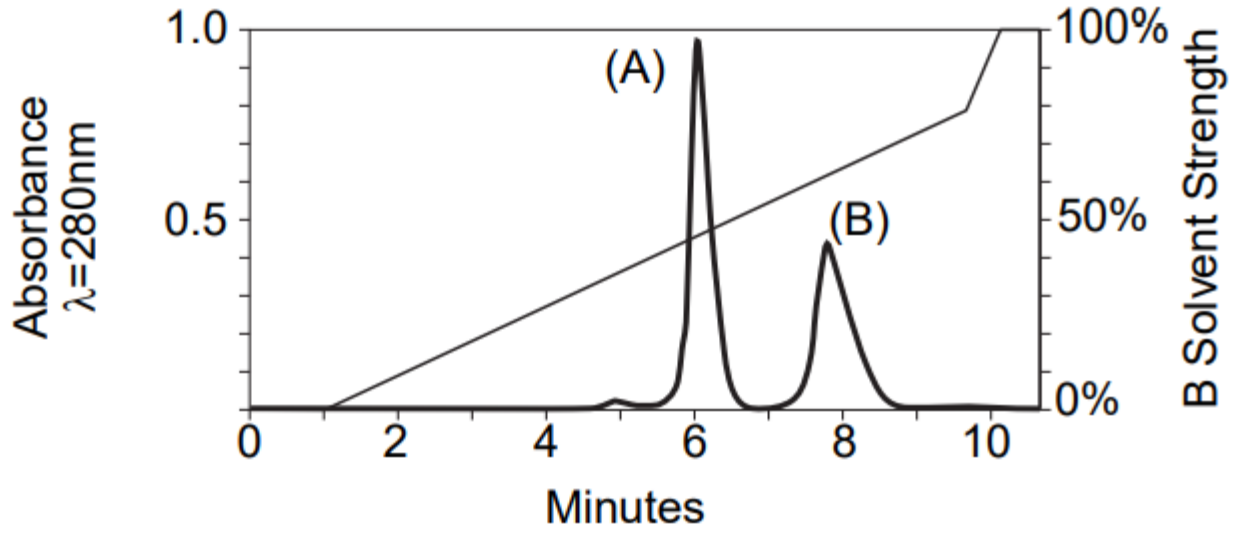


- The dry **SOLID LOAD** cartridge shows ***BETTER RESOLUTION*** than the **LIQUID INJECTION**.

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

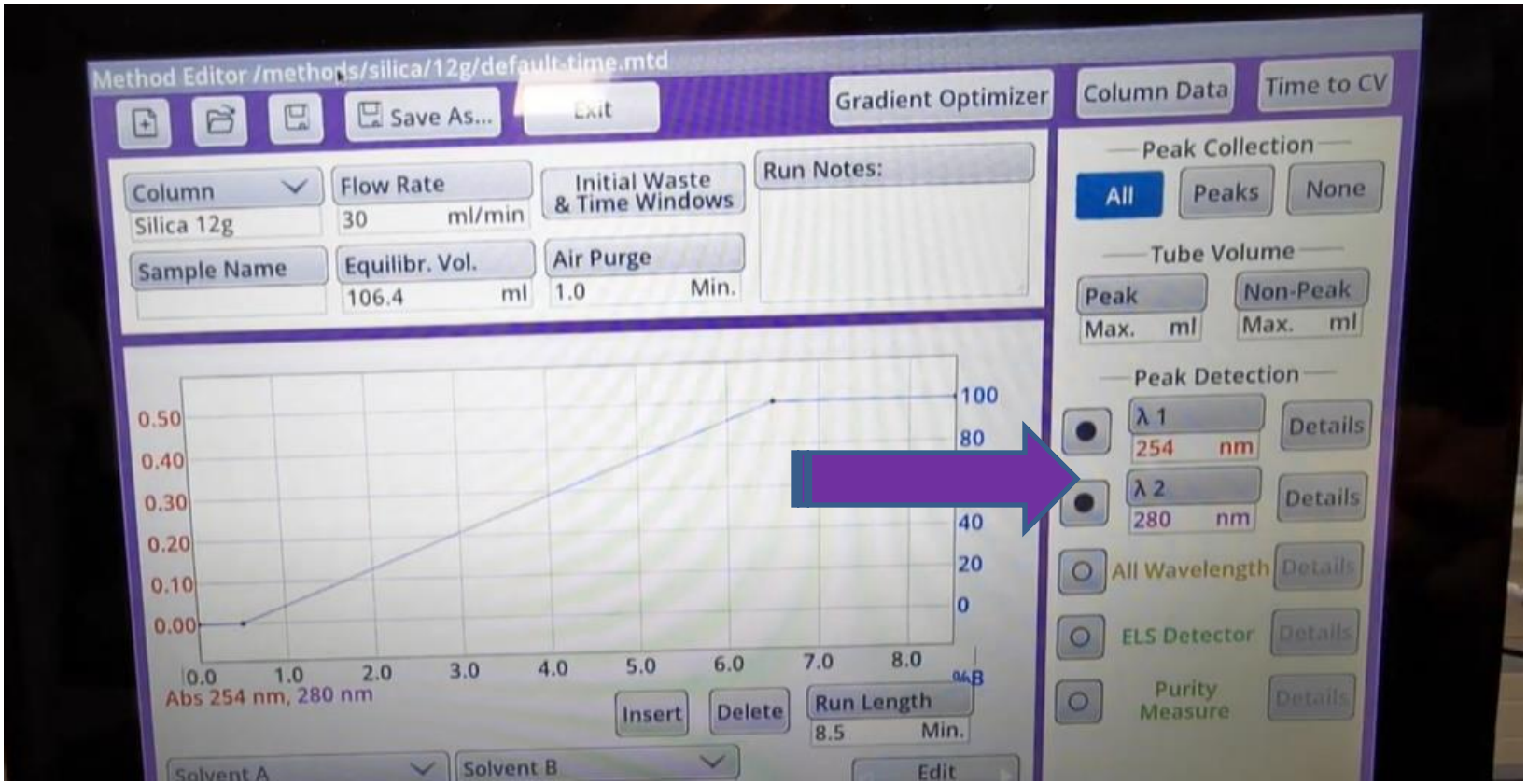
- Enable this option **to monitor or detect compounds with a PurIon mass spectrometer** system (PurIon systems only).

UV and UV-Vis Detection

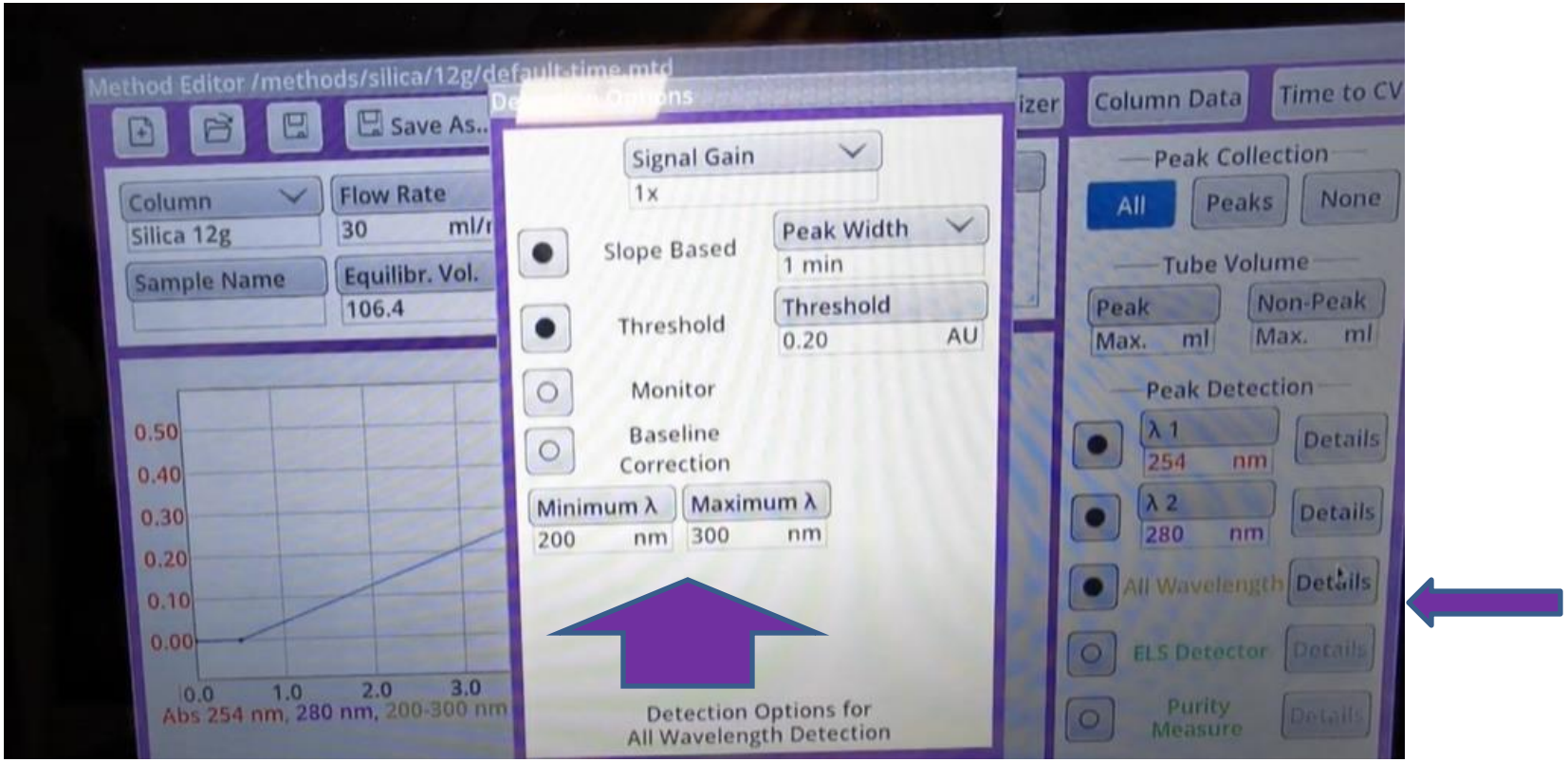
- **Non-destructive technique**
- **UV (200-400 nm) or UV-Vis (200-800 nm) configuration**
  - Requires a chromophore for detection
- Entire UV spectrum is saved throughout the chromatogram

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



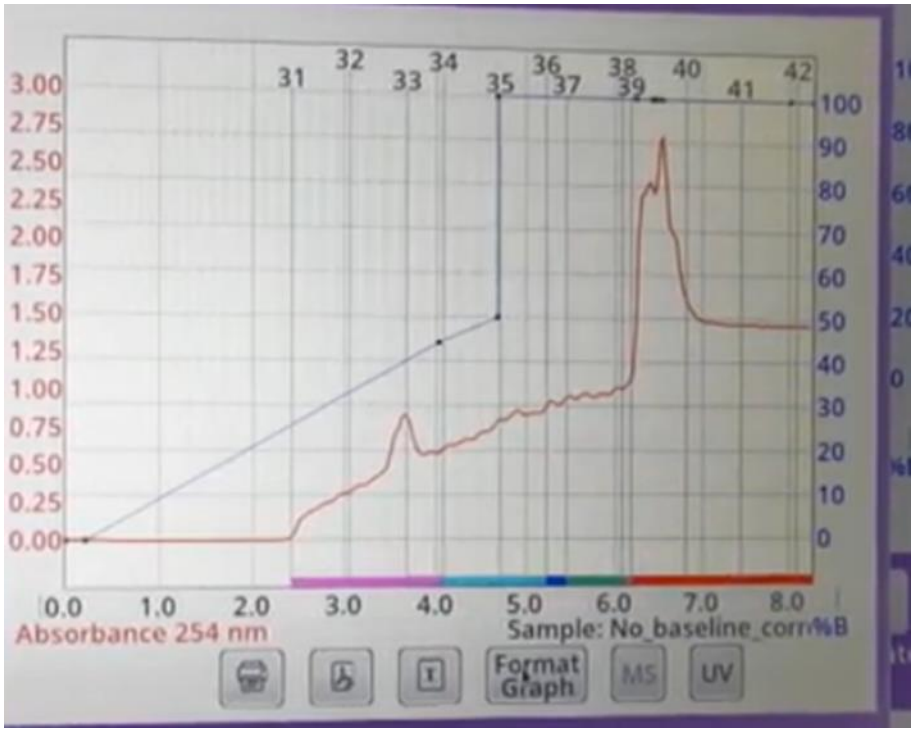
Baseline Correction :

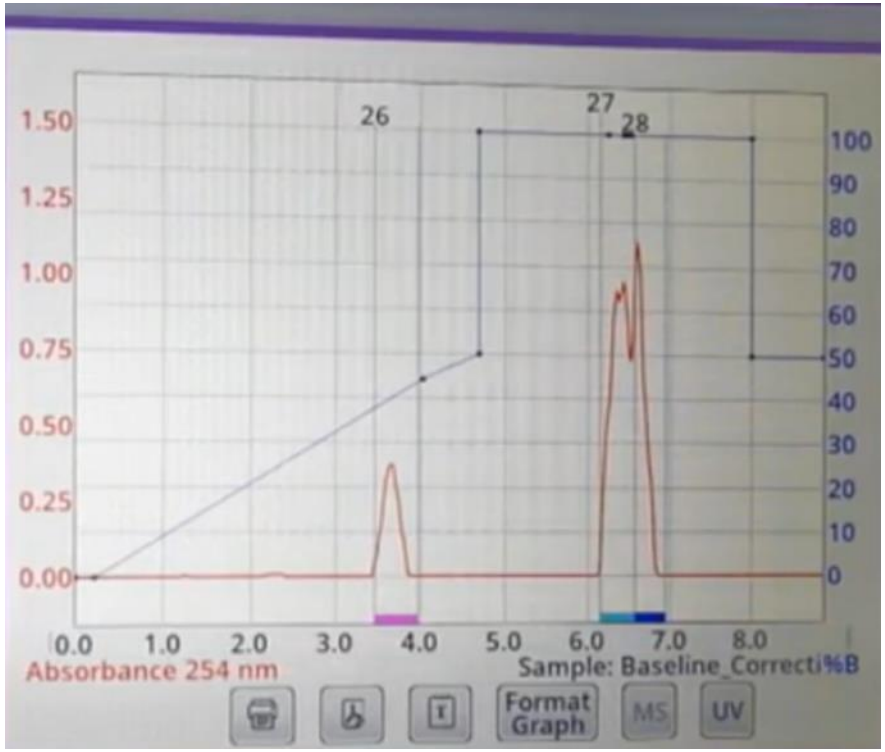
- **Its important when the solvent and the compound that absorb in the same UV region**

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

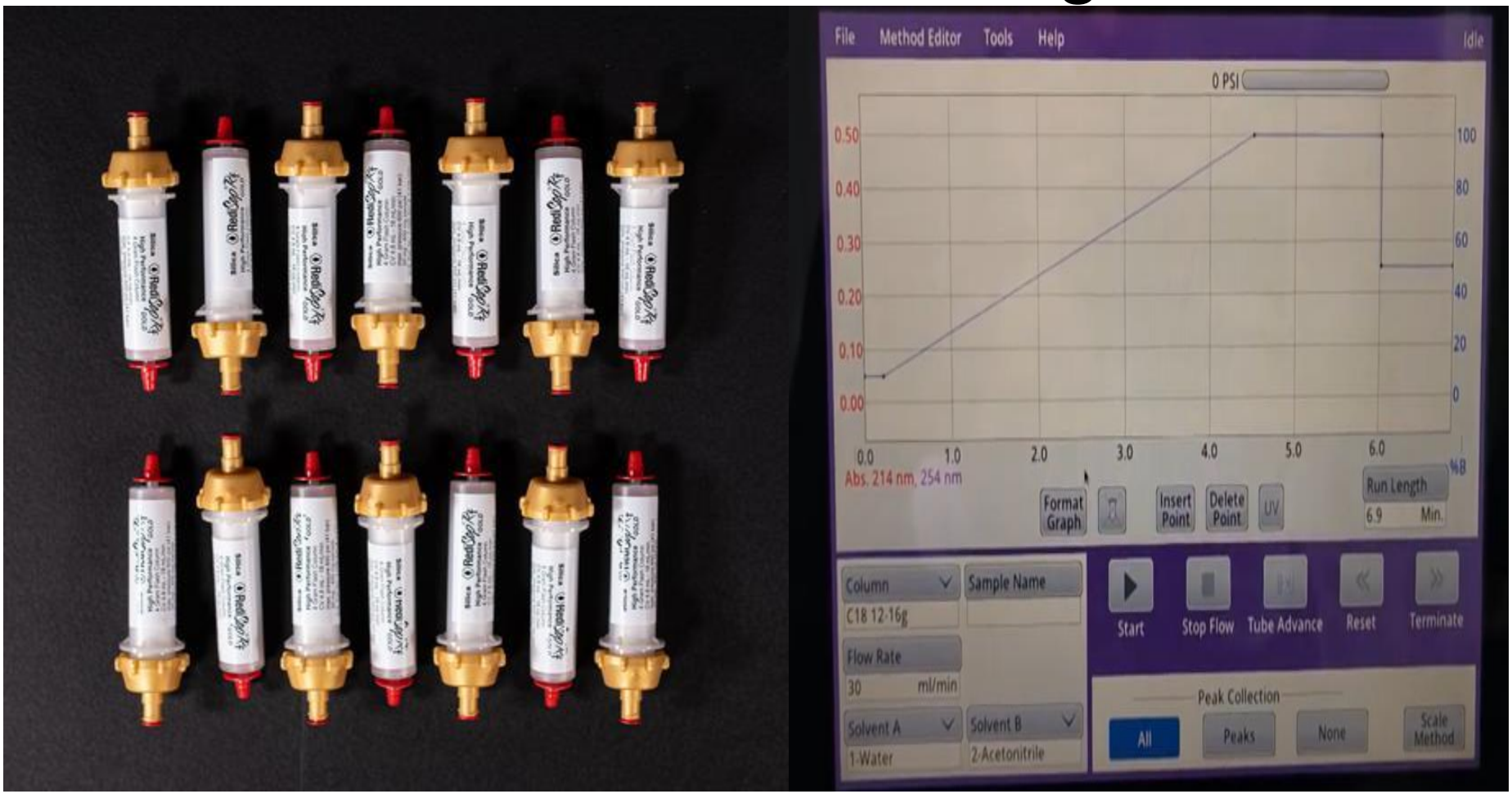




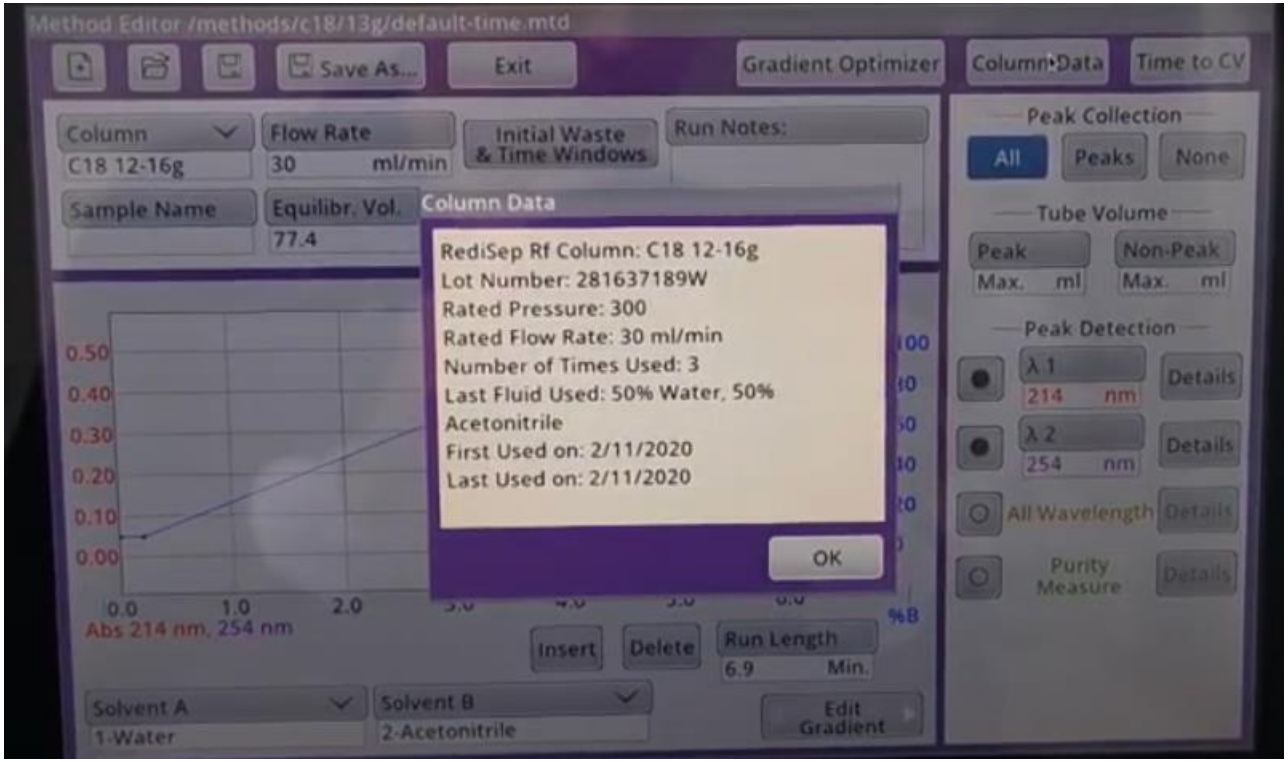
SOME FEATURES OF COMBIFLASH NEXETGEN300+

The Radio-frequency Identification RFID Tags:

- **All columns contain RFID Tag**



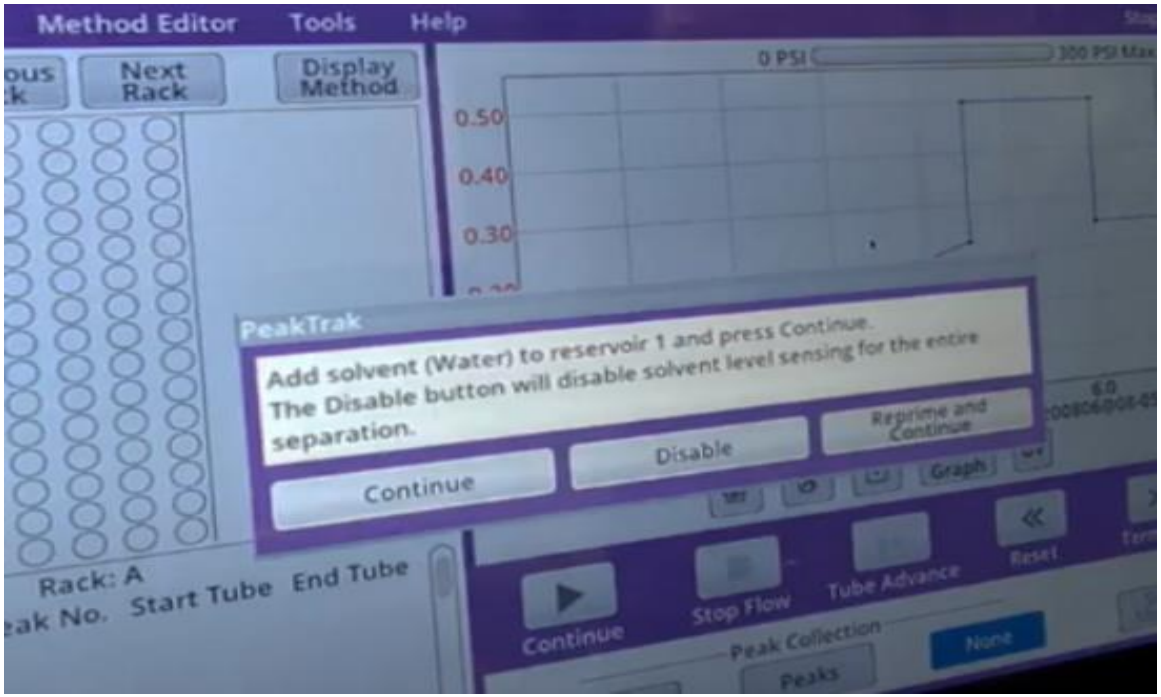
The RFID Tags



SOLVENT LEVEL SENSING:

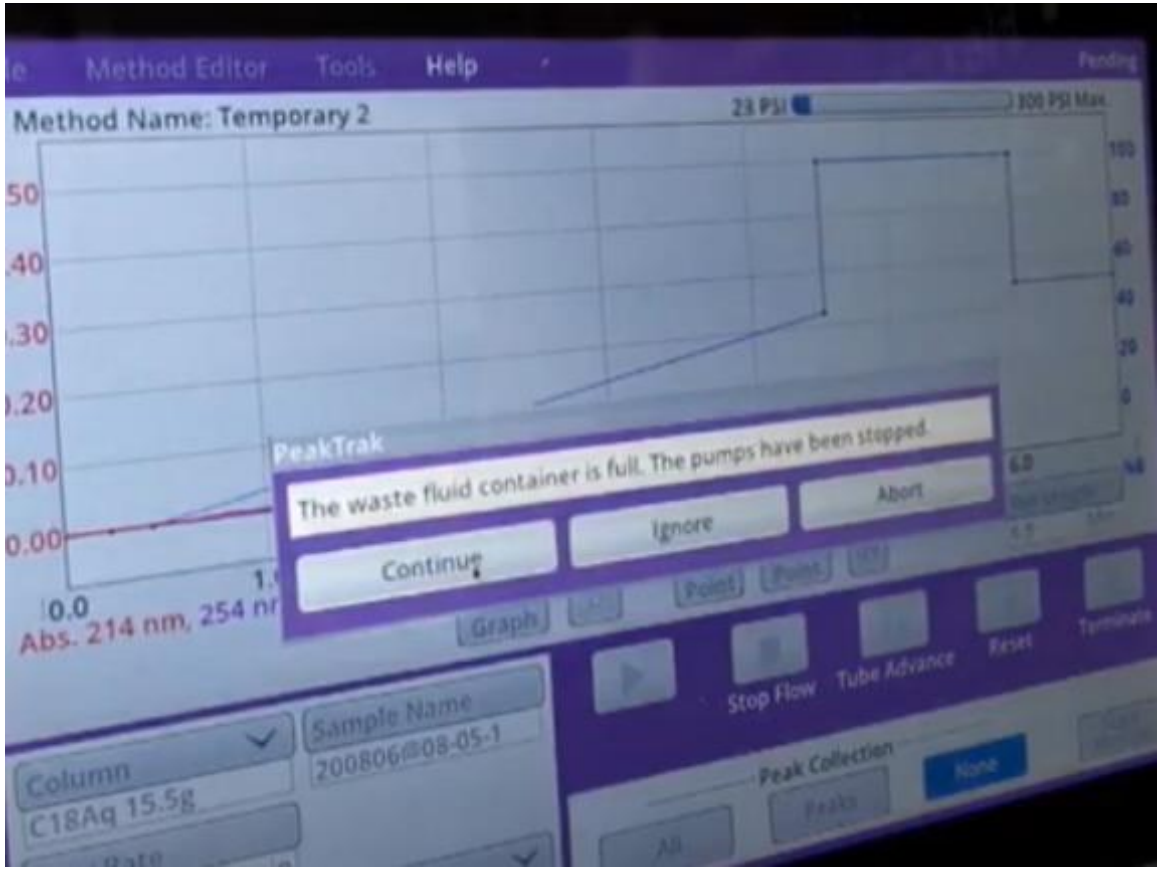
Its actively measure the depth of your inlet **solvent and depth of your waste solvent**.

SOLVENT LEVEL SENSING:



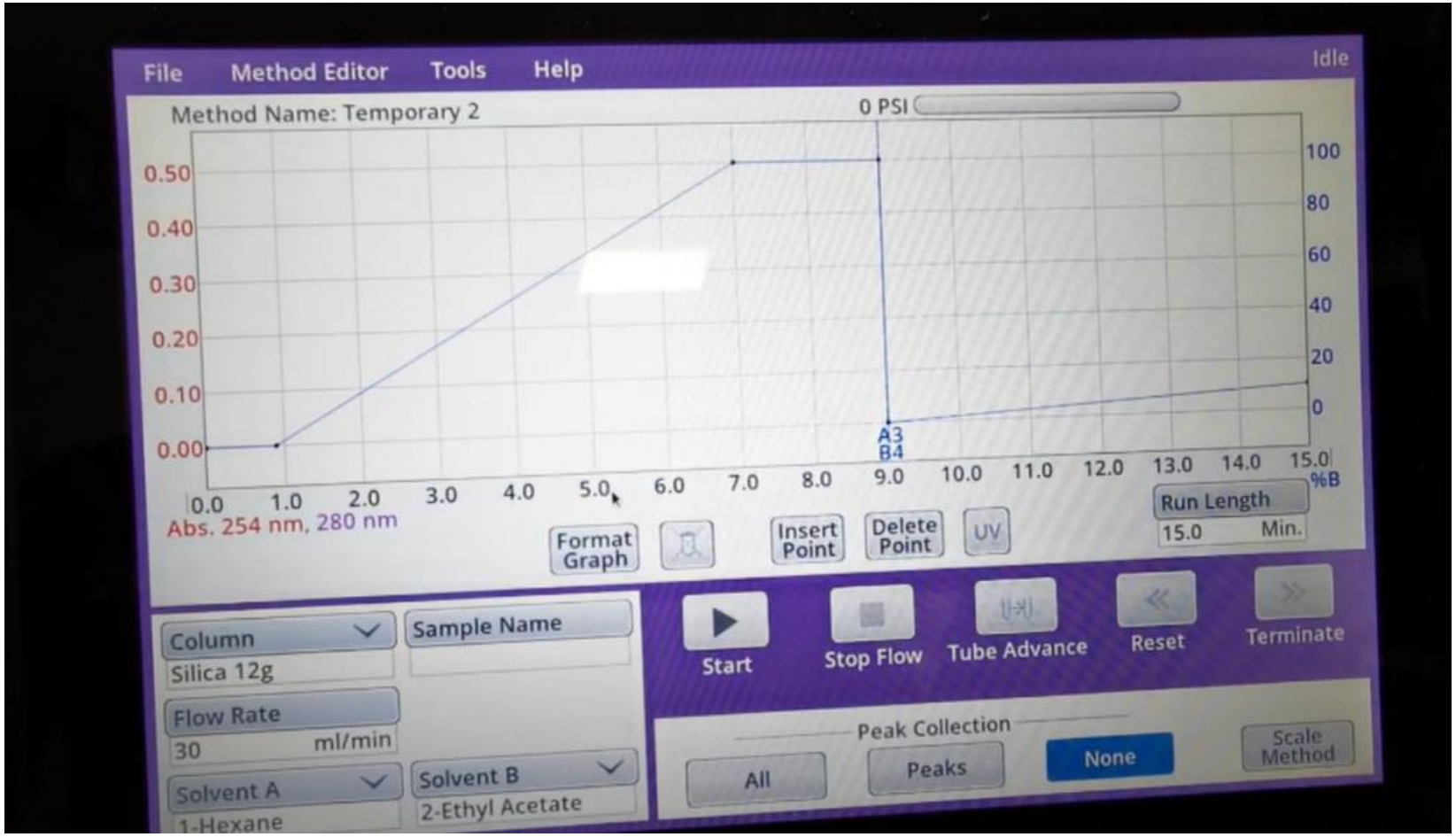
You can not run your columns dry

SOLVENT LEVEL SENSING:



Can not overfill your waste

Use the 4 Solvent Lines



THANK YOU