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Managing Method Transfer in the Pharmaceutical Laboratory

Arnie Aistars, Ph.D.

Consumables Business Development

Agenda

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- USP <621> Chromatography Change Update
  - Taking advantage of the change
  - Examples
- Strategy for Successful Method Transfer
  - Prevention
  - Troubleshooting
  - Sources of Contamination
- Method Transfer Principle
- Summary

USP <621> Chromatography Defines "Allowable Adjustments"

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- Adjustments to a USP method may be made to meet system suitability requirements
- Verification tests must be performed after changes
  - Full re-validation not required
- Must use the same L-designation of column
- Isocratic hold or dwell volume adjustments are allowed

USP 37 NF 32 S1

- Official Aug. 1, 2014

- Significant changes to Chapter <621> Chromatography

USP 37-NF 32 through First Supplement - August 1, 2014

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Variable	USP 36-NF 31	USP 37-NF 32 Through first supplement	
		Isocratic	Gradient
Particle Size	-50%	L/dp Ratio Constant or N: -25 to + 50%	No changes allowed
Column Length	±70%		No changes allowed
Flow Rate	$F2=F1\ (d2^2/d1^2)$ and ±50%	$F2=F1\ x[(dc2^2\ x\ dp1)/dc1^2\ x\ dp2)]$ and ±50%	Not applicable
Column ID	Any allowed if linear velocity is constant	Any allowed if linear velocity is constant	No changes allowed
Injection Volume	Any reduction consistent with precision and detection limits; no increase permitted	Can be adjusted as consistent with precision and detection limits	Can be adjusted as consistent with precision and detection limits
Column Temperature	±10%	±10%	±10%
Mobile Phase pH	±0.2 unit	±0.2 unit	±0.2 unit

F=Flow rate; d = internal column diameter; dc = column diameter, dp = particle size

Scaling Existing Separations

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Length (*L* , mm)	Column Diameter (*dc* , mm)	Particle Size (*dp* , µ m)	Relative Values				
			L /*dp*	*F*	*N*	Pressure	Run Time
250	4.6	10	25,000	0.5	0.8	0.2	3.3
150	4.6	5	30,000	1.0	1.0	1.0	1.0
150	2.1	5	30,000	0.2	1.0	1.0	1.0
100	4.6	3.5	28,600	1.4	1.0	1.9	0.5
100	2.1	3.5	28,600	0.3	1.0	1.9	0.5
75	4.6	2.5	30,000	2.0	1.0	4.0	0.3
75	2.1	2.5	30,000	0.4	1.0	4.0	0.3
50	4.6	1.7	29,400	2.9	1.0	8.5	0.1
50	2.1	1.7	29,400	0.6	1.0	8.5	0.1

For example, if a monograph specifies a 150-mm × 4.6-mm; 5-µm column operated at 1.5 mL/min, the same separation may be expected with a 75-mm × 2.1-mm; 2.5-µm column operated at 1.5 mL/min × 0.4 = 0.6 mL/min, along with a pressure increase of about four times and a reduction in run time to about 30% of the original.

■ 1S (*USP37*

Taking Advantage of 'Allowable Adjustments' in USP <621>

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Isocratic Methods	- Improve analysis speed and quality with UPLC and sub-2-µm columns - Improve methods with CORTECS 2.7 µm or 2.5 µm ***XP*** columns on HPLC systems - No re-validation required
Gradient Methods	- Any change requires re-validation - Fully optimize methods using sub-2-µm particles and UPLC - Develop better methods faster with ACQUITY QDa mass detector
System	- Future-proof the lab: Both HPLC and UPLC methods can be run on the ACQUITY UPLC H-Class system
Software	- ACQUITY Columns Calculator for proper transfers - Streamline verification and validation testing with Empower Method Validation Manager

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- USP <621> Chromatography Change Update
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  - Examples
- **Strategy for Successful Method Transfer**
  - **Prevention**
  - **Troubleshooting**
  - **Sources of Contamination**
- Method Transfer Principle
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USP Compendial Methods: Potential Issues

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- Mobile phase
  - High buffer salt concentration in mobile phase
  - High concentrations of modifiers (phosphoric acid, TEA etc.)
  - pH may be close to the limits of traditional column packing materials
- Method
  - Not always an optimized method
- Sample
  - Complex formulations: tablets, suspensions, ointments
  - Minimal, inadequate sample preparation
  - New excipients may not be compatible with USP method
  - Sample may not be fully soluble in diluent/mobile phase
  - Methods (often isocratic) may not elute all sample components
    - API, related substances, formulation excipients

Strategy for Successful Method Transfer to UPLC

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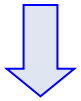
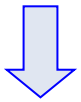
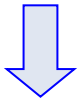
Determine Method Compatibility



Prepare UPLC System & Mobile Phase



Method Transfer and Routine Analysis



Successful Transfer

Prevention

Determining UPLC Method Compatibility

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- Did the separation ever work well on an HPLC system?
  - Check buffer salt solubility with bench top experiment
- Does the HPLC method properly elute ALL excipients as well as the API and related substances?
  - Look at the properties of formulation components and their compatibility with the original USP method
    - Solubility in diluent and mobile phase
    - Stability (temperature, pH)
  - Modifications to sample preparation or method MAY be required, to prevent sample build-up on column

System Preparation and Mobile Phase

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- System
  - Ensure the system has been cleaned regularly
  - Sonicate or replace solvent filters to ensure cleanliness
    - Sinkers are significant source of bacteria
  - Run a system performance standard to ensure system is in proper working condition

- Mobile Phase
  - Prepare fresh mobile phase
  - Use high quality, branded mobile phase solvents
  - Use high purity mobile phase buffers and reagents
  - Consider filtering or purchasing pre-filtered mobile phase solvents
  - Ensure that the mobile phase pH is compatible with the column

Strategy for Successful Method Transfer

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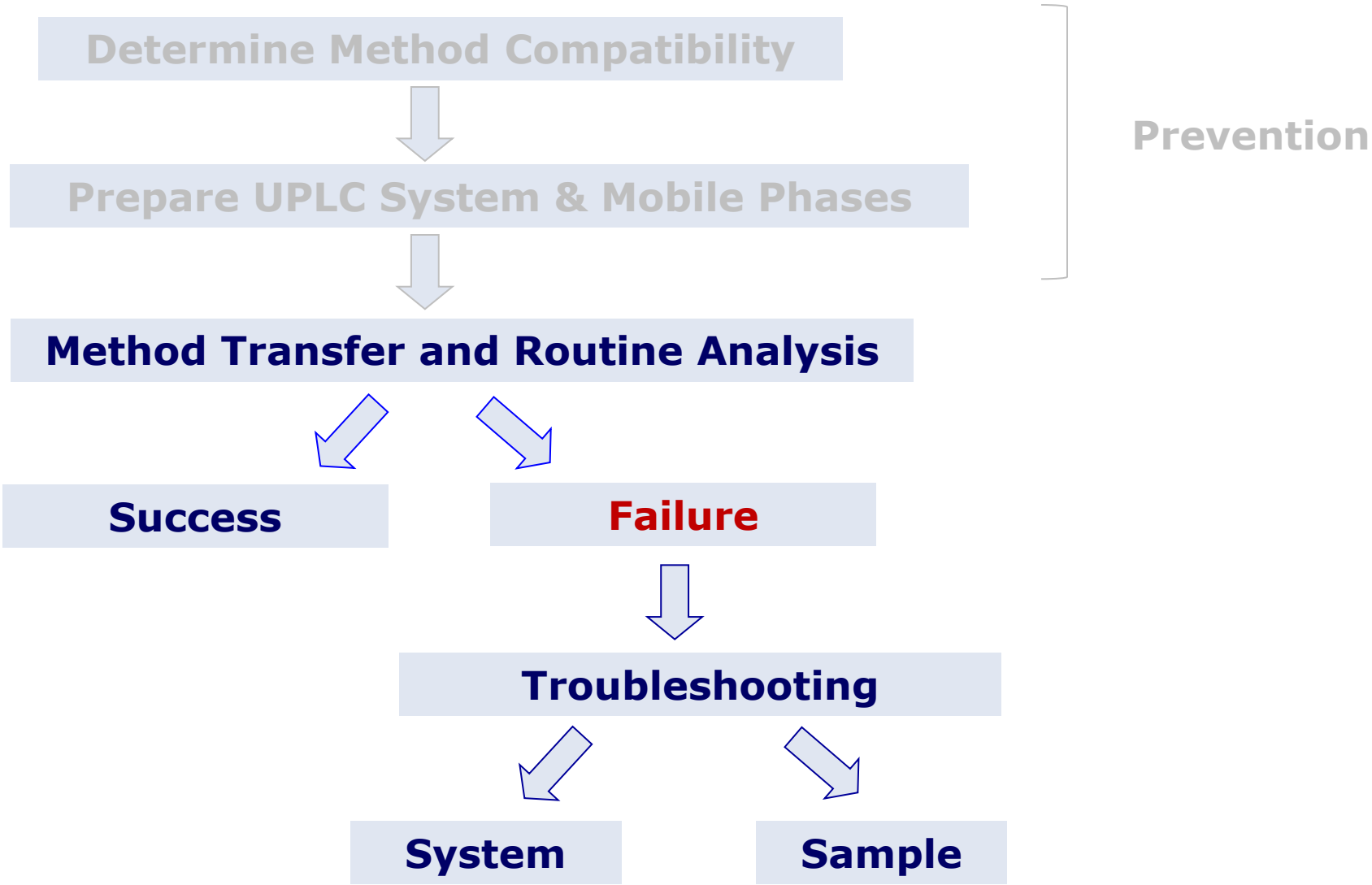

Quick Troubleshooting

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Failing chromatography
- Poor peak shape
- Tailing
- RT shifts
- Drop in plate count
- System overpressure

Does a fresh sample help?

YES → **Sample Stability**
-check sample stability

NO

Does column washing help?

YES → **Insufficient Wash**
-build a column washing step into each sample set or run

NO

Does replacing column inlet/outlet frits help? (for troubleshooting only)

YES → **Particulates/Bacteria**
-clean system
-replace sinkers
-make fresh mobile phase
-filter mobile phase
-use pre-column filter

-re-examine sample matrix solubility and sample prep

NO

Column Bed Contamination

Bacterial Contamination

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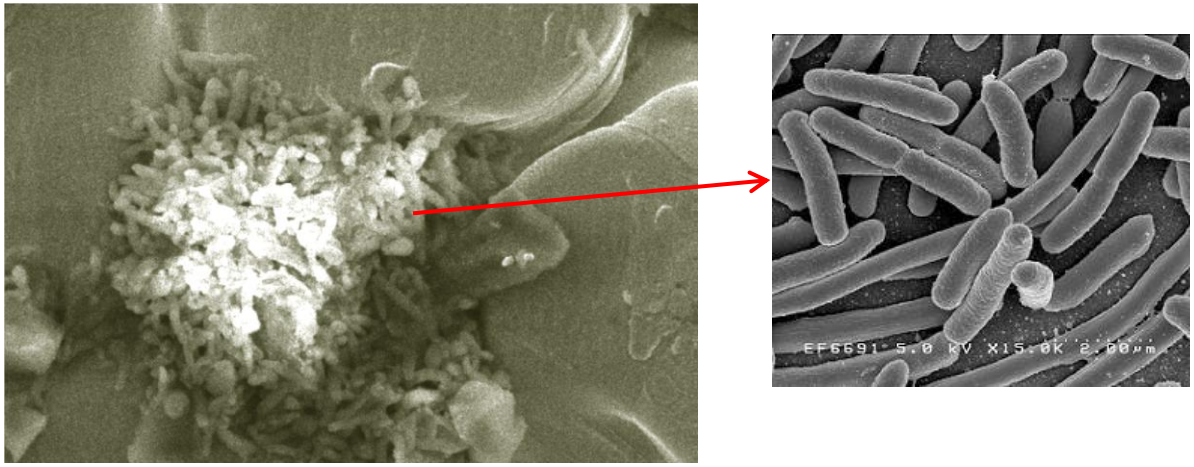
- Bacteria in the system is a common cause of column failure
- Microbial build-up occurs in all systems running aqueous mobile phase
- Does not only occur at neutral pH
- 15 minutes is all it takes when conditions are right



SEM: column inlet frit, inlet side

Effect of Bacterial Contamination on Chromatography

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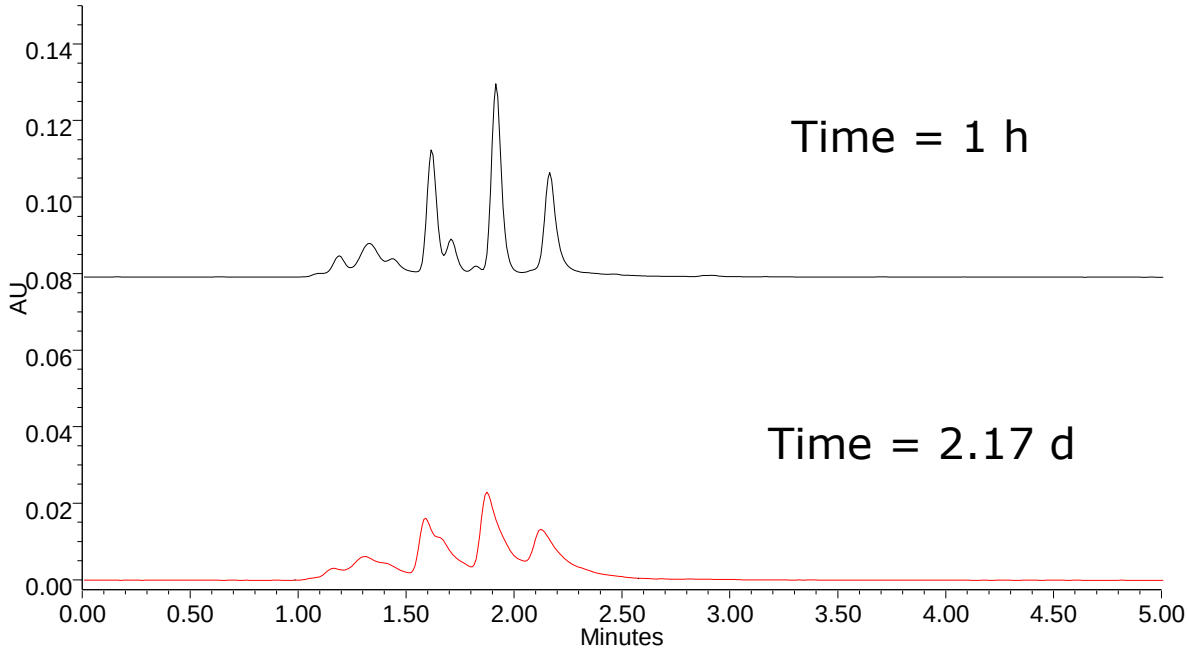

Protein Mix

Column contaminated with bacteria (confirmed by SEM analysis of column frit)

Conditions: 25mM sodium phosphate, 150 mM sodium chloride, pH 6.8, 0.4 mL/min, Injection vol: 4 µL, Wavelength: 280 nm

Preventing Bacterial Contamination

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- Tips
  - Replace sinkers (high source of bacteria)
  - Prepare mobile phase fresh daily
  - Pre-mix isocratic mobile phase with organic solvents
  - Add starting % organic to aqueous mobile phases and adjust the gradient accordingly
  - Flush the system regularly (with column removed) using a series of water/acid/organic washes to eliminate microbial growth.
    - See "Controlling Contamination in UltraPerformance LC®/MS and HPLC/MS Systems" PN 715001307
  - Do NOT perform a hot water wash: this will worsen contamination

Column Bed Contamination: Is it from the System or Sample?

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Failing chromatography
- Poor peak shape
- Tailing
- RT shifts
- Drop in plate count
- System overpressure

Not related to sample stability, washing or particulates

Column Bed Contamination

- Clean system
- PM system
- Fresh mobile phase
- New sinkers

Is a routine use study with diluent blanks on a NEW column succesful? (standard every 20 injections)

NO

System issue

Further investigate system contamination
- Bacteria (less than 0.2 µm)
- Mobile phase impurities
- Filter compatibility
- Column stability under method pH and temperature conditions

Preventing System-related Column Bed Contamination

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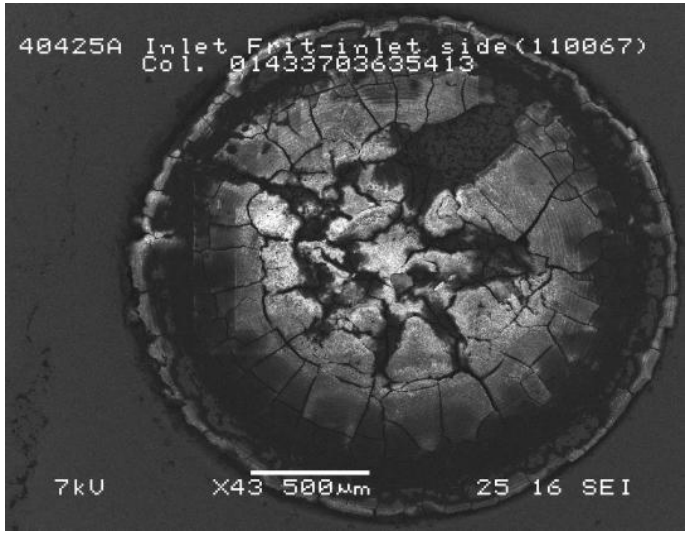
- Tips
  - Prevent bacterial growth in system
  - Use high quality, high purity reagents for mobile phase
  - Consider using a VanGuard pre-column
  - Ensure that the column being used is stable at the pH and temperature specified in the method
  - Check chemical compatibility of filters (sample/mobile phase) with the solvents being used

Polysulphone material from filter membranes plugging inlet frit



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Method Transfer Scenarios

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HPLC Method

Transferred to UPLC Platform

Scaled to UPLC Method

Scaled to HPLC Method

Differences in System Volume: Low *vs.* High Pressure Mixing

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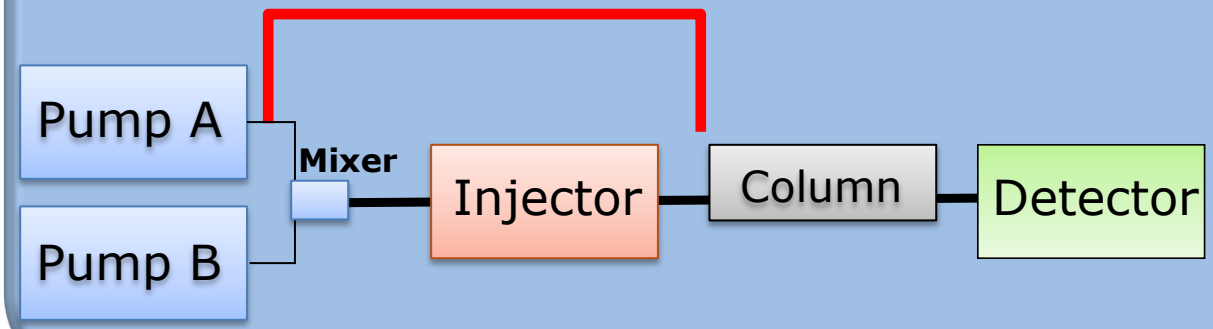
Multiple/Dual Pumps (Binary) – High Pressure Mixing

Smaller System Volume = Smaller Dwell volume



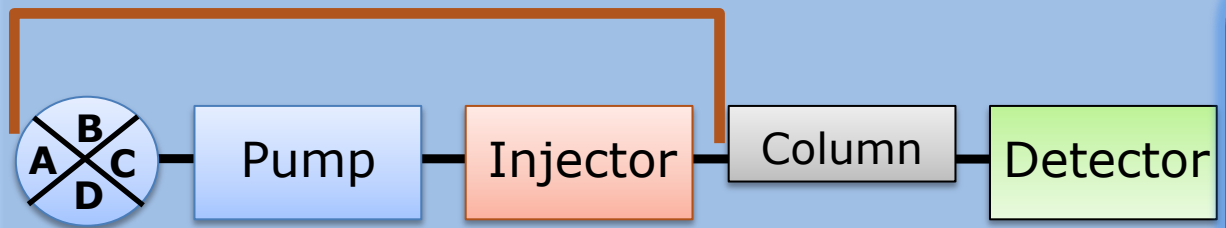
Solvent mixing:
After going through the pump
Or pre-mixed solvents only

Smaller system volume

Minimal dispersion

Single Pump (Quaternary) - Low Pressure Mixing

Larger System Volume = Larger Dwell volume



Gradient Proportioning Valve

Solvent mixing:
Before going through the pump

Larger system volume

Auto·Blend® :
For mobile phase generation

System Volume Timing Offset

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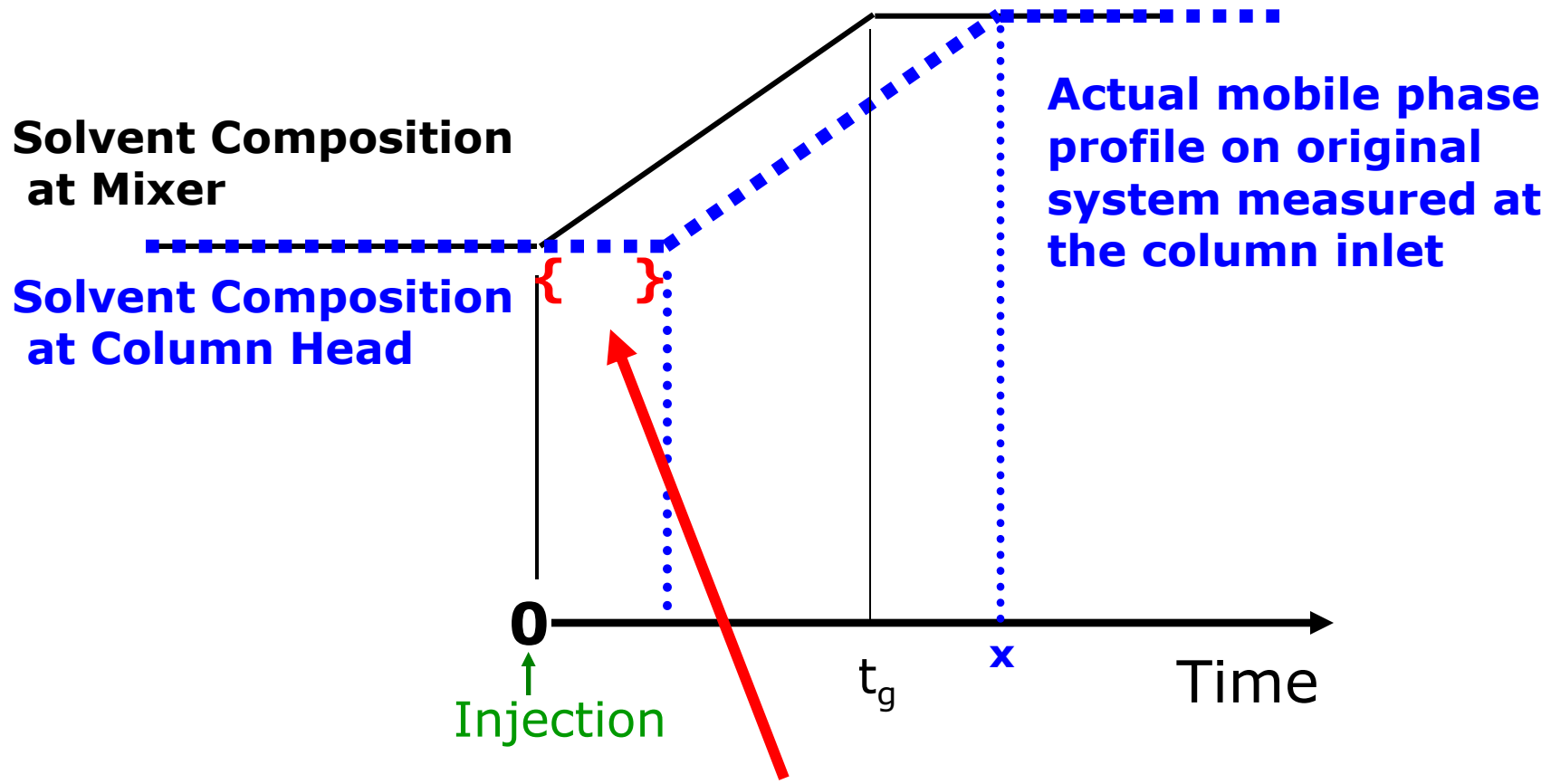

System volume creates an offset before the solvent composition change reaches the inlet of column (*i.e.,* an "isocratic hold" at the beginning of every gradient)

Different System Volumes Effect on Separation

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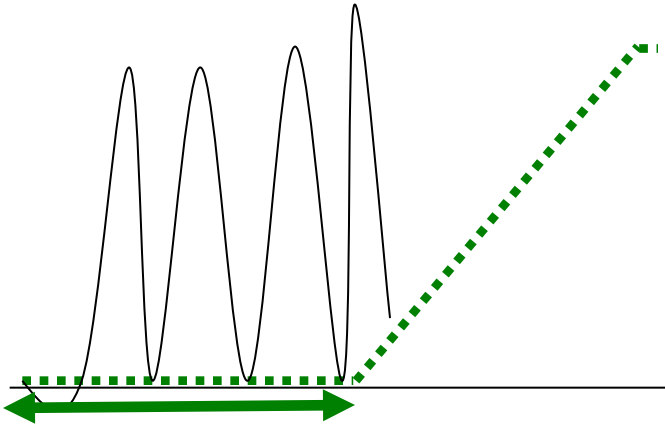
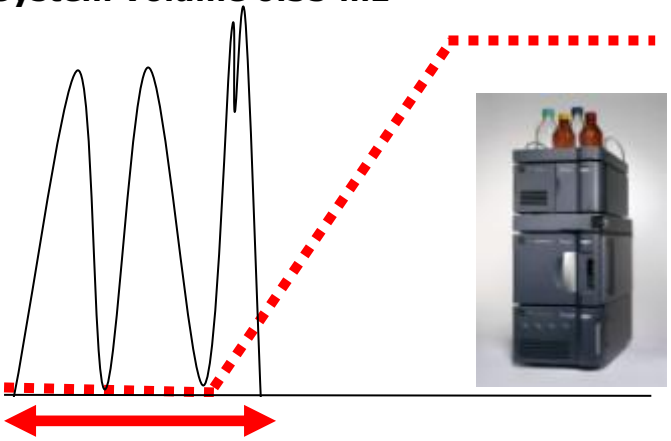
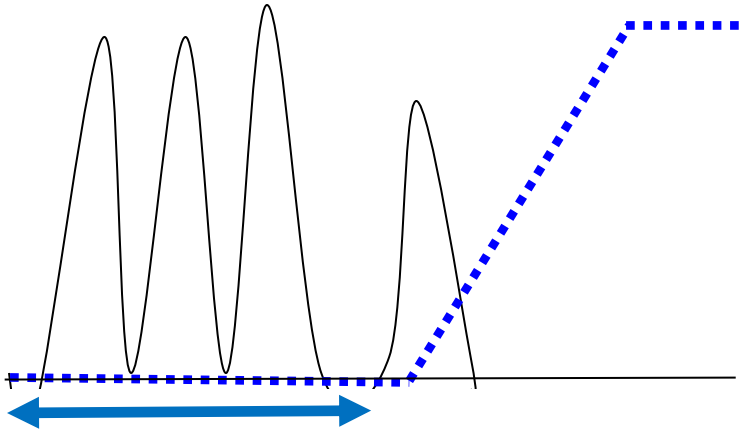


Original Instrument
System Volume 0.9 mL

Smaller Volume
System Volume 0.35 mL



Larger Volume
System Volume 1.4 mL



Target System with smaller volume (less isocratic hold time)

Target System with larger volume (longer isocratic hold time)

Gradient Type: Compensating for System Volumes

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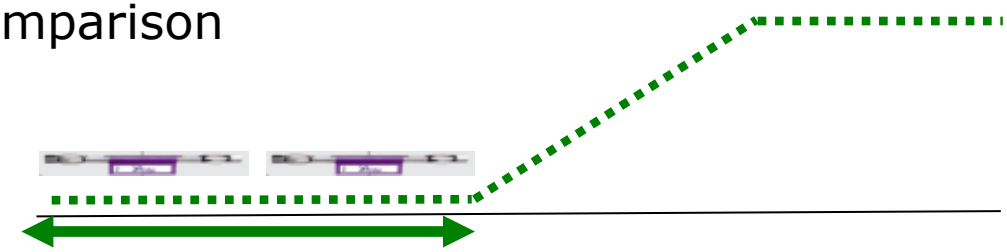
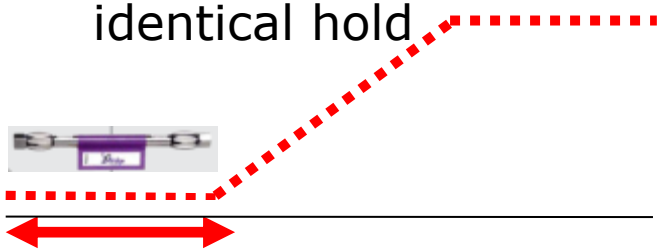
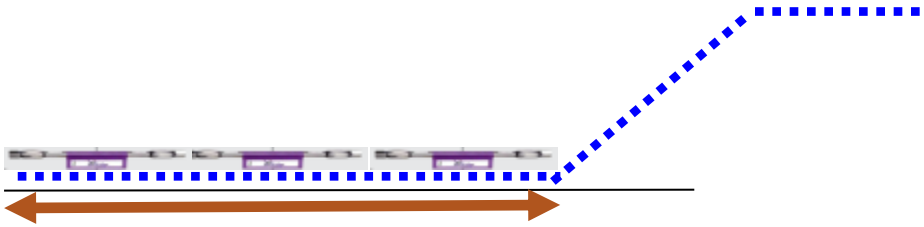
- Compare system volumes
  - This volume should be converted to "column volumes (CVs)" for the best comparison



- If target system gives smaller isocratic segment
  - ADD an initial hold to the gradient table to give the identical hold



- If target system gives larger isocratic segment
  - Use the pre-injector volume feature



Scaling Injection Volume

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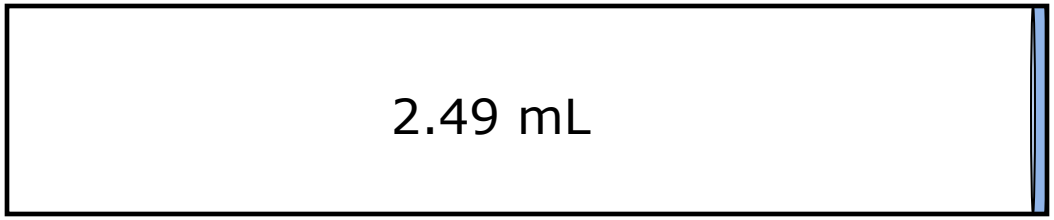
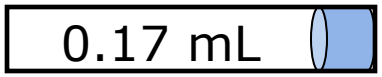
Guideline:
Injection volume should be less than 5% of column volume.
Aim for <1% and experimentally determine if you can go higher based on chromatographic conditions.

4.6 x 150 mm



20 µL injection/2.49 mL = 0.8%

2.1 x 50 mm



20 µL injection/0.17 mL = 12%

If you inject too much, the result will be poor peak shape due to volume overload

ACQUITY UPLC Columns Calculator

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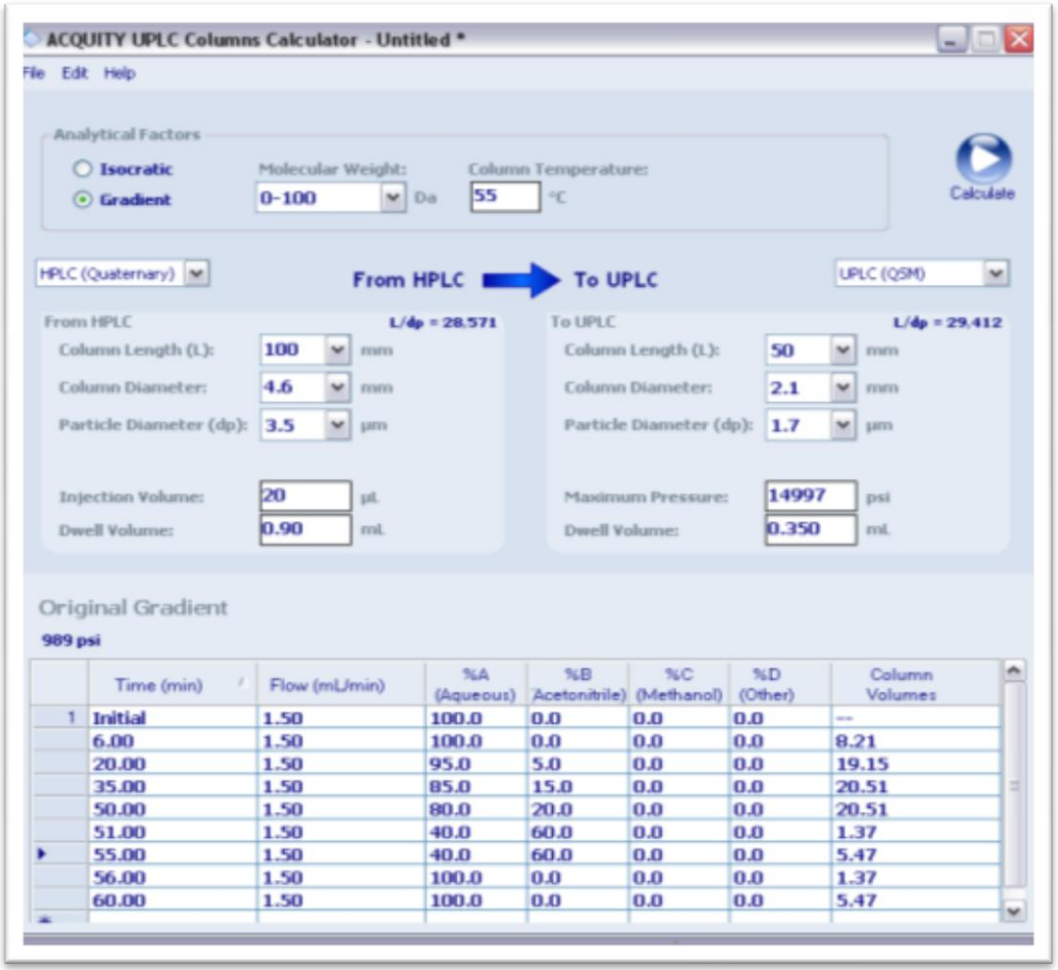

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B Acetonitrile)	%C (Methanol)	%D (Other)	Column Volumes
1	Initial	1.50	100.0	0.0	0.0	0.0	--
	6.00	1.50	100.0	0.0	0.0	0.0	8.21
	20.00	1.50	95.0	5.0	0.0	0.0	19.15
	35.00	1.50	85.0	15.0	0.0	0.0	20.51
	50.00	1.50	80.0	20.0	0.0	0.0	20.51
	51.00	1.50	40.0	60.0	0.0	0.0	1.37
	55.00	1.50	40.0	60.0	0.0	0.0	5.47
	56.00	1.50	100.0	0.0	0.0	0.0	1.37
	60.00	1.50	100.0	0.0	0.0	0.0	5.47

ACQUITY UPLC® Columns Calculator handles these calculations

Three Method Transfer Scenarios

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- **Scenario 1** : Maximizing Asset Utilization
  - Applying existing HPLC methods onto HPLC and ACQUITY UPLC H-Class systems
  - Adapting HPLC methods to different system designs

- **Scenario 2** : Transferring a HPLC method to UPLC systems
  - Converting a legacy HPLC method into a UPLC method

- **Scenario 3**: Transferring a UPLC method to HPLC systems
  - Taking advantage of UPLC for quickly and efficiently developing a method
  - Transfer this method to labs still equipped with HPLC systems

Scenario 1 - Maximizing Asset Utilization: Adapting HPLC Methods to Different Systems Design

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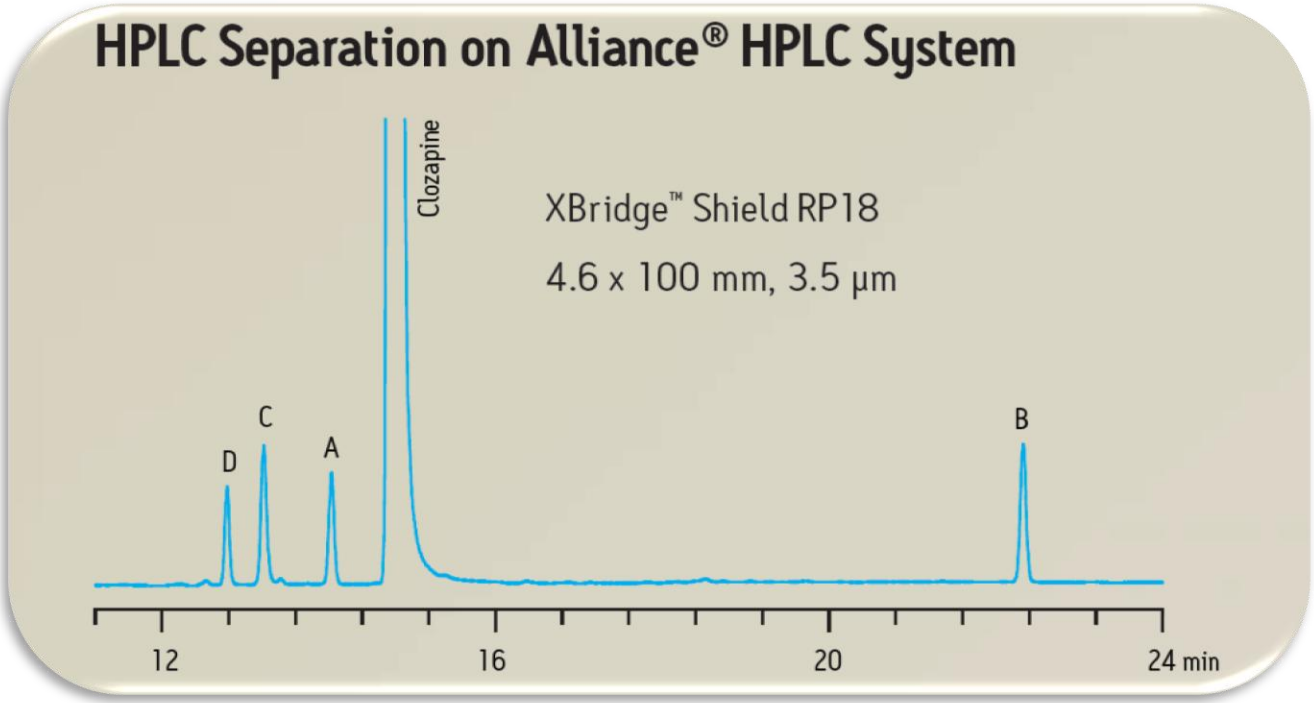

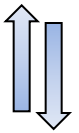

Future-proofing your lab

Invest in new instrumentation that can run both legacy HPLC methods and UPLC methods for new projects

Goal
Transfer existing HPLC method to a different LC system
Must compensate for system dwell volume differences

Scenario 1:
LC Instrument Transfer

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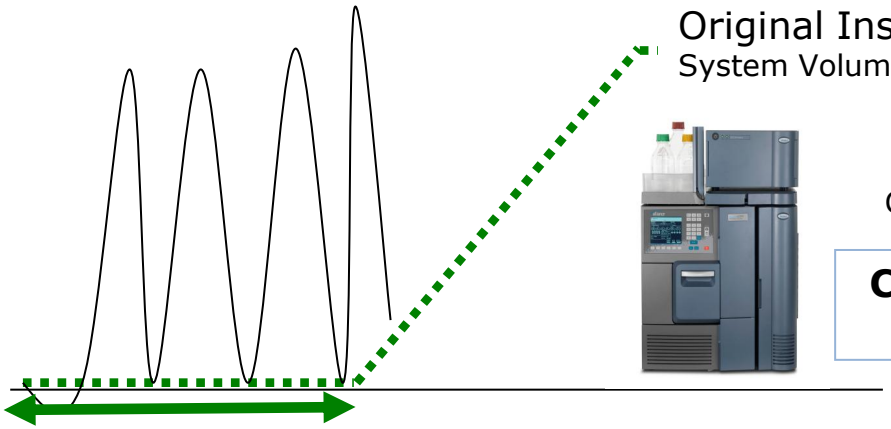
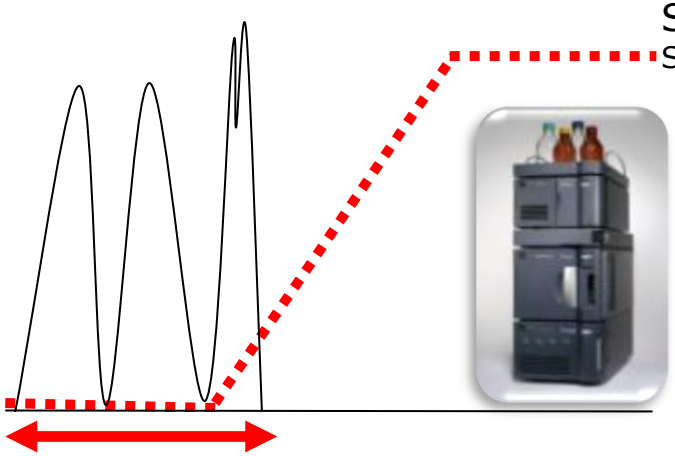


Original Instrument
System Volume **0.9 mL**

Column Volume 4.6 x100 mm : **1.66 ml**

Conversion in column volumes :
0.9/1.66 =0.54 cv

Target System with smaller volume (less isocratic hold time)
Must compensate with an isocratic hold to preserve separation



Smaller Volume
System Volume **0.35 mL**

Column Volume 4.6 x100 mm : **1.66 ml**

Conversion in column volumes :
0.35/1.66 =0.21 cv

Scenario 1:
HPLC to HPLC Method Transfer

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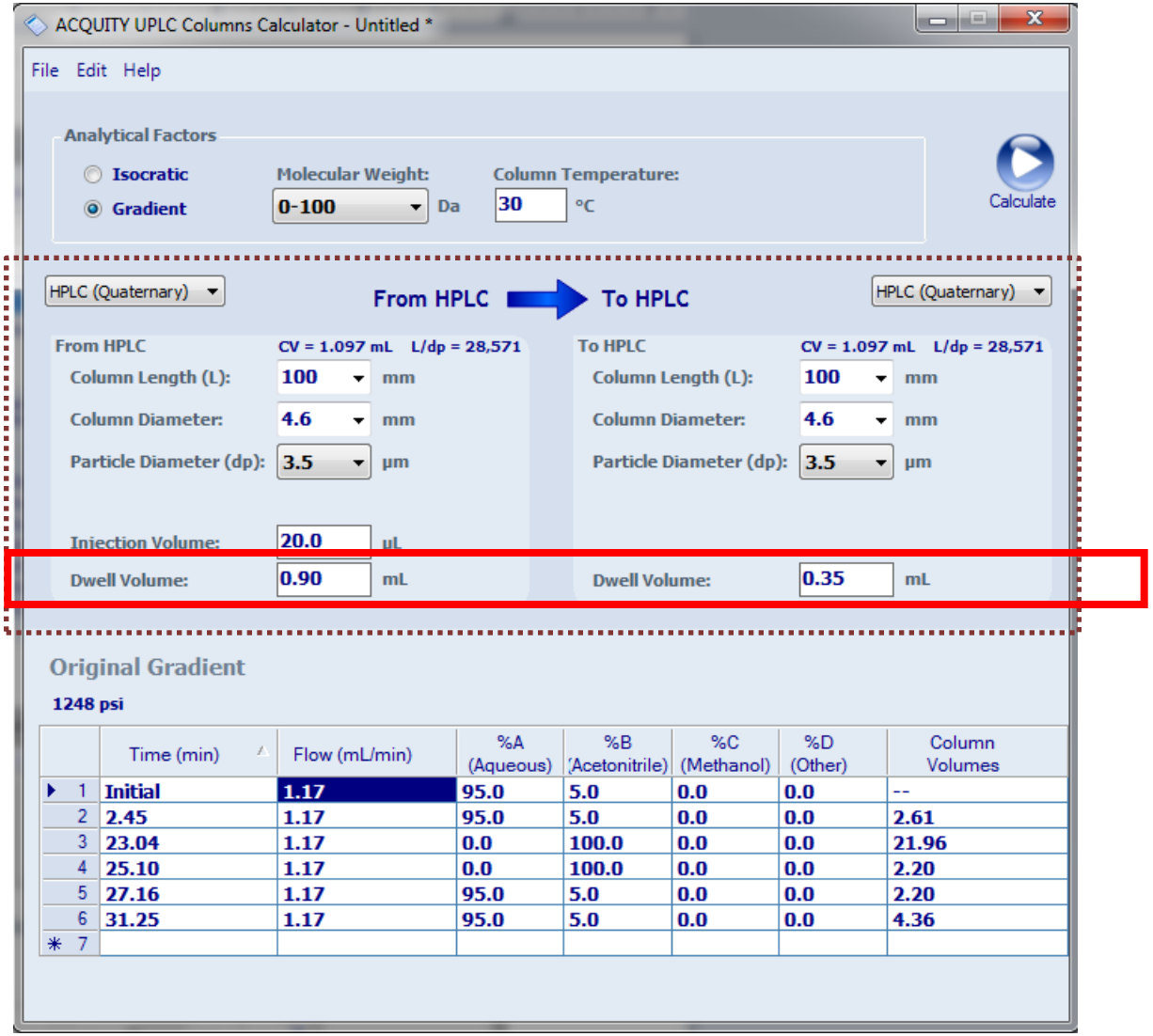

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B (Acetonitrile)	%C (Methanol)	%D (Other)	Column Volumes
1	Initial	1.17	95.0	5.0	0.0	0.0	--
2	2.45	1.17	95.0	5.0	0.0	0.0	2.61
3	23.04	1.17	0.0	100.0	0.0	0.0	21.96
4	25.10	1.17	0.0	100.0	0.0	0.0	2.20
5	27.16	1.17	95.0	5.0	0.0	0.0	2.20
6	31.25	1.17	95.0	5.0	0.0	0.0	4.36
7							

Dwell Volume

To preserve the gradient profile when transferring from one instrument to another, the system dwell volume must be considered.

Scenario 1:
HPLC to HPLC Method Transfer

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Original Gradient

1248 psi

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B (Acetonitrile)	%C (Methanol)	%D (Other)	Column Volumes
1	Initial	1.17	95.0	5.0	0.0	0.0	--
2	2.45	1.17	95.0	5.0	0.0	0.0	2.61
3	23.04	1.17	0.0	100.0	0.0	0.0	21.96
4	25.10	1.17	0.0	100.0	0.0	0.0	2.20
5	27.16	1.17	95.0	5.0	0.0	0.0	2.20
6	31.25	1.17	95.0	5.0	0.0	0.0	4.36
7							

Gradient Results - Untitled

Column	Run Time (min)	Peak Capacity	Flow Rate (mL/min)	Pressure (psi)	Injection Volume (uL)
Original HPLC column conditions					
100 mm x 4.6 mm, 3.5 µm	31.25	98	1.17	1248	20.0
New HPLC conditions with scaled gradient (accounting for particle size)					
100 mm x 4.6 mm, 3.5 µm	31.72	98	1.17	1248	20.0
New HPLC conditions with scaled gradient (disregarding particle size)					
100 mm x 4.6 mm, 3.5 µm	31.72	98	1.17	1248	20.0

Print

Flow Rate

New HPLC Gradient

New HPLC conditions with scaled gradient (accounting for particle size), 100 mm x 4.6 mm, 3.5 µm column

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B (Acetonitrile)	%C (Methanol)	%D (Other)	Column Volumes
1	Initial	1.17	95.0	5.0	0.0	0.0	--
2	2.92	1.17	95.0	5.0	0.0	0.0	3.11
3	23.51	1.17	0.0	100.0	0.0	0.0	21.96
4	25.57	1.17	0.0	100.0	0.0	0.0	2.20
5	27.63	1.17	95.0	5.0	0.0	0.0	2.20
6	31.72	1.17	95.0	5.0	0.0	0.0	4.36

Gradient Column Volumes

To preserve the gradient profile, the number of gradient column volumes for each step, should be maintained.

As a result, the gradient time table has been adjusted.

Why Add an Isocratic Hold?

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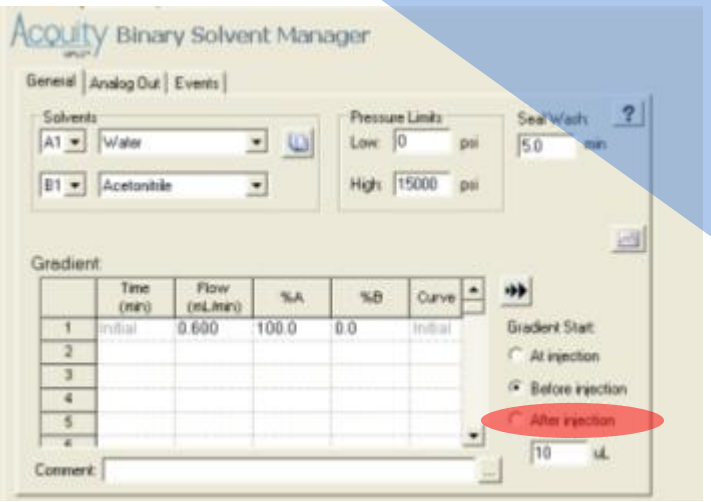
Injection

Alliance HPLC

System with larger dwell volume
(longer isocratic hold time)



Injection

ACQUITY
UPLC H-Class

System with smaller dwell volume
(less isocratic hold time)

1. Isocratic Hold
2. Tubing

Scenario 1 - The Result: HPLC to HPLC Method Transfer

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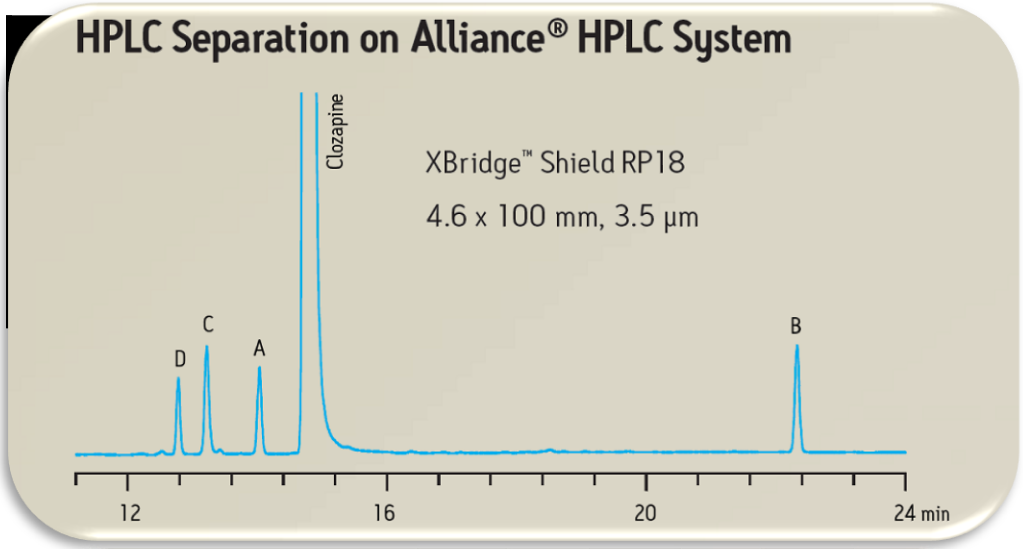

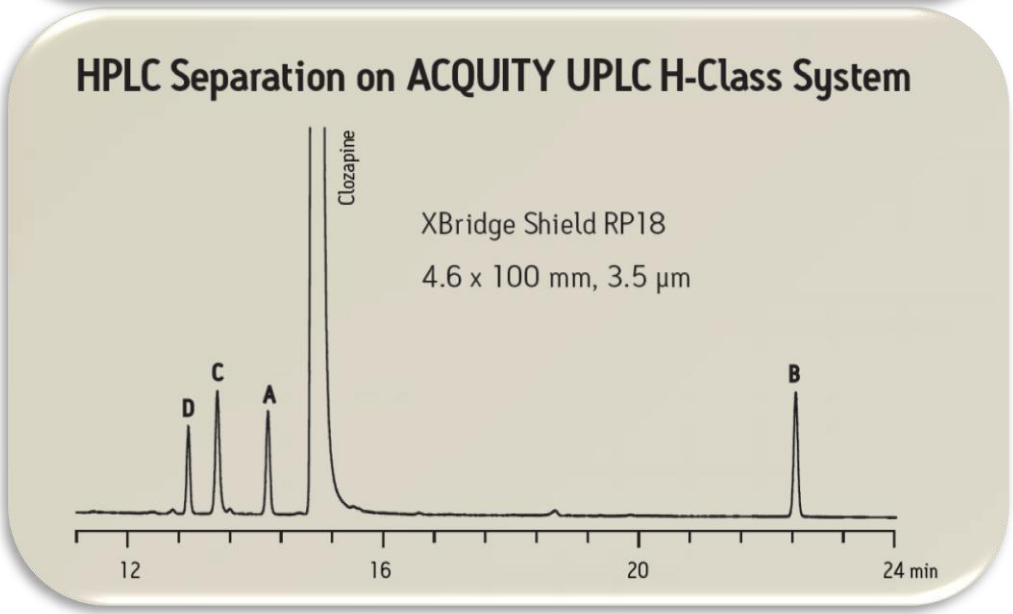

Future-proof your lab

Run HPLC methods on ACQUITY UPLC H-Class

Flexibility to run both HPLC and UPLC methods

Ease of Method Transfer Between LC Platforms

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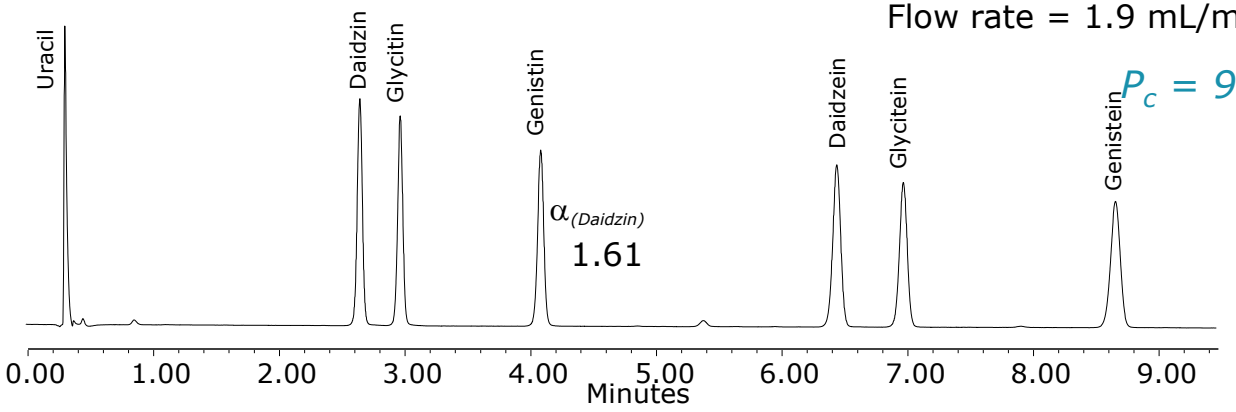
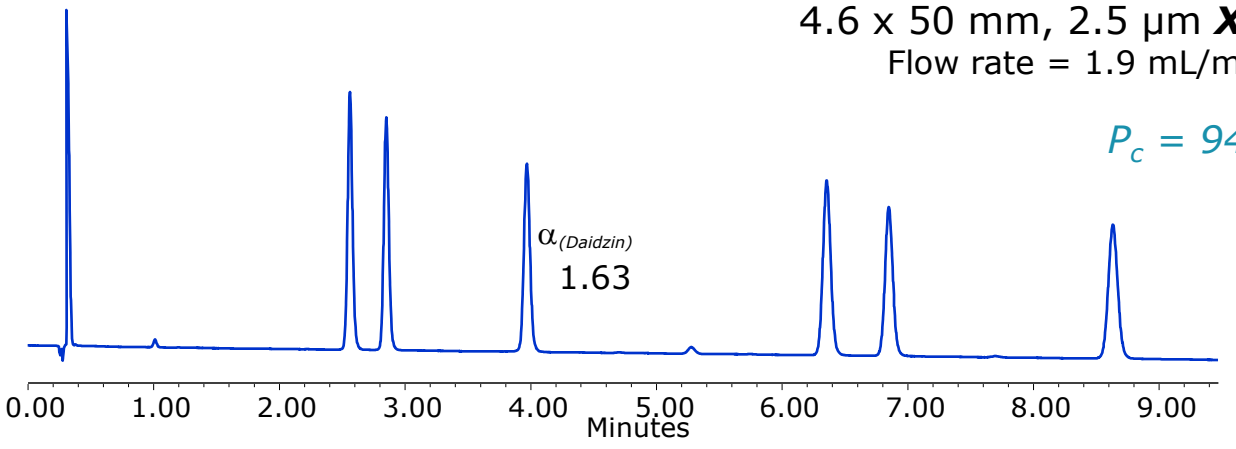
HPLC

XSelect HSS T3
4.6 x 50 mm, 2.5 µm ***XP***
Flow rate = 1.9 mL/min



ACQUITY UPLC H-Class

XSelect HSS T3
4.6 x 50 mm, 2.5 µm ***XP***
Flow rate = 1.9 mL/min



Transfer methods between different LC systems

Three Method Transfer Scenarios

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- **Scenario 1** : Maximizing Asset Utilization
  - Applying existing HPLC methods onto HPLC and ACQUITY UPLC H-Class systems
  - Adapting HPLC methods to different system designs

- **Scenario 2** : Transferring a HPLC method to UPLC systems
  - Converting a legacy HPLC method into a UPLC method

- **Scenario 3**: Transferring a UPLC method to HPLC systems
  - Taking advantage of UPLC for quickly and efficiently developing a method
  - Transfer this method to labs still equipped with HPLC systems

Scenario 2: Turning a Legacy HPLC Method Into a UPLC Method

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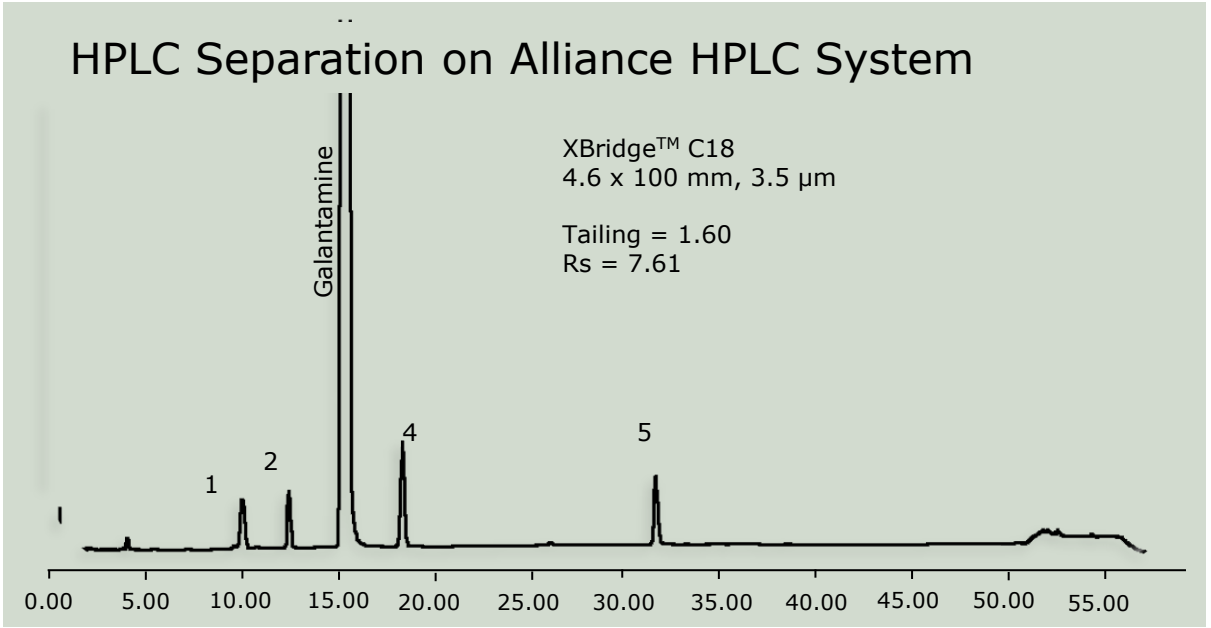

Method Transfer - Reduce Analysis Time

Take advantage of sub-2-µm particle technology and transfer to ACQUITY UPLC H-Class system

Criteria

USP Tailing <2.0, Rs (galantamine/impurity 4) >4.5

Scenario 2:
LC Instrument Transfer

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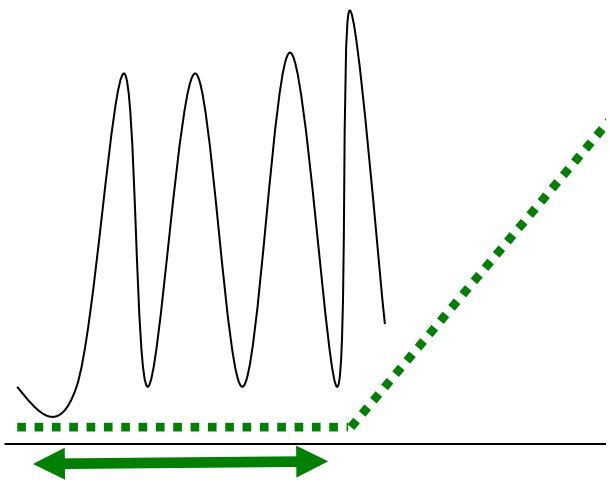
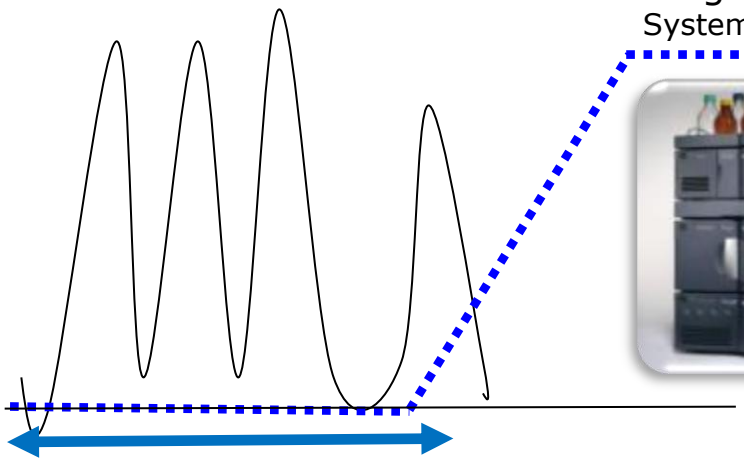


Original Instrument
System Volume **0.9 mL**

Column Volume 4.6 x100 mm : **1.66 ml**

Conversion in column volumes : 0.9/1.66 =0.54 cv

Target System with larger volume in cv (longer isocratic hold time)



Larger Volume
System Volume **0.35 mL**

Column Volume 2.1 x50 mm : **0.17 ml**

Conversion in column volumes : 0.35/0.17 =2.06 cv

Scenario 2: Turning a Legacy HPLC Method Into a UPLC Method

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ACQUITY UPLC Columns Calculator - Untitled *

File Edit Help

Analytical Factors

Isocratic
Gradient

Molecular Weight: 0-100 Da

Column Temperature: 30 °C

Calculate

HPLC (Quaternary) From HPLC → To UPLC UPLC (QSM)

From HPLC	CV = 1.097 mL L/dp = 28,571		To UPLC	CV = 0.114 mL L/dp = 29,412	
Column Length (L):	100	mm	Column Length (L):	50	mm
Column Diameter:	4.6	mm	Column Diameter:	2.1	mm
Particle Diameter (dp):	3.5	µm	Particle Diameter (dp):	1.7	µm
Injection Volume:	20.0	µL	Maximum Pressure:	14997	psi
Dwell Volume:	0.90	mL	Dwell Volume:	0.350	mL

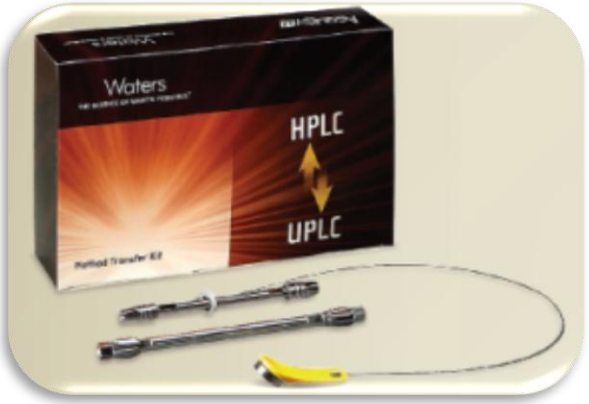
Original Gradient

1600 psi

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B (Acetonitrile)	%C (Methanol)	%D (Other)	Column Volumes
1	Initial	1.50	100.0	0.0	0.0	0.0	--
3	6.00	1.50	100.0	0.0	0.0	0.0	8.21
4	20.00	1.50	95.0	5.0	0.0	0.0	19.15
5	35.00	1.50	85.0	15.0	0.0	0.0	20.51
6	50.00	1.50	80.0	20.0	0.0	0.0	20.51
7	51.00	1.50	40.0	60.0	0.0	0.0	1.37
8	55.00	1.50	40.0	60.0	0.0	0.0	5.47
9	56.00	1.50	100.0	0.0	0.0	0.0	1.37
10	60.00	1.50	100.0	0.0	0.0	0.0	5.47
* 11							

L/dp
(Column Length to Particle Size Ratio)

To preserve the separation power of the gradient, L/dp must be matched



Scenario 2: Turning a Legacy HPLC Method Into a UPLC Method

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Injection Volume

To preserve the mass and volume load on column, the injection volume must be scaled appropriately

Column	Run Time (min)	Peak Capacity	Flow Rate (mL/min)	Pressure (psi)	Injection Volume (uL)
Original HPLC column conditions					
100 mm x 4.6 mm, 3.5 µm	60.00	68	1.500	989	20.0
New UPLC conditions with scaled gradient (accounting for particle size)					
50 mm x 2.1 mm, 1.7 µm	14.17	69	0.644	4850	2.1

Original Gradient

989 psi

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B Acetonitrile)	%C (Methanol)	%D (Other)	Column Volumes
1	Initial	1.50	100.0	0.0	0.0	0.0	--
	6.00	1.50	100.0	0.0	0.0	0.0	8.21
	20.00	1.50	95.0	5.0	0.0	0.0	19.15
	35.00	1.50	85.0	15.0	0.0	0.0	20.51
	50.00	1.50	80.0	20.0	0.0	0.0	20.51
	51.00	1.50	40.0	60.0	0.0	0.0	1.37
	55.00	1.50	40.0	60.0	0.0	0.0	5.47
	56.00	1.50	100.0	0.0	0.0	0.0	1.37
	60.00	1.50	100.0	0.0	0.0	0.0	5.47

Pre-Injector Volume

To preserve the gradient profile, the pre-injector volume must be used.

This allows the gradient to start before the injection is triggered.

New UPLC Gradient

Use pre-injector volume = 256 µL

New UPLC conditions with scaled gradient (accounting for particle size), 50 mm x 2.1 mm, 1.7 µm column

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B Acetonitrile)	%C (Methanol)	%D (Other)	Column Volumes
1	Initial	0.644	100.0	0.0	0.0	0.0	--
2	1.06	0.644	100.0	0.0	0.0	0.0	5.96
3	4.46	0.644	95.0	5.0	0.0	0.0	19.15
4	8.10	0.644	85.0	15.0	0.0	0.0	20.51
5	11.74	0.644	80.0	20.0	0.0	0.0	20.51
6	11.99	0.644	40.0	60.0	0.0	0.0	1.37
7	12.96	0.644	40.0	60.0	0.0	0.0	5.47
8	13.20	0.644	100.0	0.0	0.0	0.0	1.37
9	14.17	0.644	100.0	0.0	0.0	0.0	5.47

Why Use a Pre-Injection Volume?

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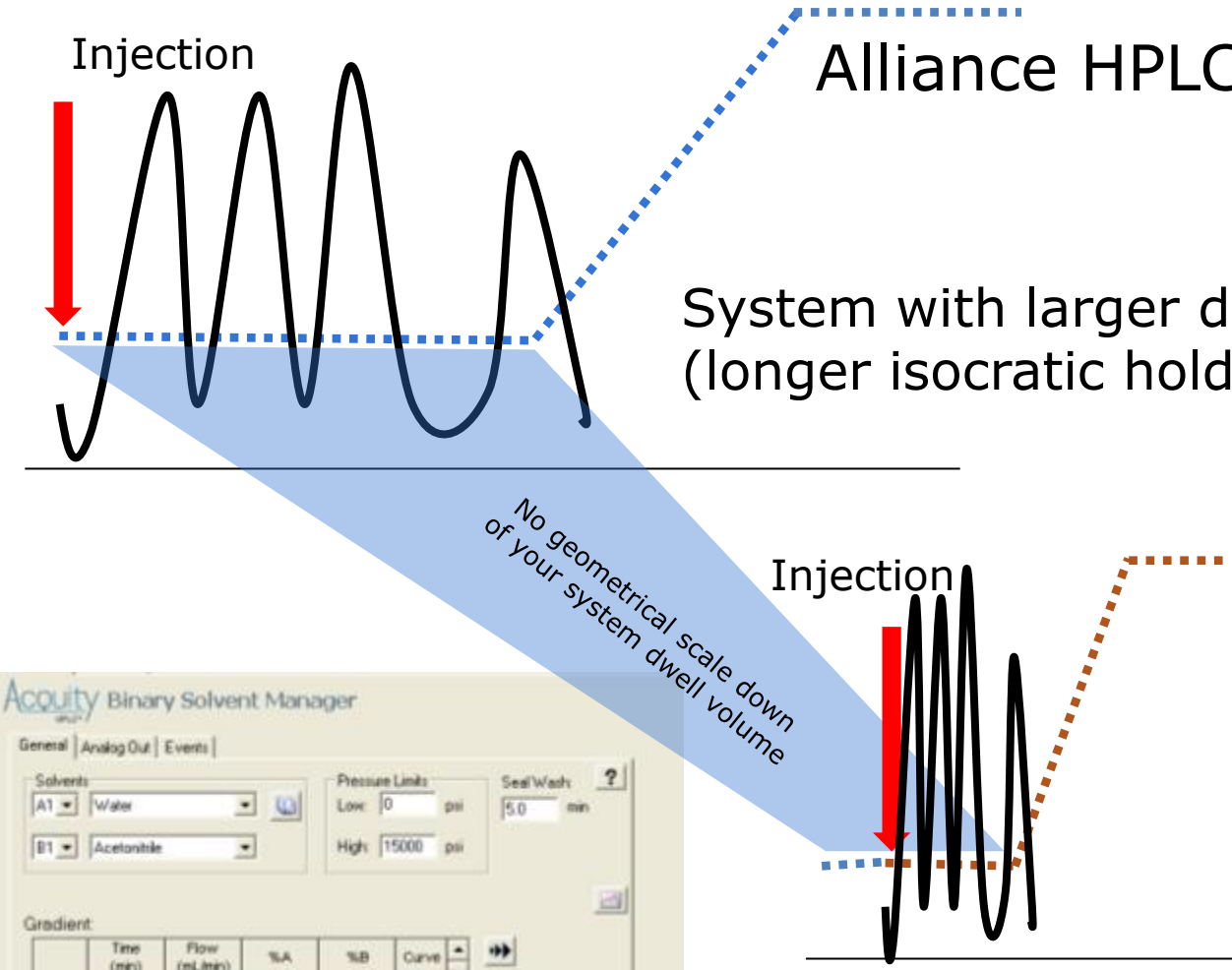

Scenario 2 - The Result: Turning a Legacy HPLC Method Into a UPLC Method

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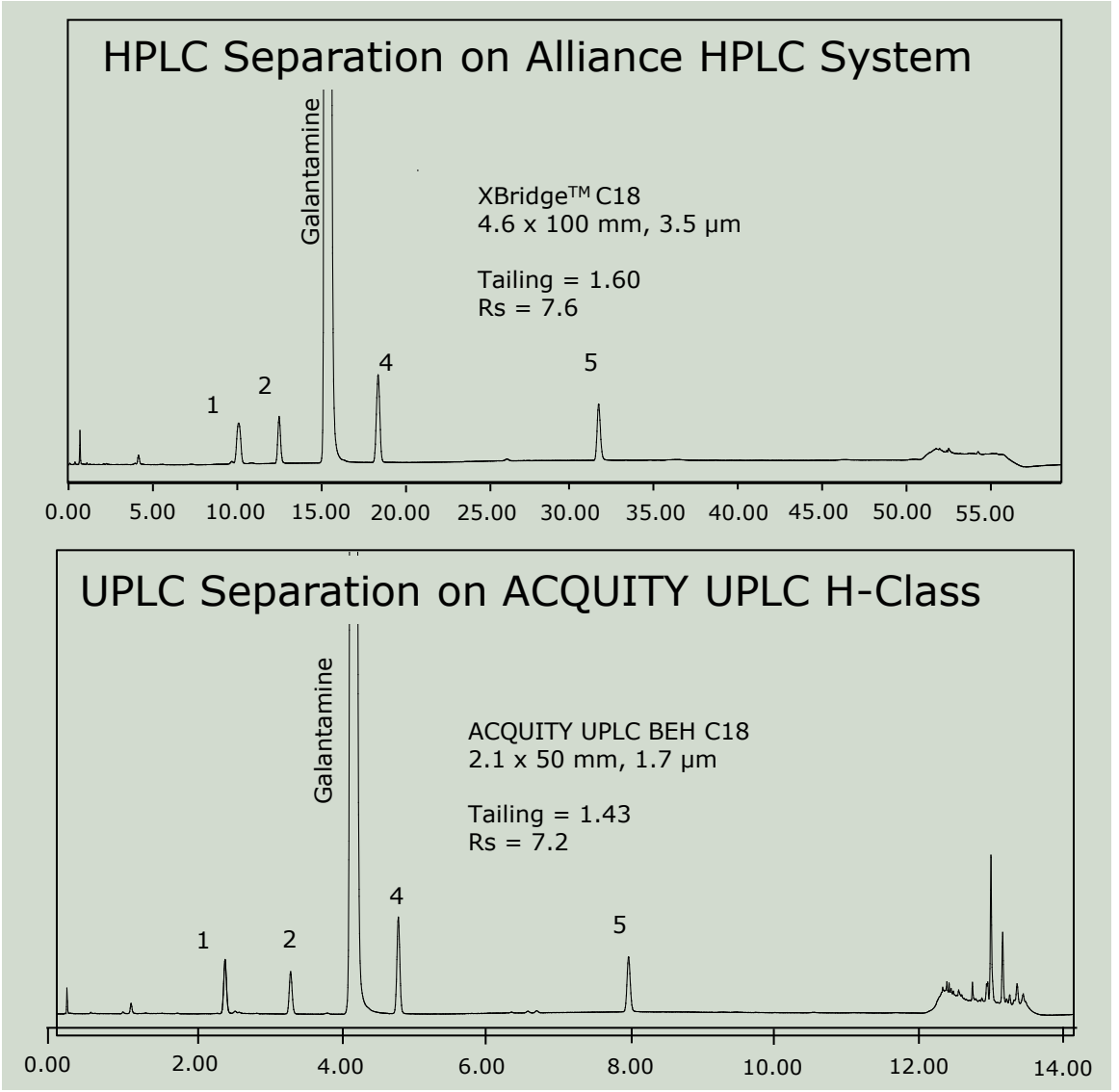
Adjustment of method
Reduce analysis time 4.3X while preserving separation integrity

Tailing and Resolution Criteria were met

Criteria
USP Tailing < 2.0
Rs (galantamine/impurity 4) > 4.5



Three Method Transfer Scenarios

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- **Scenario 1** : Maximizing Asset Utilization
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Scenario 3 :
UPLC to HPLC Method Transfer

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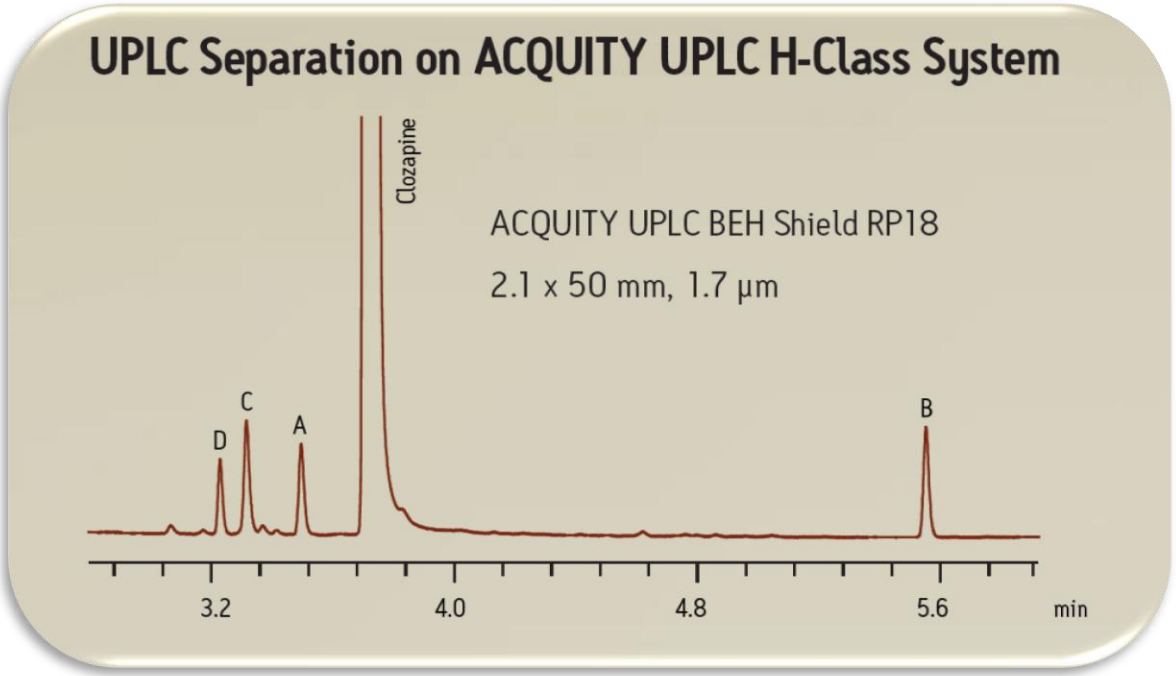

Maximize Asset Utilization

Transfer from UPLC to another department/contract partner that has a bank of HPLC instruments

Goal

Transfer UPLC method to HPLC while maintaining selectivity

Scenario 3 :
UPLC to HPLC Method Transfer

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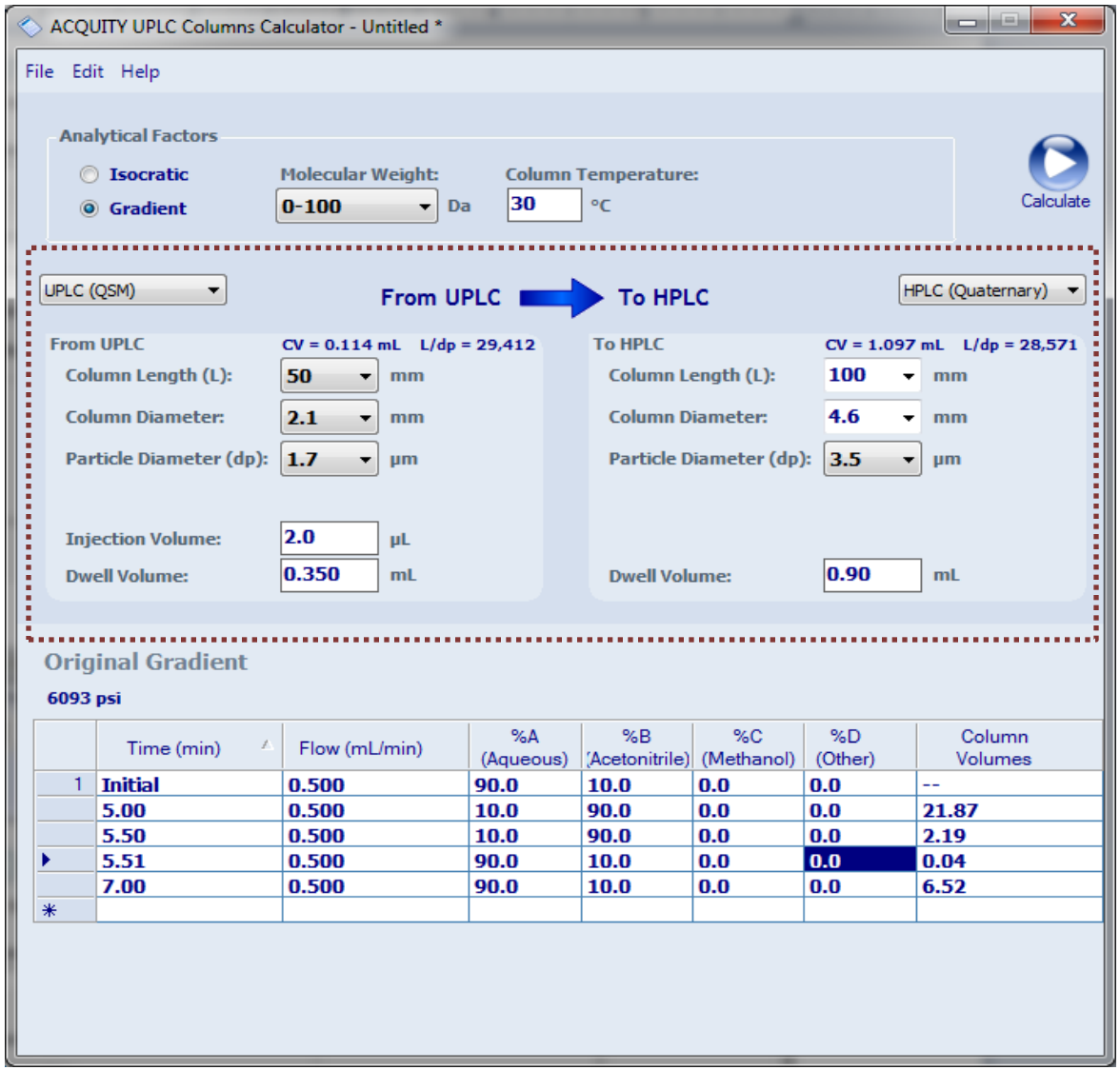

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B (Acetonitrile)	%C (Methanol)	%D (Other)	Column Volumes
1	Initial	0.500	90.0	10.0	0.0	0.0	--
	5.00	0.500	10.0	90.0	0.0	0.0	21.87
	5.50	0.500	10.0	90.0	0.0	0.0	2.19
▶	5.51	0.500	90.0	10.0	0.0	0.0	0.04
	7.00	0.500	90.0	10.0	0.0	0.0	6.52
*							

Easy Method Transfer

The ACQUITY UPLC Columns Calculator will provide target method key parameters automatically.

Scenario 3:
UPLC to HPLC Method Transfer

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- **ACQUITY UPLC Columns Calculator**
  - For automatic calculations
- **Injection Volume**
  - Injection volume properly scaled
- **Extra Gradient Hold**
  - Automatically calculated and inserted into gradient table
- **N_{cv} (Column Volumes)**
  - Kept consistent throughout gradient steps

Original Gradient

6093 psi

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B (Acetonitrile)	%C (Methanol)	%D (Other)	Column Volumes
1	Initial	0.500	90.0	10.0	0.0	0.0	--
	5.00	0.500	10.0	90.0	0.0	0.0	21.87
	5.50	0.500	10.0	90.0	0.0	0.0	2.19
▶	5.51	0.500	90.0	10.0	0.0	0.0	0.04
	7.00	0.500	90.0	10.0	0.0	0.0	6.52
*							

Gradient Results - Untitled

Column	Run Time (min)	Peak Capacity	Flow Rate (mL/min)	Pressure (psi)	Injection Volume (uL)
Original UPLC column conditions					
50 mm x 2.1 mm, 1.7 µm	7.00	86	0.50	6093	2.0
New HPLC conditions with scaled gradient (accounting for particle size)					
100 mm x 4.6 mm, 3.5 µm	30.93	84	1.17	1243	19.2
New HPLC conditions with scaled gradient (disregarding particle size)					
100 mm x 4.6 mm, 3.5 µm	14.00	82	2.40	2559	19.2

Print

Flow Rate

New HPLC Gradient

Undo gradient hold = 2459 µL

New HPLC conditions with scaled gradient (accounting for particle size), 100 mm x 4.6 mm, 3.5 µm column

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B (Acetonitrile)	%C (Methanol)	%D (Other)	Column Volumes
▶ 1	Initial	1.17	90.0	10.0	0.0	0.0	--
2	2.11	1.17	90.0	10.0	0.0	0.0	2.24
3	22.70	1.17	10.0	90.0	0.0	0.0	21.87
4	24.76	1.17	10.0	90.0	0.0	0.0	2.19
5	24.80	1.17	90.0	10.0	0.0	0.0	0.04
6	30.93	1.17	90.0	10.0	0.0	0.0	6.52

Scenario 3 - The Result: Transfer a UPLC Method to HPLC Systems

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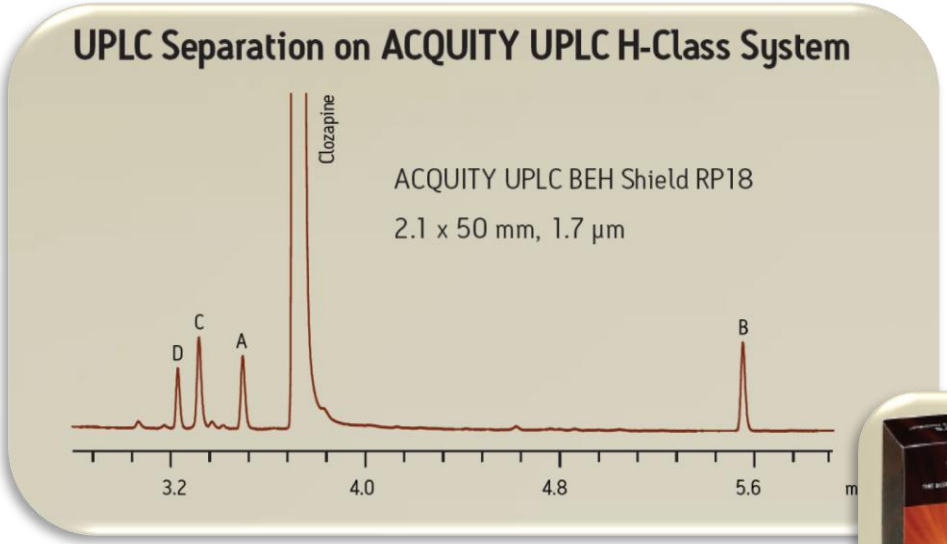
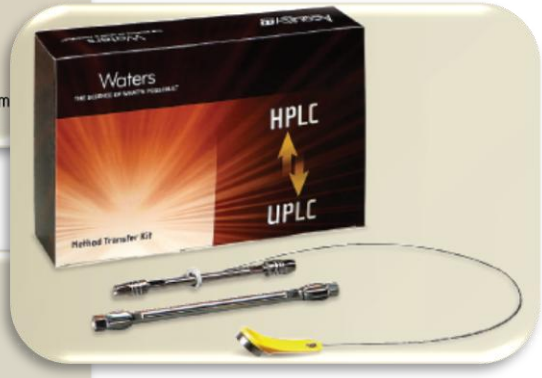
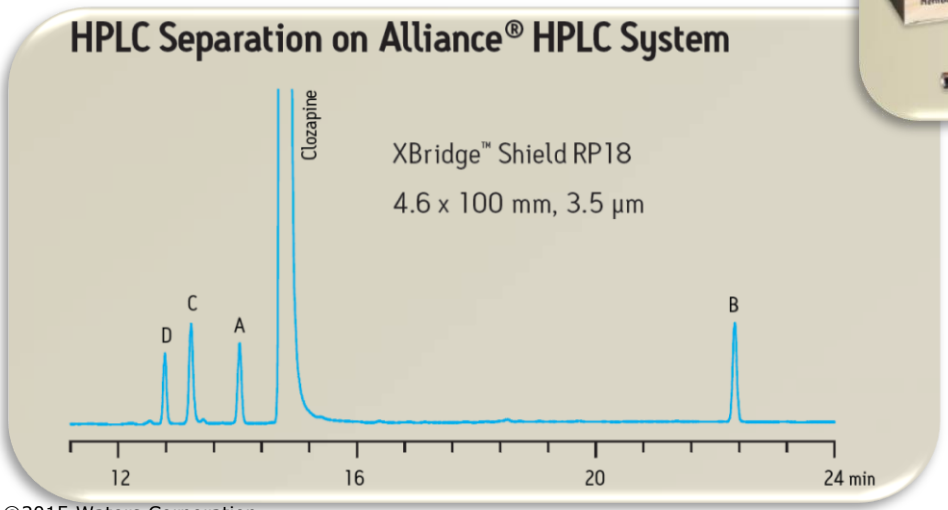


Relative RT to Clozapine		
Peak	**H-Class**	**HPLC**
Impurity D	0.867	0.865
Impurity C	0.890	0.895
Impurity A	0.939	0.950
Clozapine	1.000	1.000
Impurity B	1.500	1.513





Maximize Asset Utilization

Transfer between HPLC and UPLC

Sustained selectivity between particle sizes

Summary

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- USP Chapter <621> Chromatography updated Aug-2014
- Other Pharmacopoeias (eg. EU) expected to follow. USP and EurP to harmonize both chapter <621> and 2.2.46 to the extent possible
- Isocratic methods: more flexibility in column dimensions
- Gradient methods changes more limited
- Most methods can be transferred seamlessly from HPLC to UPLC
- Take advantage of these changes:
  - Improve isocratic methods with sub-2-µm columns and ACQUITY UPLC H-Class
  - Moderate savings with sub-3-µm columns with HPLC
  - Use Column Selectivity Chart to select the best UPLC column
  - Use the ACQUITY Columns Calculator for proper transfers per L/dp
  - Utilize ACQUITY UPLC H-Class to run both HPLC and UPLC methods
- For successful UPLC transfer, always ensure:
  - Method compatibility
  - System cleanliness (avoid bacteria)
  - Fresh, high quality mobile phases are prepared

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