GI2

Systematic Look at the Affects of Various Solvents, Injection Techniques and Sample Amounts on Preparative HPLC Columns

Gary Scharrer and Joan Stevens, Ph.D.

Gilson, Inc
Middleton, WI
www.gilson.com

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Outline

- Preparative Instrumentation
- Performance Factors
  - Solvent Selection
  - Mobile Phases
  - Sample Solubility
  - Sample Size
  - Column Loading
  - Particle Size
  - Sample Carryover
  - Injection Techniques
- Summary
- Questions?

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Prep System Configurations

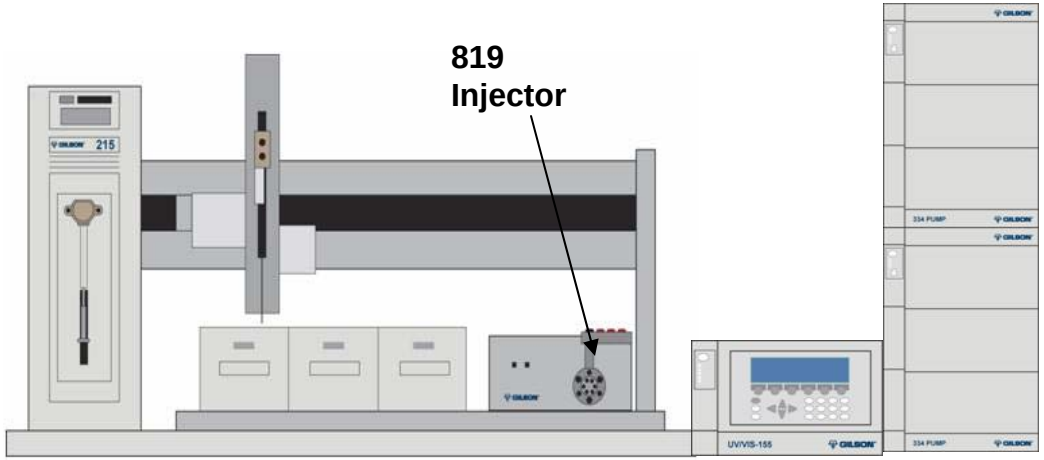

Figure 1.

System A

- Gilson 215 Liquid Handler/Fraction Collector, equipped w/175-mm Z-arm, 819 Injection Module with 5.0-mL SS loop and bevel-tip probe (269x1.3x0.8 mm ID)
- Gilson 333/34 HPLC Pump, equipped with H3 pump heads (flow rates up to 200 mL/min.)
- Gilson 155 UV/Vis Dual-wavelength Detector (0.2-mm pathlength, 1.0 AUFS)

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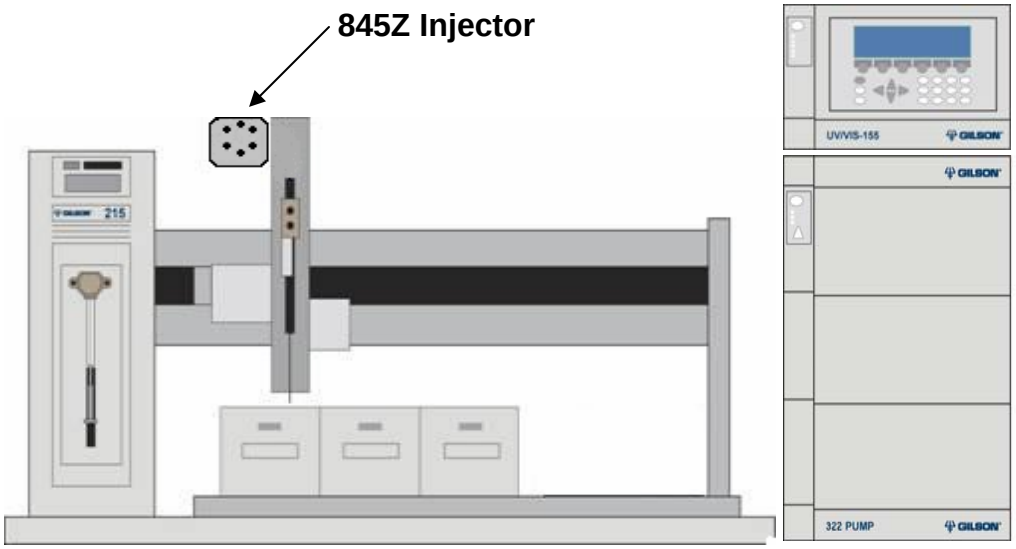
Prep System Configuration



System B

Figure 2.

- Gilson 215 Liquid Handler/Fraction Collector, equipped w/175-mm Z-arm, 845Z Injection Module w/5.0-mL SS loop, and bevel-tip probe (269x1.3x0.8mm ID)
- Gilson 322 HPLC Pump, equipped with H2 heads (flow rate up to 30 mL/min.)
- Gilson 155 UV/Vis Dual-wavelength Detector (0.2-mm pathlength, 1.0 AUFS)

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System/Injectors Used In Study

- 819 Injector – System A
  - Dispenses sample through an injection port to the sample loop
- 845Z Injector – System B
  - Aspirates sample directly into the sample loop (Z-Mounted Valve)
- Above systems/injectors were both used interchangeablely in this study.

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Solvent Selection

- All compounds in mixture must be soluble in the solvent selected at the desired concentration.
  - For polar compounds use acetonitrile, methanol or water individually or in combination.
  - For more non-polar compounds use DMSO or DMF.
- Select container material which will not attract or adhere material (i.e. glass or polypropylene).
  - Losses due to this situation are difficult to quantify since the loss occurs before sample injection.
- Select a solvent which matches the initial mobile phase as closely as possible.

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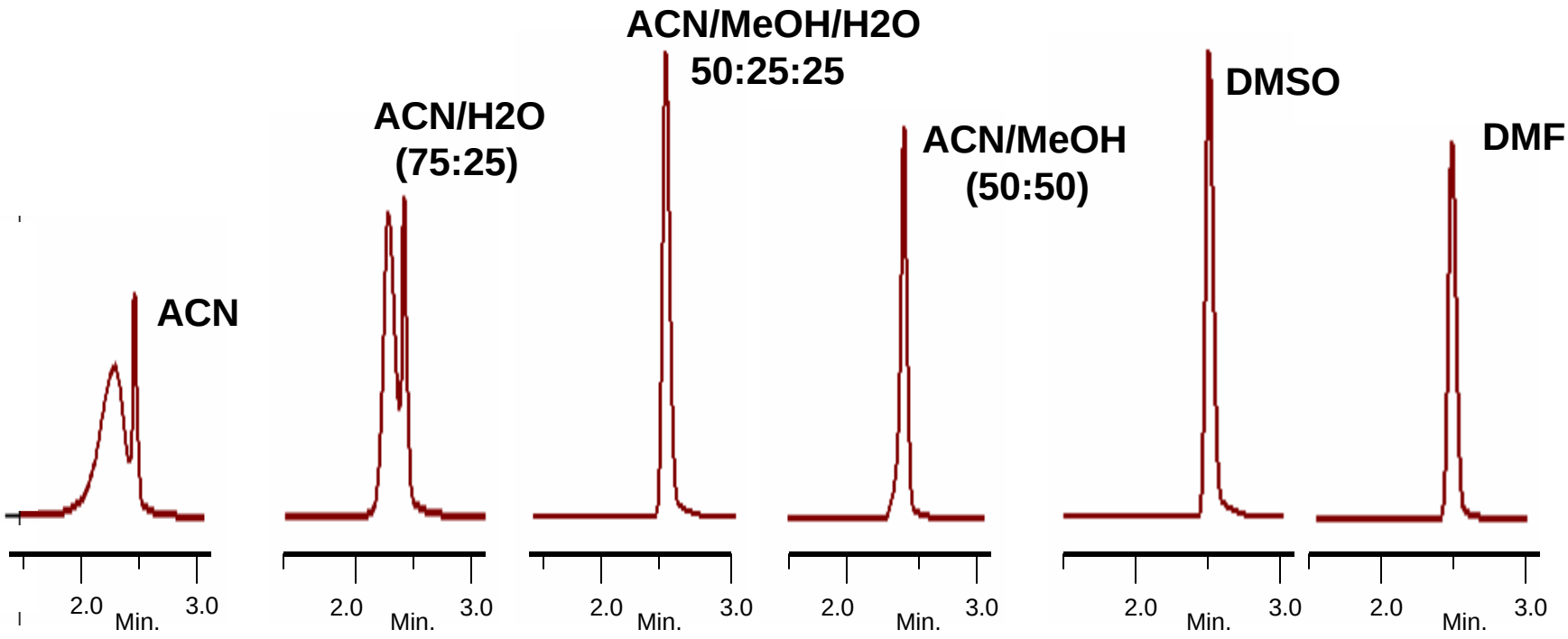
Solvent Effects on Chromatography



Graph 1. Choosing a solvent and how it effects the chromatography is important for system design.

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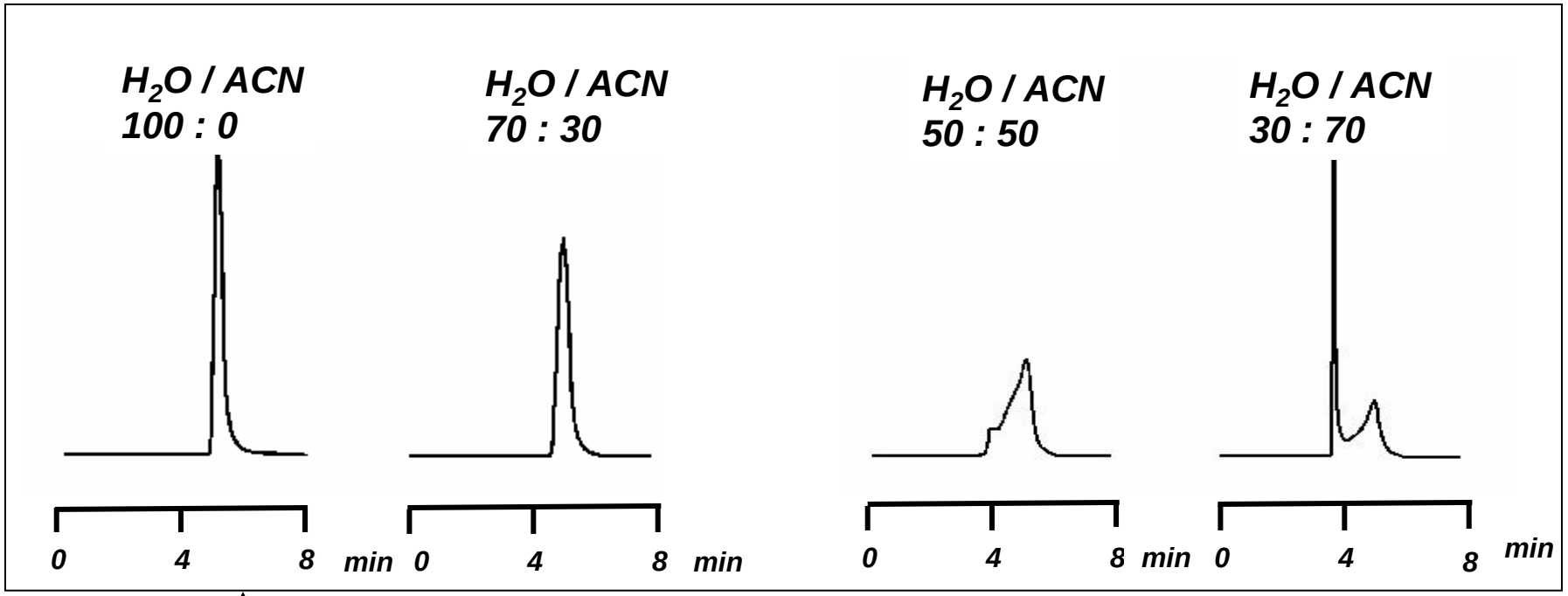
Solvent Selection



Graph 2. The sample should - whenever possible - be dissolved in the mobile phase or a weaker solvent.

If the sample is dissolved in a too strong solvent, significant disturbances can occur in the chromatogram.

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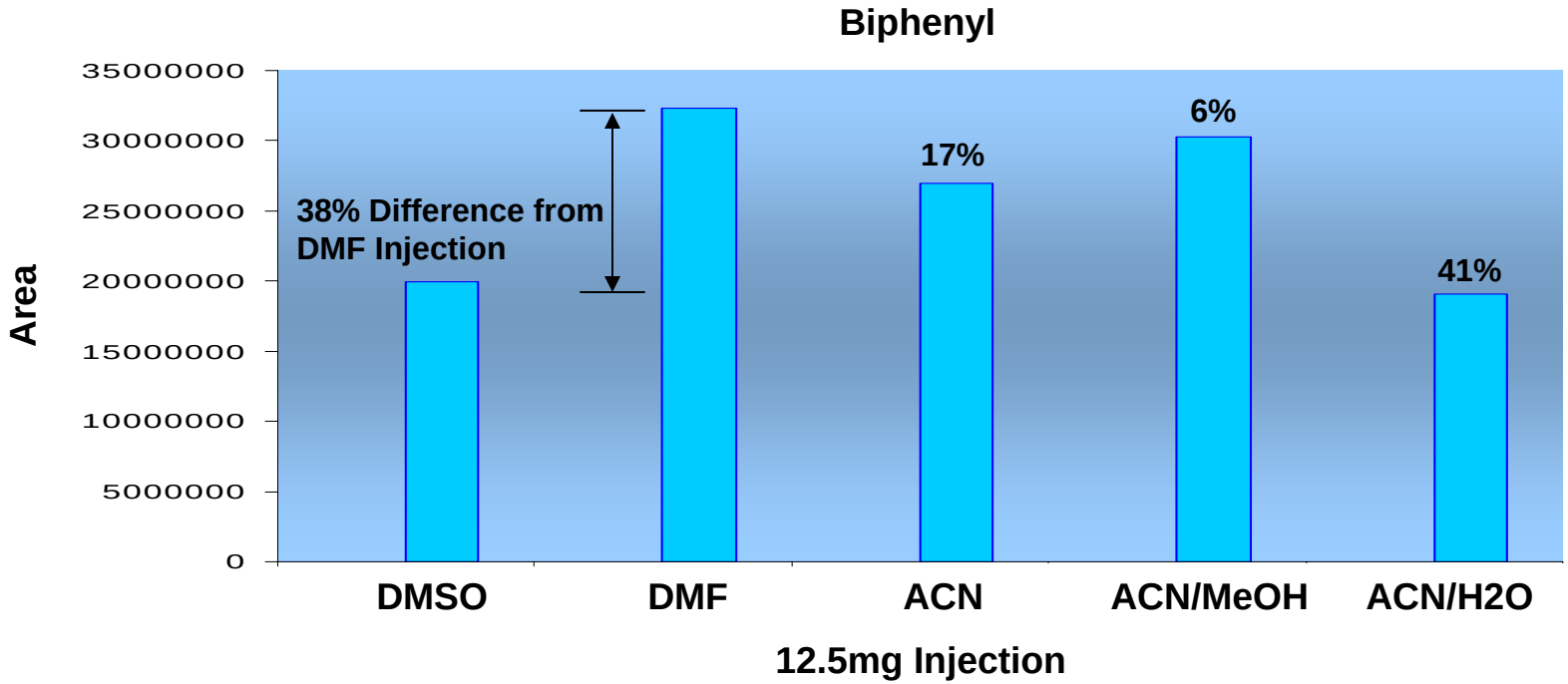
Area Decreases Due to Solvent Selection



Graph 3. This chart illustrates the response differences that occur with different solvents or solvent combinations. Assuming the DMF area as 100%, differences from 6% with ACN/MeOH to 41% with ACN/H2O are found.

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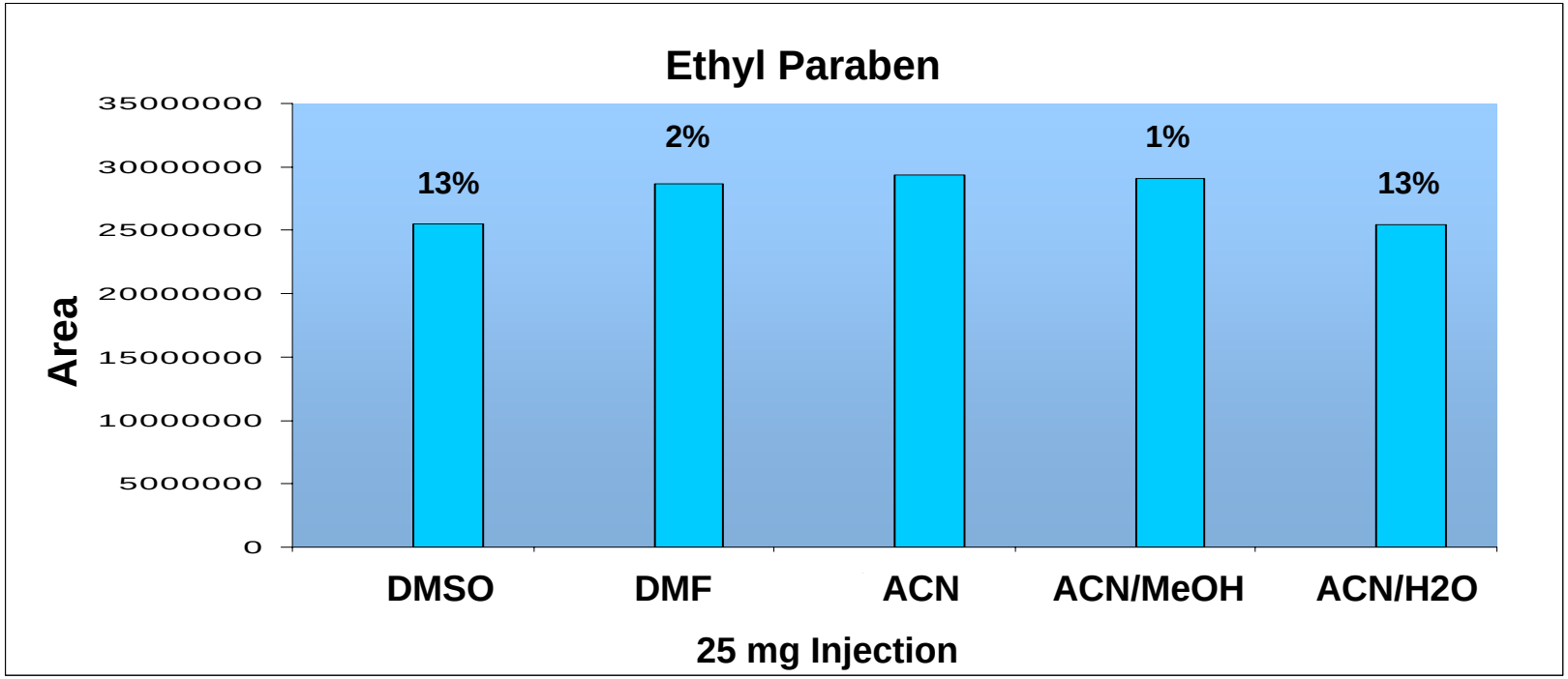
Response Differences Due to Solvent Selection



Graph 4. Another example of response differences due to solvent selection, but in this case the acetonitrile was the optimum solvent.

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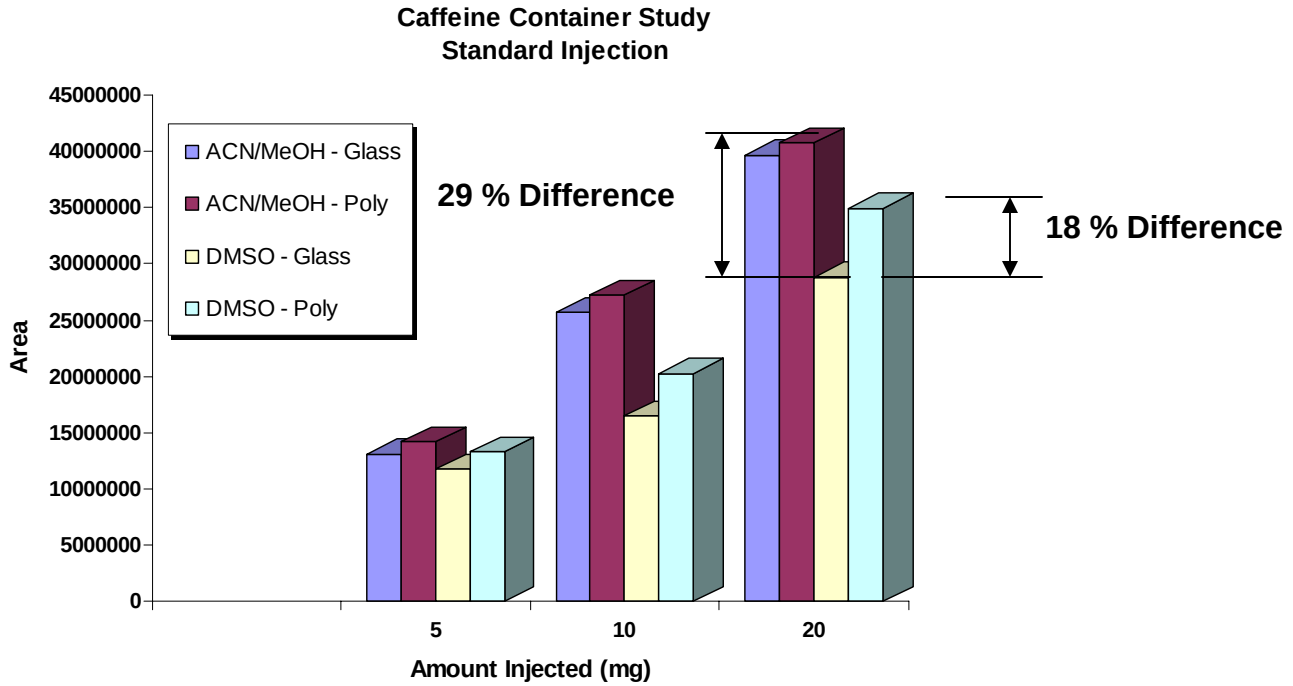
Sample Loss Due to Container Materials



Graph 5. The container material and solvent can affect sample transfer into the analysis/purification system. In this case, caffeine transfer decreases of up to 29% are possible. Even with the same solvent, decreases of up to 18% are found.

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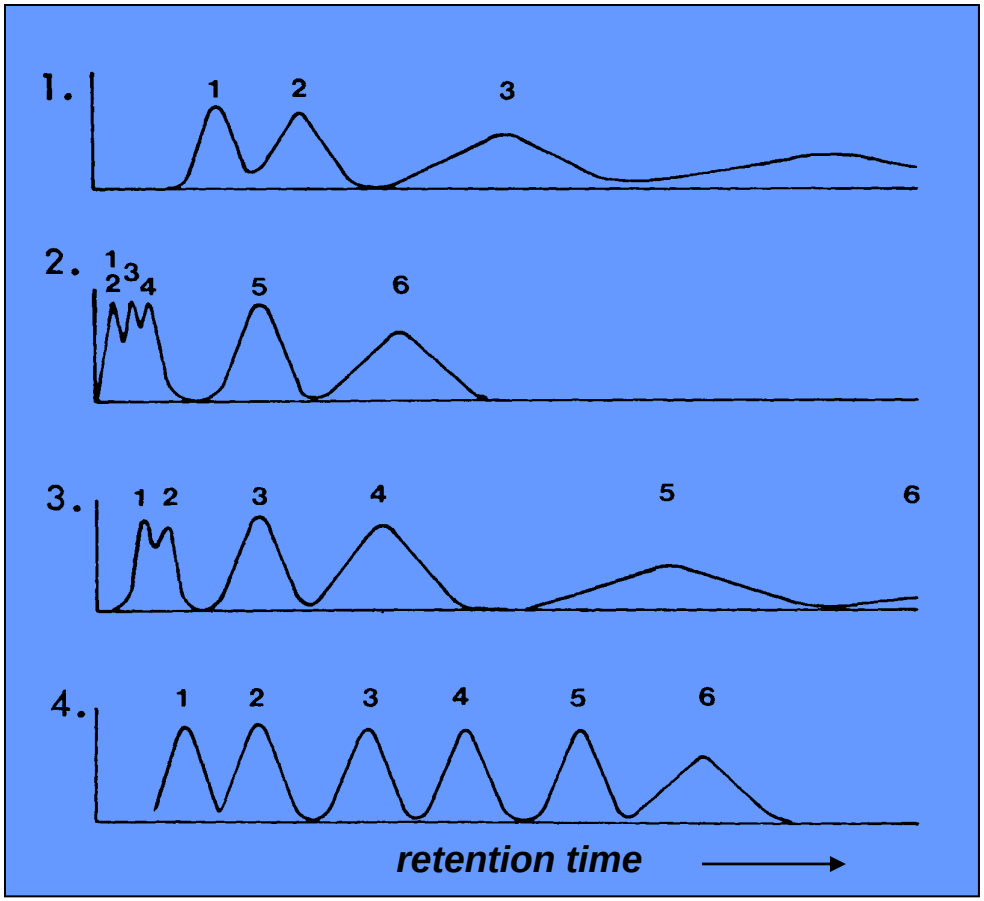
Mobile Phases



Graph 6.

Weak Mobile Phase

Strong polar or slightly soluble compounds may not elute

Strong Mobile Phase

Single peaks may co-elute or are poorly resolved

Solvent Mixtures

Only specific compound elutions are optimized

Gradient Elution

Optimized chromatogram with enhanced resolution and reduced retention time

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Sample Solubility

- Determine sample polarity.
  - Use polarity of sample as a guide for solubility.
- Mobile phase compatibility
  - Compounds could precipitate in the mobile phase if concentrations are high.
- Container material absorption
  - The possibility of the sample adhering to the container surface must be considered.

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Reverse Phase Solvent Polarity Chart

Polarity	Solvent	Polarity Index (P)*	UV Cutoff (nm)
Non- Polar	n-Propanol	4.0	210
	Tetrahydrofuran	4.2	215
	Isopropanol	4.3	210
	Ethanol	5.2	210
	Acetone	5.4	330
	Acetonitrile	6.2	190
	Acetic Acid	6.2	230
	Dimethylformamide	6.4	268
	Methanol	6.6	205
	Methyl sulfoxide	7.2	268
Polar	Water	9.0	200

*Polarity index (P) = Measure of ability of solvent to interact as a proton donor, proton acceptor, or dipole.

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Sample Loading

This parameter is important for obtaining the purest and most concentrated fraction cuts.

To attain optimum results:

- Use high concentration of sample
- Keep injection size small

Better to have multiple injections into a more concentrated fraction than to manipulate more fractions.

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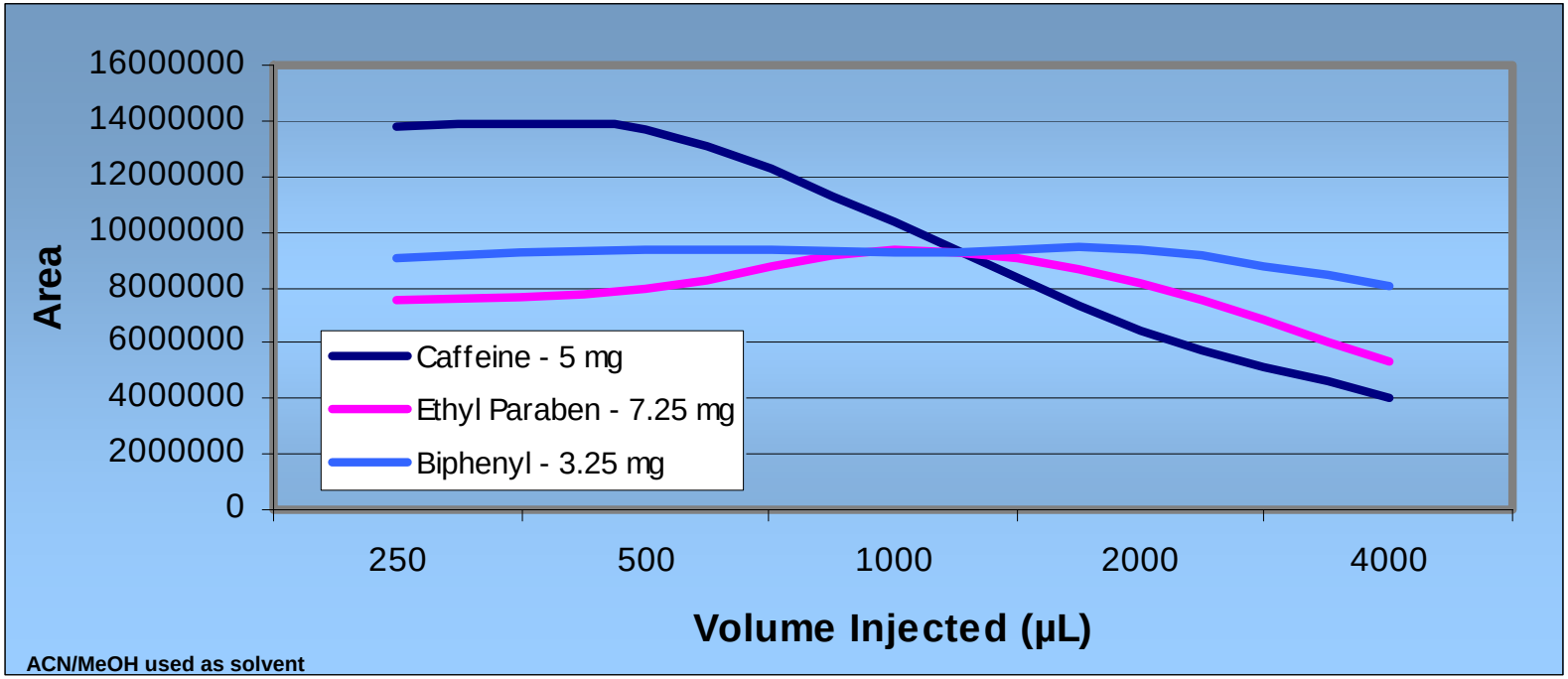
Injection Volume Effects on Peak Area



Graph 7. When the column is flooded with the injection solvent, regardless of load, peak area is suppressed until optimum injection volumes are obtained.

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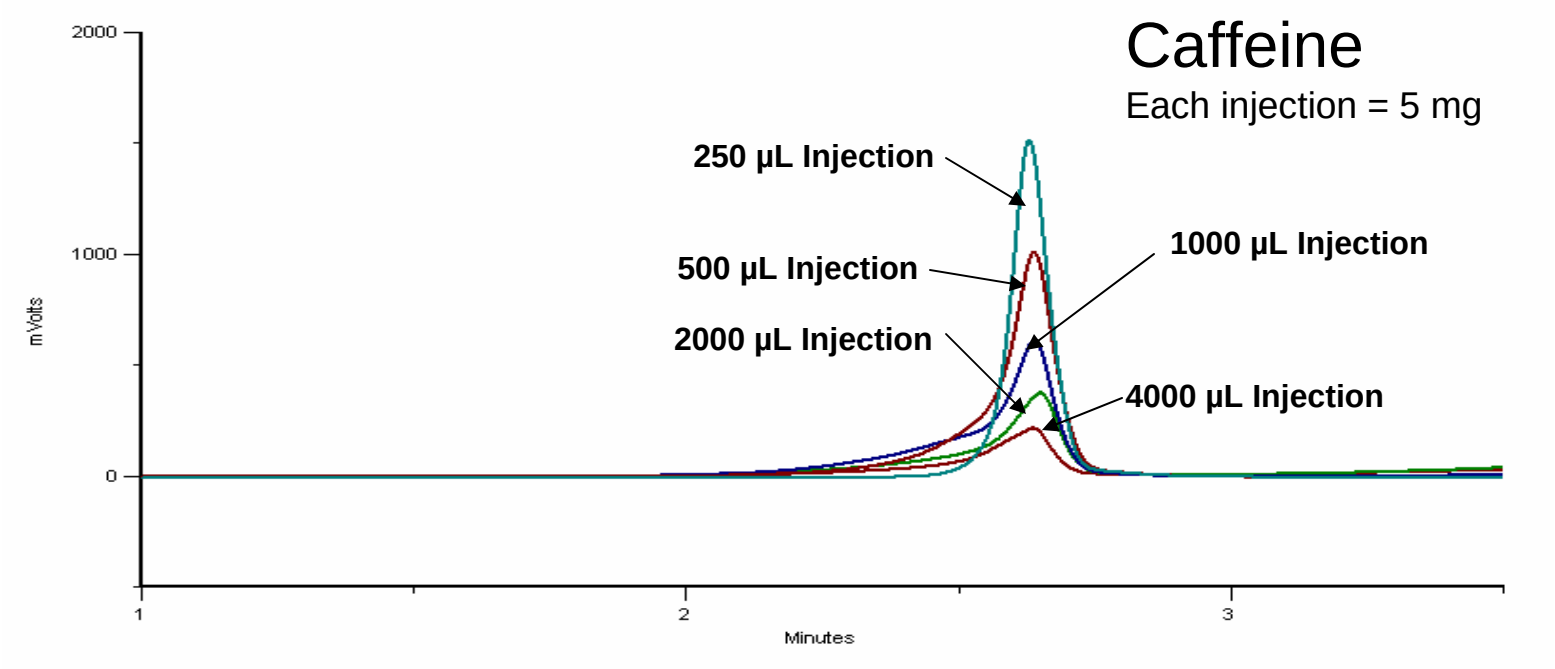
Chromatography Effects with Various Volumes



Graph 8. The effects of keeping the column load constant but varying the injection volumes can be seen as the chromatography degrades as the injection volume increases.

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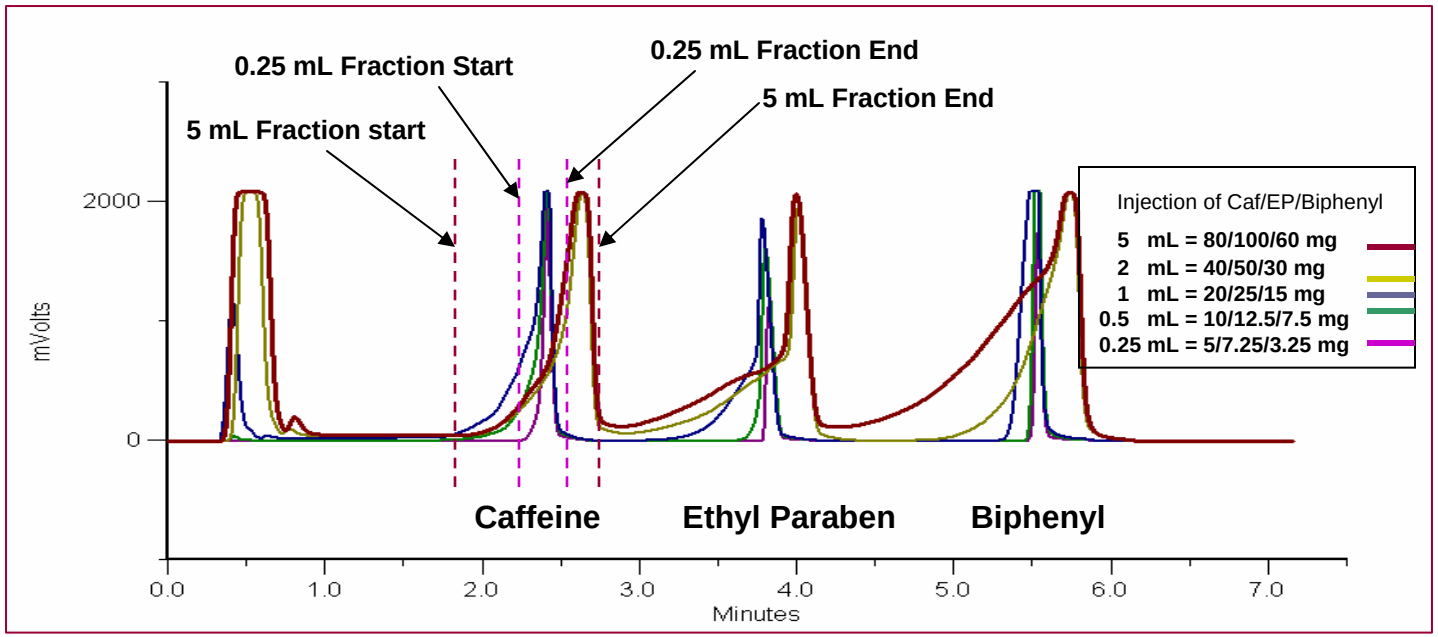
Injection Volume and Sample Loading



Graph 9. As the injection volume and column load increase at the same rate the quality of chromatography degradation is amplified. Samples can still be fractionated, but the faction cuts will be more diluted and purity could also be reduced due to closely eluting impurities.

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Particle Size

Reducing column packing particle size

- Gains resolution
- Saves solvent usage
- Reduces solvent waste.
- Reduces fraction volume.

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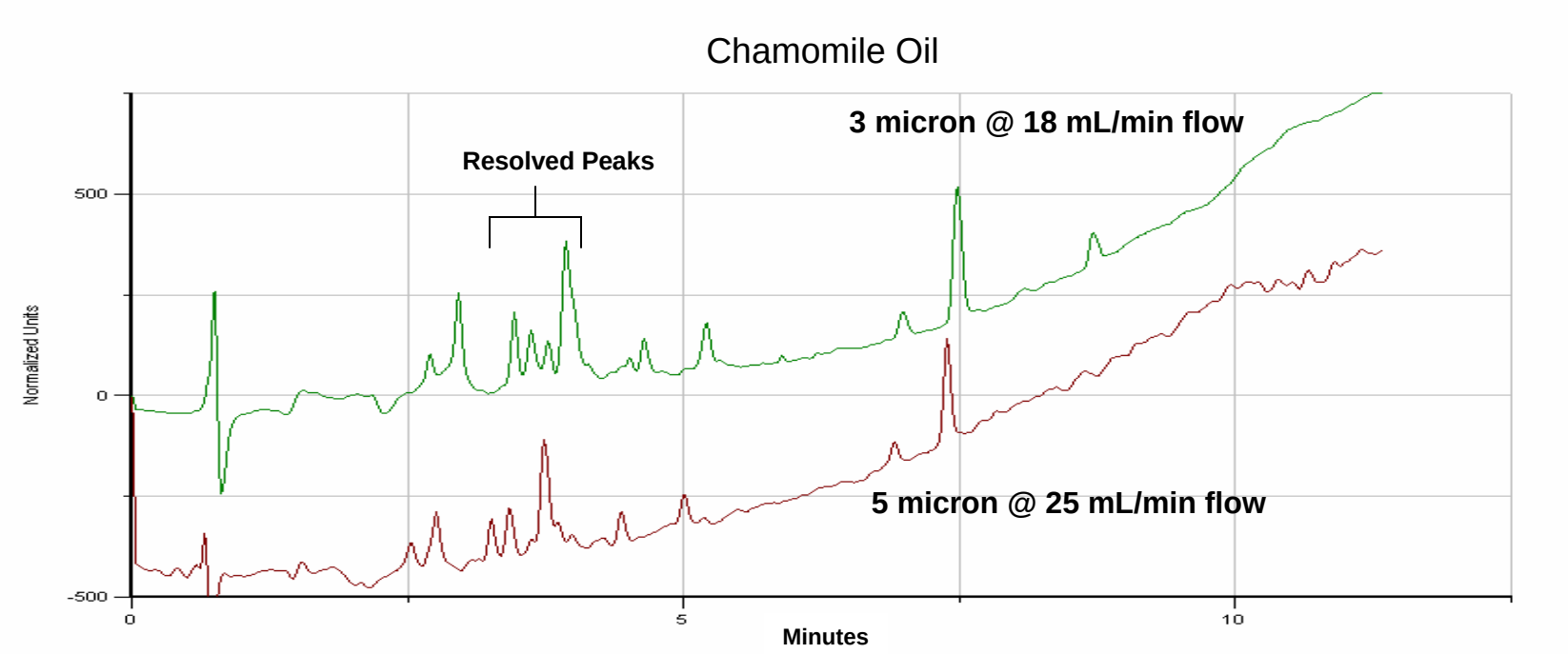
Particle Size Reduction



Graph 10. Reducing from a 5 micron to 3 micron column particle size gives resolution to closely eluting compounds and also decreases solvent consumption by 28% due to slower flow rates, without loosing sample turnaround efficiency.

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Sample Carryover

- Chemical binding
- Connections
  - Tubing
  - Injection Loop
- Physical Wear
  - Rotor seals
- Rinse station contamination
  - Probe
  - Injection Port

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Chemical Binding

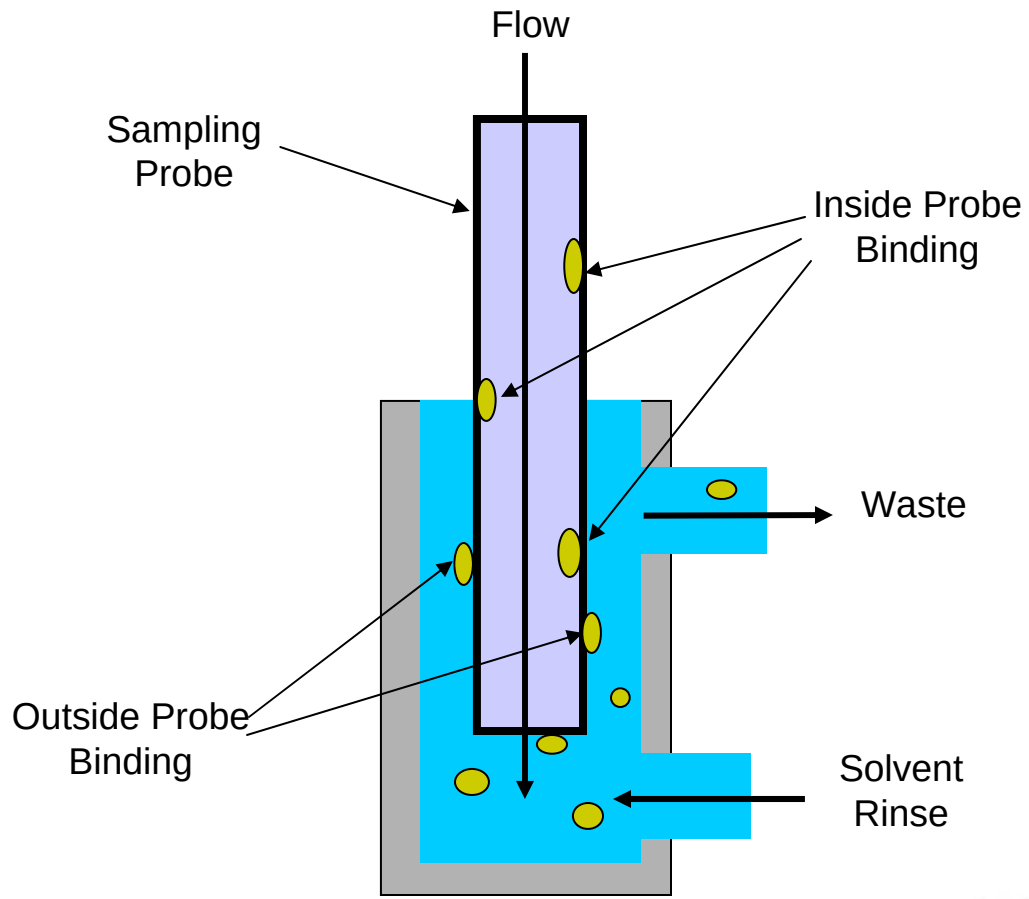
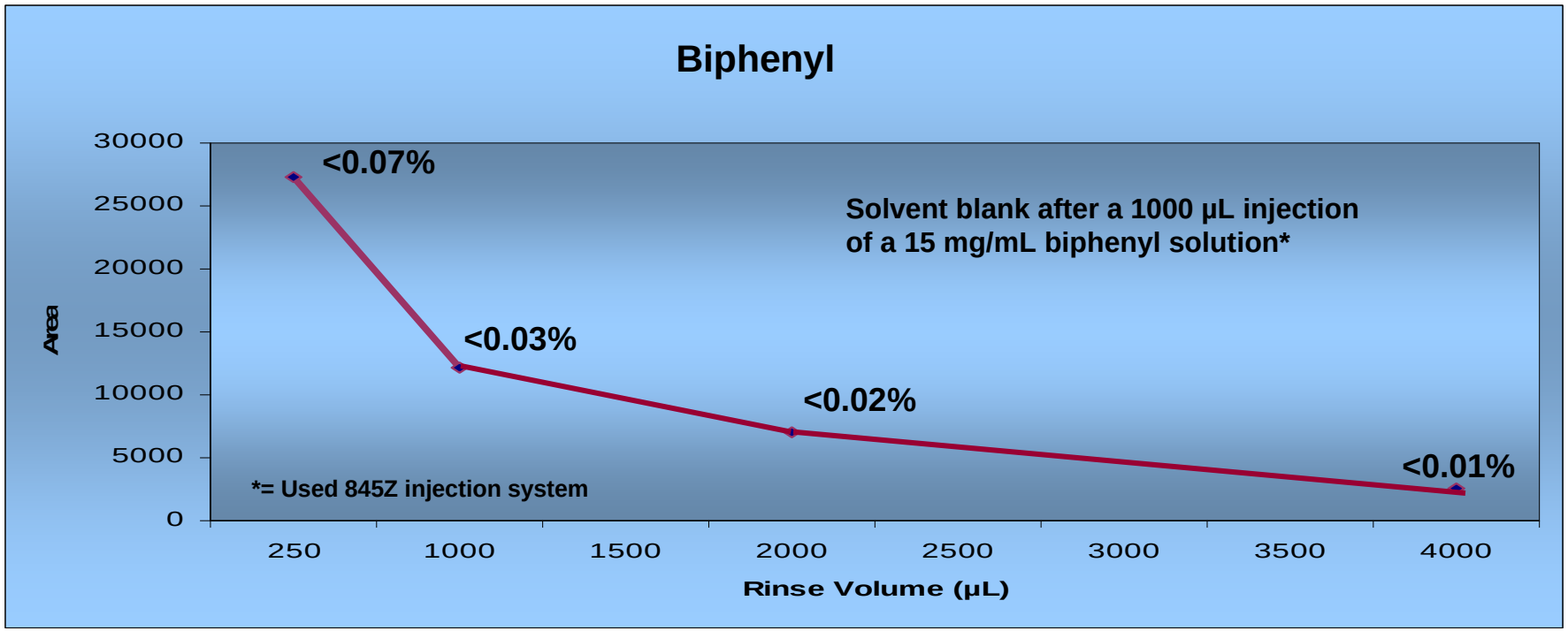


Figure 3.

Sample Carryover



Graph 11. Sample carryover is a direct result of the ability/inability of the system to rinse the sampling components correctly between injections. Different rinse routines can be configured to meet the sample situation, in this case biphenyl. The above example shows the difference in changing the rinse volume for rinsing the sample probe.

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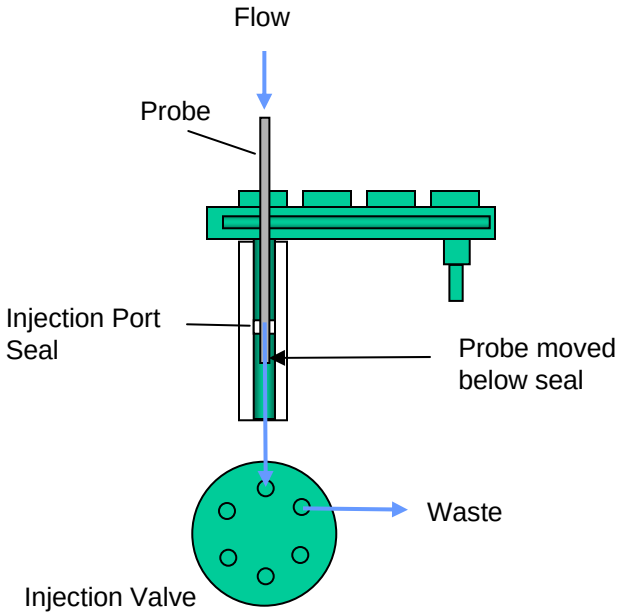
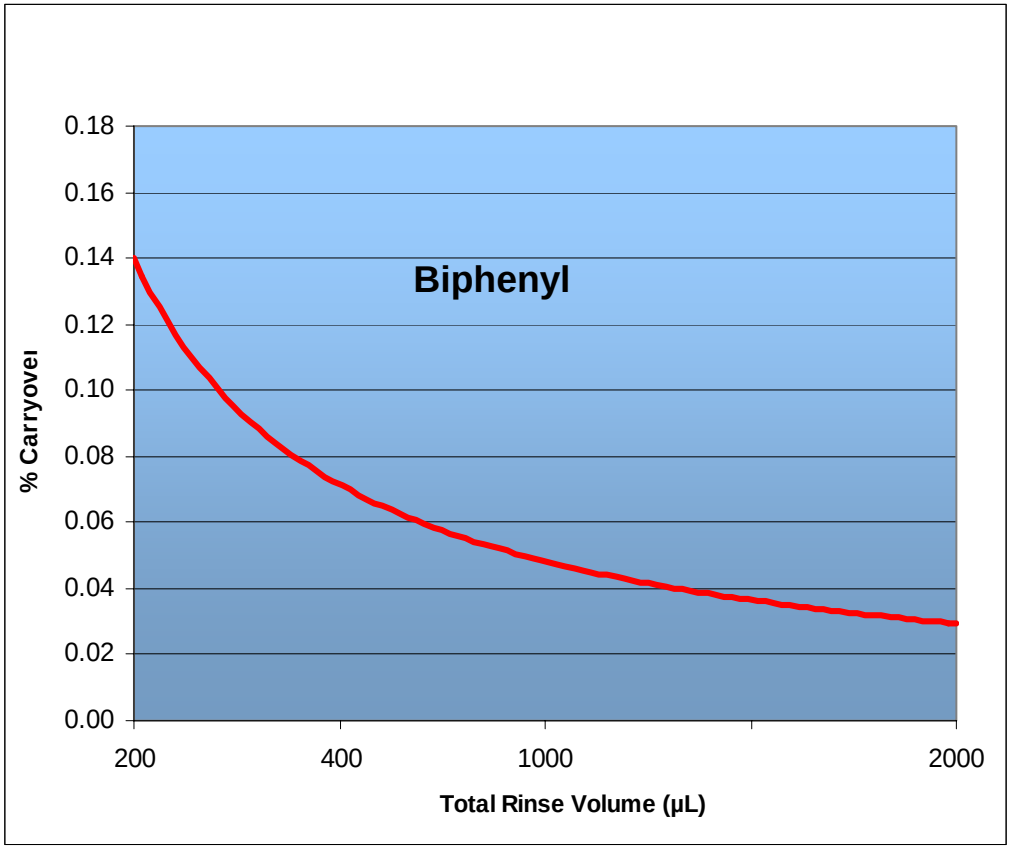
Carryover Elimination Techniques



Below seal rinse or Stationary Injection Port Rinse
Figure 3.

This procedure rinses below the seal. The waste is pushed through the injection valve to waste.



Graph 13. Percent carryover versus total rinse volume

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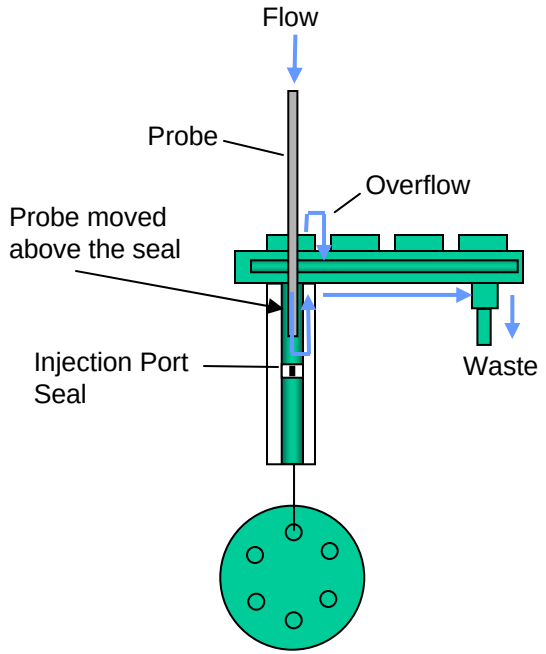
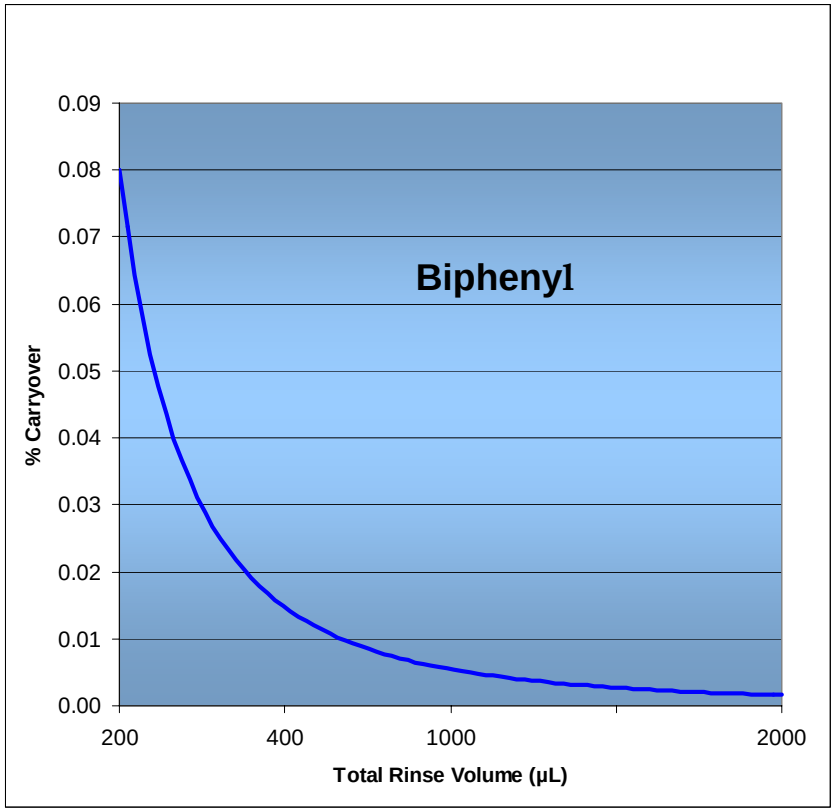
Carryover Elimination Techniques



Above seal rinse

Figure 4.

This procedure rinses above the seal. The waste is pushed out the top of the injection port to the waste channel of the injection port. This procedure is combined with the standard below seal rinse.



Graph 12. Percent carryover versus total rinse volume which Includes above and below rinses.

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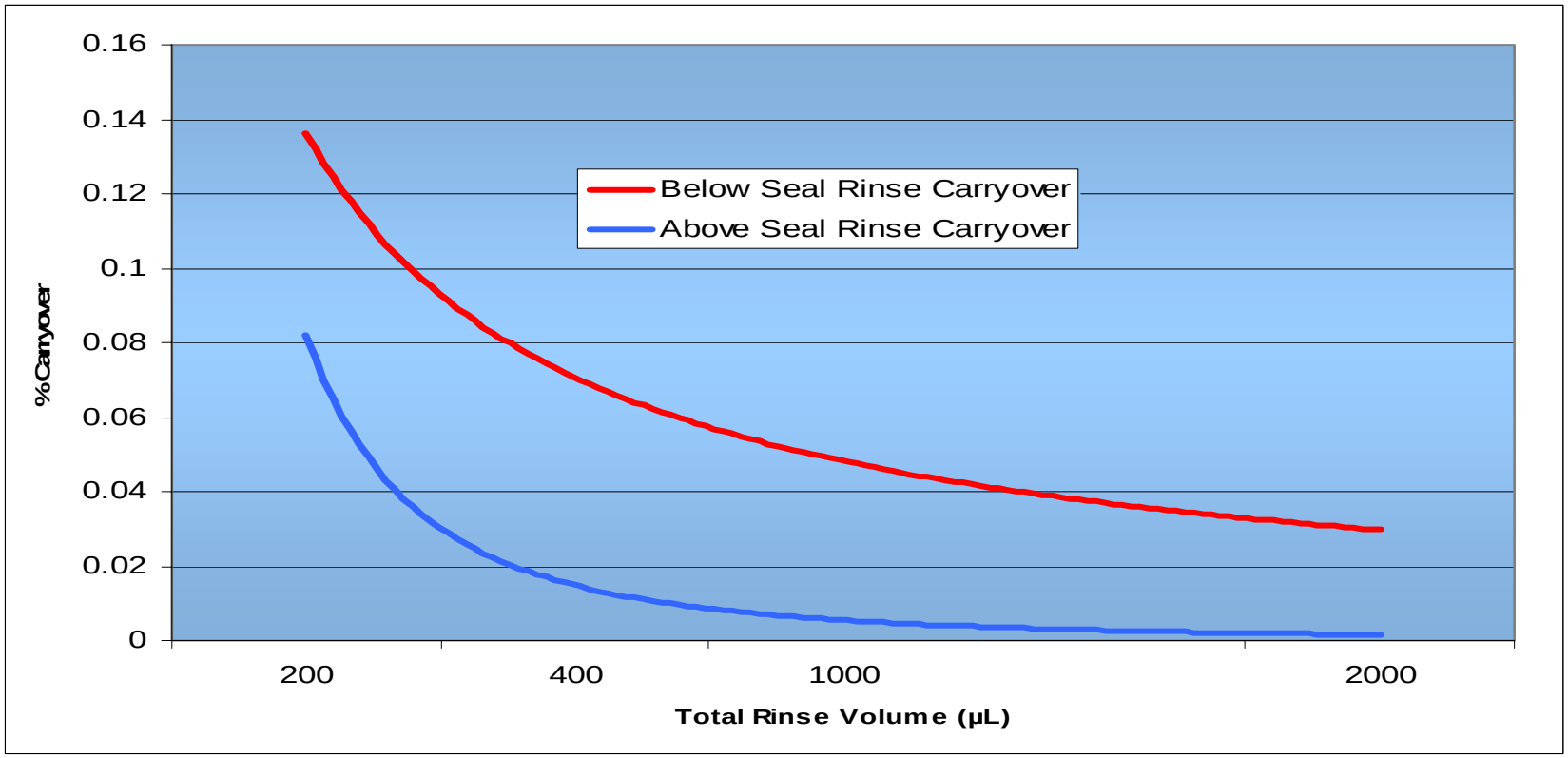
Rinse Techniques



Graph 14. Carryover can be decreased substantially if the below seal rinse is combined with an above seal rinse. The above seal rinse is a combination of one below seal rinse and three above seal rinses for the same total rinse volume as the single below seal rinse.

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Injection Techniques

- **Standard Injection**
  - Sample is aspirated into tubing, followed by dispensing into the injector sample loop (819 Injector).
- **Direct Loop Injection**
  - Sample is aspirated directly through the sample probe into the injection valve (845Z Injector).
- **Sandwich Injection**
  - Sample is aspirated into the injection path between plugs of strong solvent, typically DMSO, to protect the sample from precipitation or broadening (Either 819 or 845Z Injectors).

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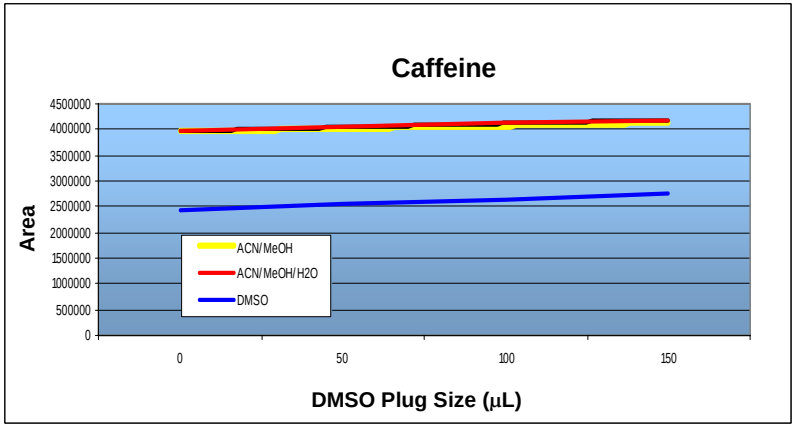
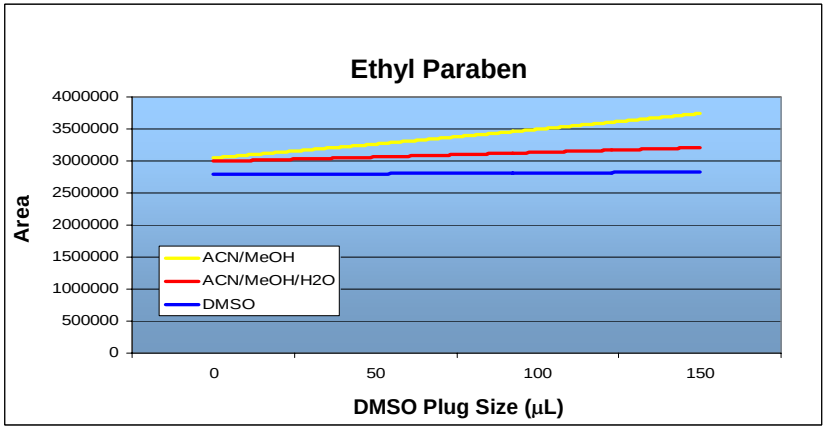
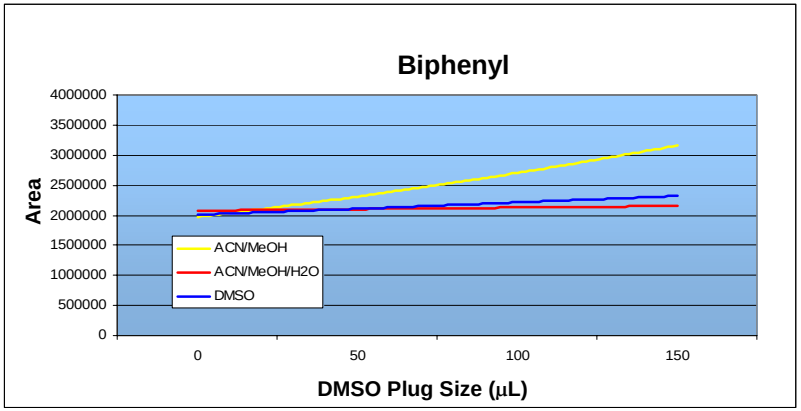
Sandwich Injection using Various Solvents and Varying DMSO Plug Size



Graph 15



Graph 17



Graph 16

Injection of various solvents and plug sizes illustrate the performance differences in solvent selection. The response increases with increasing plug size but as the plug size increase the chromatography gets poorer.

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Summary

- **Preparative HPLC Goals:**
  - Good peak shape
  - Concentrated fractions
  - Pure fractions
- **Experimental Results Conclusion**
  - Using the best suited solvent, inject the lowest amount of the most concentrated sample solution possible.
- **Preparative HPLC Reminders**
  - Use injection techniques that will allow for the sample to be injected onto the system without interference with the mobile phase prior to reaching the column
  - Optimize the solvent for solubility and chromatography.
  - Select a container material that it compatible with your solvent/sample system.

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Fact

69 of 80 human therapeutic proteins on the market in 2003 sold at prices over $10,000/gram.

Questions?

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