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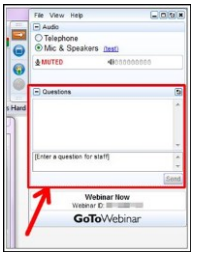

Type them into questions box!

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F. Peter Guengerich, Professor of Biochemistry and the Director of the Center in Molecular Toxicology, Vanderbilt University

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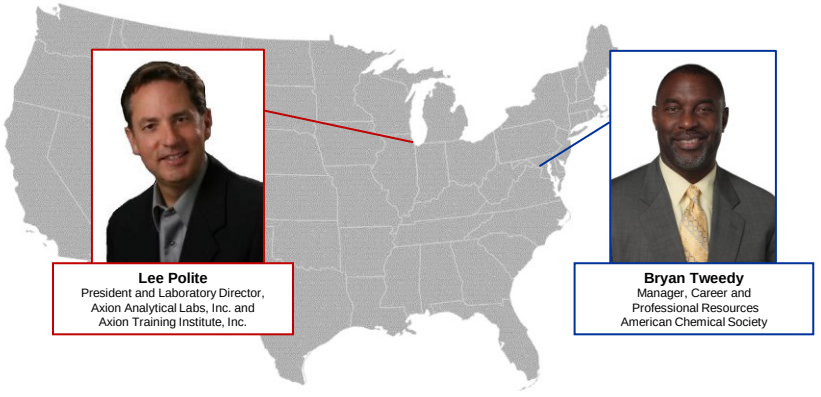
***"Unveiling the Mysteries Behind HPLC and GC Resolution**: From Theory to Practice in 30 minutes"*



Lee Polite
President and Laboratory Director, Axion Analytical Labs, Inc. and Axion Training Institute, Inc.

Bryan Tweedy
Manager, Career and Professional Resources American Chemical Society

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Unveiling the Mysteries Behind HPLC and GC Resolution

Lee N. Polite, Ph.D.
President and Founder
Axion Analytical Labs, Inc.
Axion Training Institute, Inc.

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Analytical Laboratories, Inc.

HPLC and GC – Separation Sciences

- HPLC and GC are the **most widely used chemical analysis techniques** in the world.
- Historically, HPLC and GC have been seen as very different techniques (one flow liquids, the other gasses; one has packed columns, the other does not; one vaporizes the other dissolves, etc.
- Although the instrumentation and applications are significantly different, the **underlying science is the same**: **Separation**
- This separation is based finding the set of conditions where the **analytes** (compounds of interest) **have different affinities** for the stationary phase versus the mobile phase.

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HPLC and GC – Separation Sciences

- The goal of chromatography is to separate **pure compounds from a mixture**.

- **Why do we separate?**
  - We separate to ***identify*** (Qualitative Analysis)
  - We separate to ***quantify*** (Quantitative Analysis)
  - Sometimes we separate to **purify** (Prep-Scale)

- But the first step is always to **separate**!

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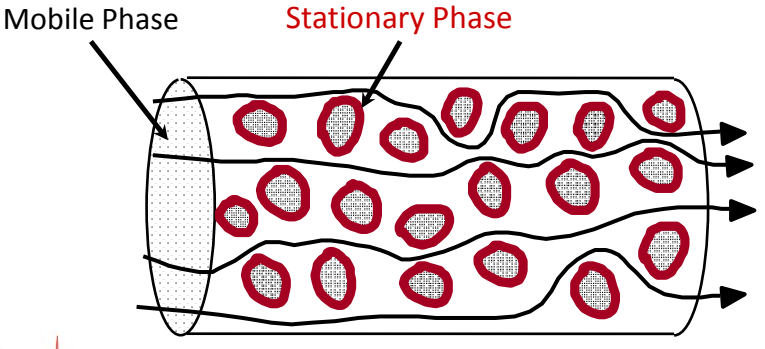
HPLC and GC – Separation Sciences

*Chromatographic separations are based on the **partitioning** or **differential migration** of molecules between two phases:*

The mobile phase and the stationary phase.



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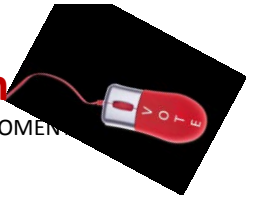
Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMEN



How familiar are you with Separations Science?

- I have never used a GC or HPLC
- I am familiar with GC or HPLC but can always gain more experience
- I work with GC or HPLC on weekly basis but am always interested in learning more.
- I could give a webinar on GC and HPLC separations science

HPLC and GC – Separation Sciences

- At first it may seem difficult to fully understand separations in HPLC and GC, but…
- There are **ONLY 3 parameters** that affect HPLC and GC separations
- If you set those 3 parameters properly, you are guaranteed a separation.
- As an added bonus, they are the **same** 3 parameters that dictate separations in **all types of chromatography** (GC, HPLC, Reversed Phase, Normal Phase, Ion Exchange, Supercritical Fluid Chromatography, etc.)

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- **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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Master Resolution Equation

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

Capacity	**Selectivity**	**Efficiency**
(Solvent Strength)	(Stationary & Mobile Phases)	(L_c, D_p, μ)

- Resolution is a function of three factors: k, α, & N
- All three factors are necessary to achieve a separation.
- These are the **only** three factors necessary to achieve a separation!

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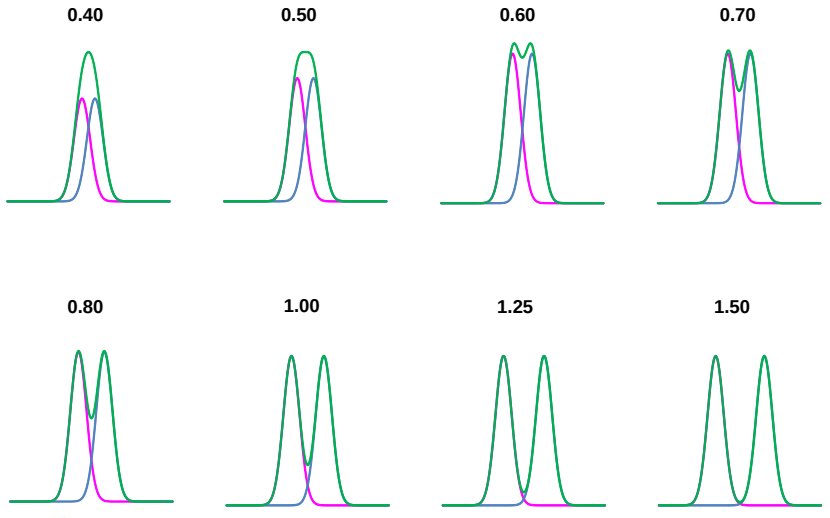
Different Resolution Values



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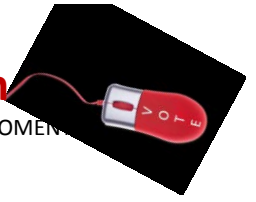
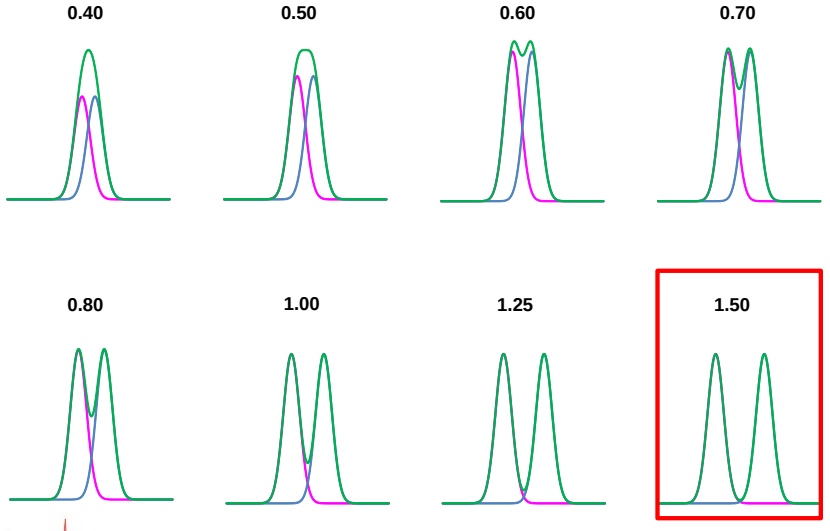


What is the definition of good resolution? It should be greater than or equal to:

- 0.50
- 0.70
- 1.00
- 1.50
- All of the values

Different Resolution Values



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GC 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 > 1.05	Avg ~ 20,000 Max ~ 400,000
	$k = (t_r - t_0)/t_0$	$\alpha = k_B/k_A$	$N = 5.545 \times \left(\frac{t_r}{W_h}\right)^2$
	Lower Temp by 25°C ~ Doubles k	Change Stationary Phase	↑ Column Length (Lc) ↓ Column Diameter (Dc) ↓ Film Thickness (D_f) Optimize Flow Rate (μ) Check Column Installation

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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 ~ 5,000 Max ~ 25,000
	$k = (t_r - t_0)/t_0$	$\alpha = k_B/k_A$	$N = 5.545 \times \left(\frac{t_r}{W_h}\right)^2$
	Weaken Mobile Phase ↑%H2O by 10% ~ 2-3 x k	Change: Mobile Phase Stationary Phase pH, Temp, buffer, additive, etc.	↑ Column Length (Lc) ↓ Particle Diameter (D_p) Optimize Flow Rate (μ) Min. Extra Col. Volume

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Audience Survey Question

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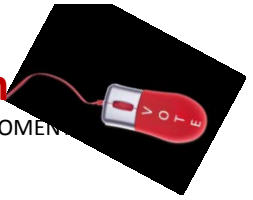
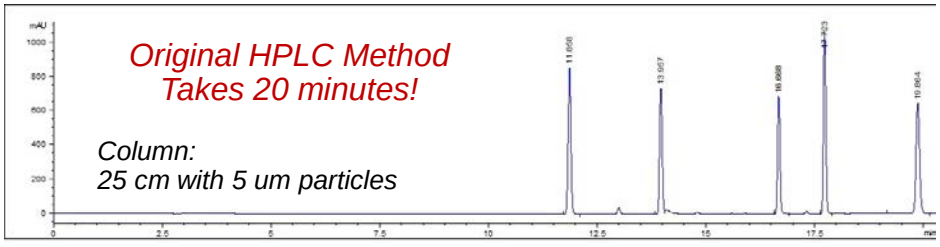
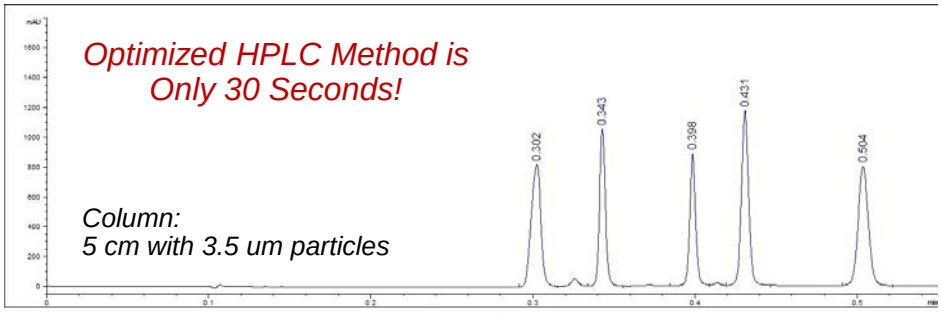


How can you imagine using the master resolution equation in your own life?

- To develop a method from scratch.
- To optimize the speed or resolution of a current method.
- To troubleshoot a current separation problem.
- To impress my friends at the next cocktail party.

Use Resolution Equation to Cut Analysis Time





Why Do We Need Such Short Analysis Times?

- Run more samples per day
- Generate More Timely Results
- Allow for more quality control (blanks, spike recoveries, duplicates, etc.)
- Enable real-time analysis

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Case Study: Cellulosic Ethanol (Renewable Energy)

- Pretreatment (key step) historically takes 30-40 minutes, leading to higher costs, lower conversion and higher levels of degradents.
- New Technology (Sweetwater Energy, Inc.) accomplishes that step in less than 10 seconds!
- Hurray for the good guys, but now analytical becomes a challenge: Either give results too late to do anything about them, or optimize methods for high speed results.
- DOE "optimized method" took 10 minutes (reduced from 55 minuets!), but that is still 600 seconds.
- Solution: 20 second HPLC analysis

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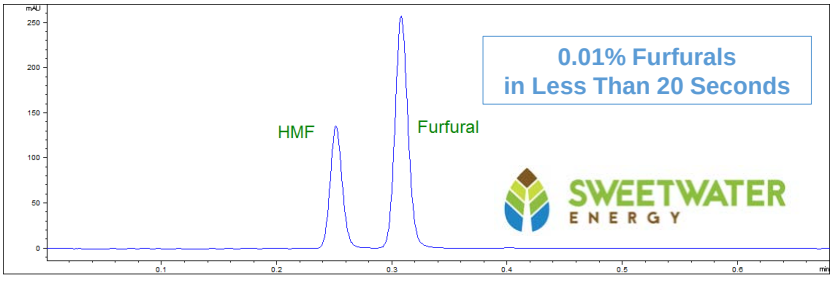
Fast HPLC of HMF and Furfural



Agilent 1200SL HPLC
Diode Array Detector @ 280nm
Zorbax Eclipse Plus C18 – 50mm x 4.6 mm x 3.5 um
Flow Rate = 5.0 ml/min
375 Bar

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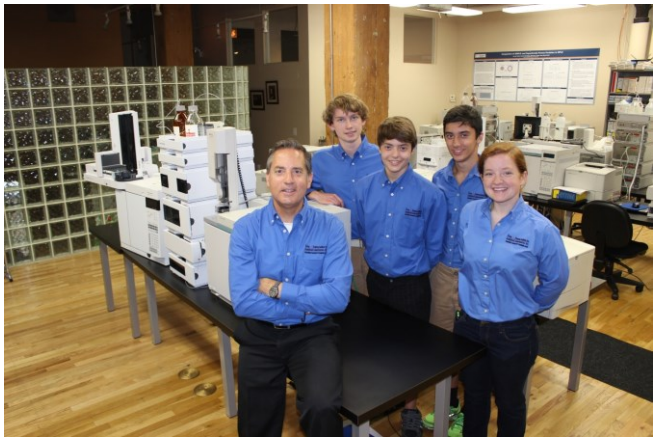
Combined HPLC and GC Master Resolution Equation

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

Resolution	Capacity / Retention Factor	Selectivity	Efficiency ("Peak Skinniness")
R>1.50	$1 < k < 5$	$\alpha > 1.2$ $\alpha > 1.05$	Avg ~ 5K / 20K Max ~ 25K / 400K
	$k = (t_r - t_0)/t_0$	$\alpha = k_B/k_A$	$N = 5.545\ x \left(\frac{t_r}{W_h}\right)^2$
Axion Training Institute, Inc.	Weaken Mobile Phase Decrease Temperature	**Change:** Stationary Phase Mobile Phase pH, Temp, buffer, additive, etc.	↑ Column Length (Lc) ↓ Particle Diameter (D_p) ↓ Column Diameter (Dc) ↓ Film Thickness (D_f) Optimize Flow Rate (μ) Min. Extra Col. Volume

Thanks…from the Axion (Summer) Team



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The Axion Summer Interns…Get Famous!

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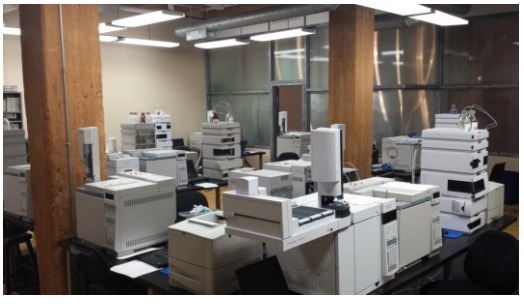
Axion's Hands-On HPLC and GC Training Facility

Axion has provided chromatography training to every major pharmaceutical, chemical and petroleum company in the US, as well as most of the larger government labs.



Axion offers comprehensive Hands-On HPLC and GC training courses at its downtown Chicago location for anyone interested in honing their chromatography skills.

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From beginner to near-expert in four days!

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Thank You for Attending!

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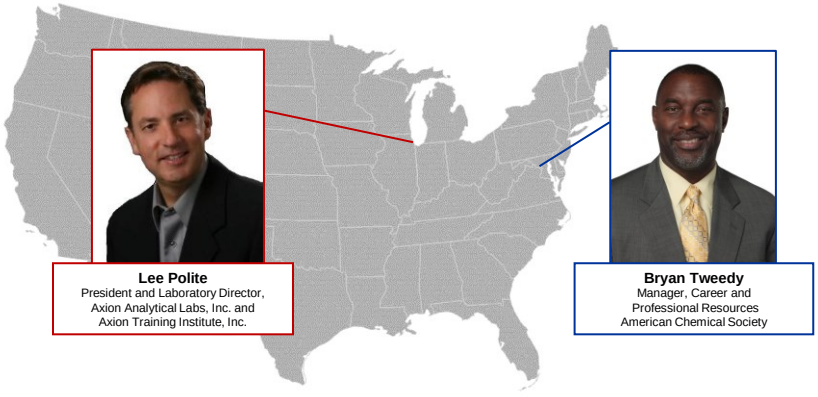

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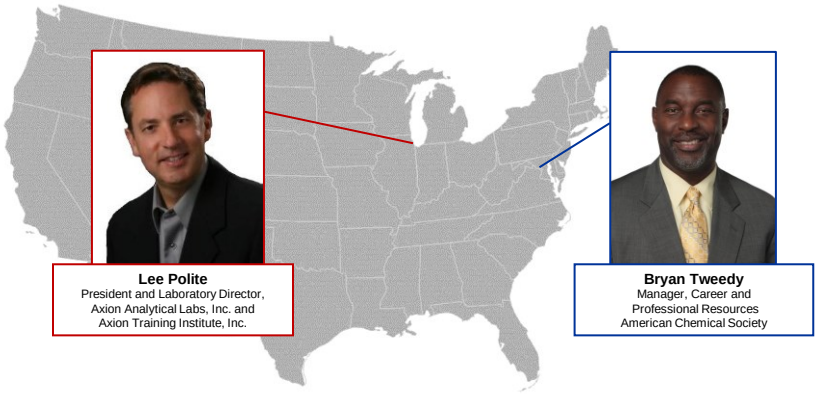

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