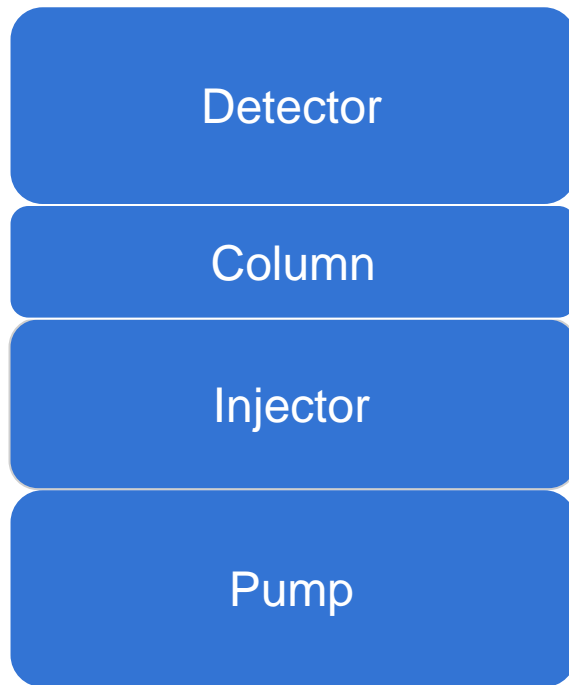


LC System Configurations: Which options should you choose?

Melanie Peguese-Richards
Technical Support Specialist

Basics of an LC System

Waters™



Outline for Today's Session

1 Pump Options

Binary Pumps
Quaternary Pumps

2 Injector Options

Flow Through Needle Injectors
Fixed Loop Injectors

3 Column Temperature

4 Detector Options

5 System Dispersion

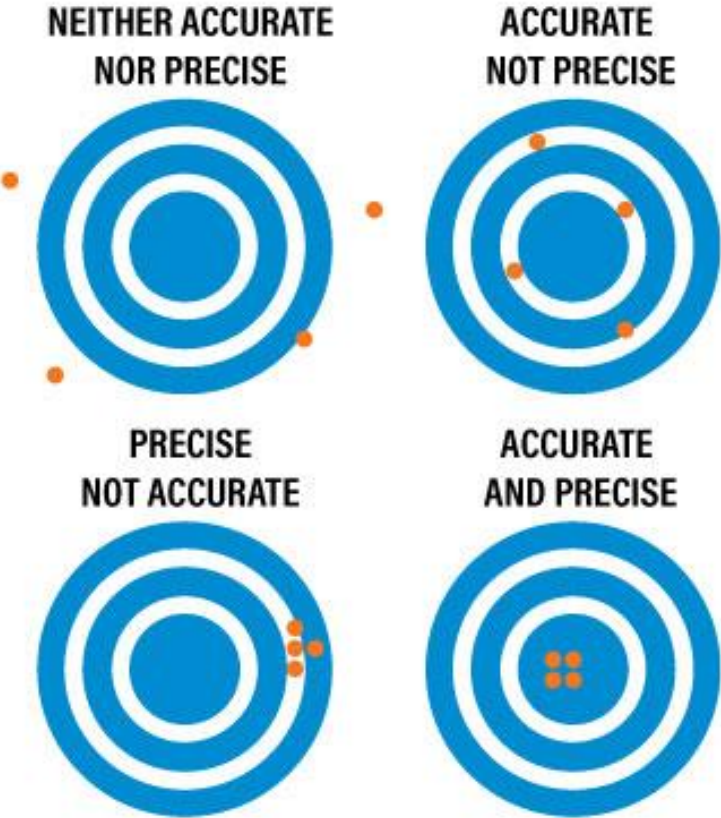
Liquid Chromatography – Terminology

- **Solvent:** Liquid
- **Mobile phase:** Solvent with or without additives
- **Pump:** Moves the mobile phase to the column
- **Solvent Management** modules includes:
 - Pump or pumps
 - In-line degasser
 - Filter/mixer
- **Types of solvent delivery**
 - **Isocratic:** Same mobile phase composition for the separation
 - **Gradient:** Mobile phase composition changes during the separation
- **Chromatographic terms**
 - **Run time:** Separation time + column re-equilibration time
 - **Re-equilibration time:** Needed to bring the column to initial condition
 - **Cycle time:** Injection to injection time – longer than run time
 - **Dwell volume:** the volume required for the change in a gradient to reach the column, or the volume difference between the point of mixing and the head of the column

An abstract graphic featuring a complex network of interconnected nodes and lines, resembling a molecular structure or a data network. The nodes are represented by small circles of varying sizes and shades of blue and grey, connected by thin, light blue lines. The background is a gradient of blue, with a darker blue horizontal band across the middle where the title is located.

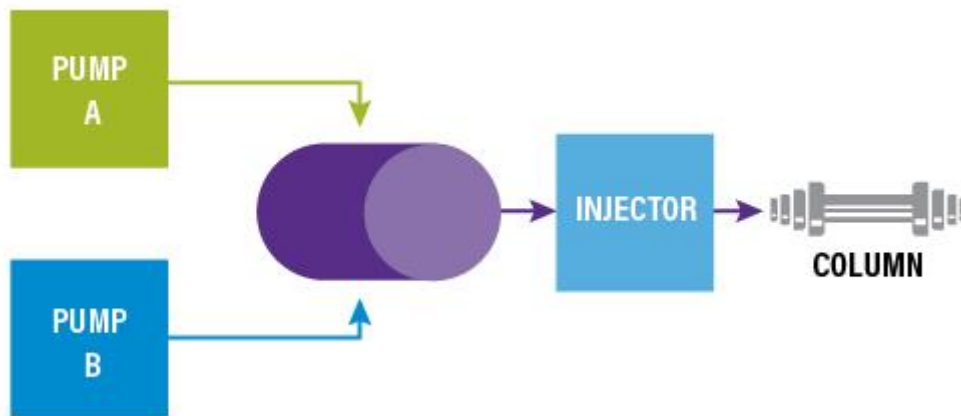
Pump Options

Precision v Accuracy



Pump options – Binary

- 2 independent pumps
- Composition determined by Mobile Phase ratio
- Solvents are mixed after pumping



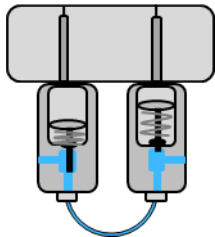
Mobile Phase Delivery of Binary Solvent Manager

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Method conditions:

Flow Rate: 0.600 mL/min

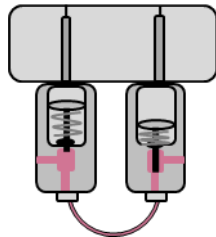
Composition: 98:2 Mobile Phase A: Mobile Phase B (Isocratic)



Flow Requirement

Pump A must deliver:

$$\begin{aligned} 0.600 \text{ mL/min} \times 0.98 \\ = 0.588 \text{ mL/min or} \\ \mathbf{588 \mu\text{L/min}} \end{aligned}$$

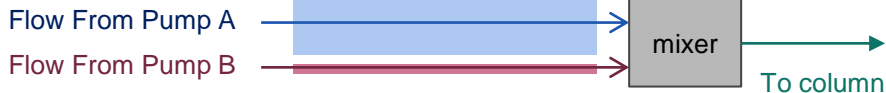


Flow Requirement

Pump B must deliver:

$$\begin{aligned} 0.600 \text{ mL/min} \times 0.02 \\ = 0.012 \text{ mL/min or} \\ \mathbf{12 \mu\text{L/min}} \end{aligned}$$

Isocratic Method



Gradient Method



Gradient Method: 98:2 A:B to 50:50 A:B

Initial A flow rate = 0.588 mL/min; Initial B flow rate: 0.012 mL/min

During gradient, linear change to final conditions:

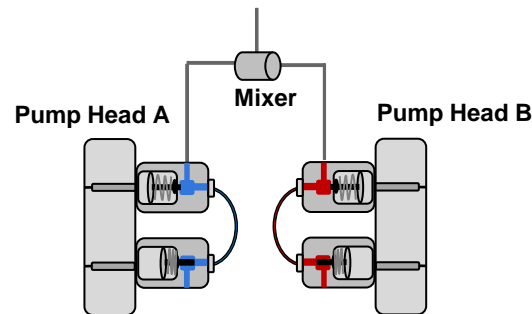
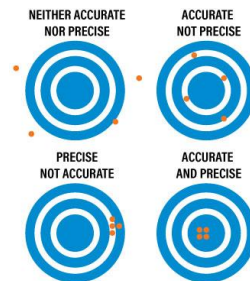
Final A flow rate = 0.300 mL; Final B flow rate = 0.300 mL/min

Compositional accuracy and precision dependent only on flow rates of A & B

Principles of Binary Solvent Manager

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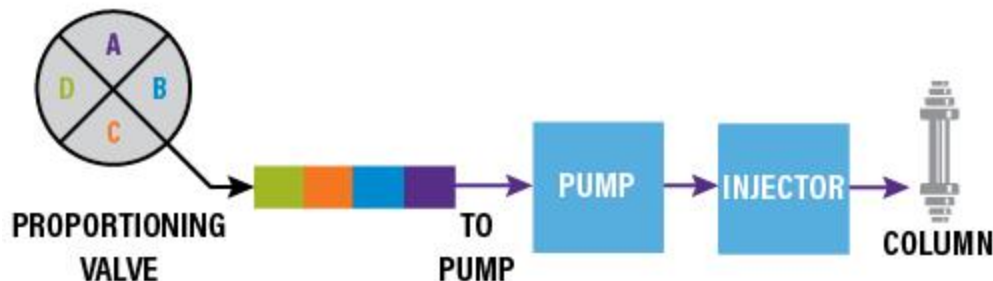
- Each pump head can impact the gradient fidelity:
 - Gradient accuracy requires the pumps deliver the **exact flow rate** each time
 - Gradient precision requires the pumps deliver the **identical flow rate** each time
- Since each mobile phase is delivered independently:
 - Flow rates are for individual solvents, viscosity changes from mixing occur after pump head
 - Superior performance at **low flow rates** and **low % of solvents**



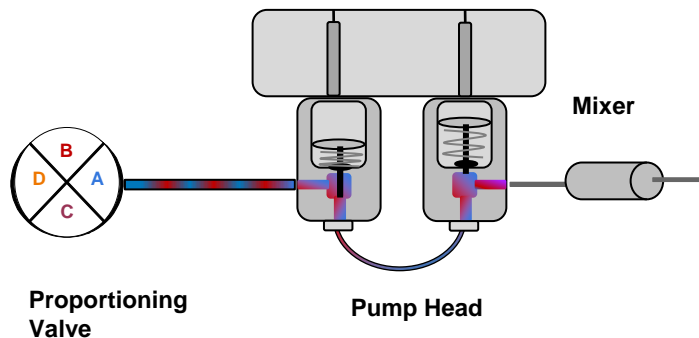
Pump Options – Quaternary

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- 1 pump with up to 4 solvents
- Mobile phase delivered via a proportioning valve before the pump
 - Low pressure mixing



Quaternary Cycle Timing



Stroke volume is the volume within each pump head (i.e. volume of a pump stroke)

Duty cycle is the time required for a single stroke

$$\text{Duty cycle} = (\text{stroke volume}) / (\text{flow rate})$$

Determine duty cycle for an isocratic method:

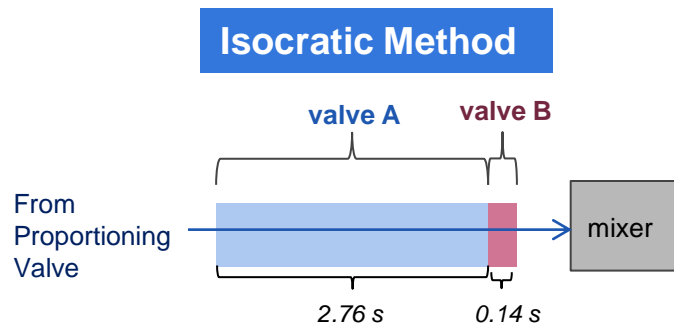
Flow Rate: 0.600 mL/min

Composition: 95:5 Mobile Phase A: Mobile Phase B (Isocratic)

$$\begin{aligned} \text{Duty cycle} &= (\text{stroke volume}) / (\text{flow rate}) \\ &= \frac{0.029 \text{ mL}}{0.600 \frac{\text{mL}}{\text{min}}} = 0.048 \text{ min} = 2.9 \text{ s} \end{aligned}$$

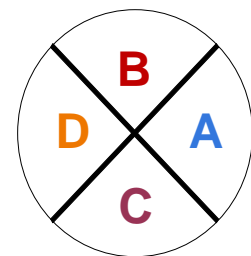
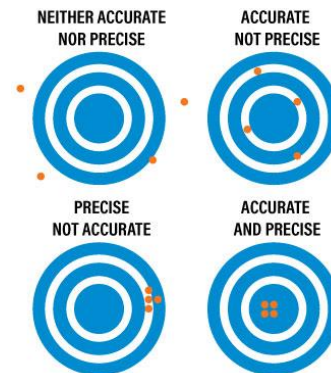
Valve A open for : $0.95 * 2.9 \text{ s (duty cycle)} = 2.76 \text{ s}$

Valve B open for : $0.05 * 2.9 \text{ s (duty cycle)} = 0.14 \text{ s!}$



Impact of Quaternary Cycle Times

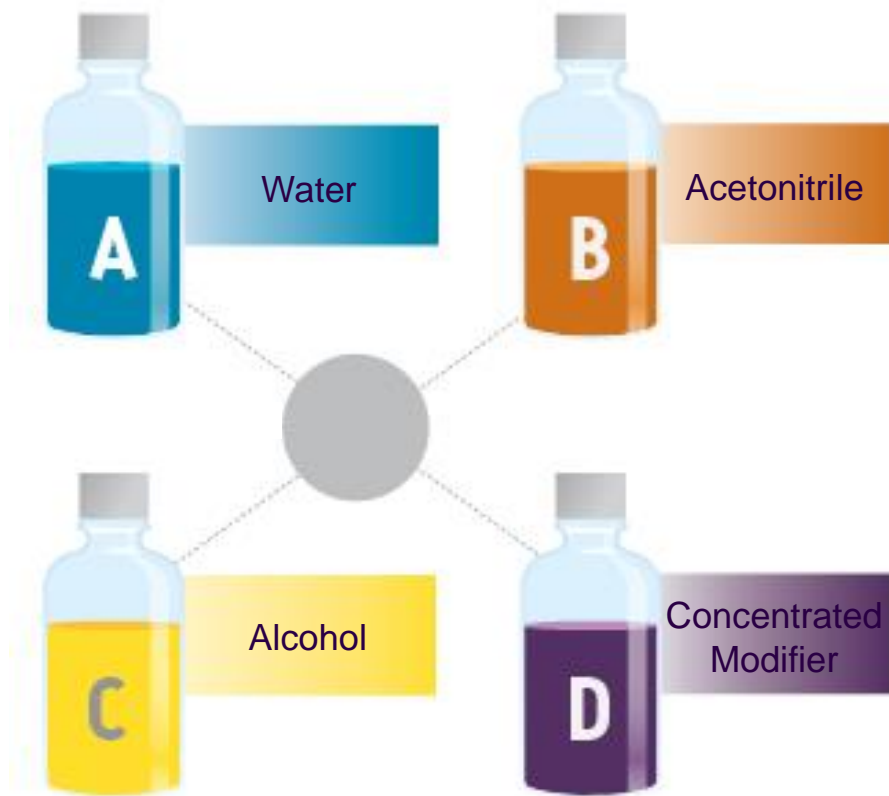
- The proportioning valve is critical to gradient formation of a quaternary pump
 - Gradient accuracy requires the proportioning valve deliver the **exact packet size** of solvent each time
 - Gradient precision requires the proportioning valve deliver the **identical packet size** each time
- Both accuracy and precision can be impacted by the cycle time
 - 5-95% of solvent typically be accurately and precisely delivered by a quaternary solvent manager
 - Very low flow rates can pose challenge for gradient delivery



Proportioning Valve

Quaternary Pump System – Auto•Blend Plus™ Technology

Waters™



Which LC Pump is Right for You?

- Are you performing developing methods or running routine screening?
 - Method development: **Quaternary** system provides the greatest flexibility
 - Routine Screening: **Binary** system may be best choice if only two mobile phases are used
- Do you need high sample throughput?
 - Speed: **Binary** system has lowest dwell volume for high throughput applications
- What kind of mobile phases options might you need?
 - Pre-mixed: **Binary** and **Quaternary** systems are both options. **Binary** may be preferred
 - Blending 3 or more solvents, Dial-a-Mix: Only performed using **Quaternary** system

Binary Pump

- Limited to 2 mobile phase options
- High pressure mixing
- Gradient changes can be made rapidly
- Better for high-throughput/screening analysis

Quaternary Pump

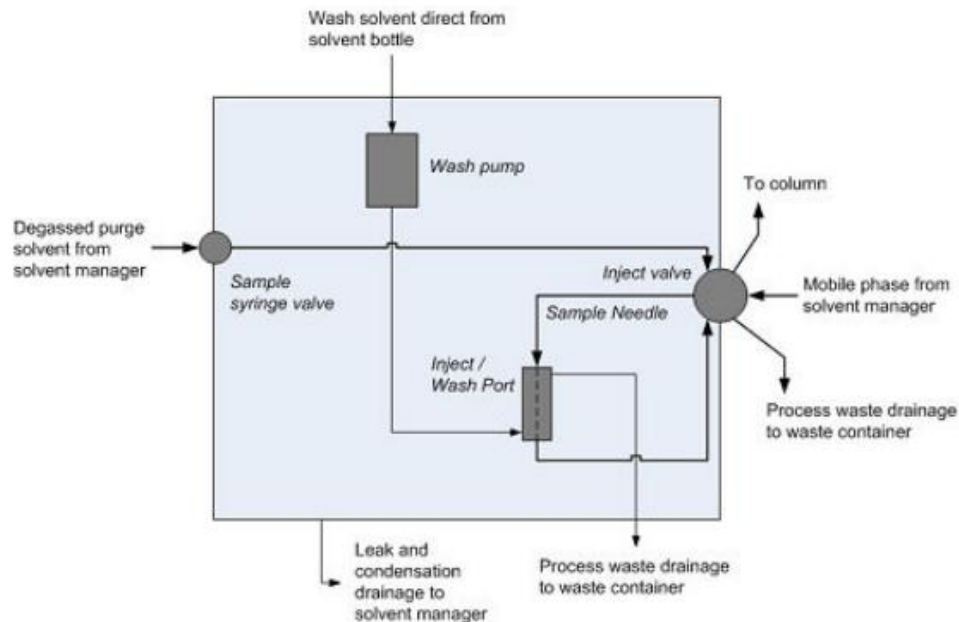
- Up to 4 mobile phases
- Low pressure mixing
- Gradient changes will be slower
- Better for method development



Injector Options

Flow Through Needle (FTN) Injectors

- Needle is part of the flow path
- Mobile phase flow controlled via valves
- Inside of needle washed by mobile phase as part of the flowpath



Purge Solvent

- This does not contact sample, so composition is less important
- Use a mixture that has some organic solvent to prevent bacterial growth
- Try to avoid additives

Needle Wash Solvent

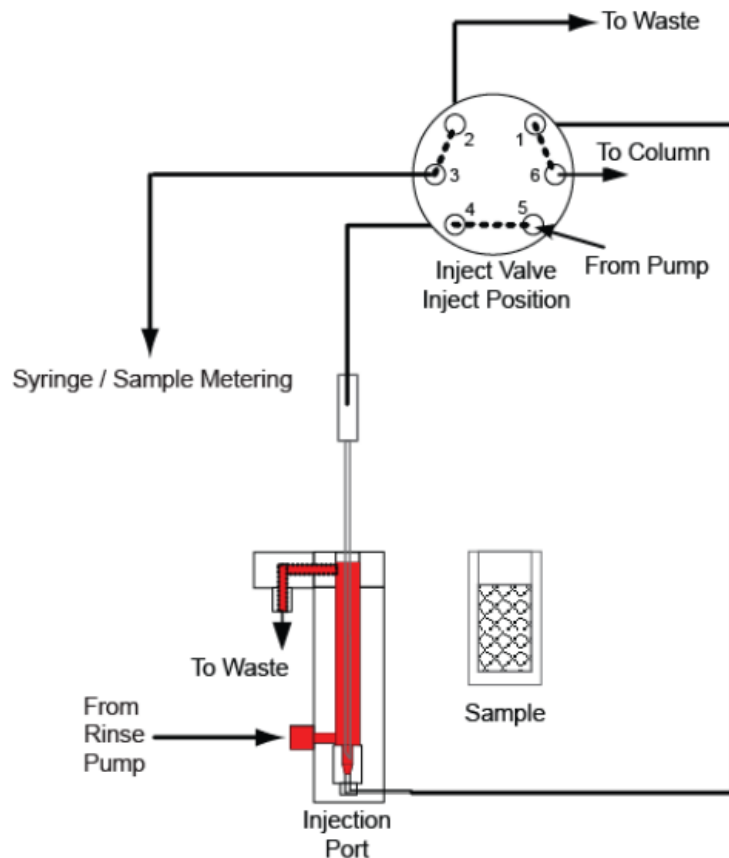
- Sample should be entirely soluble in the wash solvent
- Should be compatible with the sample diluent
- Can contain modifiers
- Very poor selection of wash solvent can lead to contamination
- 50/50 Water/Acetonitrile is typically a good starting point – But check solubility

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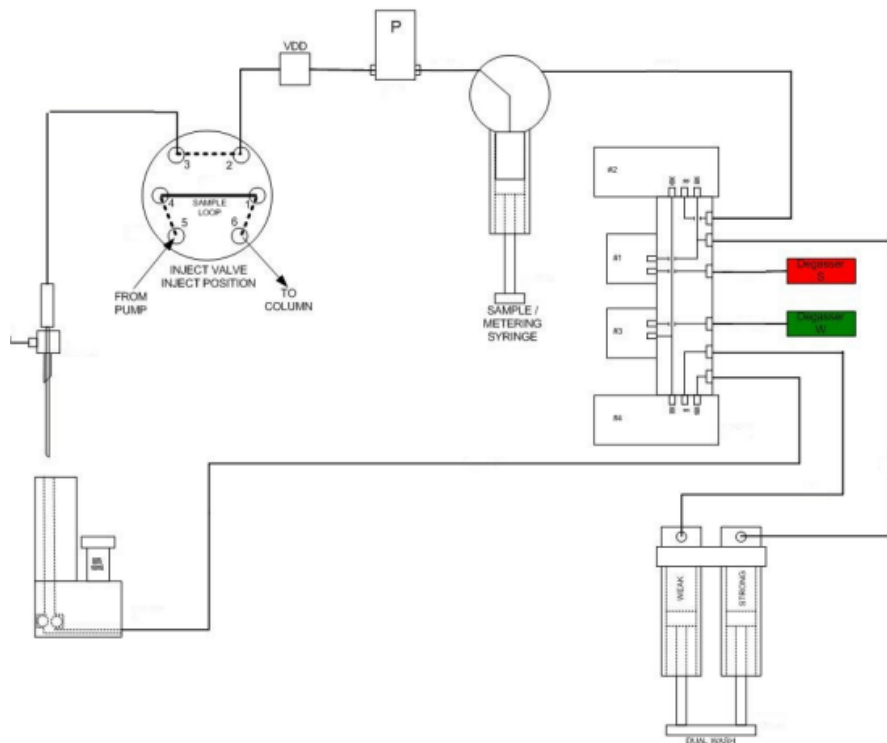
Flow Through Needle – Sample Injection

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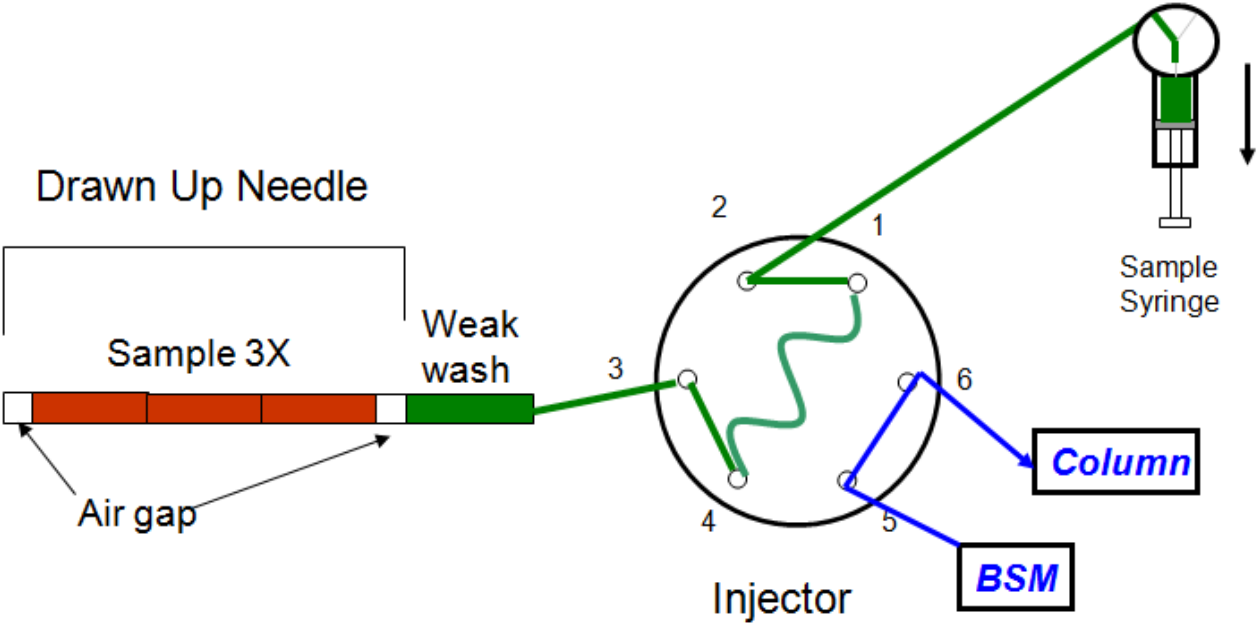


Injector Options – Fixed Loop

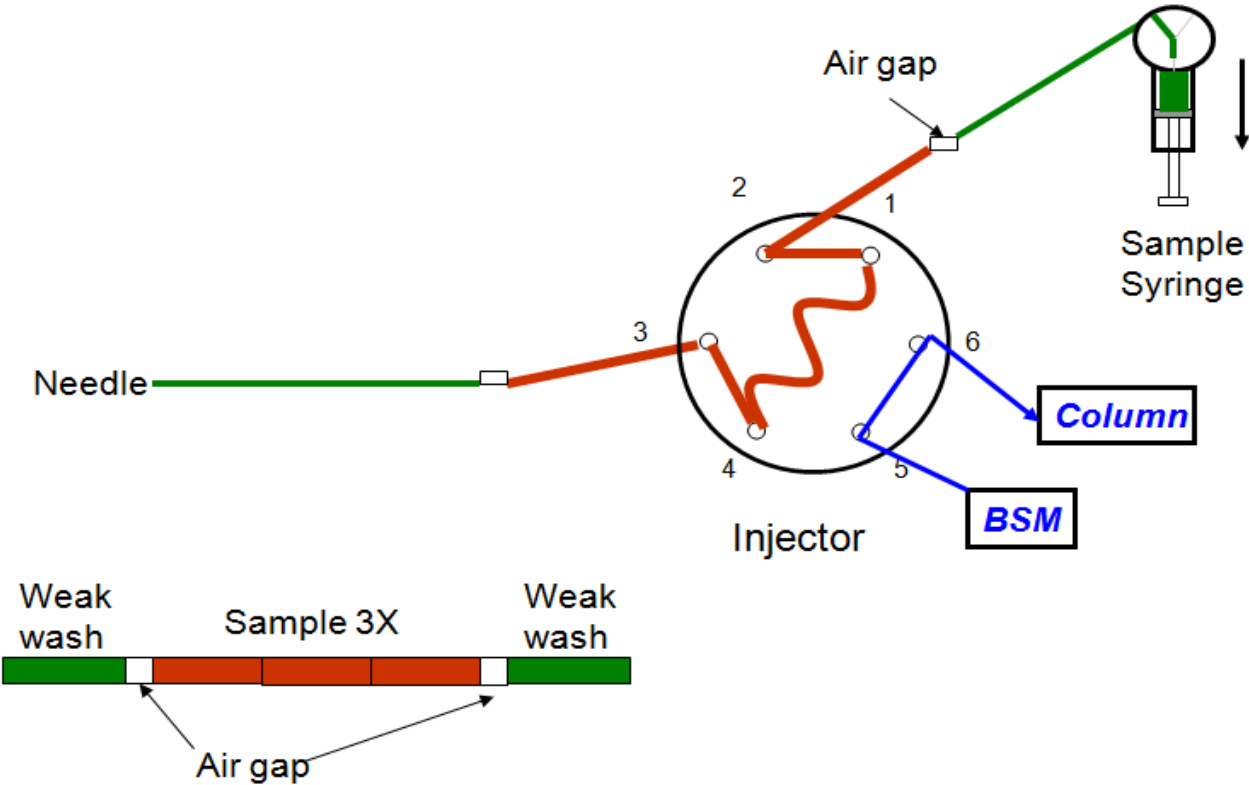
- Needle is not part of the flow path
- Variety of injection options available
 - Full loop
 - Partial Loop Pressure Assist
 - Partial Loop with Needle Overfill
 - Default injection type for Waters UPLCs



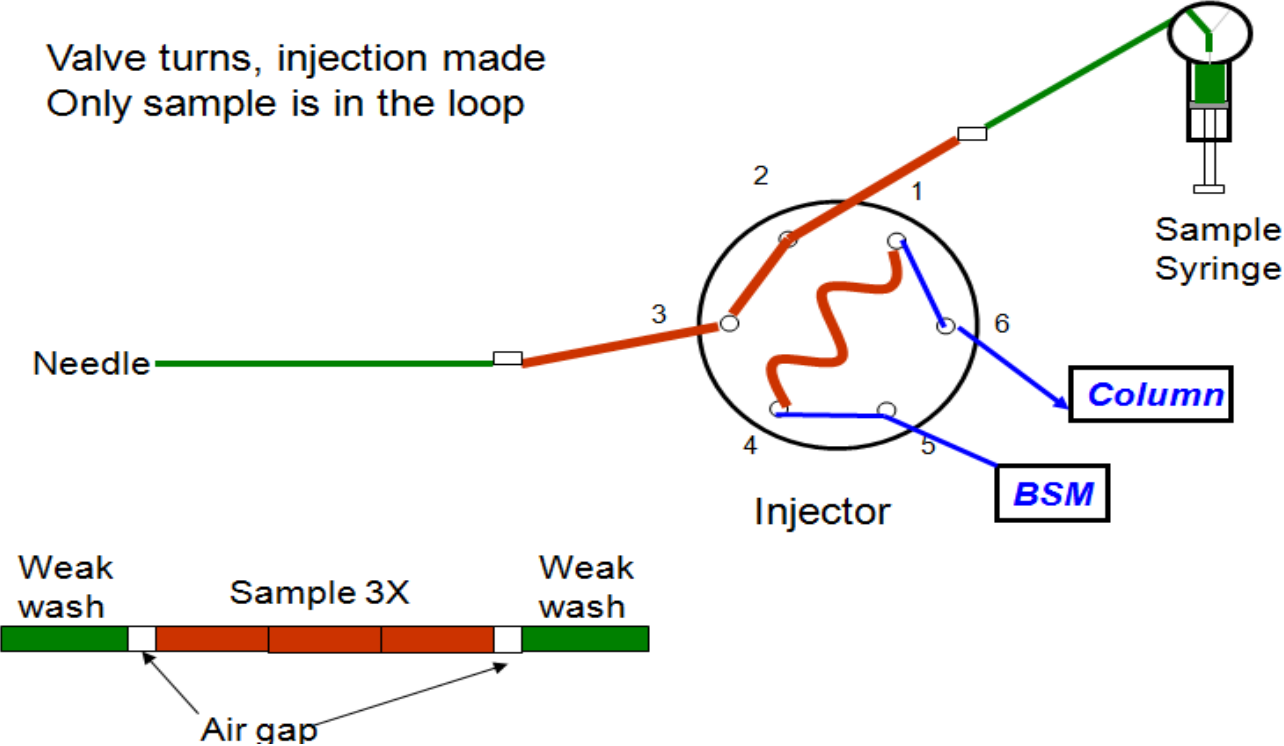
Full Loop Injection – Sample Draw



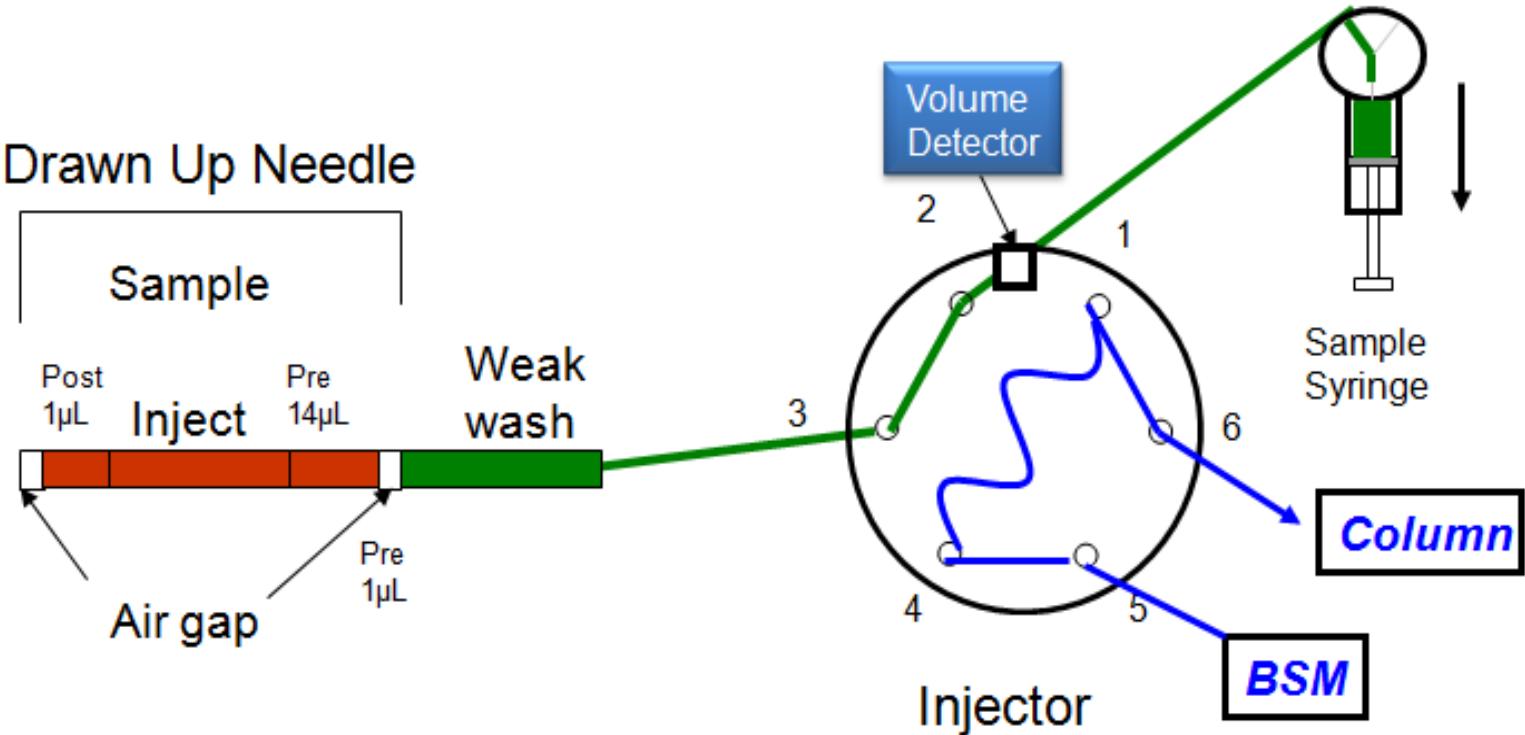
Full Loop Injection – Sample Loaded



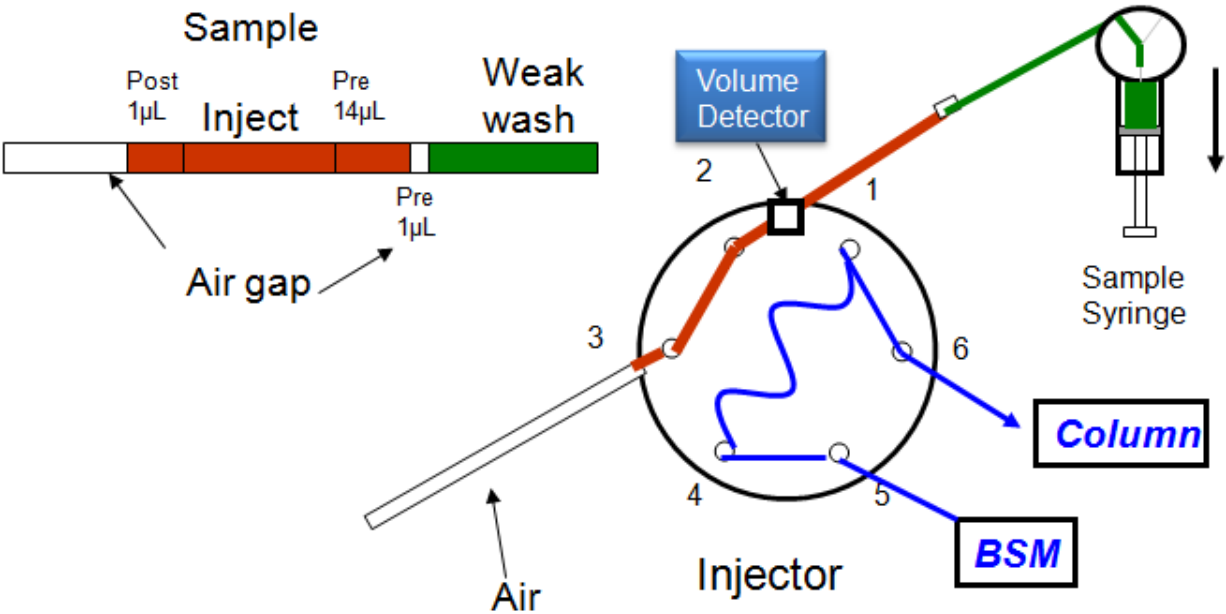
Full Loop Injection – Sample Injected



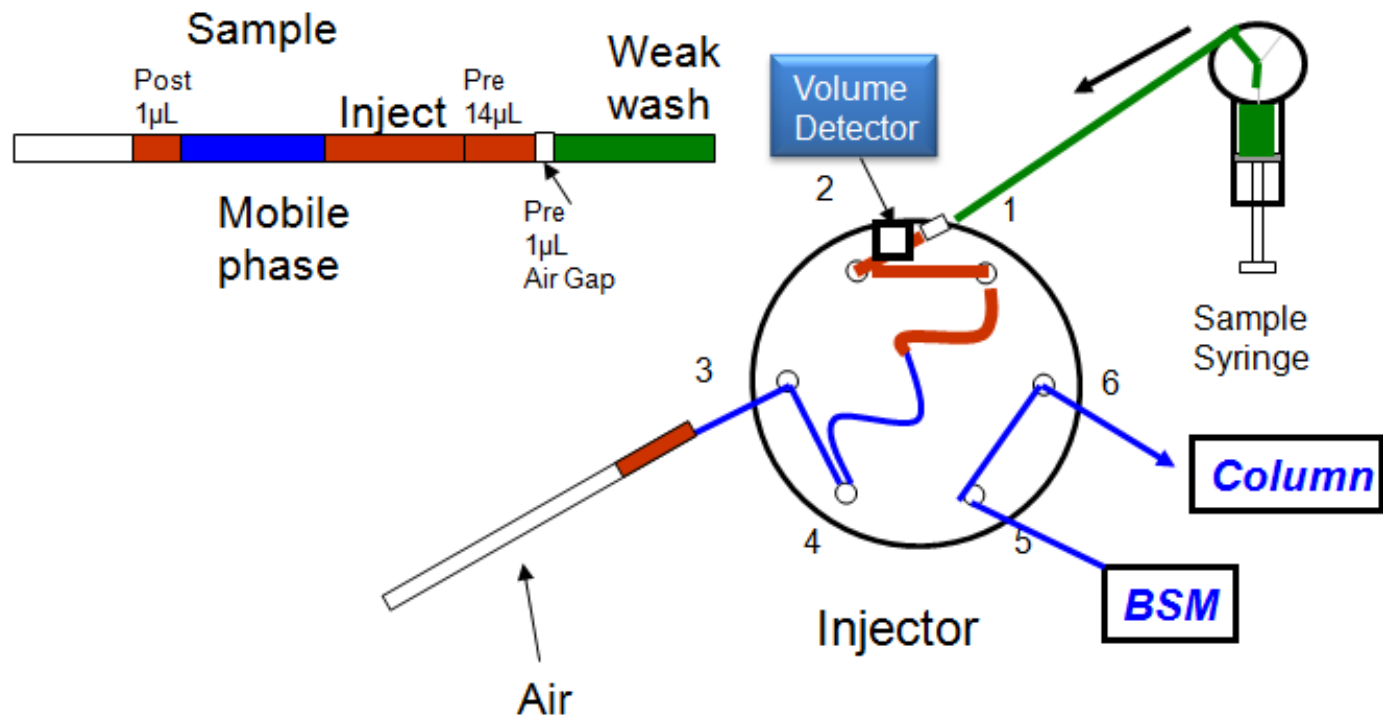
Partial Loop with Needle Overfill (PLUNO) – Sample Drawn



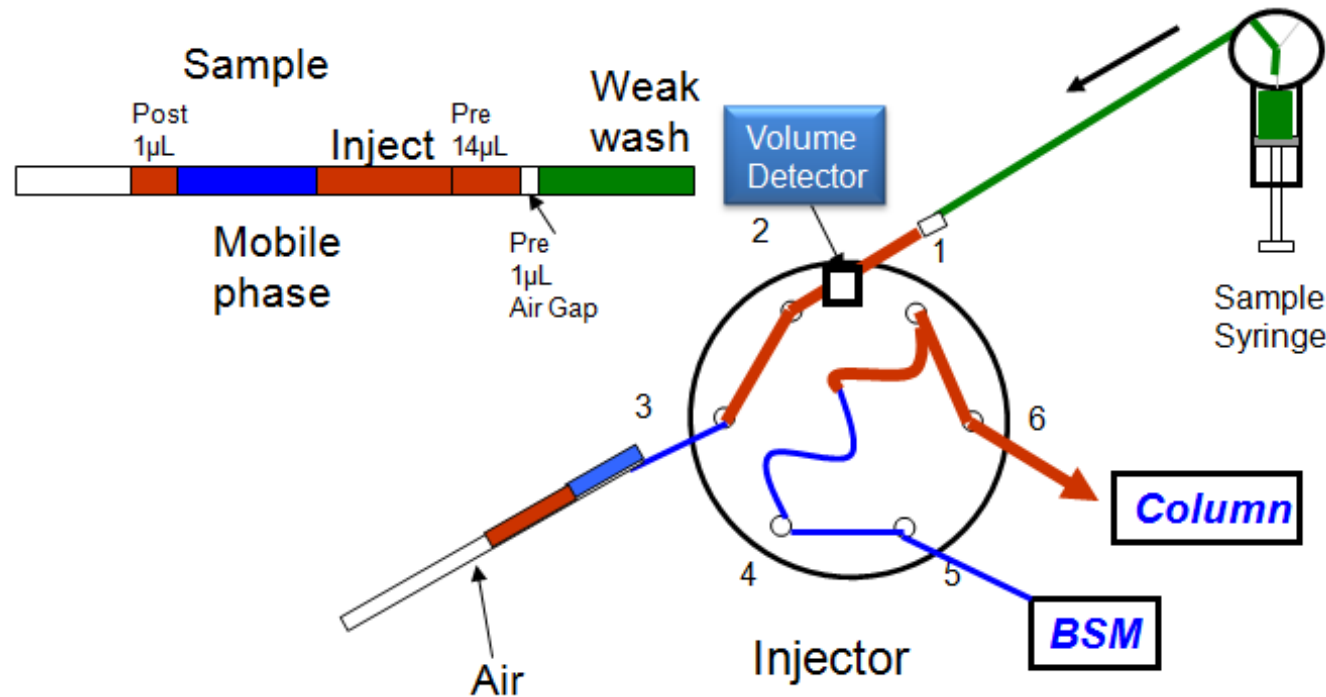
Partial Loop with Needle Overfill (PLUNO) – Sample Positioned



Partial Loop with Needle Overfill (PLUNO) – Sample Loaded



Partial Loop with Needle Overfill (PLUNO) – Sample Injected



- Strong Wash
 - Washes outside of the needle
 - Higher organic content than weak wash
 - Must be compatible with weak wash and mobile phase

- Weak Wash
 - Washes the inside of the needle
 - Could be injected onto the column depending on injection type
 - Composition should be close to your mobile phase initial conditions
 - Must contain some enough organic to be bacteriostatic

Which Injector Should You use?

- Sample limited?
 - Flow through Needle. Only injects what you program

- If you know you have very sticky compounds, prone to carryover and cycle time is critical?
 - Fixed loop. More flexibility in washing solution
 - Faster cycle time as sample can be loaded during the previous injection saving time.

- Both sample limited and prone to carryover?
 - Flow through Needle. Ads cycle time as gradient washing step (high %) needs to increase.

- Methods with various injection volumes?
 - Flow Through Needle design. Injects 0.1-10 µl without configuration change. 0.1-50 µl with extension loop.

Two Solutions for Sample Loading

Fixed Loop

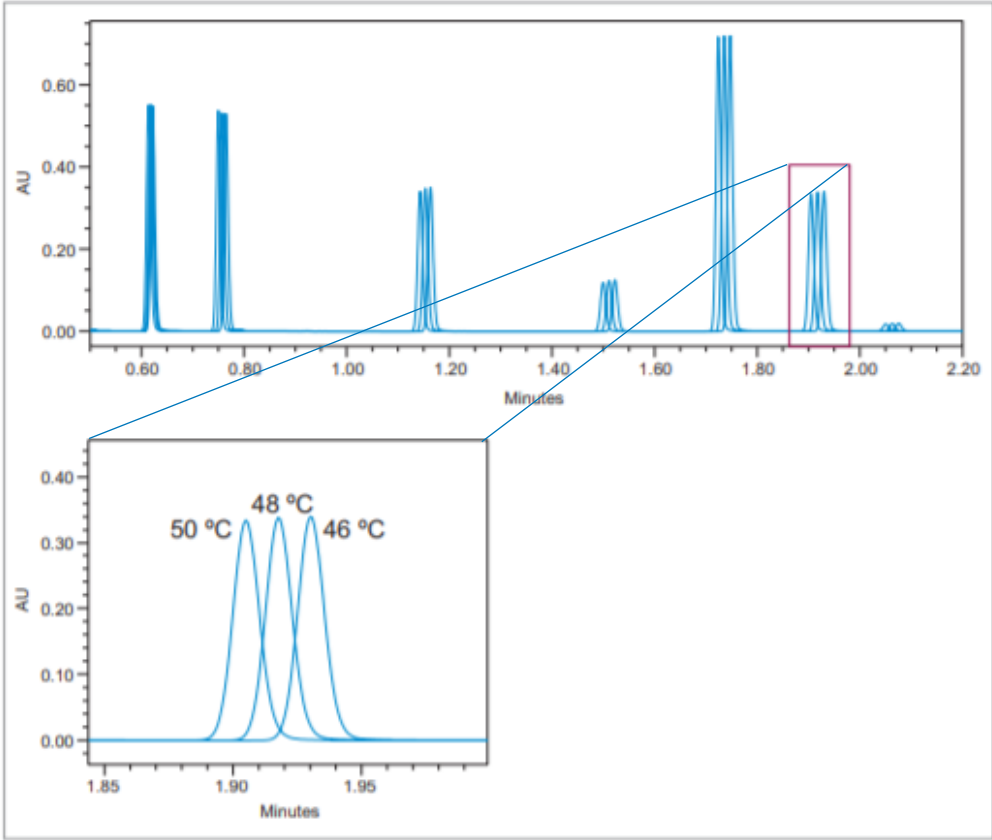
- Sample is **transferred** from the sample vial through the needle to the sample loop
- Equivalent to using a transfer pipet to place 10 mL of a standard solution in a 100 mL volumetric flask includes
 - Rinse step to manage cross contamination and dilution
 - Loading step to ensure correct volume is taken
 - Delivery step to ensure the correct volume is dispensed
- Benefits – Speed, low dispersion, small contribution to dwell volume
- Issues ...
 - Needle and Sample Loop volumes must be calibrated for best results
 - Sample solution is exposed to surface of the needle *before* arriving at sample loop – potential loss of analytes via adsorption ... **hydrophobia!**

Flow Through Needle

- Sample is **drawn directly** into the sampling needle which is part of the sample loop
- Equivalent to loading a manual sample syringe
- Single step process with minimal sample loss
- Benefits
 - Minimal loss of sample
 - Volumetric accuracy
 - Full chromatographic gradient is pumped through the needle
 - Small contact area for carryover
- Issues ... dispersion is greater for small injection volumes

Column Temperature Control

Impact of Column Temperature



<http://www.waters.com/webassets/cms/library/docs/720006236en.pdf>

Types of Mobile Phase Heating

- Passive Pre-heating

- System tubing is held inside the column oven at the same temperature as the column

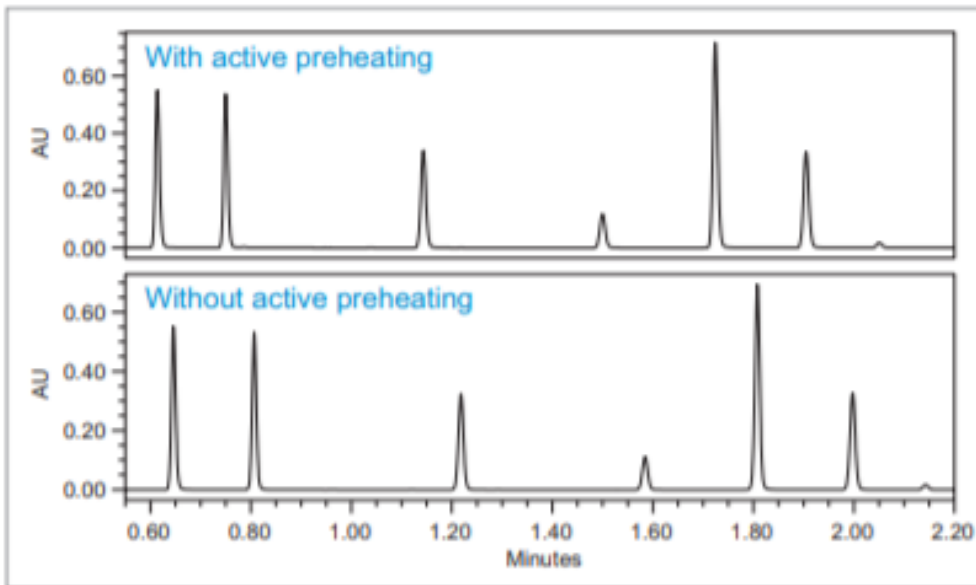


- Active Pre-heating

- Mobile phase passes through a heat sink with a temperature applied prior to hitting the column



Active v Passive Preheating

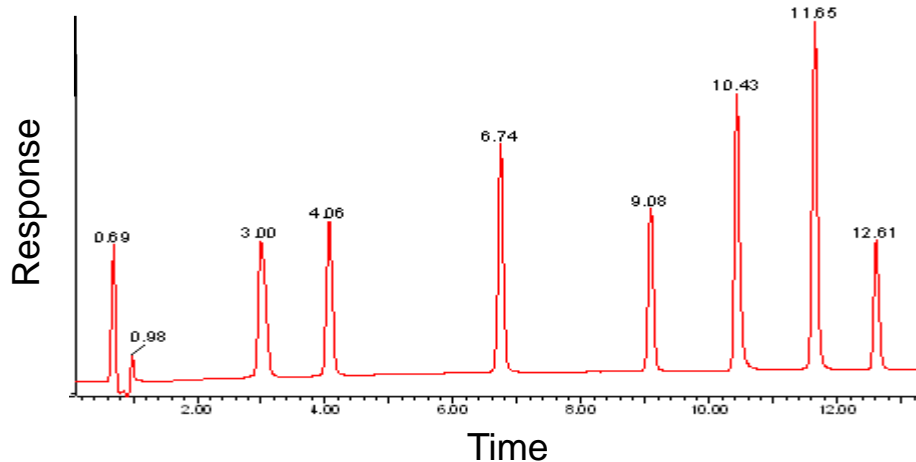


Peak	RT with AP (min)	RT without AP (min)	RT Δ (min)
1	0.614	0.646	0.030
2	0.750	0.807	0.057
3	1.143	1.218	0.075
4	1.500	1.585	0.085
5	1.724	1.808	0.084
6	1.905	1.998	0.093
7	2.051	2.143	0.092

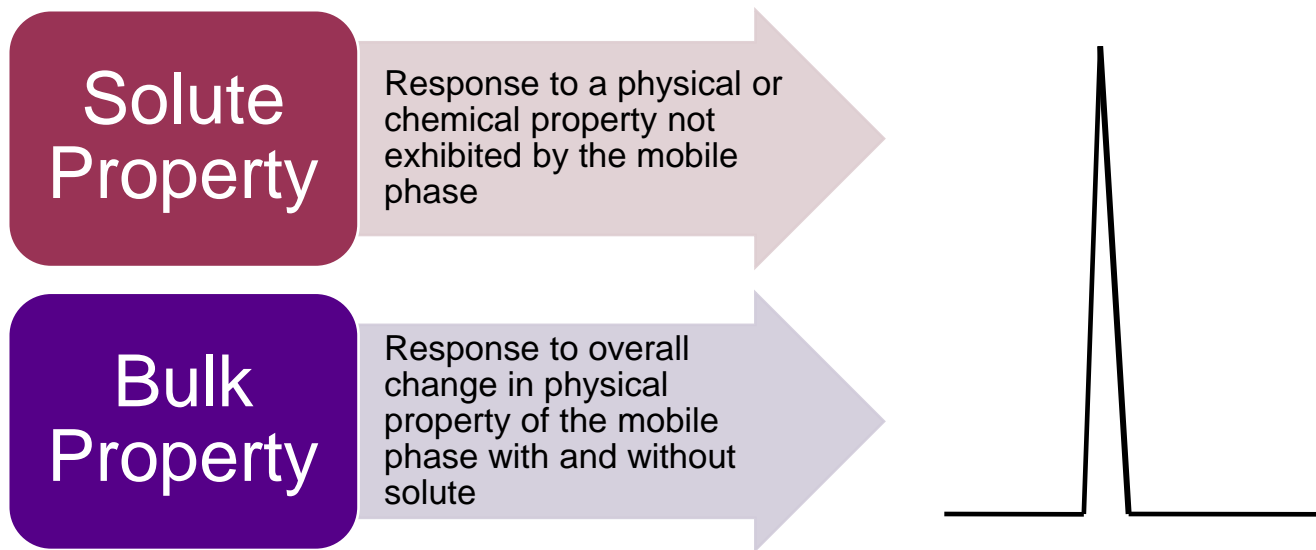
The background of the slide features a complex, abstract network diagram. It consists of numerous small, light blue circular nodes connected by thin, light blue lines, creating a web-like structure. Some nodes are slightly larger and darker blue. The network is denser on the left side and fades out towards the right. A solid dark blue horizontal band runs across the middle of the slide, serving as a backdrop for the title text.

LC Detection Options

- Instrument in the chromatographic system which senses the presence of a compound passing through and provides an electronic signal to a computer data station or recorder.
- Output is a chromatogram where analytes appear as peaks when detected.



Selecting the Right Detection Technique

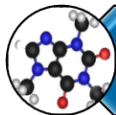


- There is no single detector that can be employed for all LC separations – no 'black box' or 'universal detector'
- Combining detection techniques can improve detection capabilities

Selecting the Right Detection Technique

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Points to
consider when
selecting
detection
techniques



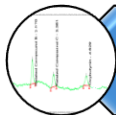
Chemical Properties of Sample



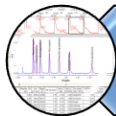
Mobile Phase Constraints



Gradient or Isocratic Separation



Limits of Detection

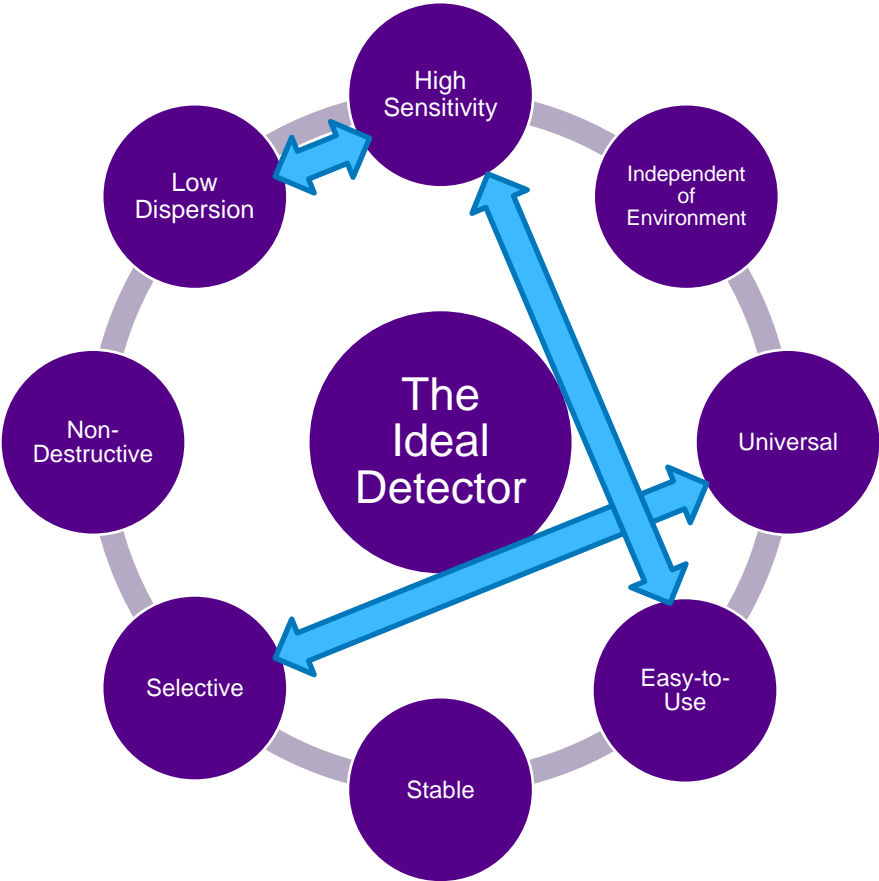


Peak Identification



Multiple Detection Techniques Required?

Desired Criteria for Detectors



Optical Detectors

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Absorbance
(UV/Vis)

Electrochemical

Laser
Interferometer

Nitrogen Specific

Mass Spectrometry

Chemiluminescence

Evaporative Light
Scattering

Laser Polarimeter

Nuclear Magnetic
Resonance

Refractive Index

Circular Dichroism
(Chiral)

Condensation
Nucleation Light
Scattering

Fluorescence

Photodiode Array

Off-line Methods

Radiochemical

Conductivity

Laser Induced
Fluorescence

Multi-Angle Light
Scattering

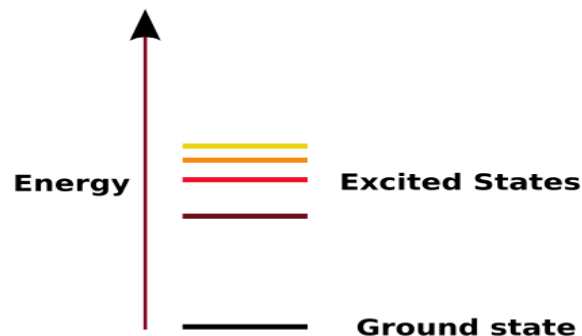
Optical Rotation

Sulfur Specific

Viscometry

Charged Aerosol
Detection

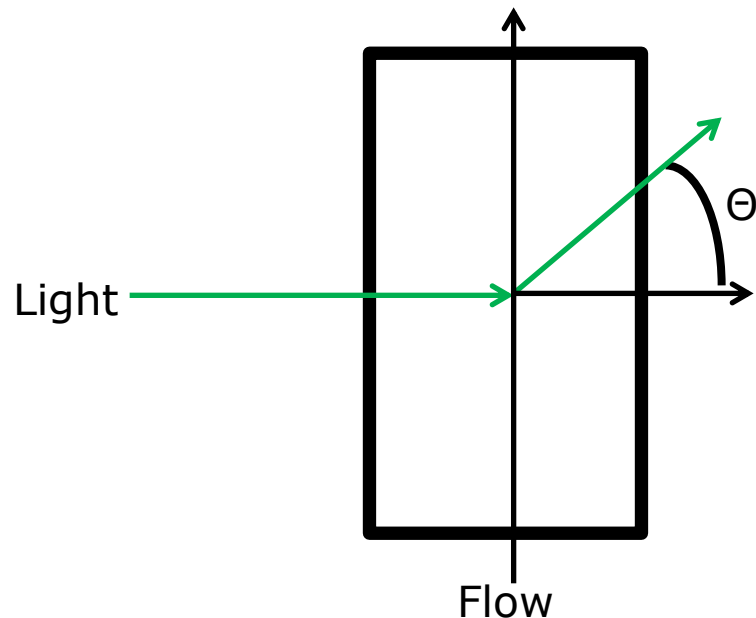
- Molecules can absorb light (energy)
 - When the molecule absorbs energy its electronic structure changes to what is called an 'excited state'
 - It is not stable in its 'excited state' so it releases the energy, usually as heat, and goes back to its original electronic structure or 'ground state'
- Molecules absorb light at specific wavelengths (energy levels)
- The amount of light absorbed depends on:
 - The molecule
 - The concentration
 - The path length the light travels through



Refractive Index Detector (RI)

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- Detection Mechanism
 - Universal analyte detector
 - Measures the change in refractive index when the sample passes through flow cell
 - Solvent must remain the same throughout separation - isocratic
 - VERY temperature sensitive - sometimes difficult to stabilize baseline
 - Less sensitivity than other detectors
 - Can not be used with gradients
- Typical Analytes: Those without chromophores
 - Lipids
 - Carbohydrates
 - Polymers

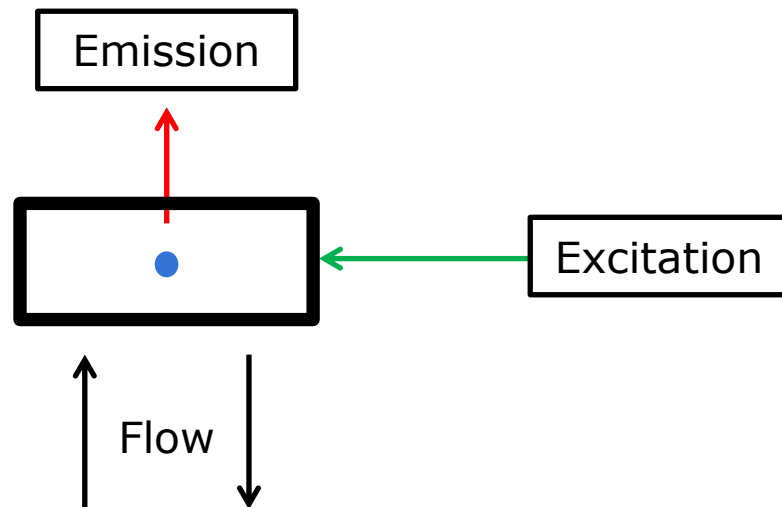


■ Detection Mechanism

- Excitation wavelength generates fluorescence, emission at a higher wavelength
- Very sensitive and selective as compared to optical detectors
 - Typical FLR sensitivity is 10-1000 times greater than that of UV/VIS
- Detector response can vary dependent upon separation conditions and lamp energy

■ Typical Analytes

- Analytes must have fluorophore group, those with ring structures
- Amino Acids
- Vitamins
- Derivatized compounds with a fluorescent tag



■ Detector Mechanism

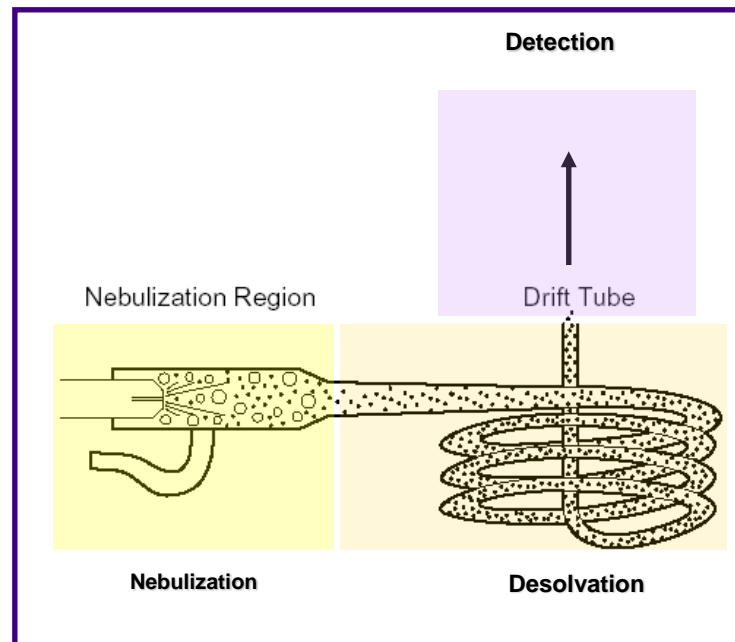
- The conductivity of the column effluent is continuously measured and the appearance of the analyte in the cell is indicated by a change in conductivity.
- Best use is made of this detector in isocratic analysis since solvent gradients will cause a proportional shift in the baseline.
- Analytes must have a positive or negative charge to be detected
- Response is linear with concentration over a wide range.

■ Typical Analytes

- Inorganic anions and cations
- Small organic acids
- Ion exchange chromatography

Evaporative Light Scattering Detector

- Broad application range for non-volatile or semi-volatile compounds
 - Fast and sensitive non-UV/Visible detection
- Nebulizer options
 - 2 nebulizers cover wide flow range
 - Temperature controlled
- 1 – 1.5 orders of magnitude linear range
- Destructive detector



■ Detection Mechanism

- Measures the mass-to-charge ratio (m/z)
 - Compounds must have a charge (positive or negative) associated with them to be detected.
 - The compound can acquire a charge
- MS detection occurs in a high vacuum environment
 - In LC/MS analytes must go from a liquid to a gaseous environment.
 - Mobile phase must be volatile so it can be removed.

■ Typical Analytes

- Proteins
- Peptides
- Drugs
- Pesticides

An abstract network diagram composed of numerous interconnected nodes and lines, creating a complex web-like structure. The nodes are represented by small circles of varying shades of blue and grey, while the lines are thin, light blue. The background is a gradient of blue, transitioning from a lighter shade on the left to a darker shade on the right. A solid dark blue horizontal band runs across the middle of the image, serving as a backdrop for the title text.

System Dispersion

Instrument (System) Dispersion

What is it & Where is it

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- Instrument dispersion is the broadening of the analytical band due to the instruments flow path volume
- Any place where the analytical band “moves” adds to an instrument’s dispersion
 - Injector
 - Tubing
 - Pre-column
 - Post-column
 - Column heater design
 - Flow cell volume

Defining the LC Categories by their System Dispersion

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**HPLC
Systems**

Dispersion > 30 μ L



Alliance™ System

**UHPLC
Systems**

Dispersion 12 - 30 μ L



ACQUITY™ Arc System

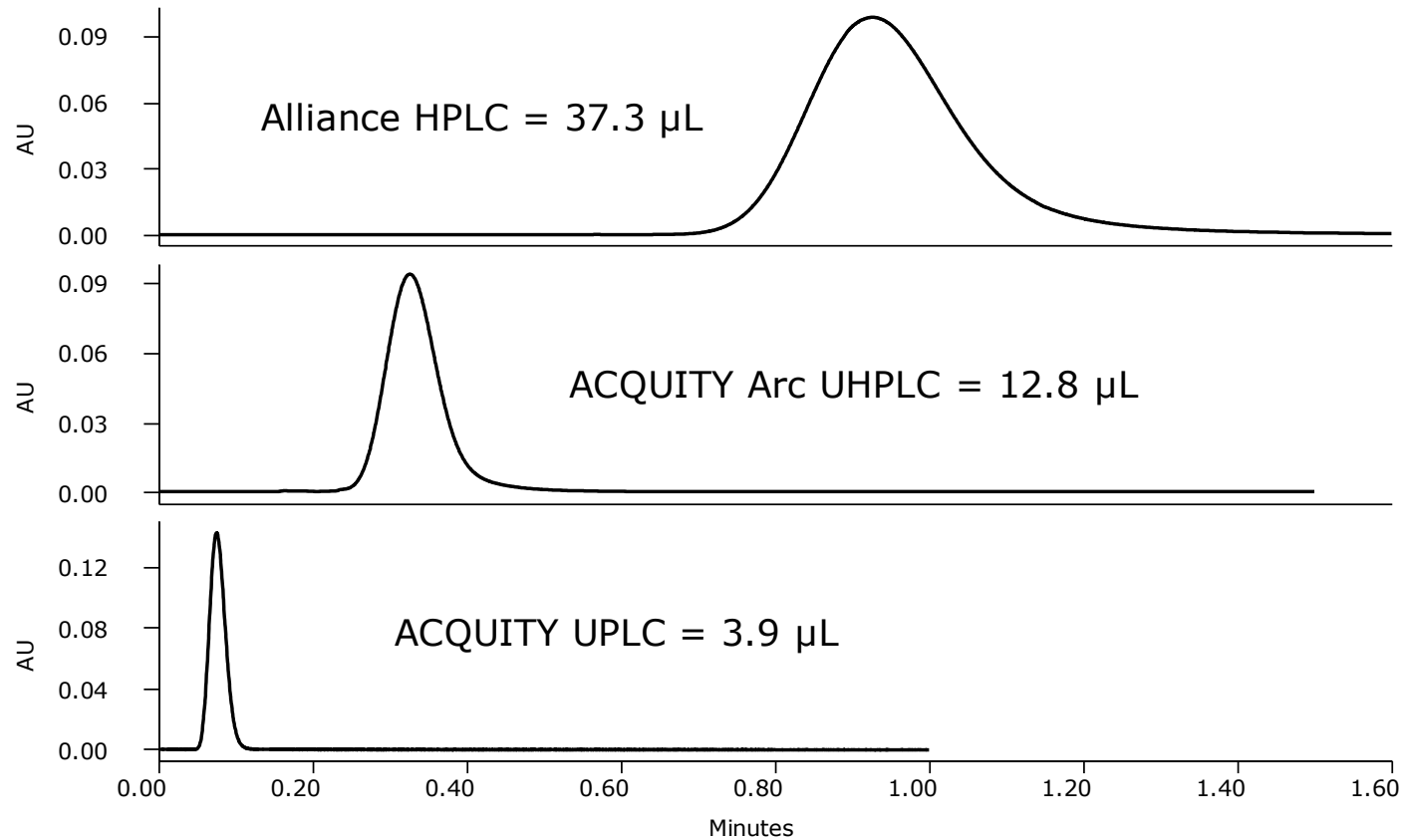
**UPLC™
Systems**

Dispersion < 12 μ L



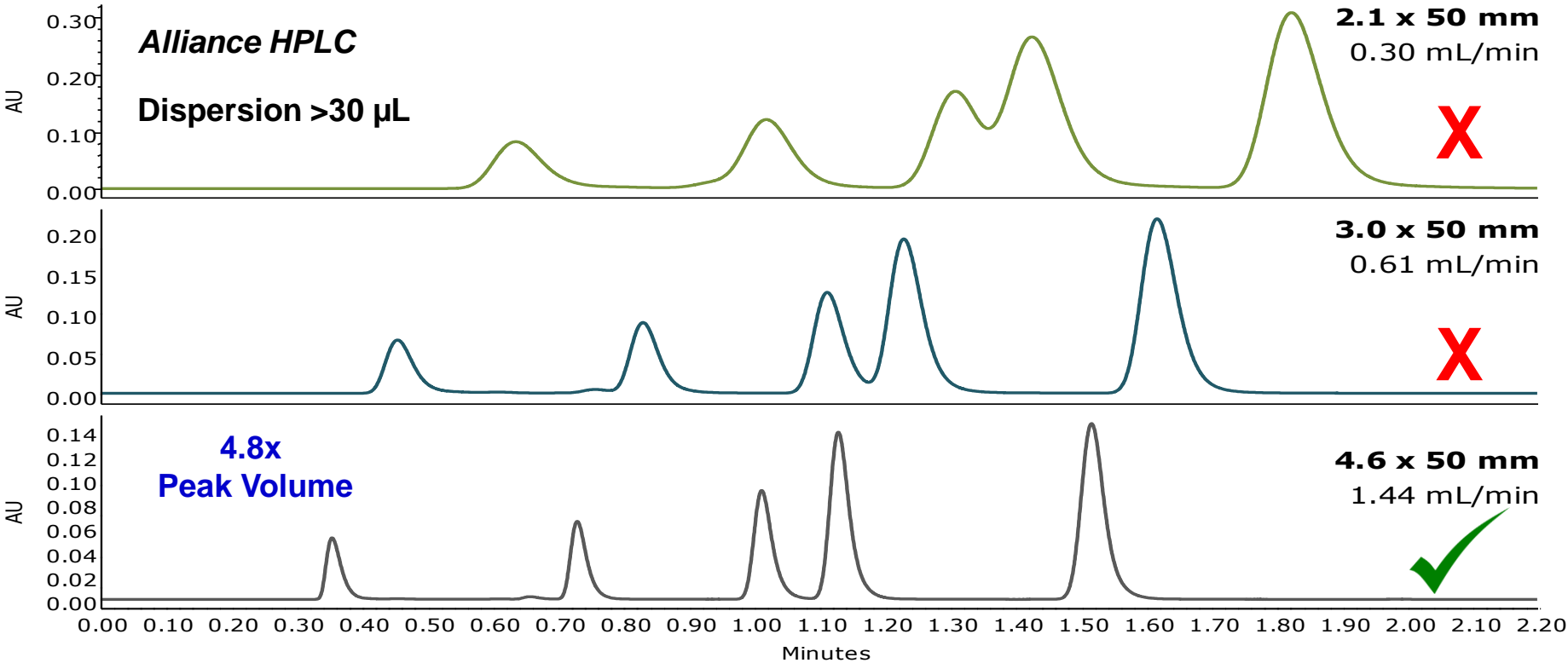
ACQUITY™ UPLC System

Calculated Instrument Dispersion Values



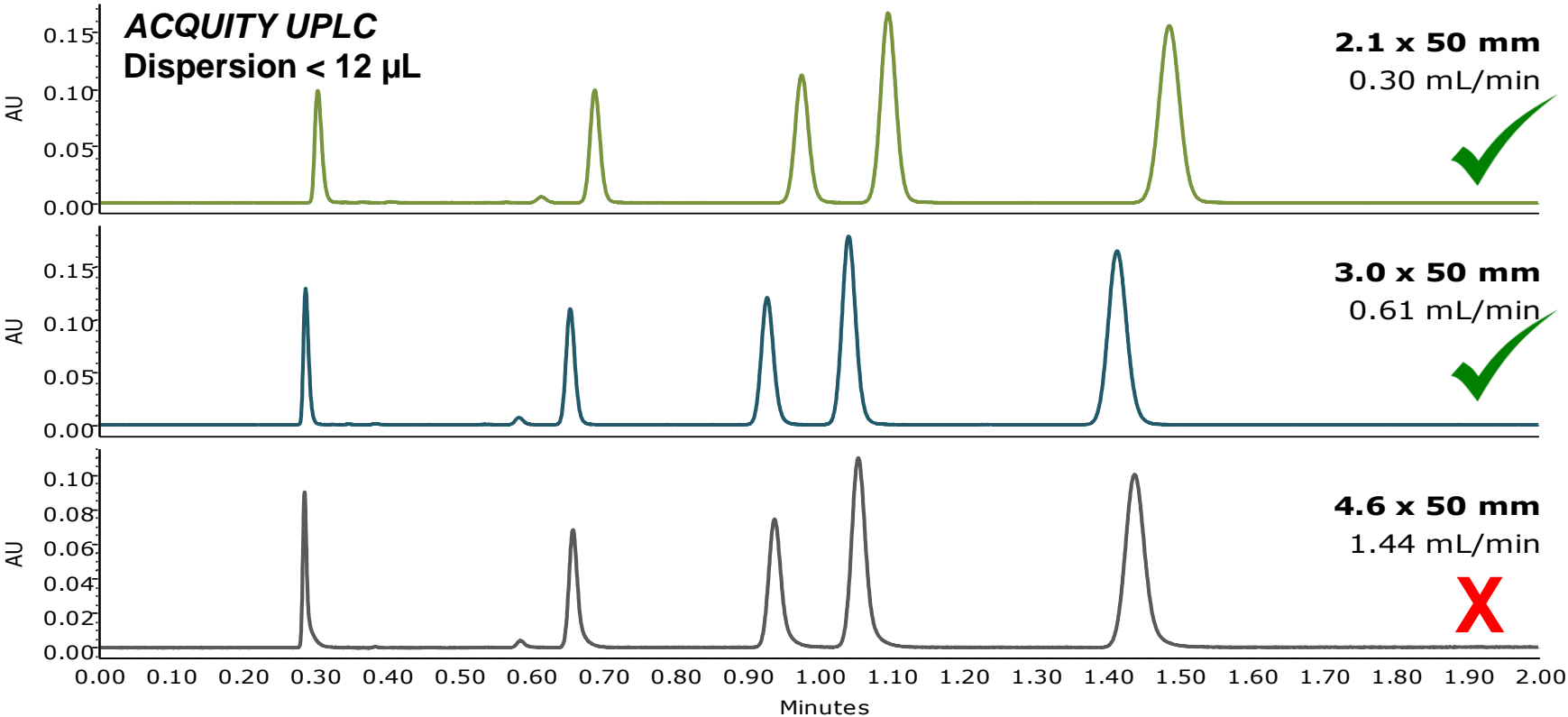
Column Internal Diameter and Instrument Dispersion

HPLC Instruments



Column Internal Diameter and Instrument Dispersion

UPLC Instruments

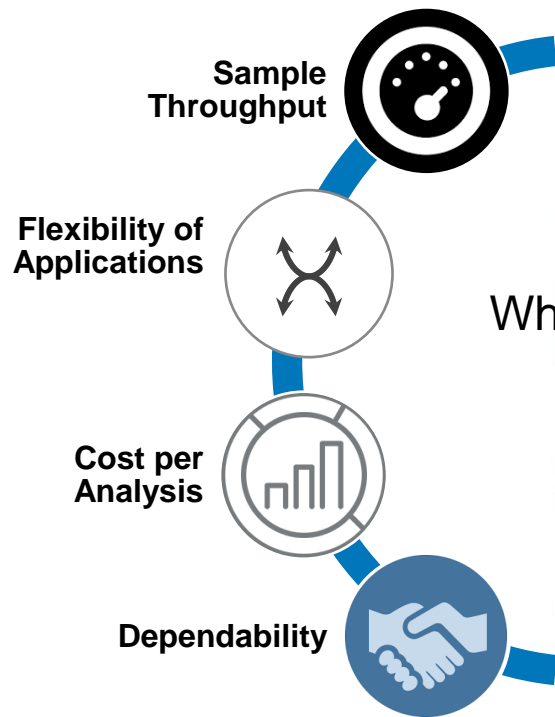


Matching Instruments and Columns

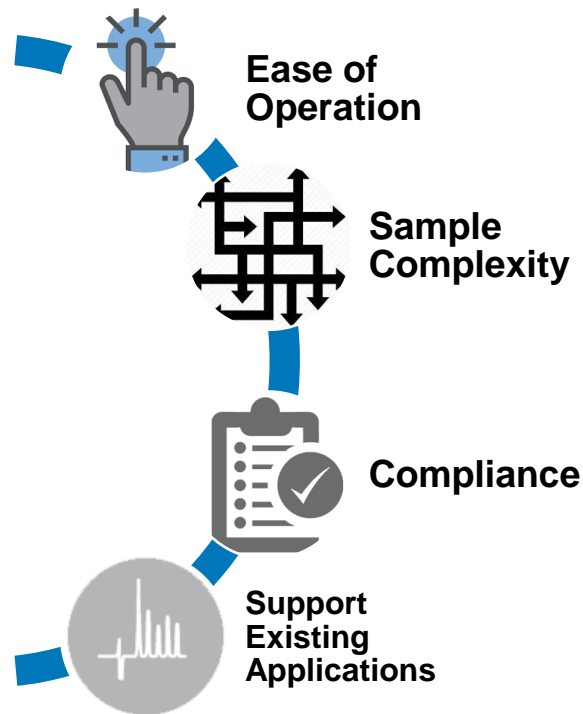
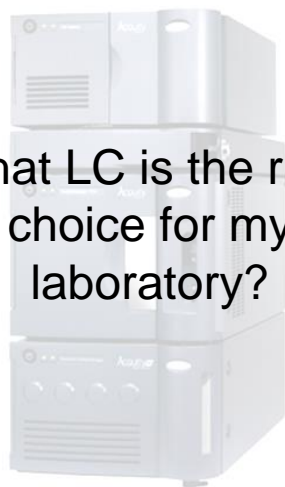
Chromatographic System	Dispersion	Particle Size	Recommended Column ID	
			Primary	Secondary
UPLC Systems	< 12 μL	<2.0 μm	2.1 mm	1.0 mm or 3.0 mm
UHPLC Systems	12 – 30 μL	2.x μm	3.0 mm	2.1 mm or 4.6 mm
HPLC Systems	>30 μL	>3.0 μm	4.6 mm	3.0 mm or > 4.6 mm

Choosing an LC System for your lab

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What LC is the right choice for my laboratory?



The background of the slide features a complex, abstract network of interconnected nodes and lines, resembling a molecular structure or a data network. The nodes are represented by small circles in various shades of blue and grey, while the lines are thin and light blue. This graphic is overlaid on a blue gradient background that transitions from a lighter shade on the left to a darker shade on the right.

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