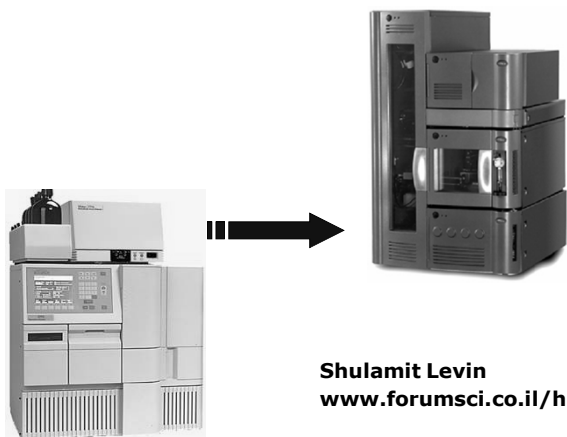


# HPLC to UPLC: Method Transfer and Development

## Method Transfer From HPLC to UPLC



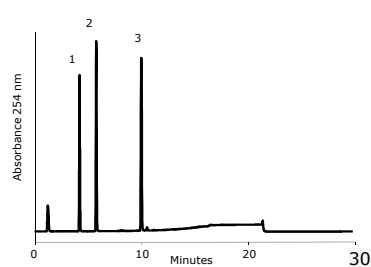
Shulamit Levin  
[www.forumsci.co.il/hplc](http://www.forumsci.co.il/hplc)

## Methods Transfer Considerations

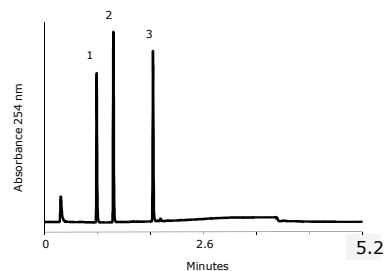
- Four classes of generic HPLC methods transfer include:
  - Replace a longer column with a shorter column
  - Replace a column brand with another column brand
  - Replace an instrument with a different instrument
  - Replace both the instrument and the column
- The major benefits of UPLC are realized when the HPLC method is transferred to a UPLC instrument with a UPLC column.
  - Need to replace both the instrument and column

## HPLC Transferred to UPLC:

- Scaling Down to smaller columns
- Changing to equivalent columns
- Changing Instrument brand



Original 30 minute HPLC



Transferred 5.2 minute UPLC

## Method Transfer Process Steps for success

- **Gather information about existing method and results**
  - Compare Instruments
  - Select new or target column
    - Chemistry
    - Dimensions
  - Select target conditions based on geometric considerations
  - Evaluate results of transfer
  - Optimize as required

## HPLC to UPLC: Method Transfer and Development

### Critical Concern of Method Transfer

- The new UPLC method will be different from the original HPLC method....
  - **Operating conditions, e.g., flow rate**
  - **Run time**
  - **Appearance**
- But, the new UPLC method must also preserve critical HPLC method parameters
  - **Complete resolution of all relevant analytes**
  - **Peak homogeneity/purity**
  - **Certainty of peak identification**
  - **Quantitative accuracy and precision**

### Required Information Original method

- Column
  - Chemistry (ligand, brand, particle size)
  - Dimensions
- Conditions
  - Mobile phase
  - Flow rate
  - Gradient profile, including regeneration and reequilibration
  - Temperature
- Sample
  - Diluent
  - Concentration
  - Molecular weight(s)
  - Injection volume

### Required Information Original results

- Chromatogram
  - Number of peaks
  - Retention
  - Resolution
- Quantitation
  - Limit of detection
  - Limit of quantitation
  - Linear dynamic range
  - Accuracy
  - Precision

### Method Transfer Process Steps for success

- Gather information about existing method and results
- **Compare Instruments**
- Select new or target column
  - Chemistry
  - Dimensions
- Scale down geometrically: flow, injection volume and gradient times
- Evaluate results of transfer
- Optimize as required

## HPLC to UPLC: Method Transfer and Development

### Instrument Comparison Solvent delivery

- We assume that:
  - Solvent is always flowing at the programmed rate
  - Percent composition is always what is programmed
  - A gradient follows the programmed profile
  - Solvent composition reaches column at the programmed time

### Required Information Original instrument

- **Mode of gradient generation**
  - single pump with gradient proportioning valve
  - dual pump
  - Brand and model number
- **System volume (dwell volume or delay volume)**
  - Value and method used to measure
- **Injection mechanism**
- **Mode of detection**

### Example Original method

Ambient temperature: 21 – 22° C  
Flow rate: 1.50 mL/min  
Sample analytes: Caffeine (100 µg/mL)  
Hydroquinidine (33 µg/mL)  
3-Aminobenzophenone (39 µg/mL)  
Molecular weight(s): Less than 500  
Sample diluent: DMSO  
Injection: 10 µL  
Detection: 254 nm  
Mobile phase: A: 0.05% TFA in water  
B: 0.05% TFA in acetonitrile

### Example Original gradient profile

Gradient Step	Time since injection	Flow Rate	%A	%B	Curve
Initial	0	1.5	95	5	*
2	15	1.5	5	95	6
3	20	1.5	5	95	1
4	30	1.5	95	5	1

# HPLC to UPLC: Method Transfer and Development

## Example Original column

Solid Phase: XTerra MS C<sub>18</sub>  
Particle Size: 5 µm  
ID: 4.6 mm  
Length: 150 mm



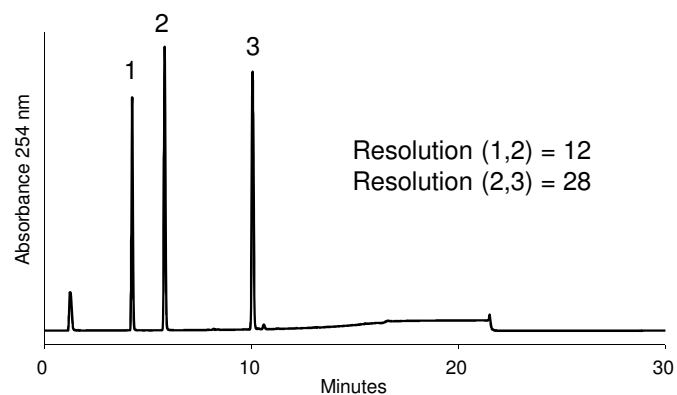
Calculate:  $\frac{L}{d_p} = \frac{150,000 \mu\text{m}}{5 \mu\text{m}} = 30,000$

## Example Original instrument

- Waters Alliance 2695 Solvent Manager  
— Single pump gradient with low pressure mixing
- Waters Alliance 2695 Sample Manager
- Waters 2487 TUV at 254 nm



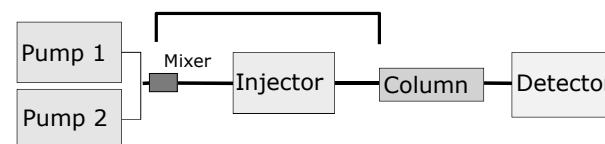
## Example Original result



## Volumes of Pumping Systems

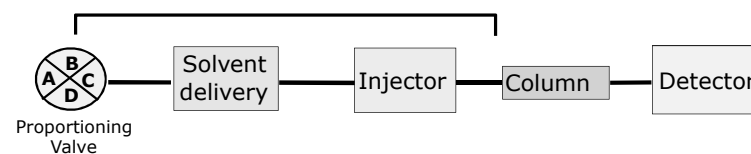
### Multi-Pump

Smaller System Volume = Dwell volume



### Single Pump

Larger System Volume = Dwell volume



## HPLC to UPLC: Method Transfer and Development

### Different Configurations Create Different Time Delays

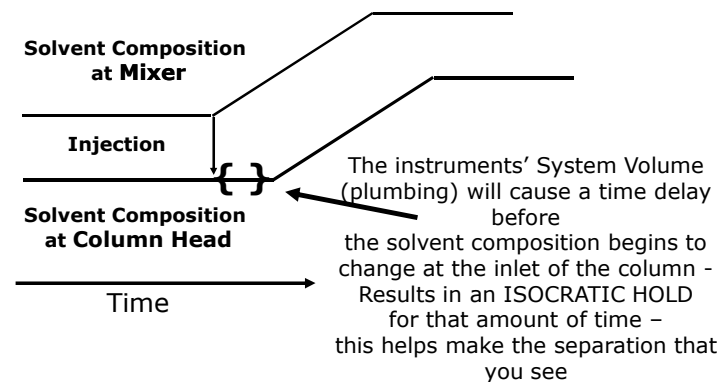


**Mixing Valve Low –  
More Pipe Volume –  
More Time Delay**

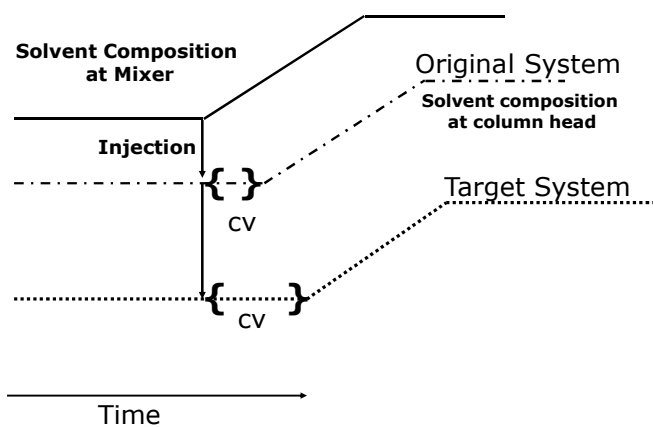


**Mixing Valve High –  
Less Pipe Volume –  
Less Time Delay**

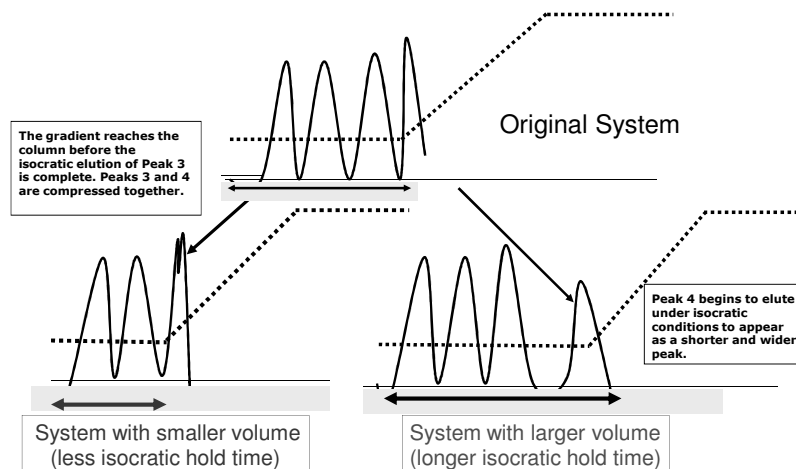
### Timing Offset of a System due to It's System Volume



### Offset from System Volume Both systems in units of column volume (cv)

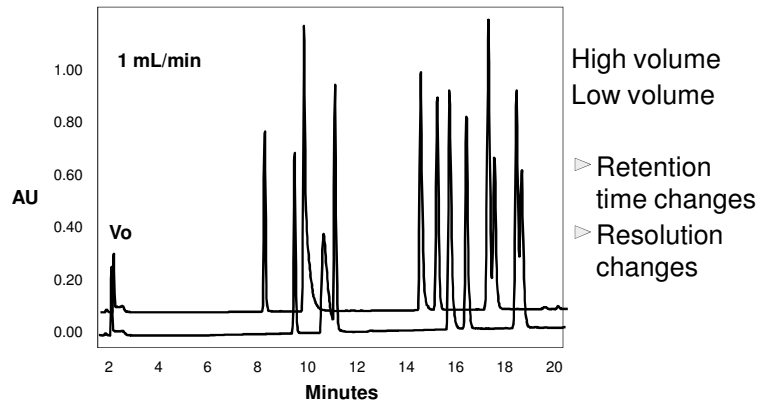


### Different System Volumes Effect on separation



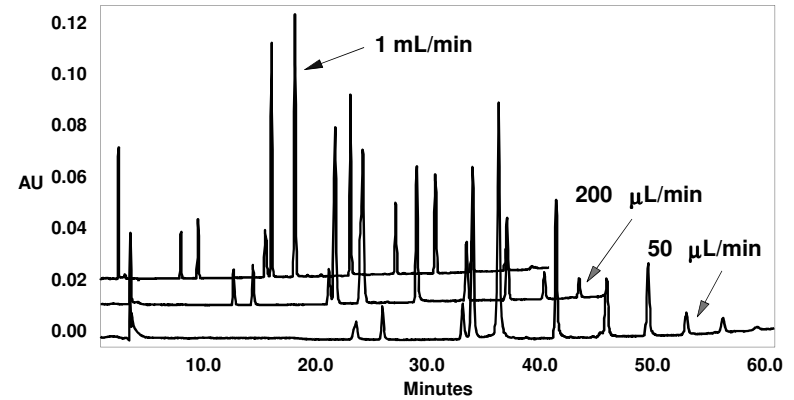
# HPLC to UPLC: Method Transfer and Development

## Effects of System Volume on Gradients

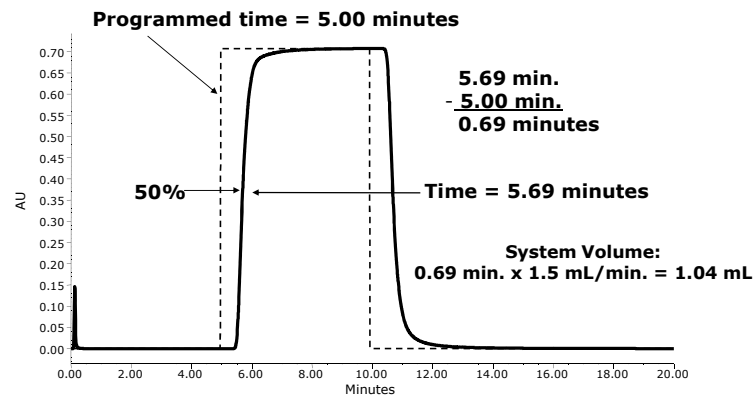


## Linear Gradient Separation 1 mL/min, 200 and 50 $\mu$ L/min

[Application](#)



## Measuring System Volume



## System Volume Recommended method for measurement

1. Remove column
2. Use Acetonitrile as A, and Acetonitrile with 0.05 mg/mL uracil as B (eliminates non-additive mixing and viscosity problems)
3. Monitor 254 nm
4. Use the flow rate in the original method and the intended flow rate on the target instrument
5. Collect 100% A baseline for 5 min
6. At 5.00 min, program a step to 100% B, and collect data for an additional 5 min
7. Measure absorbance difference between 100% A and 100% B
8. Measure time at 50% of that absorbance difference.
9. Calculate time difference between start of step and 50% point
10. Multiply time difference by flow rate yields System Volume

# HPLC to UPLC: Method Transfer and Development

## System Delay Results

- **Alliance 2695 = 1.04 mls**
- **ACQUITY UPLC = 0.109 mls**
- **Will this make a difference in the transferred method?**
  - Alliance 2695 with a 4.6 x 150 mm column
  - ACQUITY UPLC with a 2.1 x 50 mm column

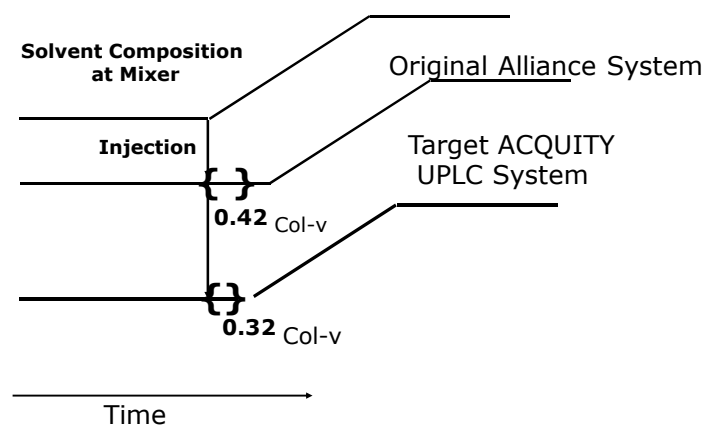
## Compensation for Different System Volumes

- Compare System Volumes (**measured in cv**)
  - 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, no exact compensation is possible unless the system provides the ability to start forming the gradient before the actual injection. **{Negative Hold}**

## Compensation for System Volume

- **Original Alliance 2695 System**
  - Measured system volume = 1.04 mL
  - 4.6 x 150 mm column volume = 2.49 mL
  - System volume = 1.04 mL / 2.49 mL = 0.42 column volumes (Col-v)
- **Target ACQUITY UPLC System**
  - Measured system volume = 0.109 mL
  - 2.1 x 100 mm column volume = 0.34 mL
  - System volume = 0.109 mL / 0.34 mL = 0.32 column volumes (Col-v)

## Offsets from Different System Volumes Expressed in column volumes (cv)



## HPLC to UPLC: Method Transfer and Development

### Calculation of Initial Hold Time for UPLC

- **Initial Hold (column volumes) =**  
Original System Volume – Target System Volume  
 $0.42 \text{ cv} - 0.32 \text{ cv} = 0.10 \text{ Col-v's Initial Hold}$
- **Initial Hold Volume (mL) =**  
Initial Hold Col-v x Target Column Volume  
 $0.12 \text{ cv} \times 0.34 \text{ mL} = 0.0408 \text{ mL}$
- **Initial Hold Time =**  
Initial Hold Volume / UPLC Flow rate =  
 $0.0408 \text{ mL} / 0.6 \text{ mL/min} = 0.068 \text{ min.}$

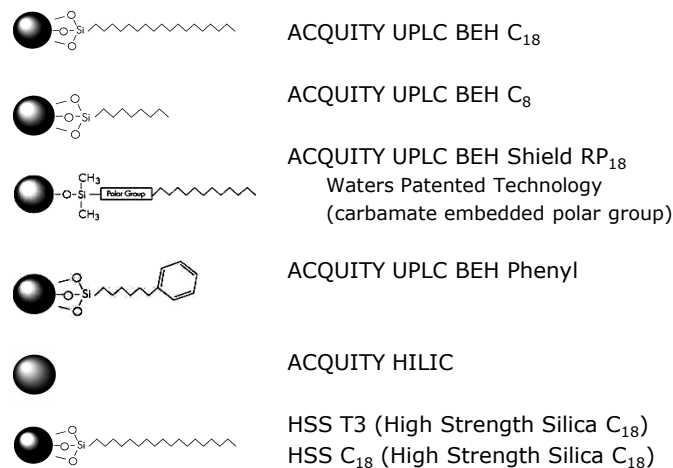
### Compensating for System Volumes

- **Compare system volumes (measured in column volumes)**
- **Target system gives smaller isocratic segment**  
– ADD an initial hold to the gradient table to give the identical hold.
- **Target system gives larger isocratic segment**  
– No exact compensation is possible  
– Chromatographic effect of extra isocratic hold usually small

### Method Transfer Process Steps for success

- Gather information about existing method and results
- Compare Instruments
- **Select new or target column**
  - Chemistry
  - Dimensions
- Scale down geometrically: flow, injection volume and gradient times
- Evaluate results of transfer
- Optimize as required

### ACQUITY UPLC Columns

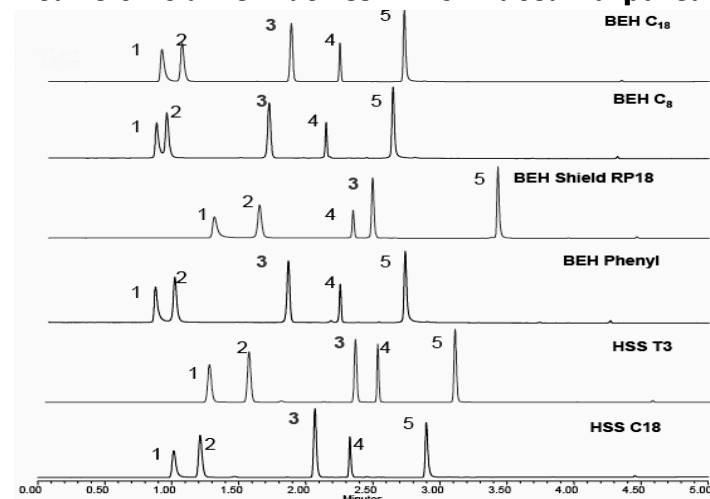


# HPLC to UPLC: Method Transfer and Development

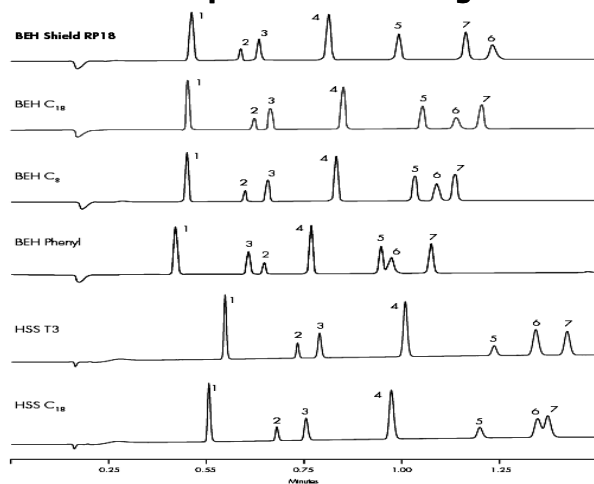
## ACQUITY BEH, & HSS chemistries

UPLC® Particle	Ethylene Bridged Hybrid (BEH)	High Strength Silica (HSS)
Available Chemistries	C18, C8, Shield RP18, Phenyl, HILIC	T3, C18
pH Range	1-12; (RP18: 2-11); (HILIC: 1-8)	2-8
Maximum Pressure	15,000 psi (~1000 bar)	15,000 psi (~1000 bar)
Particle Size	1.7 µm	1.8 µm
Pore Diameter & Volume	130Å / 0.7 mL/g	100Å / 0.7 mL/g
Surface Area	185 m²/g	230 m²/g

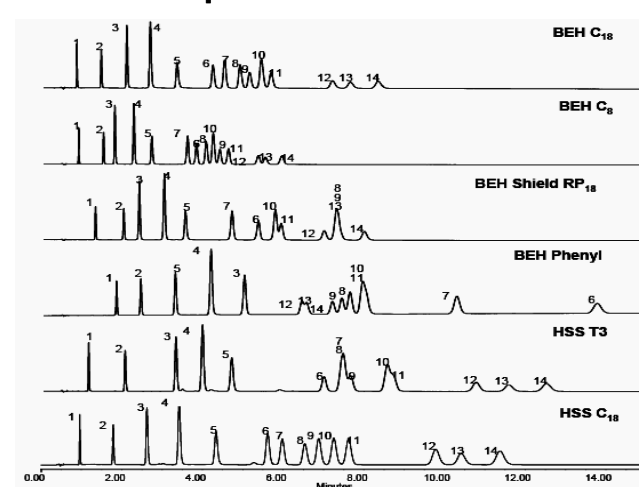
## Ligand Selectivity Caffeic Acid Derivatives in Echinacea Purpurea



## Stationary Phase Selectivity: UPLC® Separations of Analgesics



## Stationary Phase Selectivity: UPLC® Separations of Nitroaromatics



# HPLC to UPLC: Method Transfer and Development

## Classification of Reversed Phase Columns

- Pharmacopeias Definitions and Database
- Selectivity Chart Database
- ACDLabs Database
- Physical Characterization

## USP Definitions: Categories For HPLC Columns Definitions are too broad

Category	Particle Dia.(μ m)	Bonding	Description
L1	1.7 to 10	C18	Octadecyl silane chemically bonded to porous silica or ceramic particles.
L2	30 to 50	C18	Octadecyl silane chemically bonded to silica gel of a controlled surface

## Pharmacopeias Definitions and Database

The Product Quality Research Institute (PQRI) approach for selecting columns of equivalent selectivity

Column hydrophobicity			<a href="http://www.usp.org/USP/NF/columns.html">http://www.usp.org/USP/NF/columns.html</a> measured by the column parameter					H			
Column steric interaction			" "					S*			
Column hydrogen-bond acidity			" "					A			
Column hydrogen-bond basicity			" "					B			
Column ion-exchange capacity			$F_s = \frac{\{[12.5 (H_2 - H_1)]^2 + [100 (S^* - S^*)]^2 + [30 (A_2 - A_1)]^2 + [143 (B_2 - B_1)]^2 + [83 (C_2 - C_1)]^2\}^{1/2}}{[1]}$					C			
Rank	F	Column	H	S	A	B	C(2.8)	C(7.0)	Type	USP Designation	Manufacturer
0	0	Xterra MS C18	0.984	0.012	-0.143	-0.015	0.133	0.051	B	L1	<a href="#">Waters</a>
1	2.72	Ascentis C-8	0.899	0.024	-0.18	-0.002	-0.124	-0.035	B		<a href="#">Supelco</a>
2	2.87	Zorbax Eclipse Plus C18	1.03	0.007	-0.072	-0.02	-0.004	0.02	B		<a href="#">Agilent</a>
3	2.95	Zorbax Eclipse XDB-C8	0.919	0.025	-0.219	-0.008	0.003	0.012	B	L7	<a href="#">Agilent</a>
4	3.1	COSMOSIL MS-II	1.031	0.042	-0.132	-0.014	-0.118	-0.027	B		<a href="#">Nacal Tesque</a>
5	3.32	CAPCELL PAK C8 UG120	0.854	0.037	-0.097	-0.013	-0.046	-0.01	B	L7	<a href="#">Shiseido</a>
6	4.37	Synergi Max-RP	0.989	0.028	-0.008	-0.013	-0.133	-0.034	B		<a href="#">Phenomenex</a>
7	4.54	Epic C18	0.95	-0.027	-0.203	-0.007	-0.131	-0.041	B	L1	ES Industries

## Columns Classification The PQRI approach for selecting columns of equivalent selectivity

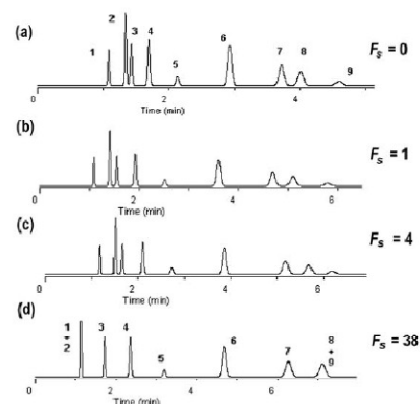


Fig. 3. Example of use of  $F_s$  values to select columns of either similar (a-c) or different (d) selectivity

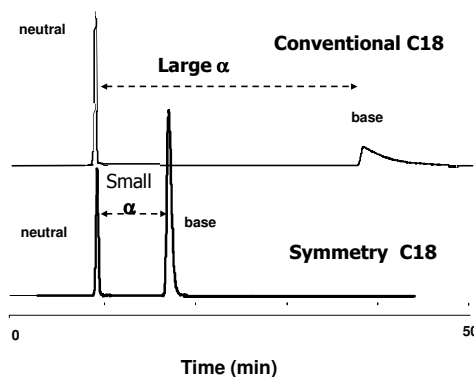
# HPLC to UPLC: Method Transfer and Development

## Selectivity Chart Database

$$K' = (RT - t_0) / t_0$$

$$\alpha = k'_2 / k'_1$$

- Large Relative Retention Factor ( $\alpha$ ) indicates high silanol activity
- Small Relative Retention Factor ( $\alpha$ ) indicates low silanol activity



## Stationary Phase Characterization

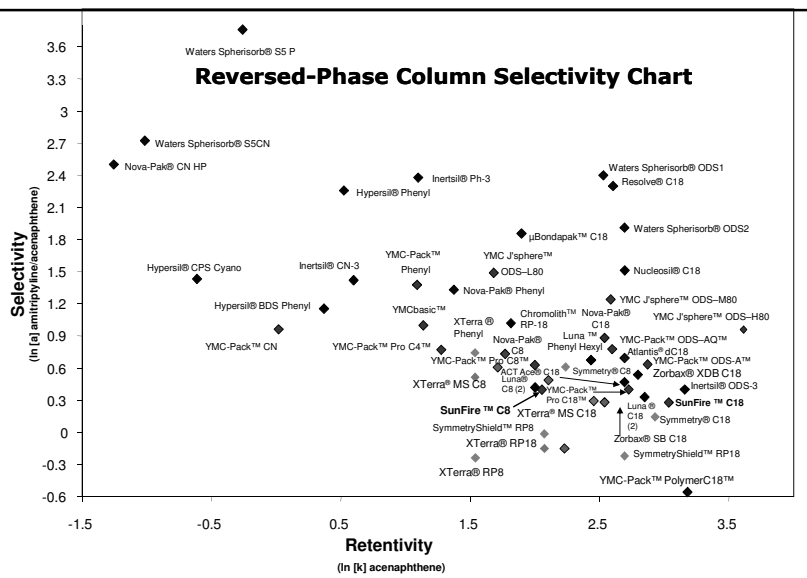
- Hydrophobicity (x - axis)**
  - Measured by the retention factor of acenaphthene
- Selectivity and Silanol Activity (y - axis)**
  - Measured by the relative retention ( $\alpha$ ) between amitriptyline and acenaphthene at pH 7.00 in phosphate buffer

Selectivity  
 $\alpha$

Selectivity Chart  
for Different Columns

Hydrophobicity k

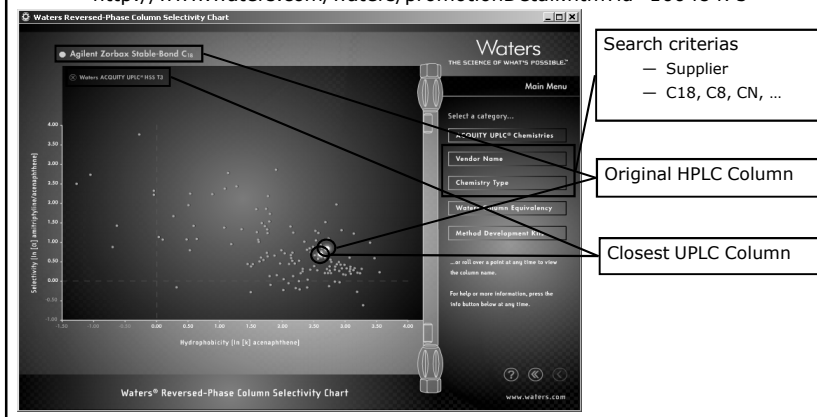
## Reversed-Phase Column Selectivity Chart



## Tool to Help Select The Right Column

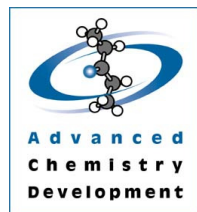
Waters Reverse-Phase Column Selectivity Chart

- Down-loaded from Waters web site (V3.0)
  - <http://www.waters.com/waters/promotionDetail.htm?id=10048475>



# HPLC to UPLC: Method Transfer and Development

## ACDLabs Database Result of 2 Columns Comparison



### HPLC Column Selector, Version 11.0

Build version: 24 April 2008

The Column Selector is based on an article by M.R. Euerby and P. Petersson published in *J. Chromatogr. A* **2003**, 994, 13-36.

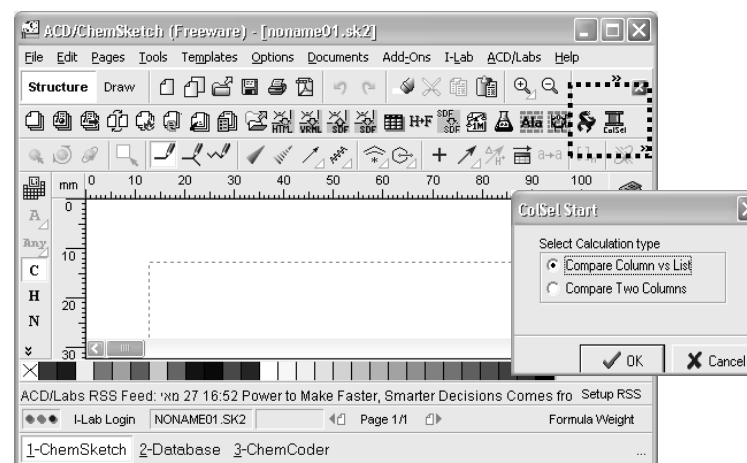
#### Single pair column comparison

CDF = 0.180

Column 1: Acquity BEH C18  
Column 2: HyPURITY C18

	Parameters					
Weightings	kPB	aCH2	aTO	aCP	aBP76	aBP27
	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
	2.81	1.46	1.36	0.36	0.26	0.14
	3.2	1.47	1.6	0.37	0.29	0.1

## ACDLabs Column Selector

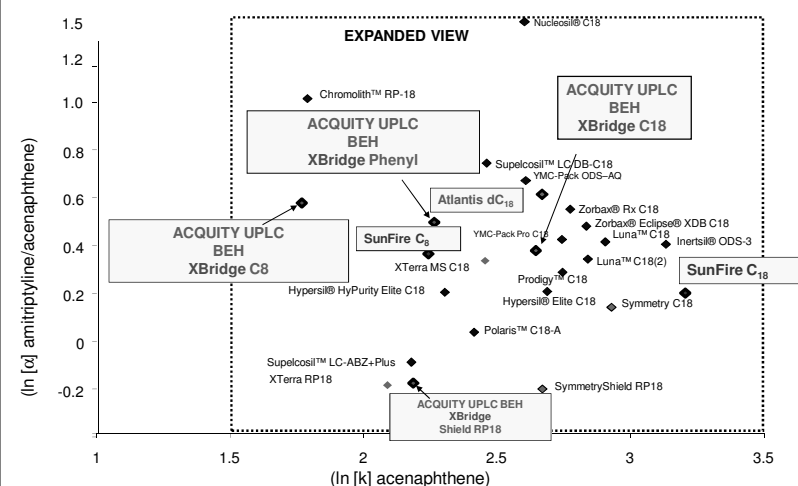


## Physical Parameters Characterizing Stationary Phases

### Physical Characteristics of HPLC and UPLC™ Packing Materials

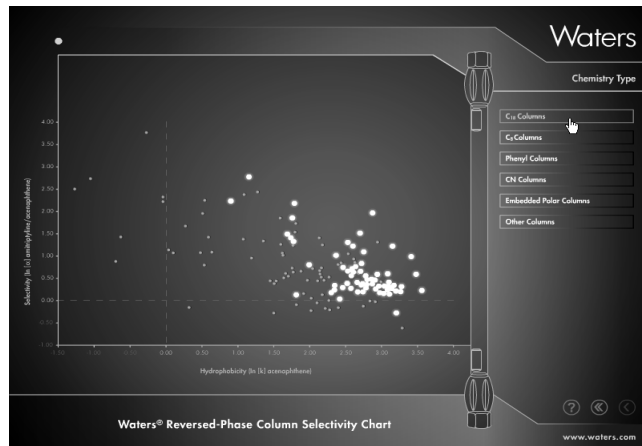
Brand	Chemistry	Particle Shape	Particle Size(s)	Pore Size	Surface Area [m²/g]	Pore Volume [cc/g]	% Carbon Load	Endcapped
SunFire™	C18	Spherical	2.5, 3.5, 5, 10 µm	100Å	340	0.90	16	yes
	C8	Spherical	2.5, 3.5, 5, 10 µm	100Å	340	0.90	11.5	yes
	Silica	Spherical	5, 10 µm	100Å	340	0.90	n/a	n/a
Symmetry®	C18	Spherical	3.5, 5 µm	100Å	335	0.90	19.1	yes
	C8	Spherical	3.5, 5 µm	100Å	335	0.90	11.7	yes
SymmetryShield™	RP18	Spherical	3.5, 5 µm	100Å	335	0.90	15.0	yes
	RP18	Spherical	5 µm	100Å	335	0.90	17.0	yes
SymmetryRap™	C18	Spherical	7 µm	100Å	335	0.90	19.1	yes
	C8	Spherical	7 µm	100Å	335	0.90	11.7	yes
Symmetry300™	C18	Spherical	3.5, 5 µm	300Å	110	0.80	8.5	yes
	C8	Spherical	3.5, 5 µm	300Å	110	0.80	2.8	yes
Waters Spharion®	Silica	Spherical	3, 5, 10 µm	80Å	220	0.50	n/a	n/a
	ODS2	Spherical	3, 5, 10 µm	80Å	220	0.50	11.5	yes
	ODS	Spherical	3, 5, 10 µm	80Å	220	0.50	6.2	no
	ODS8	Spherical	5 µm	80Å	220	0.50	11.5	yes
	C8	Spherical	3, 5, 10 µm	80Å	220	0.50	5.8	yes
	C4	Spherical	3, 5, 10 µm	80Å	220	0.50	4.7	yes
	C1	Spherical	3, 5, 10 µm	80Å	220	0.50	2.2	no
	little	Spherical	3, 5, 10 µm	80Å	220	0.50	3.1	no
	Amino	Spherical	3, 5, 10 µm	80Å	220	0.50	1.9	no
	Phenyl	Spherical	3, 5, 10 µm	80Å	220	0.50	2.5	no
NEW XBridge™	ODS/ON	Spherical	5 µm	80Å	220	0.50	5.0	yes
	SAX, SCX	Spherical	5, 10 µm	80Å	220	0.50	4.0	no
	C18	Spherical	2.5, 3.5, 5 µm	135Å	185	0.7	17.5	yes
	C8	Spherical	2.5, 3.5, 5 µm	135Å	185	0.7	17.5	yes
	Shield RP18	Spherical	2.5, 3.5, 5 µm	135Å	185	0.7	17.5	yes
Xtreme®	Phenyl	Spherical	2.5, 3.5, 5 µm	135Å	185	0.7	17.5	yes
	RP18	Spherical	3.5, 5, 10 µm	125Å	175	0.70	15.0	yes
	RP18	Spherical	3.5, 5, 10 µm	125Å	175	0.70	13.5	yes
	MS C18	Spherical	2.5, 3.5, 5, 10 µm	125Å	175	0.70	15.5	yes
	MS C8	Spherical	2.5, 3.5, 5, 10 µm	125Å	175	0.70	12.0	yes

## The Modern "C<sub>18</sub> Zone"



# HPLC to UPLC: Method Transfer and Development

## Column Selectivity Chart



## Method Transfer Process Steps for success

- Gather information about existing method and results
- Compare Instruments
- Select new or target column
  - Chemistry
  - Dimensions
- **Scale down geometrically: flow, injection volume and gradient times**
- Evaluate results of transfer
- Optimize as required

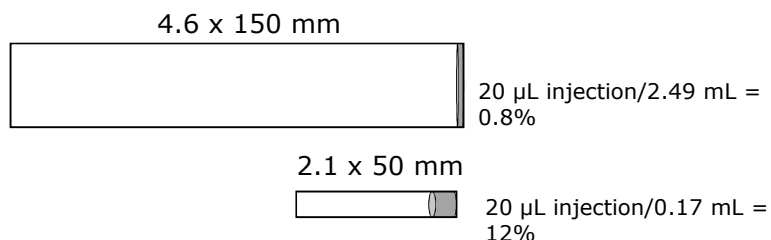
## Calculate

## Target Conditions: Injection

- Use exactly the same sample
  - Same concentration
  - Same diluents
- Scale injection volume to volume of column

## HPLC to UPLC: Method Transfer and Development

### Scale Injection Volume: Isocratic Conditions



#### 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.

### Calculation of Injection Volume

Target injection volume =

$$\text{Original injection volume} \times \frac{\text{Target Column Volume}}{\text{Original Column Volume}}$$

Scaling a **10 µL** injection on 4.6 x 250 mm to 2.1 x 50 mm

$$10 \mu\text{L} \times \frac{3.14 \times 1.1^2 \times 50}{3.14 \times 2.3^2 \times 150} =$$

$$10 \mu\text{L} \times \frac{0.17}{2.49} = 10 \mu\text{L} \times 0.068$$

$$= 0.7 \mu\text{L}$$

### Target Conditions: Injection

- Use exactly the same sample
  - Same concentration
  - Same diluents
- Scale injection volume to volume of column
  - Suggested minimum injection volume on the ACQUITY UPLC instrument is 0.5 – 1 µL
  - If calculated volume too small for injection
    - Dilute 5 - 10x with initial strength mobile phase
  - 5 µL maximum injection on 2.1 x 50 mm

### Target Conditions Flow rate

- Adjust flow rate proportional to column diameter squared for constant linear velocity

or

- Adjust linear velocity for smaller particle

# HPLC to UPLC: Method Transfer and Development

## Geometrically Scaled Flow Rate Calculation

$$\text{Target Flow Rate} = \text{Original Flow Rate} \times \frac{\pi \times r_{\text{target}}^2}{\pi \times r_{\text{original}}^2}$$

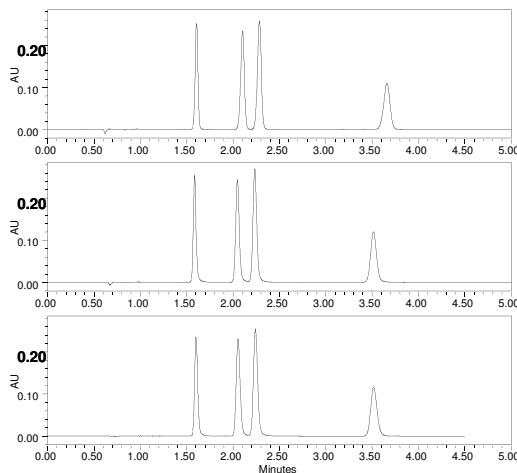
This reduces to:

$$\text{Target Flow Rate} = \text{Original Flow Rate} \times \frac{\text{diameter}_{\text{target}}^2}{\text{diameter}_{\text{original}}^2}$$

## Calculation of Linear Velocity

						Linear Velocity	
$\pi$	R (cm)	L (cm)	$r^2$	$\pi r^2 L$ (mL)	Flow rate mL/min	$t_0$	cm/min
3.14	0.25	5.00	0.06	0.98	1.00	0.98	5.10
3.14	0.10	5.00	0.01	0.16	0.16	0.98	5.10

## Constant Linear Velocity Constant Length, Smaller ID, Scaled Down Flow Rate and Injection Volume



**4.6 x 100 mm, 3.5 µm**  
Injection = 15.8 µL  
Flow Rate = 1.439 mL/min  
 $R_{s(2,3)} = 2.38$

**3.0 x 100 mm, 3.5 µm**  
Injection = 6.7 µL  
Flow Rate = 0.612 mL/min  
 $R_{s(2,3)} = 2.57$

**2.1 x 100 mm, 3.5 µm**  
Injection = 3.3 µL  
Flow Rate = 0.3 mL/min  
 $R_{s(2,3)} = 2.32$

## Geometrically Scaled Flow Rate Calculation

$$\text{Target Flow Rate} = \text{Original Flow Rate} \times \frac{\pi \times r_{\text{target}}^2}{\pi \times r_{\text{original}}^2}$$

This reduces to:

$$\text{Target Flow Rate} = \text{Original Flow Rate} \times \frac{\text{diameter}_{\text{target}}^2}{\text{diameter}_{\text{original}}^2}$$

Scaling a **1.5 mL/min** flow rate on 4.6 x 150 mm to 2.1 x 50 mm

$$1.5 \text{ mL/min} \left( \frac{2.1^2}{4.6^2} \right) = 0.31 \text{ mL/min}$$

# HPLC to UPLC: Method Transfer and Development

## Original Gradient Profile

Gradient Step	Time Since Injection	Flow Rate	%A	%B	Curve
Initial	0	0.31	95	5	*
2	15	0.31	5	95	6
3	20	0.31	5	95	1
4	30	0.31	95	5	1

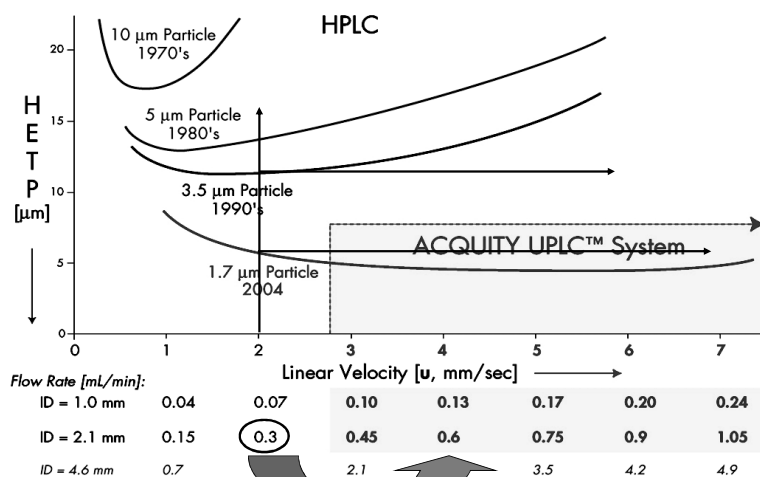
## Target Conditions Flow rate

- Adjust flow rate proportional to column diameter squared for constant linear velocity

or

- Adjust linear velocity for smaller particle

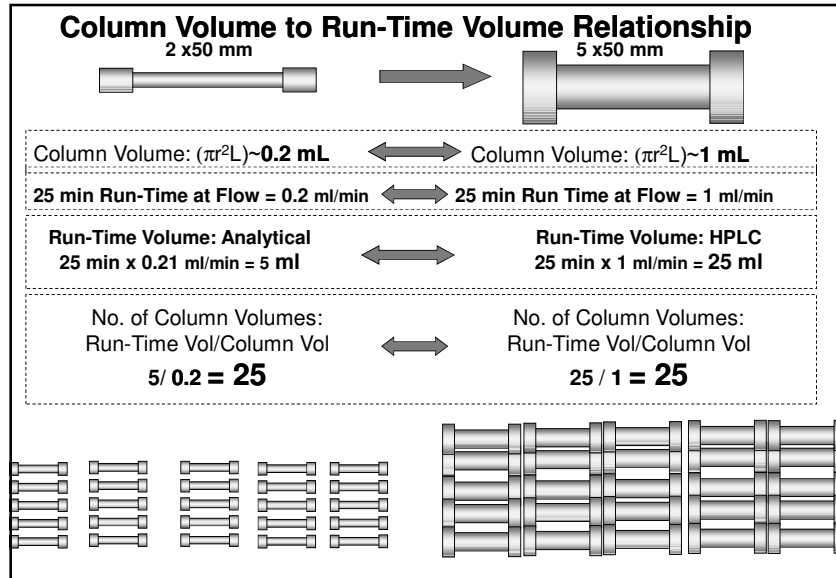
## Traditionally "Doubled" Flow Rate



## Target Conditions Gradient profile

- Express gradient duration in percent change per column volume (Col-v) units
- Calculate each segment as a number of column volumes
- Calculate time required to deliver the same number of column volumes to the target column at the geometrically scaled flow rate
- Calculate time required to deliver the same number of column volumes to the target column at the UPLC flow rate

## HPLC to UPLC: Method Transfer and Development



### Gradient Segments Express as column volumes

For 15 min at 1.5 mL/min on a 4.6 x 150 mm column

$$\text{Gradient Volume} = \text{Flow Rate} \times \text{Time} = 1.5 \text{ mL/min} \times 15 \text{ min} = 22.5 \text{ mL}$$

$$\text{Column Volume} = \pi \times r^2 \times L = 3.14 \times 2.3^2 \times 150 = 2.49 \text{ mL}$$

$$\text{Gradient Duration (Col-v)} = \frac{\text{Gradient Volume}}{\text{Column Volume}}$$

$$\text{Gradient Duration} = \frac{22.5 \text{ mL}}{2.49 \text{ mL}} = 9.03 \text{ cv}$$

### Target Conditions Gradient profile

- Express gradient duration in percent change per column volume (Col-v) units

- Calculate each segment as a number of column volumes

- Calculate time required to deliver the same number of column volumes to the target column at the geometrically scaled flow rate

- Calculate time required to deliver the same number of column volumes to the target column at the UPLC flow rate

### Original Gradient Profile for Scaling

Gradient Step	Time Since Injection	Flow Rate	%A	%B	Curve	Segment Duration (min)	Segment Duration (cv)
Initial	0	1.5	95	5	*	0	0
2	15	1.5	5	95	6	15	9.03
3	20	1.5	5	95	1	5	3.01
4	30	1.5	95	5	1	10	6.02

## HPLC to UPLC: Method Transfer and Development

### Target Conditions Gradient profile

- Express gradient duration in percent change per column volume (Col-v) units

- Calculate each segment as a number of column volumes

- Calculate time required to deliver the same number of column volumes to the target column at the geometrically scaled flow rate

- Calculate time required to deliver the same number of column volumes to the target column at the UPLC flow rate

### Scaled Gradient Profile 2.1 x 50 mm column

Adjust flow rate for 2.1 x 50 at constant linear velocity

Gradient Step	Time Since Injection	Flow Rate	%A	%B	Curve	Segment Duration (min)	Segment Duration (cv)
Initial	0	0.31	95	5	*	0	0
2	5	0.31	5	95	6	5.0	9.03
3	6.67	0.31	5	95	1	1.67	3.01
4	10.00	0.31	95	5	1	3.33	6.02

### Target Conditions Gradient profile

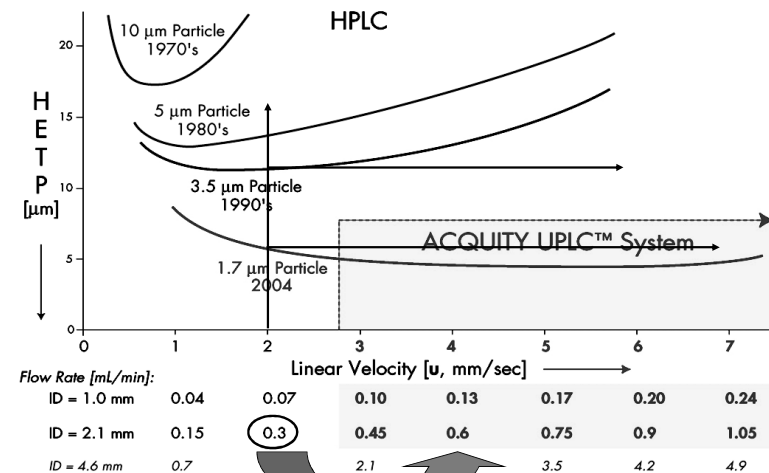
- Express gradient duration in percent change per column volume (Col-v) units

- Calculate each segment as a number of column volumes

- Calculate time required to deliver the same number of column volumes to the target column at the geometrically scaled flow rate

- Calculate time required to deliver the same number of column volumes to the target column at the UPLC flow rate

### Traditionally "Doubled" Flow Rate



## HPLC to UPLC: Method Transfer and Development

### Estimate Optimum Flow Rate UPLC

- Consider 1.7-1.9  $\mu\text{m}$  target particle (2 mm ID column)
- Assume temperature and viscosity transferred
- Adjust flow rate based on van Deemter curve and approximate molecular weight
  - $\sim 0.6$  mL/min for smaller molecules
    - average 500 dalton (molecular weight) molecules
  - $\sim 0.1$  mL/min for larger molecules because diffusion is slower
    - e.g.,  $\sim 2,000$  dalton peptides

### UPLC Scaling

Scale to a 2.1 x 50 mm column at a flow rate of 0.6 mL/min

Gradient Step	Time Since Injection	Flow Rate	%A	%B	Curve	Segment Duration (min)	Segment Duration (cv)
Initial	0	0.6	95	5	*	0	0
2		0.6	5	95	6		9.03
3		0.6	5	95	1		3.01
4		0.6	95	5	1		6.02

### Scaling for UPLC Flow Rate Step time to maintain duration (cv)

Original Step 2: 15 min @ 1.5 mL/min with duration of 9.03 cv  
Calculate Target Step 2: ? min @ 0.60 mL/min keeping duration @ 9.03 cv

Target Column Volume (2.1 x 50) = 0.17 mL

Gradient Step Volume = Duration (cv) x Target Column Volume  
= 9.03 cv x 0.17 mL = 1.54 mL

Gradient Step Time = Gradient Step Volume / UPLC Flow Rate  
= 1.54 mL / 0.60 mL/min. = 2.61 min

### UPLC Scaling

Scale to a 2.1 x 50 mm column at a flow rate of 0.6 mL/min

Gradient Step	Time Since Injection	Flow Rate	%A	%B	Curve	Segment Duration (min)	Segment Duration (cv)
Initial	0	0.6	95	5	*	0	0
2	2.6	0.6	5	95	6	2.61	9.03
3	3.4	0.6	5	95	1	0.87	3.01
4	5.1	0.6	95	5	1	1.74	6.02

# HPLC to UPLC: Method Transfer and Development

## Summary of Gradient Scale-Down

HPLC 2.49 column vol								
Gradient Step	Time Since	Flow Rate	%A	%B	Curve	Segment Duration (min)	Segment Duration (Col.Vol.)	
Initial	0	1.5	95	5	*	0	0	
2	15	1.5	5	95	6	15	9.04	
3	20	1.5	5	95	1	5	3.01	
4	30	1.5	95	5	1	10	6.02	

Geometric UPLC 0.17 column vol								
Gradient Step	Time Since	Flow Rate	%A	%B	Curve	Segment Duration (min)	Segment Duration (Col.Vol.)	
Initial	0.0	0.31	95	5	*	0	0	
2	5.0	0.31	5	95	6	5.0	9.04	
3	6.6	0.31	5	95	1	1.7	3.01	
4	9.9	0.31	95	5	1	3.3	6.02	

UPLC-scale UPLC 0.17 column vol								
Gradient Step	Time Since	Flow Rate	%A	%B	Curve	Segment Duration (min)	Segment Duration (Col.Vol.)	
Initial	0.0	0.6	95	5	*	0	0	
2	2.6	0.6	5	95	6	2.6	9.04	
3	3.4	0.6	5	95	1	0.9	3.01	
4	5.1	0.6	95	5	1	1.7	6.02	

## Calculate

Gradient Separations  
Select your existing HPLC conditions here.

Column Length: 30 cm, 25 cm, 15 cm  
Column Diameter: 4.6 mm, 4.0 mm, 3.9 mm  
Particle Size: 10 µm, 8 µm, 5 µm  
MW of Sample: 100, 200, 300  
Flow Rate: 1.50 [mL/min]  
Temperature: 30 [Celsius]  
Injection Volume: 10 [µL]  
Instrument Delay: 1.00 [mL]

Organic Modifier in B: MeCN 100%, MeOH 0%  
Pressure Units: bar, PSI, MPa  
Results With Existing Column: Peak Capacity 123, Pressure 1176 PSI

Step	Time [min]	Flow [mL/min]	%A	%B	Time Segment [min]	Column Volumes
Init Cond.	0.00	1.50	95	5	0.00	-
Init Hold	0.00	1.50	95	5	0.00	0.00
3	15.00	1.50	5	95	15.00	13.68
4	20.00	1.50	5	95	5.00	4.56
5	30.00	1.50	95	5	10.00	9.12
6						
7						
8						
9						
10						

Select Maximum Column Pressure 15000 PSI Change

Click here for your ACQUITY UPLC® column choices.

Calculate

## Change Flow

Gradient Results  
Here are your UPLC® methods.

Geometrically Scaled UPLC Method Choices:  
Scaled Gradient 2.1 mm Column  
Scaled Gradient 1.0 mm Column

Optimally Scaled UPLC Method Choices:  
Maximum Peak Capacity at Equal Run Time  
Shortest Analysis Time at Equal Peak Capacity

Results With Existing Column:  
Peak Capacity: 123  
Pressure (PSI): 1176  
Column Length: 15 cm  
Column Diameter: 4.6 mm  
Particle Size: 5 µm  
MW of Sample: 200

Scaled Gradient 2.1 mm Column  
(HPLC Linear Velocity)

Length [cm]	ID [mm]	Flow [mL/min]	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume [µL]	Detailed Gradient Profile
3	2.1	0.313	91	6.0	1815	0.4	View
5	2.1	0.313	122	10.0	3025	0.7	View
10	2.1	0.313	178	20.0	6049	1.4	View
15	2.1	0.313	220	30.3	9073	2.1	View

Change Flow: 0.6 Calculate HPLC Linear Velocity UPLC Linear Velocity

Print Results ...

## New UPLC Flow Rate

Scaled Gradient 2.1 mm Column  
(HPLC Linear Velocity)

Length [cm]	ID [mm]	Flow [mL/min]	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume [µL]	Detailed Gradient Profile
3	2.1	0.313	91	6.0	1815	0.4	View
5	2.1	0.313	122	10.0	3025	0.7	View
10	2.1	0.313	178	20.0	6049	1.4	View
15	2.1	0.313	220	30.3	9073	2.1	View

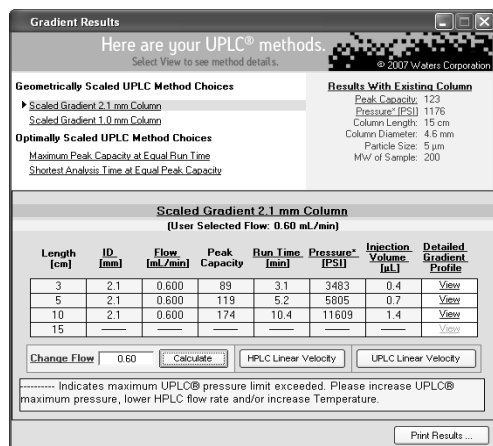
Change Flow: 0.6 Calculate HPLC Linear Velocity UPLC Linear Velocity

Print Results ...

UPLC Flow Rate of 0.6 ml/min

# HPLC to UPLC: Method Transfer and Development

## 2.1 mm Geometrically Scaled Gradient Results



## 2.1 x 50 mm Geometrically Scaled UPLC Gradient table

Geometrically Scaled Gradient: 2.1 X 50 mm

Step	Time (min)	Flow (mL/min)	%A	%B	Time Segment (min)	Column Volumes
Init Cond.	0.00	0.600	95	5	0.00	-
Init Hold	0.00	0.600	95	5	0.00	0.00
3	2.61	0.600	5	95	2.61	13.68
4	3.47	0.600	5	95	0.87	4.56
5	5.21	0.600	95	5	1.74	9.12
6						
7						
8						
9						
10						

Injection Volume: 0.7 µL  
Pmax = 5805 PSI

Print Results ...

© 2007 Waters Corporation

## Method Transfer Process Steps for success

- Gather information about existing method and results
- Compare Instruments
- Select new or target column
  - Chemistry
  - Dimensions
- Scale down geometrically: flow, injection volume and gradient times
- Evaluate results of transfer
- Optimize as required

## Target Conditions: Fine Tuning Mobile phase

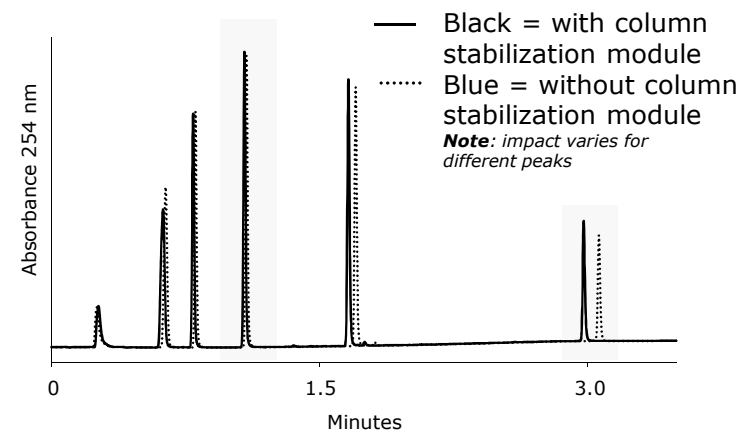
- Use exactly the same mobile phase
  - Modifier
  - pH
  - Ionic strength
  - Organic solvent
  - Percentage composition
- Change conditions only after evaluating geometric transfer **if optimization is required**

## HPLC to UPLC: Method Transfer and Development

### Target Conditions Temperature

- Temperature directly affects every chromatographic mechanism
- In method transfer temperature must be kept constant
- Pre-heating of the sample before entering the column is essential
  - Solvent is in column for 1.66 min in the original
  - Solvent is in column for 15 sec in the optimized target column
  - Less time for heat transfer
- ACQUITY UPLC Column Thermal Stabilization Kit is tuned for flow rates of about 0.5 to 0.75 mL/min

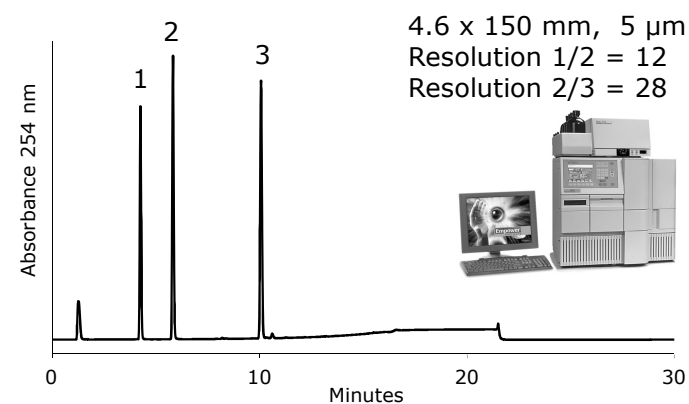
### Effect of Pre-heating



### Evaluate Results

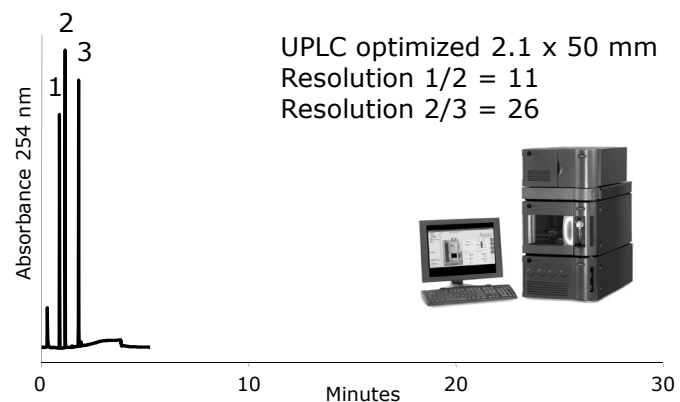
- Match Peaks
  - Count peaks
  - Examine spacing
  - Critically examine baseline for appearance or absence of small peaks
  - Match elution order and resolution
- Proceed to usual quantitative evaluation
  - Resolution(s)
  - Limits of detection (LOD)
  - Limits of quantitation (LOQ)

### Original HPLC Method

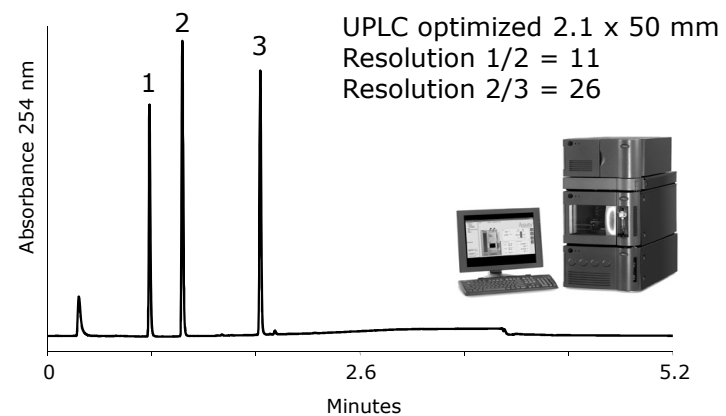


## HPLC to UPLC: Method Transfer and Development

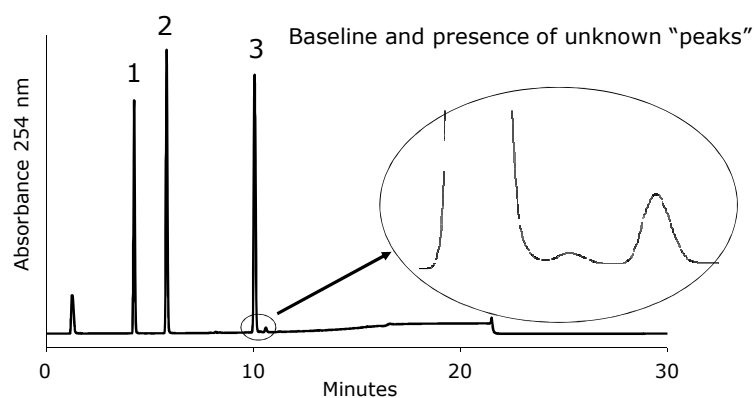
### UPLC Resolution Time scale as HPLC



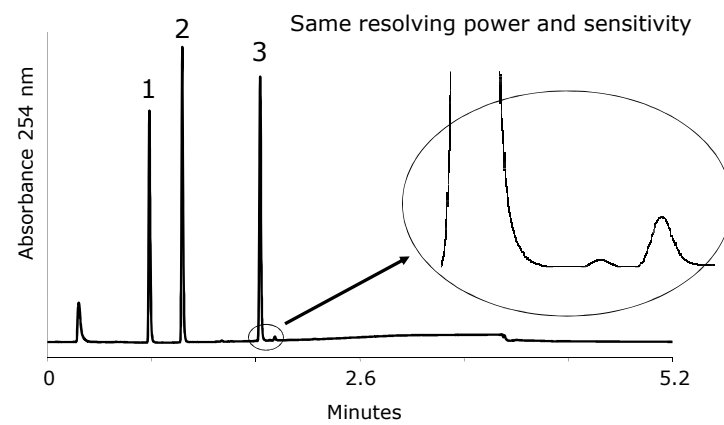
### Proportionately Magnified View



### Original 30 minute HPLC Method Minor components



### 5.2 Minute UPLC Magnified View Minor components



# HPLC to UPLC: Method Transfer and Development

## Original HPLC Method Caffeic acid derivatives found in *Echinacea sp.*

### Chromatographic Conditions:

Columns: XTerra MS C<sub>18</sub> 4.6 x 150 mm, 5.0 µm

Mobile Phase A: 0.1% CF<sub>3</sub>COOH in H<sub>2</sub>O

Mobile Phase B: 0.08% CF<sub>3</sub>COOH in ACN

Flow Rate: 1.0 mL/min

Gradient:	Time (min)	Profile %A	%B	Curve
	0.0	92	8	6
	2.0	92	8	7
	32.0	50	50	6
	35.0	10	90	6
	36.0	92	8	6
	41.0	92	8	6

Injection Volume: 10.0 µL

Weak Needle Wash: 0.1% CF<sub>3</sub>COOH in 8% ACN

Sample: Caffeic Acid Derivatives in *Echinacea purpurea*

Sample Diluent: 50:50 H<sub>2</sub>O: MeOH with 0.05% CF<sub>3</sub>COOH

Sample Concentration: 100 µg/mL

Temperature: 40° C

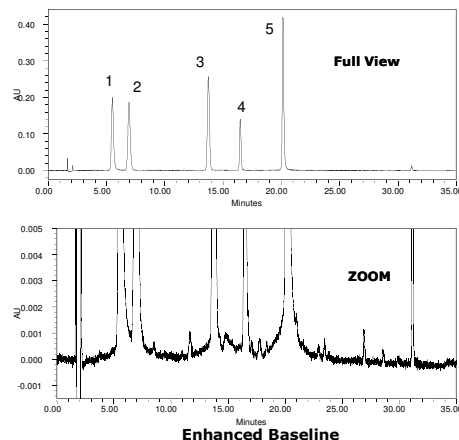
Detection: UV @ 330 nm

Sampling rate: 10 pts/sec

Time Constant: 0.1

Instrument: Alliance 2695 Separations Module with 2996 PDA detector

Analyte
1. Caffeic acid
2. Chlorogenic acid
3. Cynarin
4. Echinacoside
5. Cichoric acid



## Transferred UPLC Method Caffeic acid derivatives found in *Echinacea sp.*

### Chromatographic Conditions:

Columns: ACQUITY UPLC BEH C<sub>18</sub> 2.1 x 50 mm, 1.7 µm

Mobile Phase A: 0.1% CF<sub>3</sub>COOH in H<sub>2</sub>O

Mobile Phase B: 0.08% CF<sub>3</sub>COOH in ACN

Flow Rate: 0.5 mL/min

Gradient:	Time (min)	Profile %A	%B	Curve
	0.0	92	8	6
	0.1	92	8	7
	4.45	50	50	6
	4.86	10	90	6
	5.0	92	8	6
	6.0	92	8	6

Injection Volume: 1.0 µL

Weak Needle Wash: 0.1% CF<sub>3</sub>COOH in 8% ACN

Sample: Caffeic Acid Derivatives in *Echinacea purpurea*

Sample Diluent: 50:50 H<sub>2</sub>O: MeOH with 0.05% CF<sub>3</sub>COOH

Sample Concentration: 100 µg/mL

Temperature: 40° C

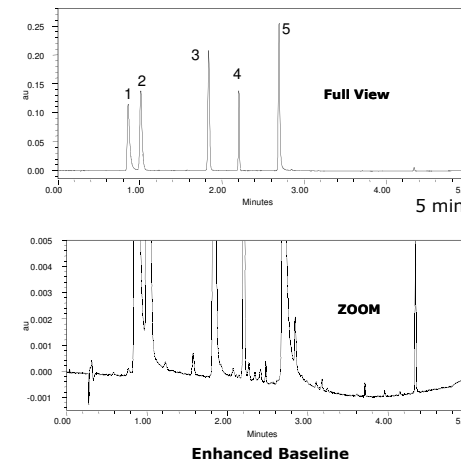
Detection: UV @ 330 nm

Sampling rate: 40 pts/sec

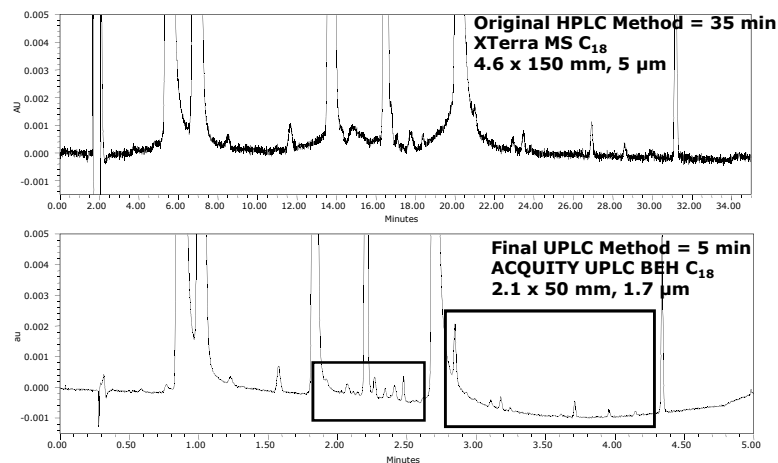
Time Constant: 0.1

Instrument: Waters ACQUITY UPLC, with TUV detector

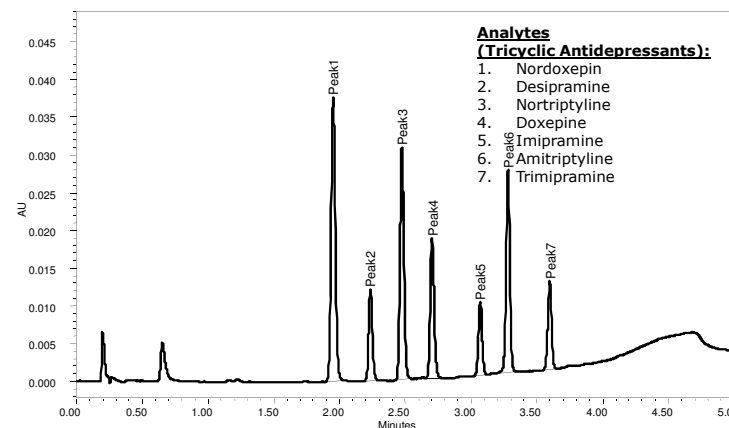
Analyte
1. Caffeic acid
2. Chlorogenic acid
3. Cynarin
4. Echinacoside
5. Cichoric acid



## HPLC to UPLC Transfer Caffeic acid derivatives found in *Echinacea sp.*



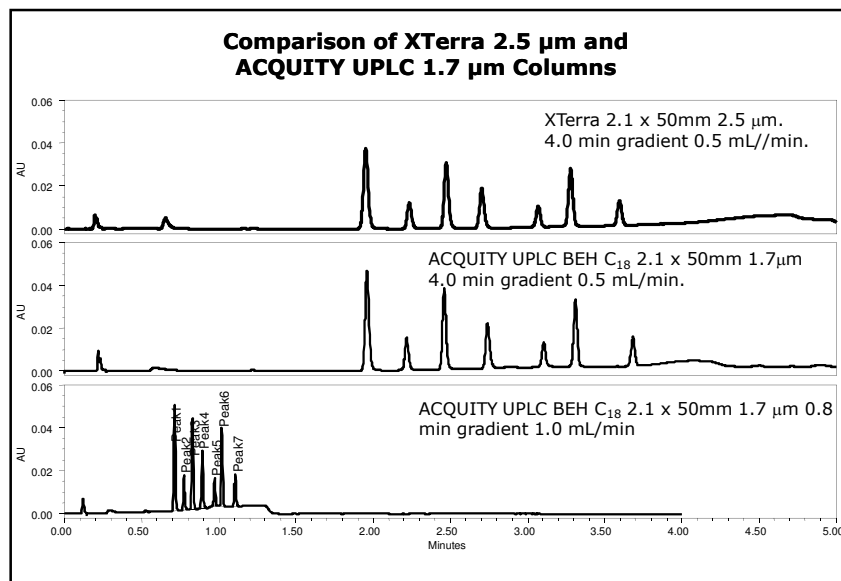
## XTerra 2.1 x 50 mm 2.5 µm on ACQUITY UPLC System



### Analytes (Tricyclic Antidepressants):

1. Nordoxepin
2. Desipramine
3. Nortriptyline
4. Doxepine
5. Imipramine
6. Amitriptyline
7. Trimipramine

# HPLC to UPLC: Method Transfer and Development

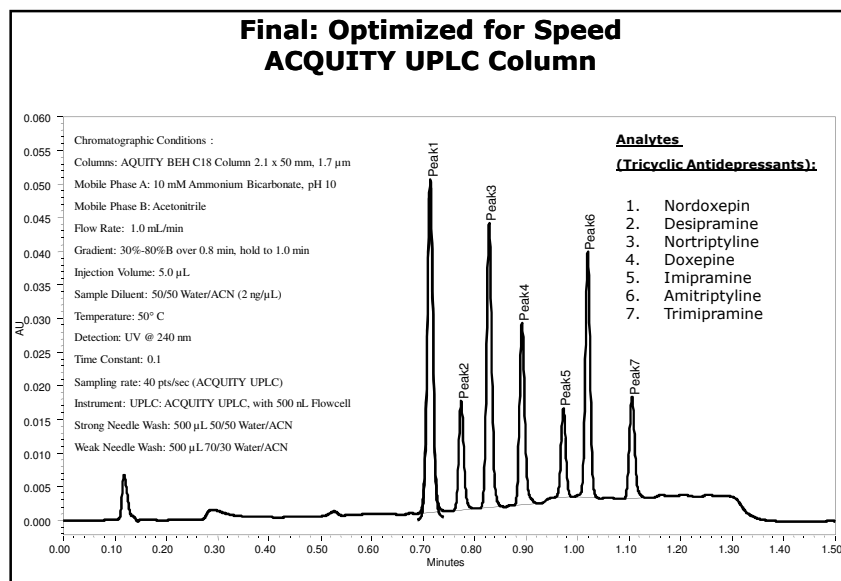


**Comparison of XTerra 2.5 µm and ACQUITY UPLC 1.7 µm Columns**

	Name	R. T.	Area	Height	USP Resolution	Peak Capacity 4Sigma	5 Sigma	Width @ 13.4%	width in seconds @ 13.4%
XTerra 2.5um	Nordoxepin	1.951	81126	37589		69.70	16809	0.0582	3.49
XTerra 2.5um	Desipramine	2.234	23818	12136	5.19	76.53	26749	0.0530	3.18
XTerra 2.5um	Nortriptyline	2.474	62884	30809	4.53	73.92	30779	0.0549	3.29
XTerra 2.5um	Doxepin	2.703	38054	18567	4.21	72.42	36005	0.0560	3.36
XTerra 2.5um	Imipramine	3.069	18669	9737	6.94	78.34	53769	0.0517	3.10
XTerra 2.5um	Amitriptyline	3.280	52903	26863	4.09	76.74	57605	0.0528	3.17
XTerra 2.5um	Trimipramine	3.597	22891	11774	6.11	77.16	71551	0.0525	3.15

	Name	R.T.	Area	Height	USP Resolution	Peak Capacity 4Sigma	5 Sigma	Width @ 13.4%	width in seconds
ACQUITY 1.7um	Nordoxepin	1.960	81613	46507		85.56	25484	0.0473	2.84
ACQUITY 1.7um	Desipramine	2.217	24695	15177	5.83	92.37	33455	0.0438	2.63
ACQUITY 1.7um	Nortriptyline	2.460	62689	37797	5.71	90.68	44729	0.0446	2.68
ACQUITY 1.7um	Doxepin	2.741	38559	21248	6.18	80.54	45538	0.0503	3.02
ACQUITY 1.7um	Imipramine	3.106	20229	11482	7.97	88.21	56985	0.0459	2.75
ACQUITY 1.7um	Amitriptyline	3.311	52040	31246	4.63	91.04	84679	0.0444	2.67
ACQUITY 1.7um	Trimipramine	3.684	22749	13522	8.35	89.73	104829	0.0451	2.70



**ACQUITY UPLC BEH C<sub>18</sub> 2.1 x 50 mm**

**0.8 minute gradient**

	Name	R.T.	Area	Height	USP Resolution	Peak Capacity 4Sigma	5 Sigma	Width @ 13.4%	width in seconds
ACQUITY 1.7um	Nordoxepin	0.713	38164	49452		39.51	17431	0.0208	1.25
ACQUITY 1.7um	Desipramine	0.774	11221	16222	3.13	44.71	26329	0.0183	1.10
ACQUITY 1.7um	Nortriptyline	0.828	29852	42341	2.97	43.35	28231	0.0189	1.13
ACQUITY 1.7um	Doxepin	0.892	19186	27114	3.46	43.88	30888	0.0187	1.12
ACQUITY 1.7um	Imipramine	0.972	8543	13203	4.51	47.34	49981	0.0173	1.04
ACQUITY 1.7um	Amitriptyline	1.020	24672	36538	2.64	45.79	50165	0.0179	1.07
ACQUITY 1.7um	Trimipramine	1.106	11291	15166	4.55	41.64	42893	0.0197	1.18

## HPLC to UPLC: Method Transfer and Development

### Method Transfer Process Steps for success

- Gather information about existing method and results
- Compare Instruments
- Select new or target column
  - Chemistry
  - Dimensions
- Select target conditions based on geometric considerations
- Evaluate results of transfer
- Optimize as required for Time and/or Resolution

### Optimization of Transferred Method

- ACQUITY UPLC is governed by the same principles reversed phase HPLC
- Everything that you used to know is still true
  - All the chemical manipulations still apply
  - All your strategies remain the same
  - Simulation software has proven useful for some cases, just like it has in conventional HPLC
- Optimize as always, but the process goes faster

### Summary

- Methods can be moved directly from HPLC to ACQUITY UPLC for
  - Improved resolution
  - Improved speed
  - Improved detection
- Many parameters can and must be manipulated to preserve results
- Attention to detail leads to success

### Method Conversion Significantly Different Column Chemistry

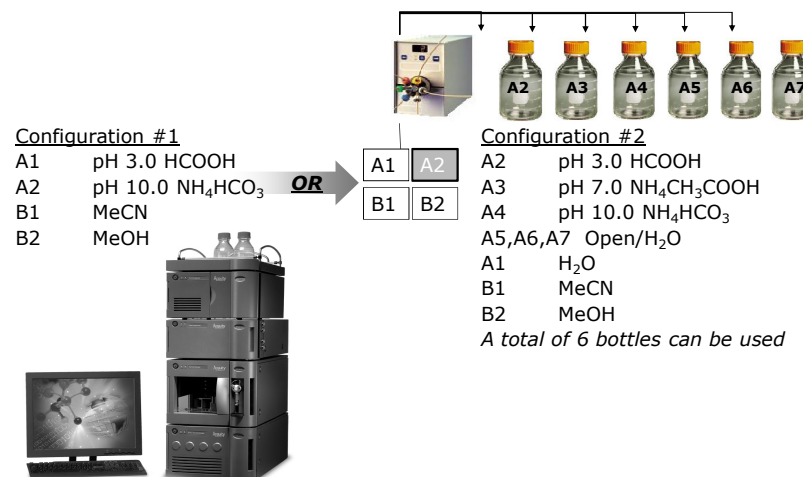
- At what point does method conversion become method development?
- Use same strategy as using today
  - Choosing the column type that you have typically used with the compounds of interest
  - Generic gradients: 10 to 90% organic solvent
  - Run a short gradient (3 min) and a long gradient (6 min)
  - Make decisions based on results to optimize method
- Optimization tools (simulation software) can be used

# HPLC to UPLC: Method Transfer and Development

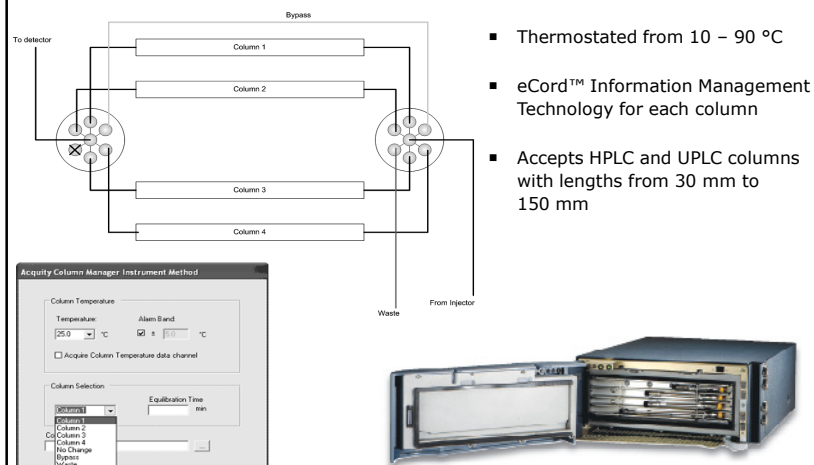
## Practical Approach To Efficient Method Development

Where to begin?

### Flexibility for Buffer Scouting



### ACQUITY UPLC Column Manager



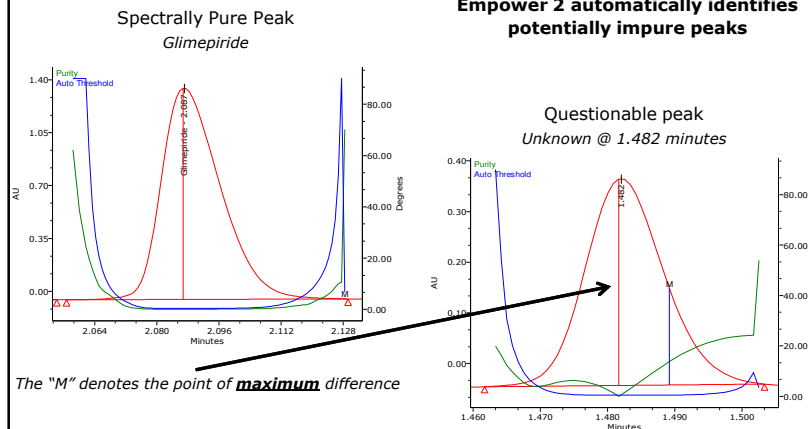
### ACQUITY UPLC PDA Detector for Peak Purity

- Support for data rates up to 80 Hz
  - Featuring independent data rate and filter constants
- Improved sensitivity
- Superior noise performance
- Light guiding flow cell design
  - Multiple flow cell options for a wider application range
- Lamp optimization



# HPLC to UPLC: Method Transfer and Development

## PDA Peak Purity Plot

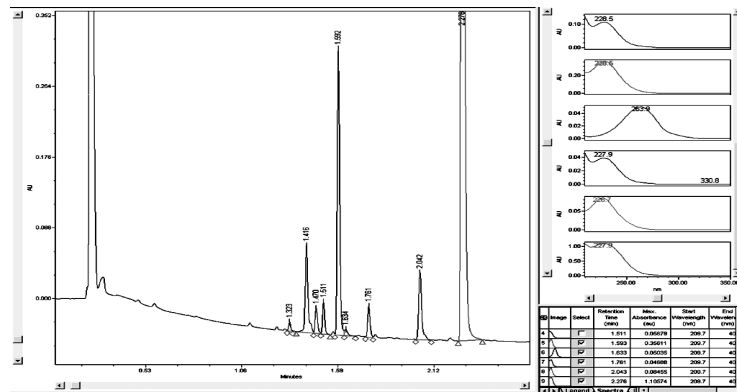


## Why Use Mass Spectrometry?

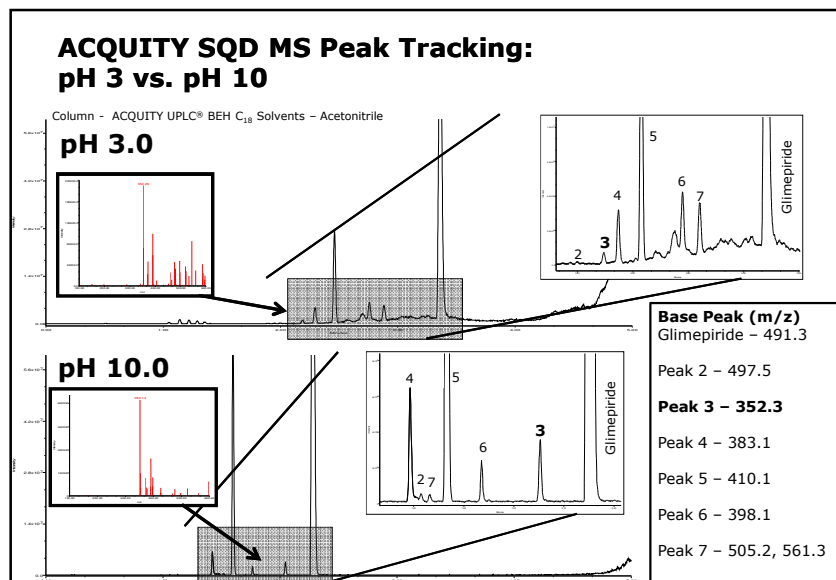
- Ability to **track peaks**
- Assists as **orthogonal** analysis
- **Confirm identity** of knowns
- **Verify purity** of the peak(s) of interest
- Help to **identify unknowns**

## UV Peak Tracking

Tracking peaks by UV during the method development process may be difficult as the spectra of related substances can be similar



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## UPLC Systematic Scouting

### Methodology

- Four ACQUITY UPLC chemistries 2.1 x 50 mm, 1.7 µm
  - ACQUITY UPLC BEH C<sub>18</sub>
  - ACQUITY UPLC BEH Phenyl
  - ACQUITY UPLC BEH Shield RP18
  - ACQUITY UPLC HSS T3
- Solvents
  - Acetonitrile
  - Methanol
- Buffers
  - pH 3 (Ammonium Formate)
  - pH 10 (Ammonium Bicarbonate)

**Setting Up Method Scouting  
Experiments with Empower Templates**

Essential for streamlining sequential workflow

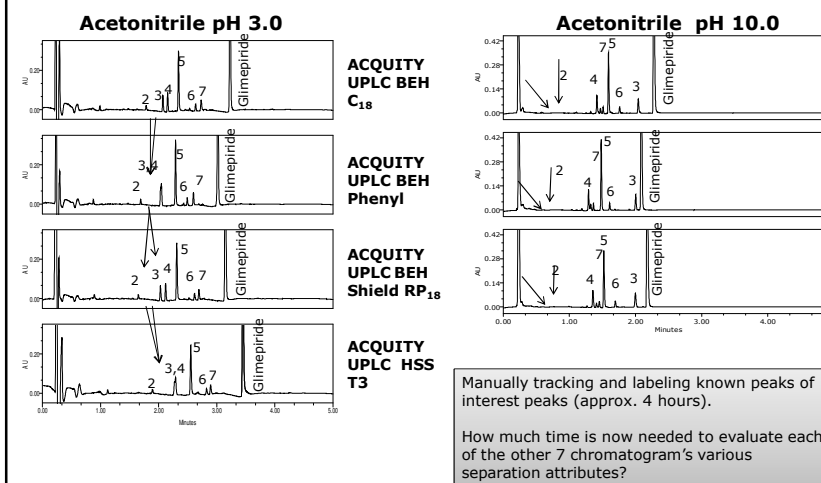
Key to data visualization

Plate/Well	SampleName	Inj Vol (µL)	# of Injs	Function	Run Time (Minutes)	Next Inj. Delay (Minutes)	Column Position	Buffer_type	Column_Type	Solvent_Type	pH
1:A,1	Blank	2.0	1	Condition Column	5.00	1.00	Position 1	10mM Ammonium Formate	ACQUITY BEH C18	ACN	pH=3.00
1:A,2	Glimepiride MS	2.0	1	Inject Samples	5.00	1.00		10mM Ammonium Formate	ACQUITY BEH C18	ACN	pH=3.00
1:A,1	Blank	2.0	1	Condition Column	5.00	1.00	Position 2	10mM Ammonium Formate	ACQUITY BEH Phenyl	ACN	pH=3.00
1:A,2	Glimepiride MS	2.0	1	Inject Samples	5.00	1.00		10mM Ammonium Formate	ACQUITY BEH Phenyl	ACN	pH=3.00
1:A,1	Blank	2.0	1	Condition Column	5.00	1.00	Position 3	10mM Ammonium Formate	ACQUITY BEH Shield RP18	ACN	pH=3.00
1:A,2	Glimepiride MS	2.0	1	Inject Samples	5.00	1.00		10mM Ammonium Formate	ACQUITY BEH Shield RP18	ACN	pH=3.00
1:A,1	Blank	2.0	1	Condition Column	5.00	1.00	Position 4	10mM Ammonium Formate	ACQUITY HSS T3	ACN	pH=3.00
1:A,2	Glimepiride MS	2.0	1	Inject Samples	5.00	1.00		10mM Ammonium Formate	ACQUITY HSS T3	ACN	pH=3.00

User Defined

Template Defined

## Importance of Scouting with Various Selectivity Tools



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## Data Processing and Interpretation with Empower 2

### ■ Sorting and visualization to evaluate:

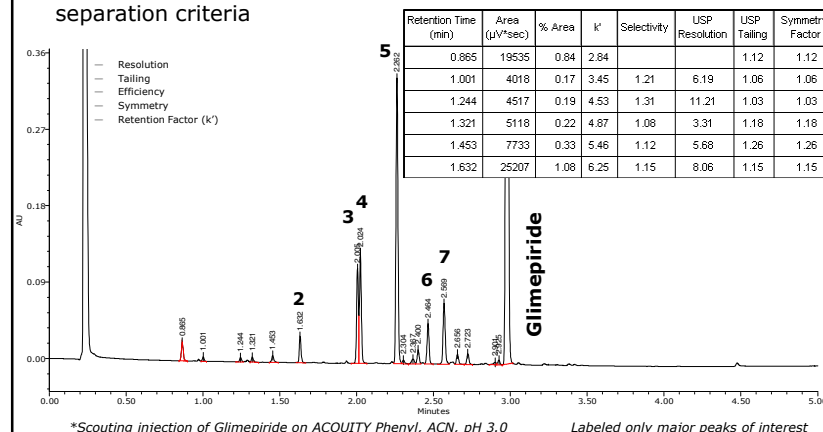
- Peak attributes
  - Area, RT, width, number of peaks
  - System suitability results
    - Resolution, tailing,  $k'$
- Purity
  - Flags maximum point of impurity

### ■ Decision Making Tools

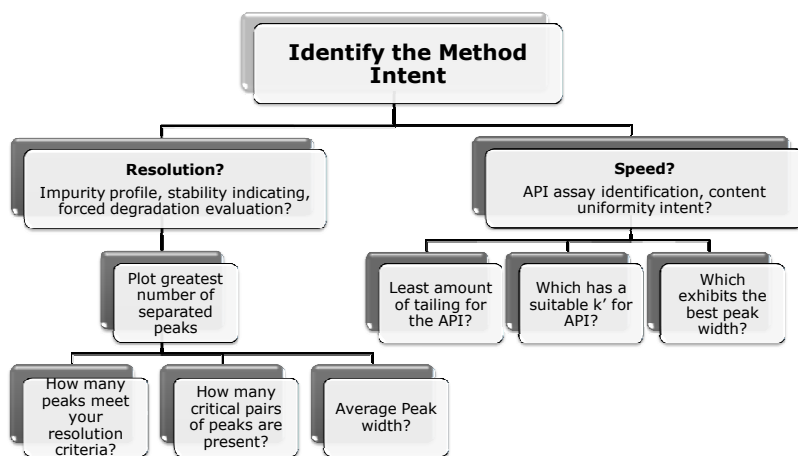
- Appropriate calculation and reporting templates

## Obtaining Separation Attributes

System Suitability is used to obtain information related to quality of separation criteria

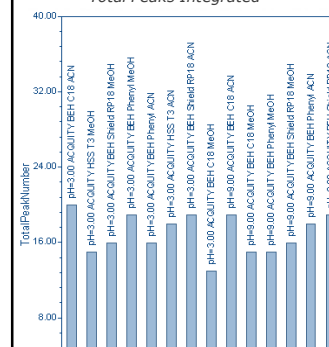


## Using Separation Attributes to Evaluate and Interpret Separation Conditions



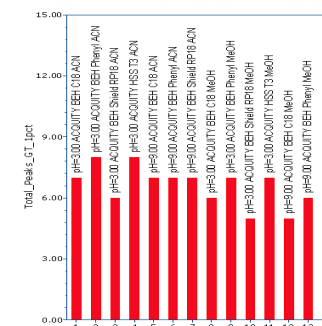
## Total Peak Number Plots

Total Peaks Integrated



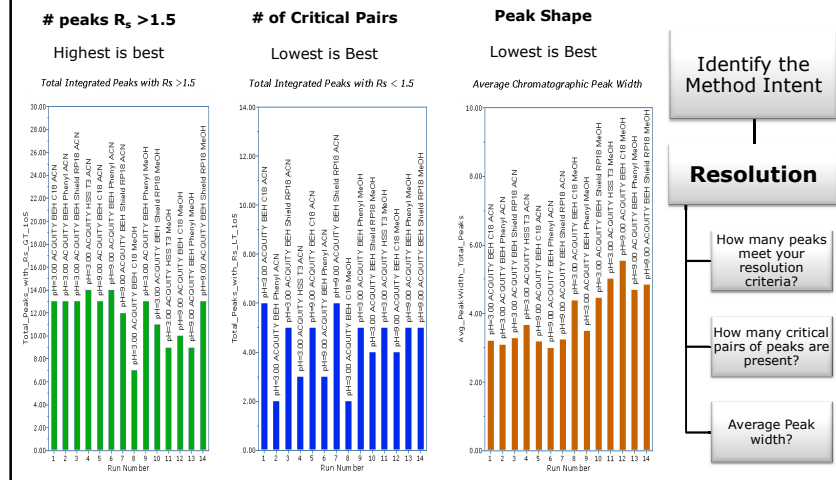
One of the fastest ways to roughly mine the method scouting data is to determine the **most number of peaks** or by **most number of peaks of interest > x%**

Total Peaks > 1% area

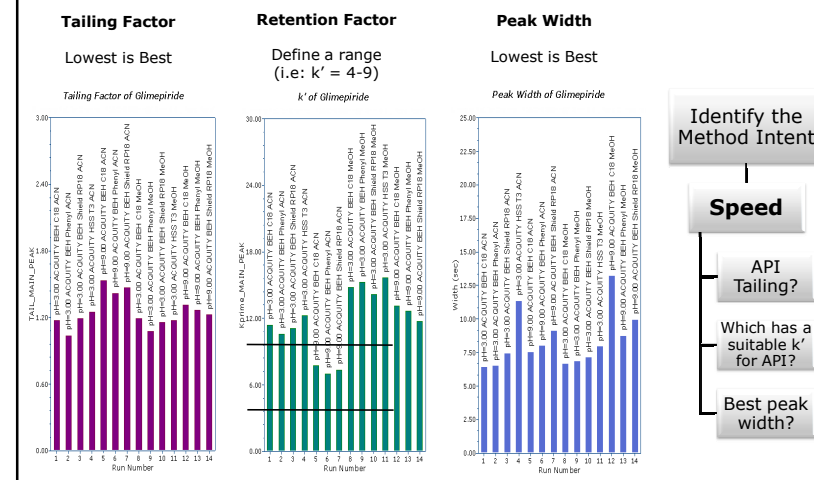


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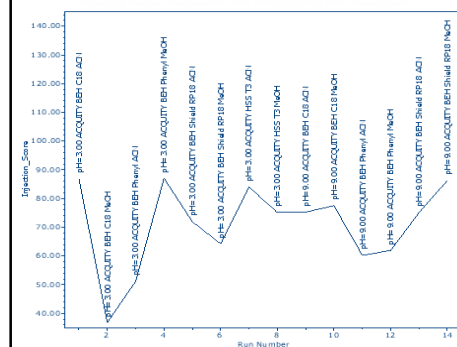
## Multiple Component Plots



## API Specific Plots



## Injection Score



The "Injection Score" is an estimation of which conditions are suitable to explore for further optimization.

In this particular example, the injection score equation included factors about:

- Total peaks found
- Total peaks above 1% area
- Run time
- Average peak width
- Separation space

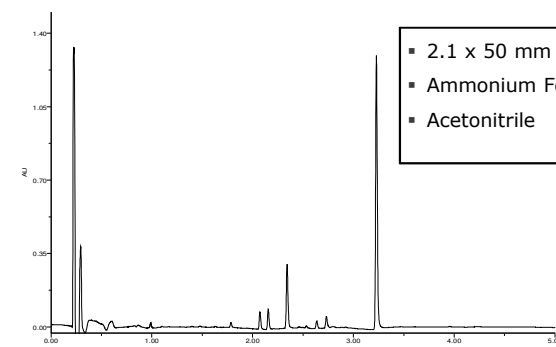
Injection score equations are recommended to include a weighted relationship of the user's goals and criteria

Best run(s) for this set

	pH	Solvent Type	Column Type	Buffer type	Total Peak Number	Total Resolution	Injection Score
1	pH3.00	ACN	ACQUITY BEH C18	10mM Ammonium Formate	20	71	87
2	pH3.00	MeOH	ACQUITY BEH Phenyl	10mM Ammonium Formate	19	63	87
3	pH3.00	ACN	ACQUITY HSS T3	10mM Ammonium Formate	19	58	84
4	pH9.00	MeOH	ACQUITY BEH Shield RP18	10mM Ammonium Bicarbonate	16	39	86

## Optimization Choice

- Based on total number of peaks and injection score, the conditions to optimize are:



- 2.1 x 50 mm ACQUITY BEH C<sub>18</sub>
- Ammonium Formate pH 3
- Acetonitrile

# HPLC to UPLC: Method Transfer and Development

## Overview

- Points to Consider
  - Quality by Design
  - Intended Use of the Method
  - Sample Selection
- Method Scouting
  - Available Tools
  - Experimental set-up
  - Case Study: Glimepiride
    - Peak Purity
    - Peak Tracking
  - Practical Approaches to Mining the Data
- Method Optimization
  - Gradients and Temperature
  - Modeling with Simulation Software

## Method Optimization: Goals and Challenges

- Goals
  - Achieve a satisfactory resolution for major peaks of interest
  - Perform the analysis in a minimal amount of injections
  - Achieve reproducibility
- Challenges
  - Many scientists still practice a very manual approach
    - Educated guess and trial and error
    - Serial injections changing one variable at a time
  - Difficult to achieve desired resolution
  - Knowing when to stop can decide the time associated with the experiment.
    - Intended use of the method
    - Was all the needed information obtained?
    - Where is it going next?

## Modeling with Simulation Software

- Ability to model in multiple dimensions
  - Reduces time needed to optimize a method
  - Models effects of multiple variables simultaneously
- There are a number of commercially-available chromatography simulation software packages
- Generally, temperature and organic modifier are linear relationships, hence only two experimental points are needed.

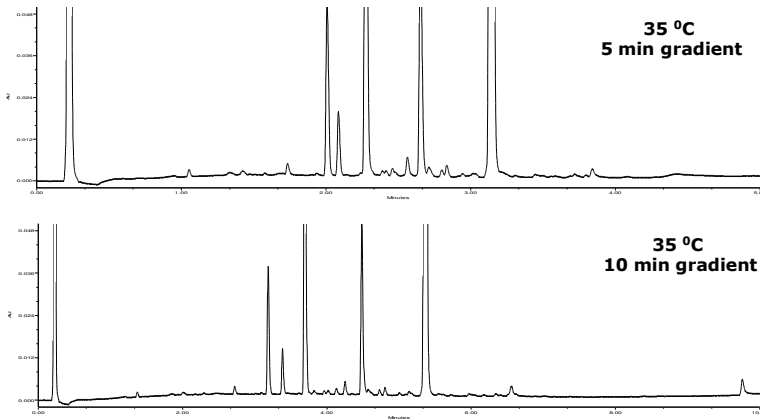
## Optimizing Glimepiride

- To model the separation for % organic and temperature, the following injections were performed on an ACQUITY BEH C<sub>18</sub>, 2.1 x 50 mm; 1.7 µm:
  - 5 minute, 5-100% B linear gradient, at 35 °C
  - 5 minute, 5-100% B linear gradient, at 50 °C
  - 10 minute, 5-100% B linear gradient, at 35 °C
  - 10 minute, 5-100% B linear gradient, at 50 °C

# HPLC to UPLC: Method Transfer and Development

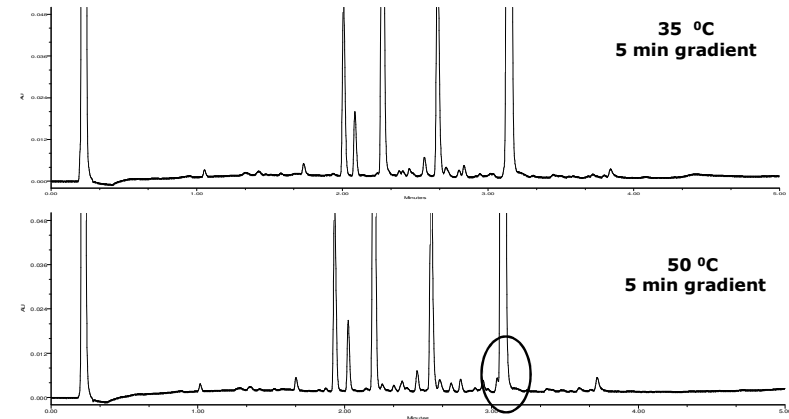
## Observing Gradient Slope Effects

Gradient slope had no effect on selectivity or emergence of coelutions...



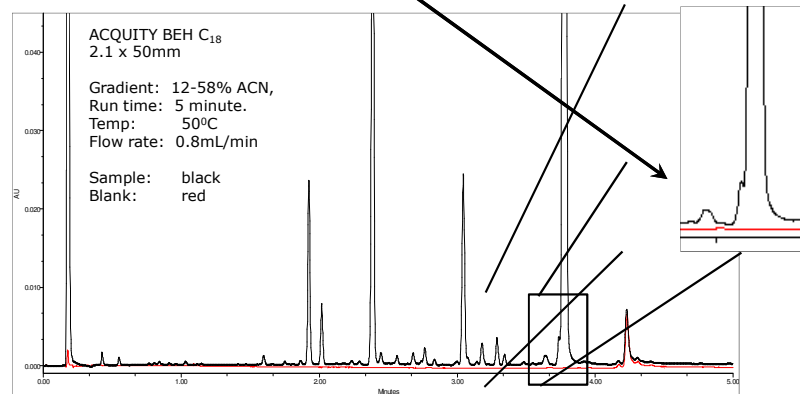
## Observing Temperature Effects

Temperature had no effect on selectivity or emergence of coelutions...



## Actual Chromatogram

...the presence of an emerging peak from the API was observed to be temperature selective in the modeling software



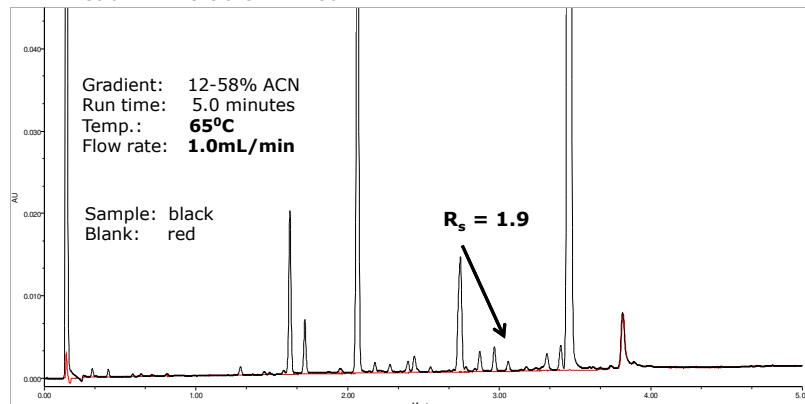
## Optimization Pathways

- How can we achieve greater resolution of the co-eluting peak with the API?
  - Increasing temperature to resolve peak
    - Using the present 50mm column
    - Adjust for higher flow rate
  - Configure for longer column dimensions (100mm)
    - Increase Temperature as necessary
    - Adjust flow rate for optimum pressure

# HPLC to UPLC: Method Transfer and Development

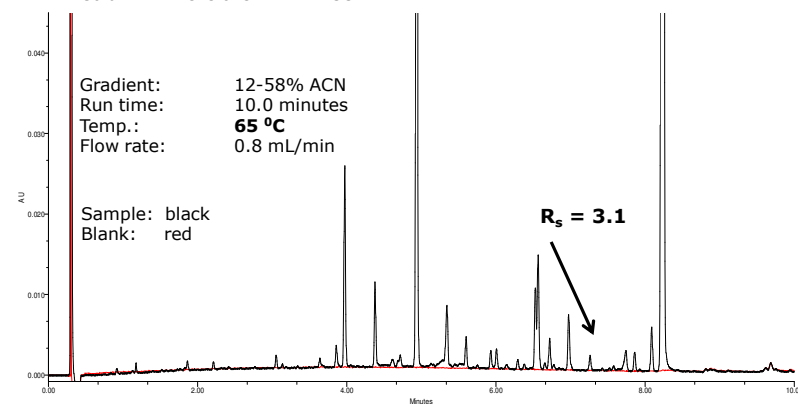
## Option #1: Increase Temperature and Flow Rate

Software Optimized for ACQUITY BEH C<sub>18</sub>  
Column Dimensions: 2.1 x 50mm



## Option #2: 100 mm Column Results

Software Optimized for ACQUITY BEH C<sub>18</sub>  
Column Dimensions: 2.1 x 100mm

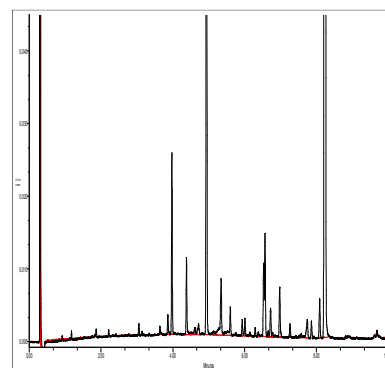
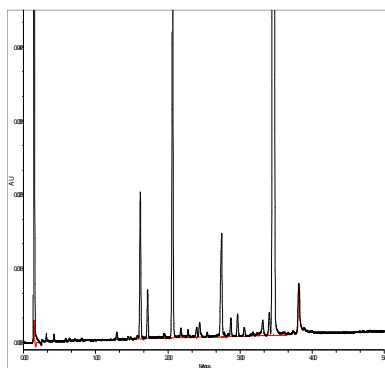


## The Final Method

*Either one can be used!*

50 mm for added **productivity**

100 mm for added **information**



## Develop Methods Faster with UPLC: Time Savings

### UPLC Method Development Protocol

2.1 x 50 mm, 1.7 µm

<u>pH 3/ acetonitrile x 4 columns</u>	<u>Time</u>
Column conditioning	5 min
Column re-equilibration	1 min
Sample injection (2 replicates)	(6min x 2) 12 min
Blank injection (2 replicates)	(6min x 2) 12 min
System purge	4 min
<u>pH 3/ methanol x 4 columns</u>	
Column conditioning	5 min
Column re-equilibration	1 min
Sample injection (2 replicates)	(6min x 2) 12 min
Blank injection (2 replicates)	(6min x 2) 12 min
System purge	4 min
<u>pH 10/ acetonitrile x 3 columns</u>	
Column conditioning	5 min
Column re-equilibration	1 min
Sample injection (2 replicates)	(6min x 2) 12 min
Blank injection (2 replicates)	(6min x 2) 12 min
System purge	4 min
<u>pH 10/ methanol x 3 columns</u>	
Column conditioning	5 min
Column re-equilibration	1 min
Sample injection (2 replicates)	(6min x 2) 12 min
Blank injection (2 replicates)	(6min x 2) 12 min
System purge	4 min
pH 3 scouting time	272 minutes
pH 10 scouting time	204 minutes
<b>SCOUTING TIME</b>	<b>476 min</b>

**TOTAL SCOUTING TIME 7.9 HOURS**

### EQUIV HPLC Method Development Protocol

4.6 x 150 mm, 5 µm

<u>pH 3/ acetonitrile x 4</u>	<u>Time</u>
Column conditioning	20 min
Column re-equilibration	10 min
Sample injection (2 replicates)	(40min x 2) 80 min
Blank injection (2 replicates)	(40min x 2) 80 min
System purge	7 min
<u>pH 3/ methanol x 4</u>	
Column conditioning	20 min
Column re-equilibration	10 min
Sample injection (2 replicates)	(40min x 2) 80 min
Blank injection (2 replicates)	(40min x 2) 80 min
System purge	7 min
<u>pH 10/ acetonitrile x 3</u>	
Column conditioning	20 min
Column re-equilibration	10 min
Sample injection (2 replicates)	(40min x 2) 80 min
Blank injection (2 replicates)	(40min x 2) 80 min
System purge	7 min
<u>pH 10/ methanol x 3</u>	
Column conditioning	20 min
Column re-equilibration	10 min
Sample injection (2 replicates)	(40min x 2) 80 min
Blank injection (2 replicates)	(40min x 2) 80 min
System purge	7 min
pH 3 scouting time	1576 minutes
pH 10 scouting time	1182 minutes
<b>SCOUTING TIME</b>	<b>2758 min</b>

**TOTAL SCOUTING TIME 46.0 HOURS**