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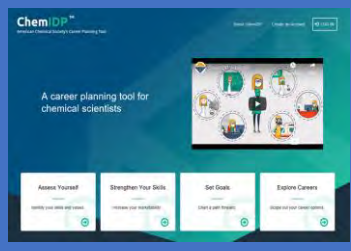

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ACS Career Navigator: Your Home for Career Services

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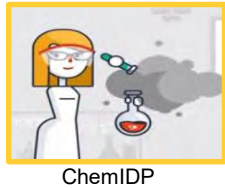
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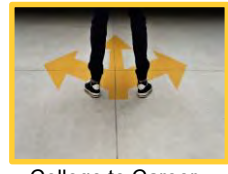
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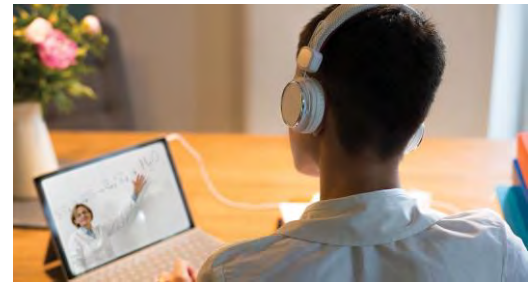

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Date: Wednesday, June 23, 2021 @ 2-3pm ET
Speakers: Carlonda Reilly, Kennametal / Serban Cantacuzene, AirLiquide / Kathleen Shelton, FMC
Moderator: Rebekah Paul, American Chemical Society

Register for Free!

What You Will Learn:

- Lessons learned from three executives' rise to the top
- Insights on how you can succeed in today's changing job market
- Advice for charting your own career in the chemical enterprise

Co-produced with: ACS Industry Member Programs

Date: Friday, June 25, 2021 @ 2-3:30pm ET
Speaker: Sir Fraser Stoddart, 2016 Nobel Laureate in Chemistry, Board of Trustees Professor of Chemistry, Northwestern University and H.N. Cheng, ACS President
Moderator: Young-Shin Jun, Washington University in St. Louis

Register for Free!

What You Will Learn:

- How mechanically interlocked molecules (MIMs) are easily made and how they can be used inn the construction of artificial molecular machines (AMMs)
- How AMMs operate under kinetic control using energy ratchets in a manner similar to that employed by our many biomotors and are at odds with how machines operate in the macroscopic world: the difference could not be more stark!

Co-produced with: ACS Committee on Science

Date: Wednesday, June 30, 2021 @ 2-3pm ET
Speakers: Caroline Trupp Gil, American Chemical Society / Karen Garcia, American Chemical Society / Carl Maxwell, American Chemical Society
Moderator: Lauren Posey, American Chemical Society

Register for Free!

What You Will Learn:

- How the Biden Administration and 117th Congress are shaping up in terms of its STEM priorities
- Which specific pieces of legislation or federal policies will be likely to impact ACS members
- How members can become involved

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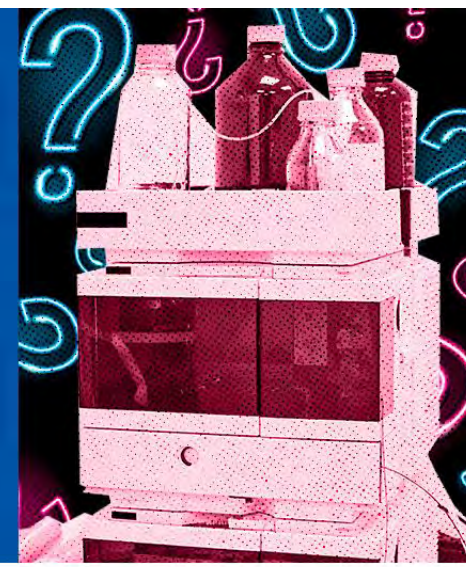
Mastering HPLC Method Development

What are all those buttons for?



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Mastering HPLC Method Development: What are all those buttons for?

LEE POLITE
President and Founder,
Axion Analytical Labs, Inc. and the Axion Training Institute, Inc.

BRYAN TWEEDY
Senior Portfolio Manager, ACS Learning and Career Development, American Chemical Society

Presentation slides are available now! The edited recording will be made available as soon as possible.

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HPLC: *The World's Most Powerful Separation Tool*

- HPLC works by separating complex mixtures into pure compounds
- Why do we separate?
- We separate in order to:
  - Identify – What is present in the sample
  - Quantify – How much is present in the sample
  - Purify – Isolate a compound from the sample
- But step one is always to separate!
- Most people expect HPLC separations to be really complicated, but there are only 3 parameters that affect the separation!
- **And here's the best part**: YOU are in charge of those 3 parameters, so YOU are in charge of the separation.
- **So let's take a closer look** at these 3 parameters and how to set them properly.

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HPLC Master Resolution Equation

$$R_s = \left(\frac{k}{1+k}\right) x \left(\frac{\alpha - 1}{\alpha}\right) x \frac{\sqrt{N}}{4}$$

Resolution	Capacity / Retention Factor	Selectivity	Efficiency ("Peak Skinniness")
R>1.50	1 < k < 5	α > 1.2	Avg ~ 10,000 Max ~ 30,000
Equation	$k = (t_r - t_0)/t_0$	$\alpha = k_B/k_A$	$N = 5.545 \times \left(\frac{t_r}{W_h}\right)^2$
How do you improve it?	Weaken the Mobile Phase: • Increase %H2O by 10% • Double the k!	Function of the Mobile and Stationary Phase, pH, Temp, buffer, additive, etc.	• Longer Column • Smaller Particles • Optimize Flow Rate • Minimize Extra Column Volume

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Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

The definition of good resolution should be greater than or equal to:

- 0.50
- 0.70
- 1.00
- 1.50
- Any of these values

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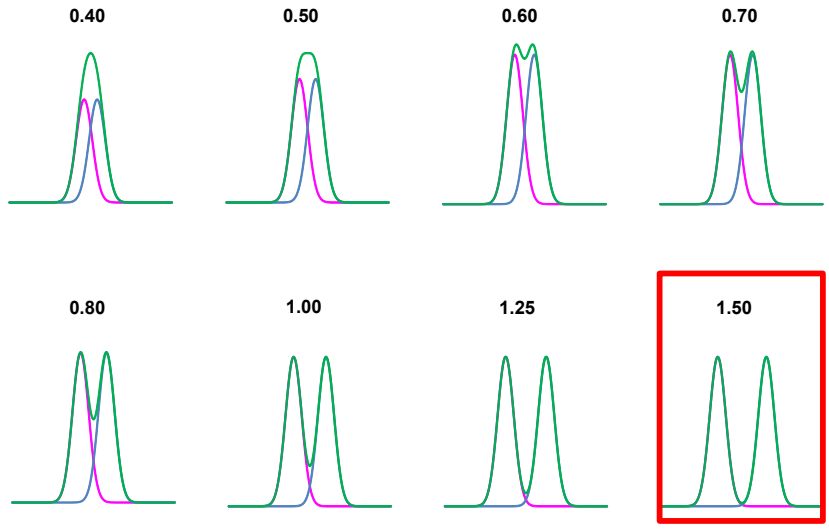
Different Resolution Values



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Method Development Step 1: Maximize Efficiency

$$R_s = \left(\frac{k}{1+k}\right) \cdot \left(\frac{\alpha - 1}{\alpha}\right) \cdot \frac{\sqrt{N}}{4}$$

Capacity Selectivity Efficiency

- Start with the highest efficiency column that you can buy
- Try a 15 cm with 3.5 um particles (~20,000 plates) or
- 10 cm with 1.8 um particles (~28,000 plates) – Requires high pressure
- Note: During method optimization, we may opt for a shorter column
- Column length is proportional to the efficiency, but also to retention time

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Method Development Step 2: *Find the Correct Selectivity*

$$R_s = \left(\frac{k}{1+k}\right) \cdot \left(\frac{\alpha - 1}{\alpha}\right) \cdot \frac{\sqrt{N}}{4}$$

Capacity **Selectivity** **Efficiency**

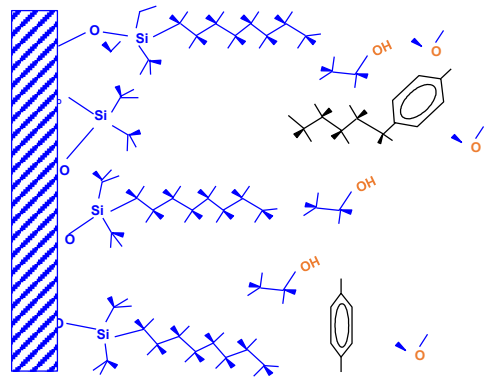
- Choose reversed phase because…
- Approximately 80% of all HPLC separations are carried out in the reversed phase mode!
- Acetonitrile or methanol blended with water on a good C18 column

Choose Reversed Phase Because… *It Just seems to work for most applications!*

Great Science Cartoon courtesy of Sidney Harris

Reversed-Phase Mechanism

- The analytes partition between the non-polar stationary phase and the polar mobile phase
- Relative affinity means there are two dimensions to the separation
- Reversed phase is especially sensitive to minor differences in hydrophobicity
- The addition or subtraction of just about any group leads to hydrophobicity changes: methyl, hydroxyl, amino, carbonyl, acid, etc.



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When to Choose Reversed Phase

- Neutral, polar and nonpolar compounds with a molecular weight less than ~2000
- Homologous series
- Organic acids and bases
- Proteins and peptides

More Challenging to do by reversed phase

- Extremely polar compounds
- Extremely non-polar compounds

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Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

Why do we usually choose reversed phase?

- The mechanism seems to work for most separations
- It allows us to analyze polar and non-polar compounds
- The solvents are less hazardous than normal phase
- To impress my friends at the next cocktail party!
- All of the above

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Method Development Step 3: Optimize Capacity Factor

$$R_s = \left(\frac{k}{1+k}\right) \cdot \left(\frac{\alpha - 1}{\alpha}\right) \cdot \frac{\sqrt{N}}{4}$$

Capacity Factor | Selectivity | Efficiency

- How do you find the correct mobile phase strength?
- Try all of the strengths!... and see where your peaks elute.
- Scouting Run: Gradient from weakest to strongest mobile phase
- Listen to your sample! The peaks will elute at their desired %B
- There are 3 simple rules for finding the correct mobile phase…but first some definitions.

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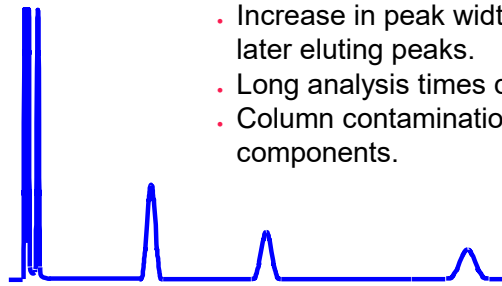
Isocratic Elution (Constant Solvent Composition)

Isocratic 50/50 Water/Methanol



Problems:

- Poor resolution of early eluting peaks.
- Increase in peak width and decrease in peak height for later eluting peaks.
- Long analysis times due to a wide range in k'.
- Column contamination with strongly retained components.

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Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

The first two peaks are coming off together near the void volume (low capacity factor). What should we do to the mobile phase in order to improve the separation?

- Make the mobile phase stronger
- Make the mobile phase weaker
- Slow down the flow rate
- Change the detector lamp

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Gradient Elution

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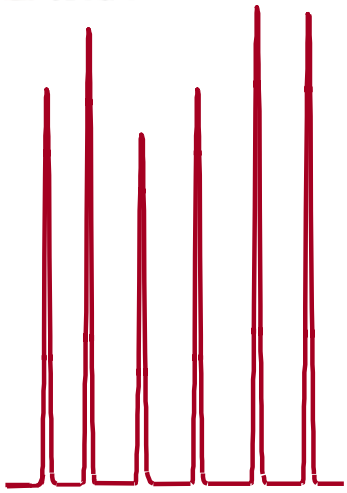

Gradient from 10-100% Methanol

Gradient Elution - Mobile phase composition is changed (strengthened) during the separation.

Advantages
- Improved *overall* resolution
- Increased detection
- Ability to separate complex samples
- Shorter analysis times
- Decrease in column deterioration due to strongly retained components

Other Uses
- Column Cleaning
- Scouting run in method development

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3 Simple Gradient Rules

10% Organic

100% Organic

3 Important Rules for Setting Gradient Parameters

1. Initial Composition – Must be weak enough to give the first peak a k' of at least 1.0
2. Final Composition – Must be strong enough to elute the last peak from the column
3. Gradient Steepness – The longer the gradient, the higher the resolution, but it takes longer. Max 30 min.

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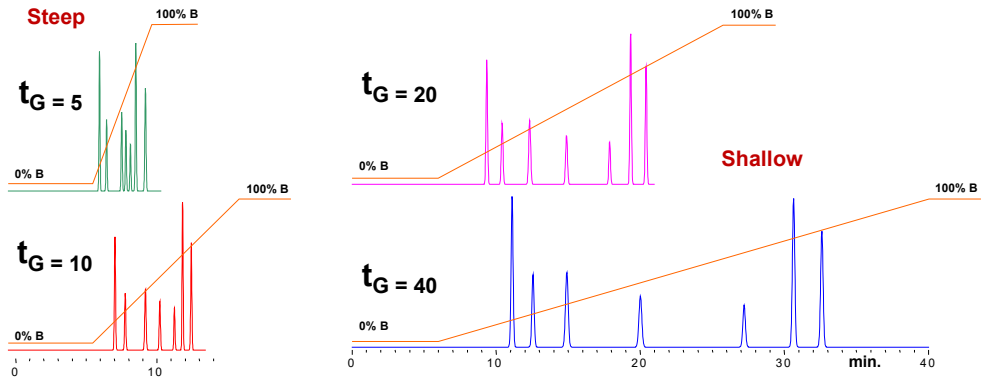
Select Gradient Steepness



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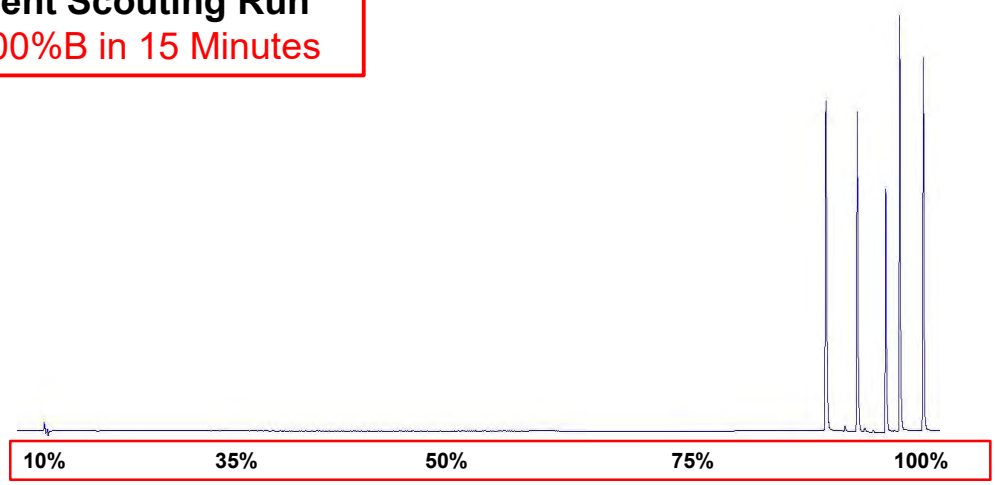
Unknown Sample #1
Gradient Scouting Run
10 - 100%B in 15 Minutes



%B (Acetonitrile or Methanol)

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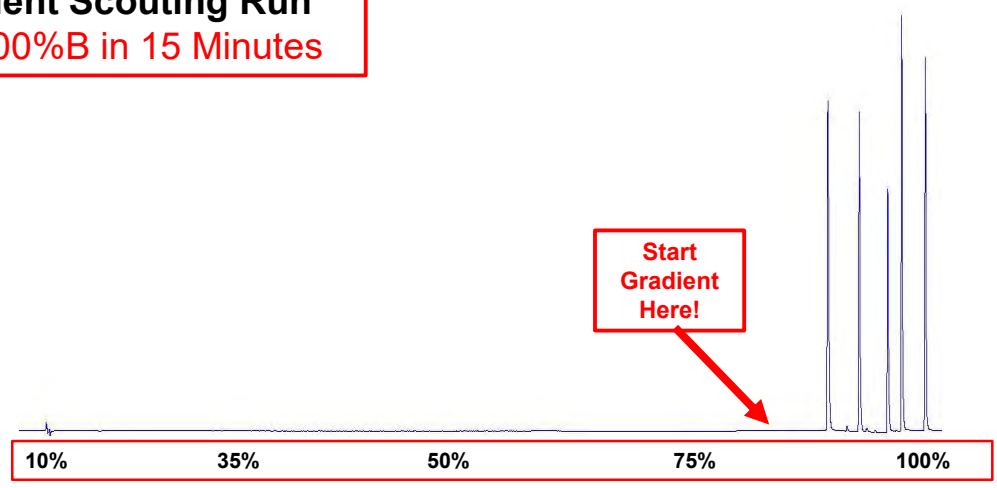
Unknown Sample #1
Gradient Scouting Run
10 - 100%B in 15 Minutes



%B (Acetonitrile or Methanol)

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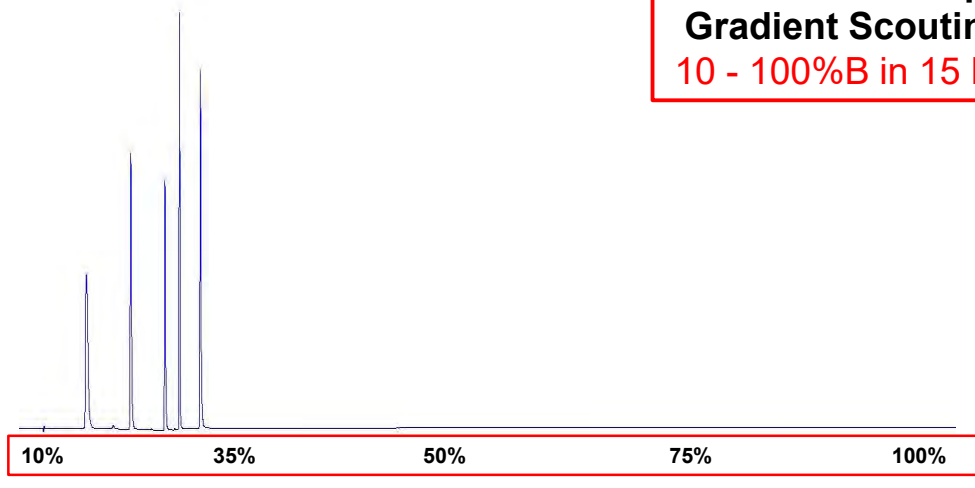
Unknown Sample #2
Gradient Scouting Run
10 - 100%B in 15 Minutes



%B (Acetonitrile or Methanol)

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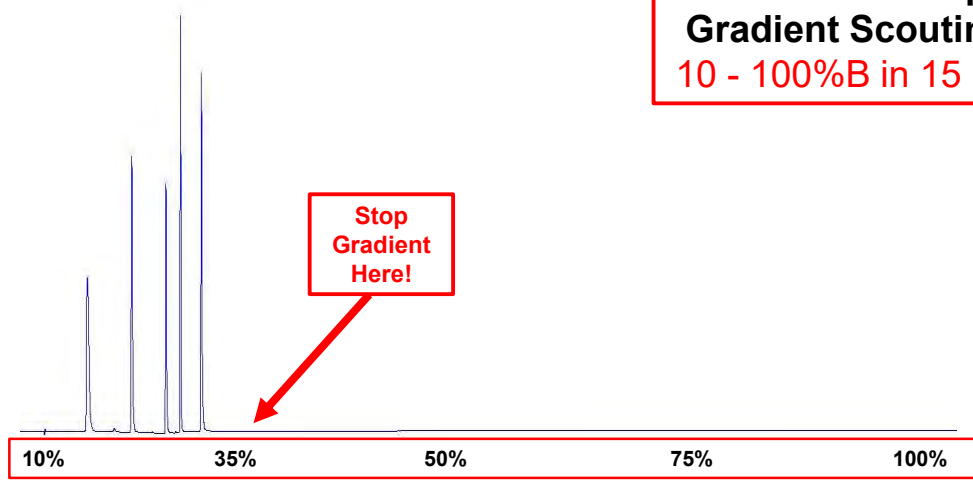
Unknown Sample #2
Gradient Scouting Run
10 - 100%B in 15 Minutes



%B (Acetonitrile or Methanol)

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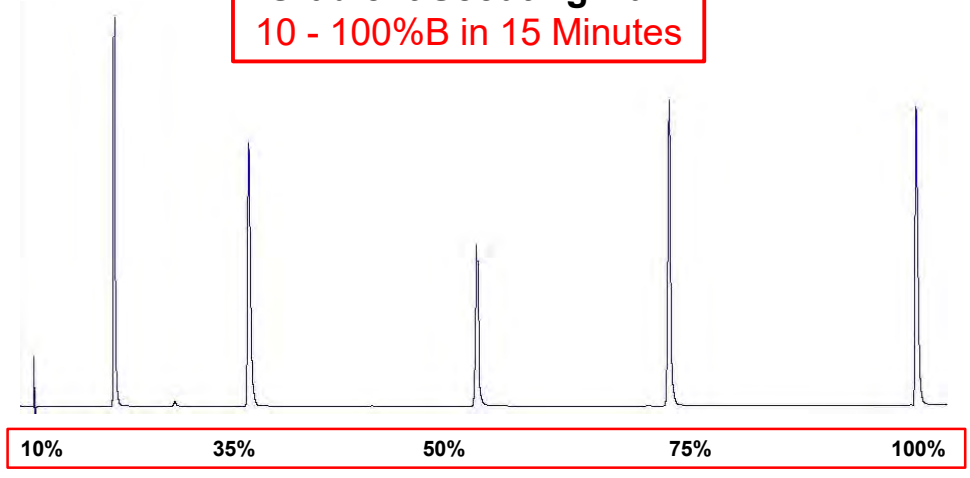
Unknown Sample #3
Gradient Scouting Run
10 - 100%B in 15 Minutes



%B (Acetonitrile or Methanol)

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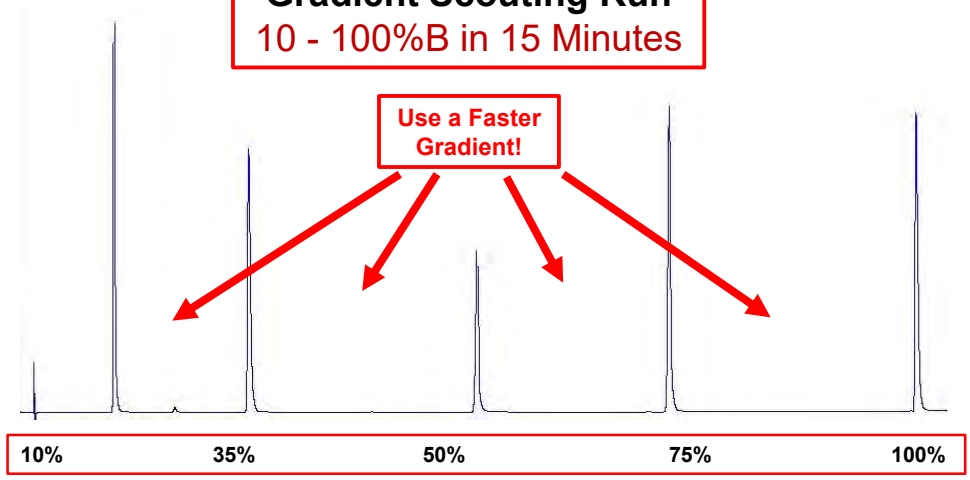
Unknown Sample #3
Gradient Scouting Run
10 - 100%B in 15 Minutes



%B (Acetonitrile or Methanol)

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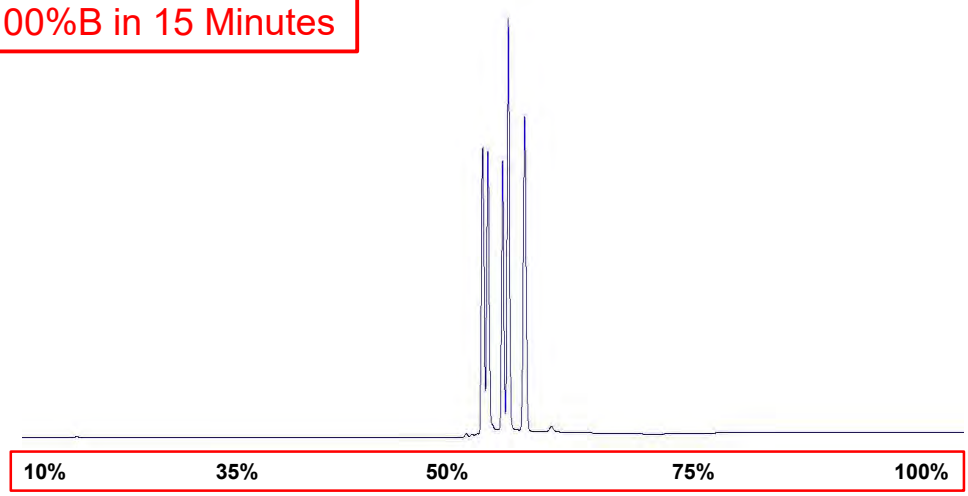
Unknown Sample #4
Gradient Scouting Run
10 - 100%B in 15 Minutes



%B (Acetonitrile or Methanol)

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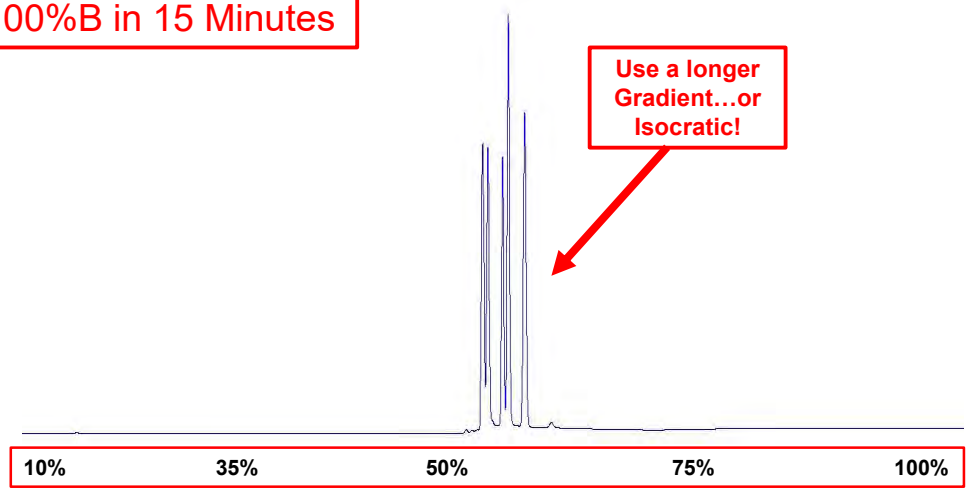
Unknown Sample #4
Gradient Scouting Run
10 - 100%B in 15 Minutes



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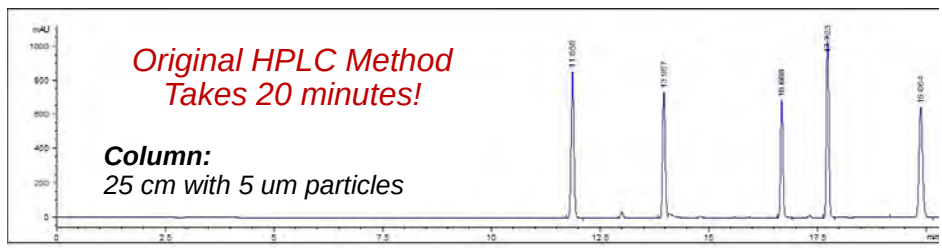
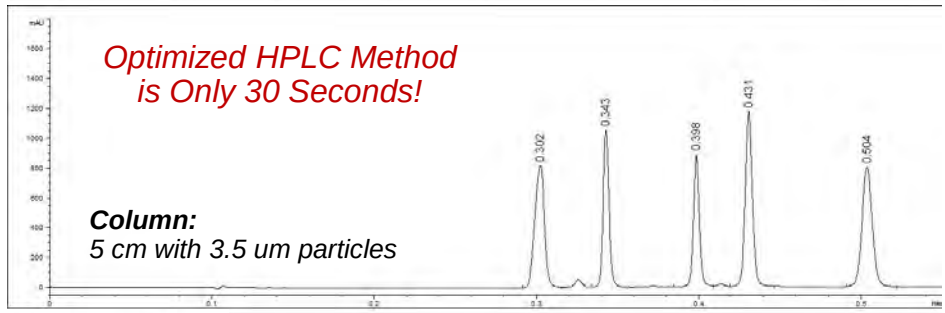
%B (Acetonitrile or Methanol)

After Method Development, Use the Resolution Equation to Cut Analysis Time





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Mastering HPLC Method Development

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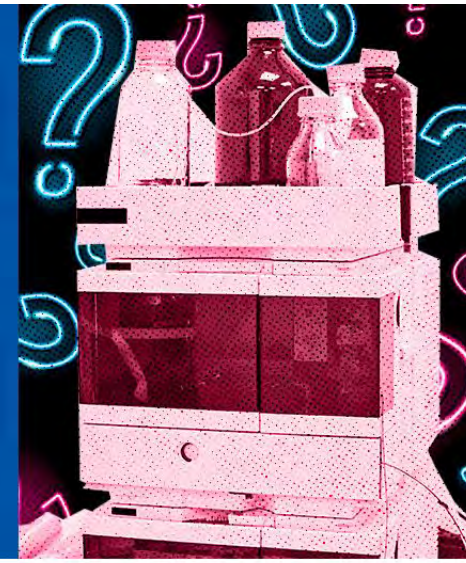

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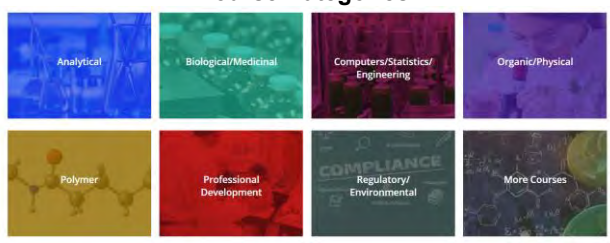

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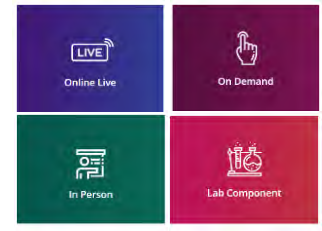

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BRYAN TWEEDY
Senior Portfolio Manager, ACS Learning and Career Development, American Chemical Society

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Moderator: Rebekah Paul, American Chemical Society

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- Lessons learned from three executives' rise to the top
- Insights on how you can succeed in today's changing job market
- Advice for charting your own career in the chemical enterprise

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Speaker: Sir Fraser Stoddart, 2016 Nobel Laureate in Chemistry, Board of Trustees Professor of Chemistry, Northwestern University and H.N. Cheng, ACS President
Moderator: Young-Shin Jun, Washington University in St. Louis

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