HPLC to UPLC® Method Migration: An Overview of Key Considerations and Available Tools

Dr. Michael Swartz, Ph. D.
Principal Consulting Scientist
Worldwide Pharmaceutical Business Operations
Waters Corporation

PittCon 2007

Evaluation Form

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

We Appreciate Your Opinion

- Please Complete Evaluation Form
- Qualify for drawing to win a $25 AMEX gift card (drawn at end of this session – must be present to win)

AND

- Qualify to win a “Gold Package” registration to Inform 2007 ($1595 value; one winner per day; winner to be notified post-Pittcon)

Thank You!

Outline

- UPLC® Overview
- Principles of Method Migration
- Examples
- Software Tools Available
- Summary

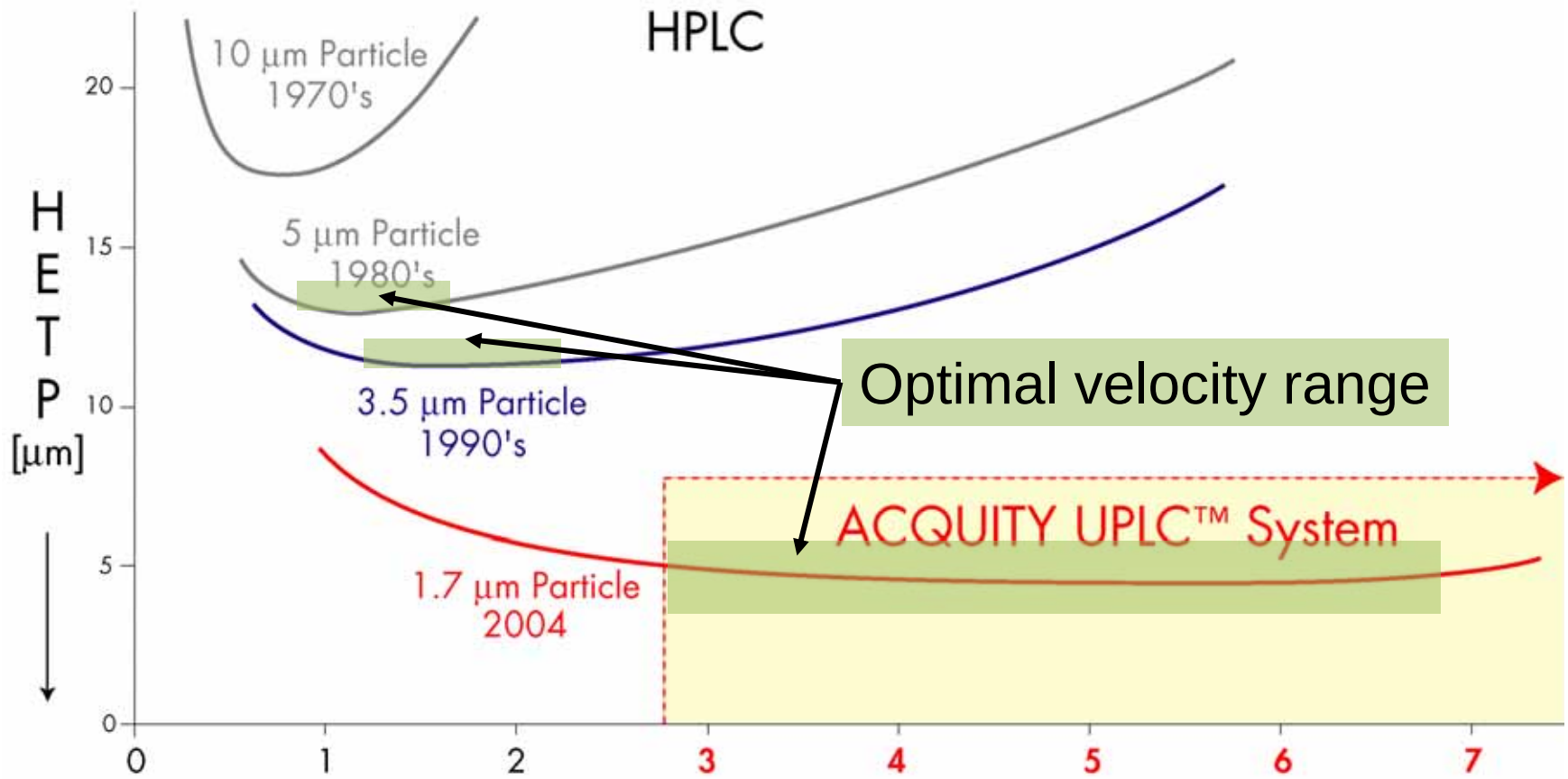
Ultra Performance LC®

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- A New Class of Separation Science
  - —Based on chromatography columns with very small particles
  - —Based on instruments designed to take advantage of the small particles
- Provides Improved Resolution, Speed, and Sensitivity with no Compromises
- Suitable for Chromatographic Applications in General
  - —Appropriate for developing new methods
  - —Appropriate for improving existing methods

Smaller Particles
The Enabler of Productivity

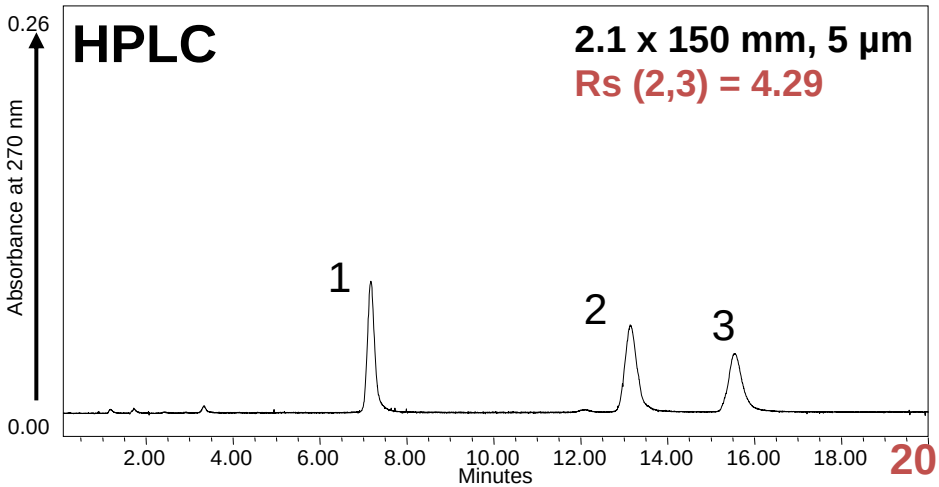
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Flow Rate [mL/min]:							
ID = 1.0 mm	0.04	0.07	0.10	0.13	0.17	0.20	0.24
ID = 2.1 mm	0.15	0.3	0.45	0.6	0.75	0.9	1.05
ID = 4.6 mm	*0.7*	*1.4*	*2.1*	*2.8*	*3.5*	*4.2*	*4.9*

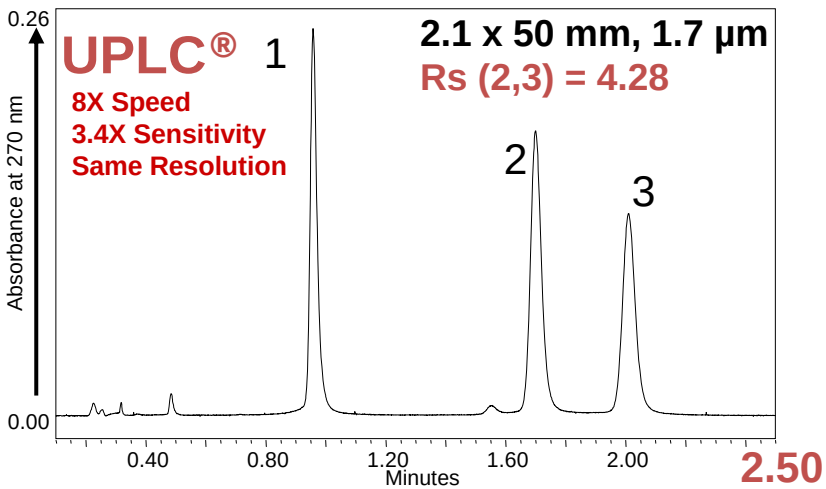
HPLC vs. UPLC®: *Speed, Sensitivity and Resolution*

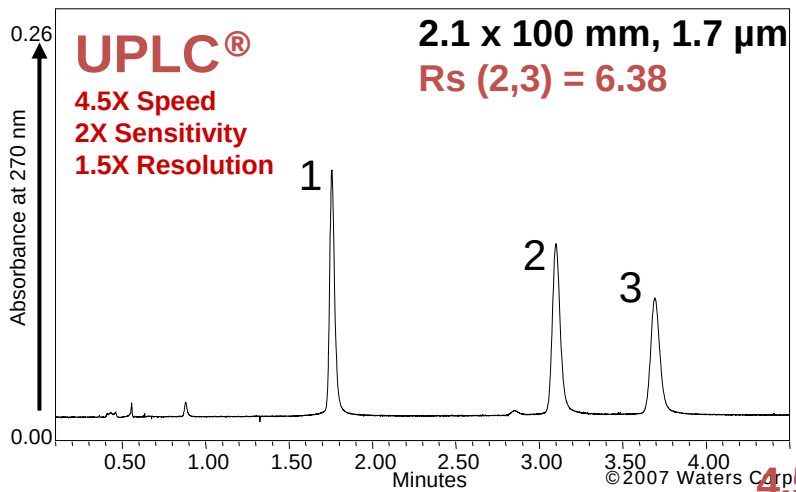
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Faster, More Sensitive Methods

Faster, More Sensitive, Higher Resolution Methods





Method Conversion From *HPLC* to ACQUITY *UPLC*®

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

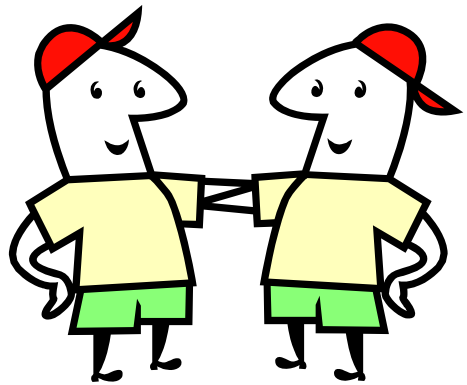
- Why Convert HPLC Methods to UPLC?
  - —Get faster results with more resolution
    - More information
    - More robust methods
    - Better situational response time (stat samples faster, research decisions with more information, process monitoring, product release)
    - More samples analyzed per system, per scientist

Increased Productivity

Migrating Methods

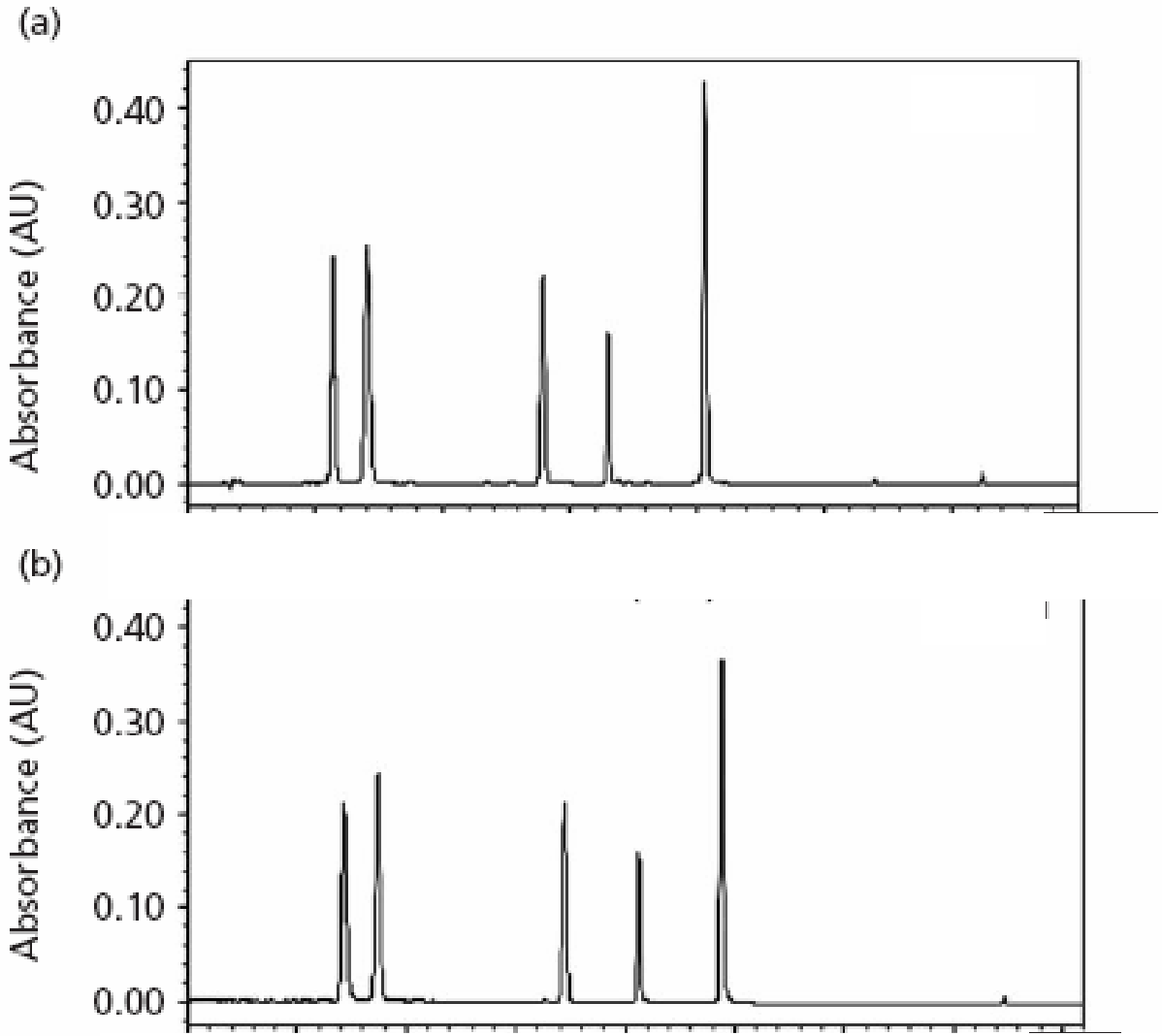
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- The New Method Must Preserve
  - Complete resolution of all relevant analytes
  - Peak homogeneity/purity
  - Certainty of peak identification
  - Quantitative accuracy and precision



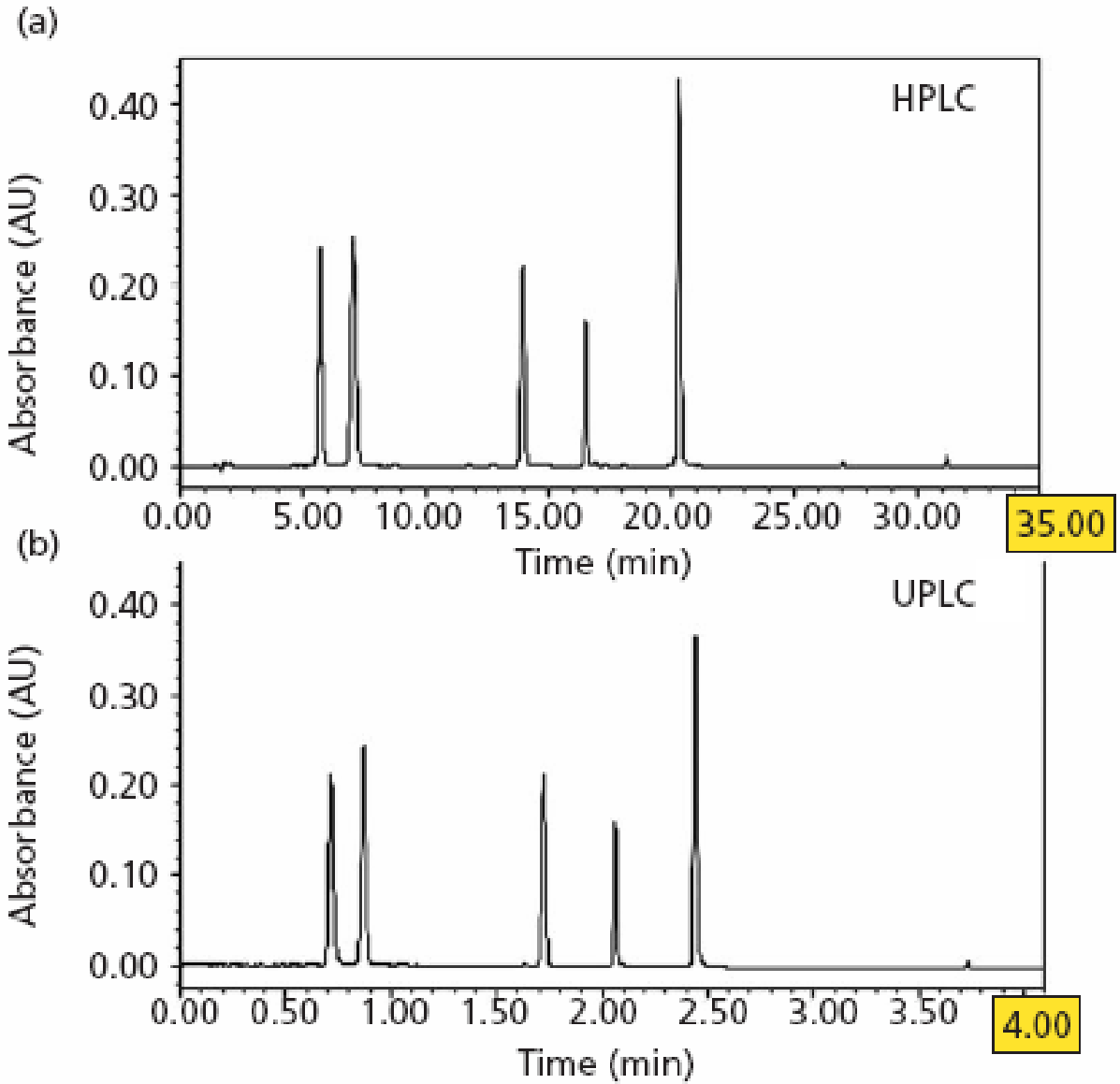
Method Migration Success?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



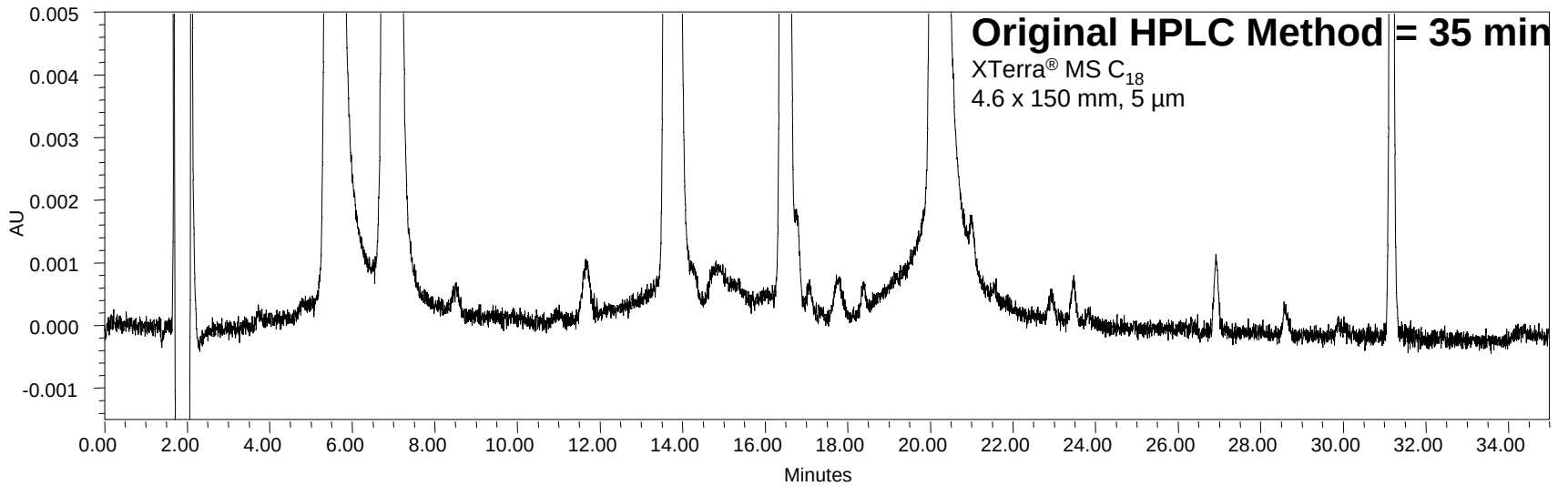
Method Migration Success!

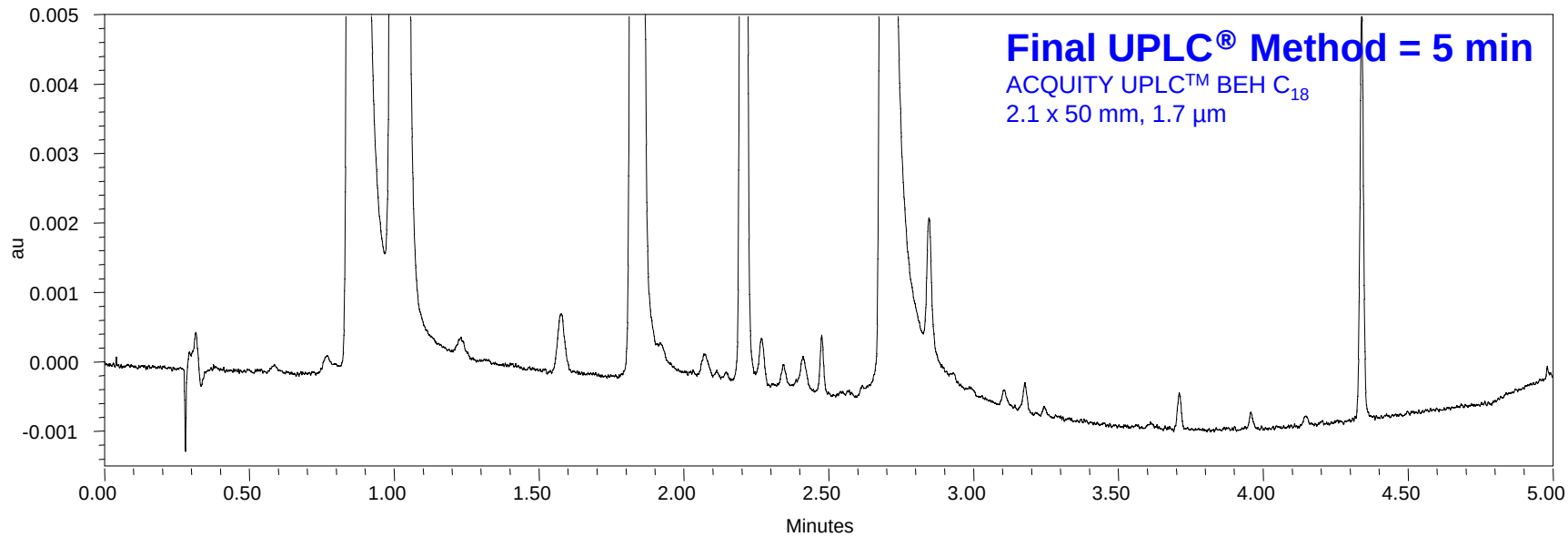
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Caffeic Acid Derivatives in Echinacea Purpurea

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

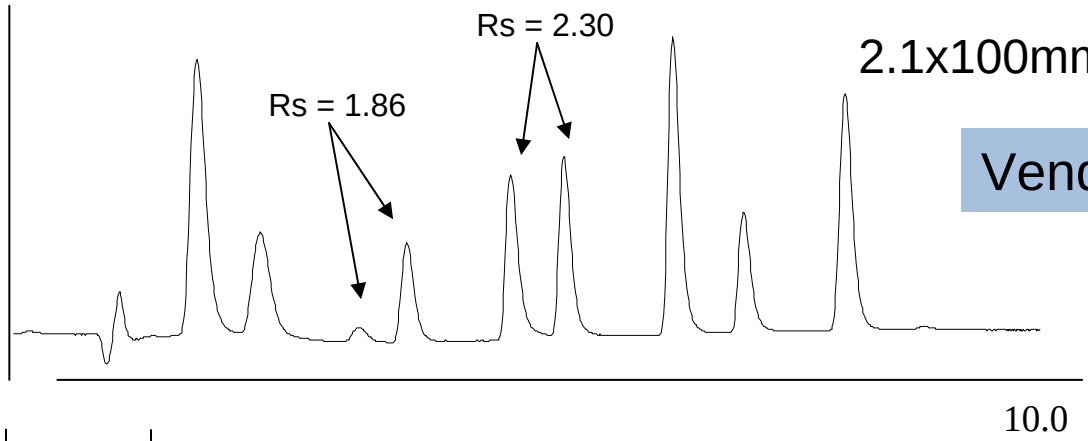




ACQUITY "HPLC"

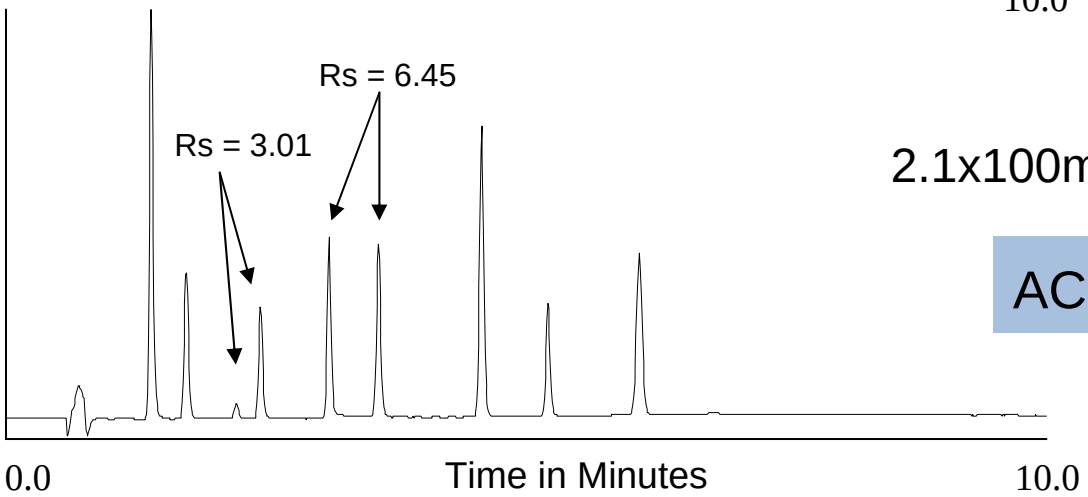
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

8 Diuretics + impurity



2.1x100mm 5µ C18

Vendor X HPLC



2.1x100mm 5µ C18

ACQUITY (HPLC)

- It is possible to transfer most HPLC methods to ACQUITY systems with attention to details
  - —Instrument design and performance differences could result in result differences
  - —Some adjustments may be required

- ***But:*** This is not taking advantage of ***UPLC!!***

Migrating Methods From *HPLC to UPLC®*

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

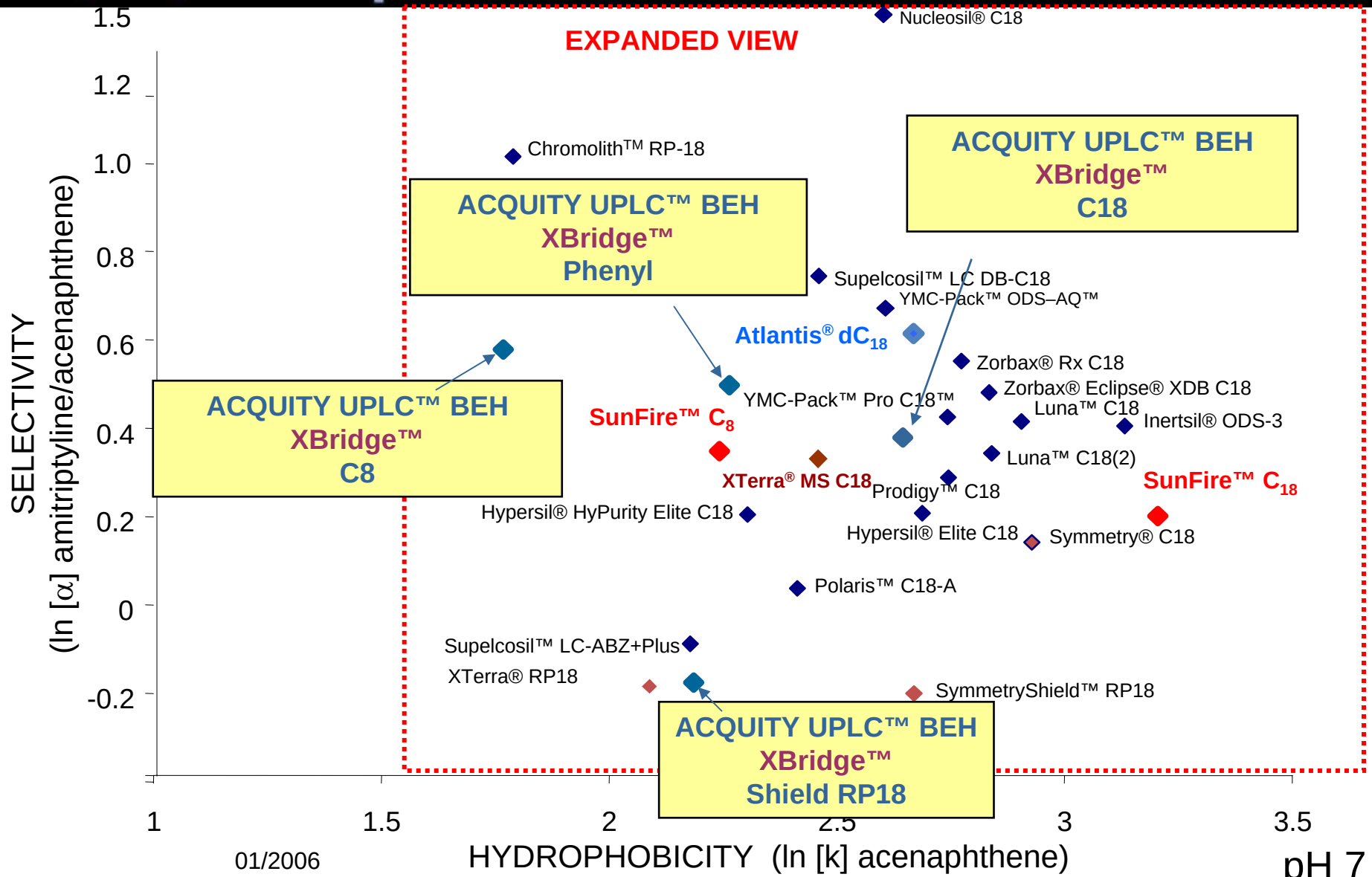
- Method Transfer Between HPLC Systems
  - First step towards method conversion
  - HPLC to ACQUITY as HPLC
- Method Migration or Conversion
  - —HPLC separation to an ACQUITY UPLC® separation
- Method Optimization
  - —Separation goals
  - —Method verification
- Method Development
  - —Systematic approach

Keys for HPLC to UPLC® Method Migration Success

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Proper Column Choice
  - Chemistry
  - Dimensions
- Keep in Mind Instrument Differences
  - Gradient delay volume
  - Detector data rates
- Proper Geometric Scaling
- Optimize for UPLC
- Don't Forget LC Theory

Determination of Proper Column Chemistry



Determination of Proper Column Dimensions

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Internal diameter
  - —Generally prefer 2.1 mm
  - —Only use 1 mm for specific reason
    - o Severely sample limited
    - o Direct flow to mass spectrometer
- Length
  - —If primary goal is SPEED
    - o 50 mm length to start
  - —If primary goal is RESOLUTION
    - o 100 mm length to start

Instrument Differences: Compensating for System Volumes

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Compare system volumes
  - —This volume should be converted to column volumes 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
  - —No exact compensation is possible
  - —Chromatographic effect of extra isocratic hold usually small

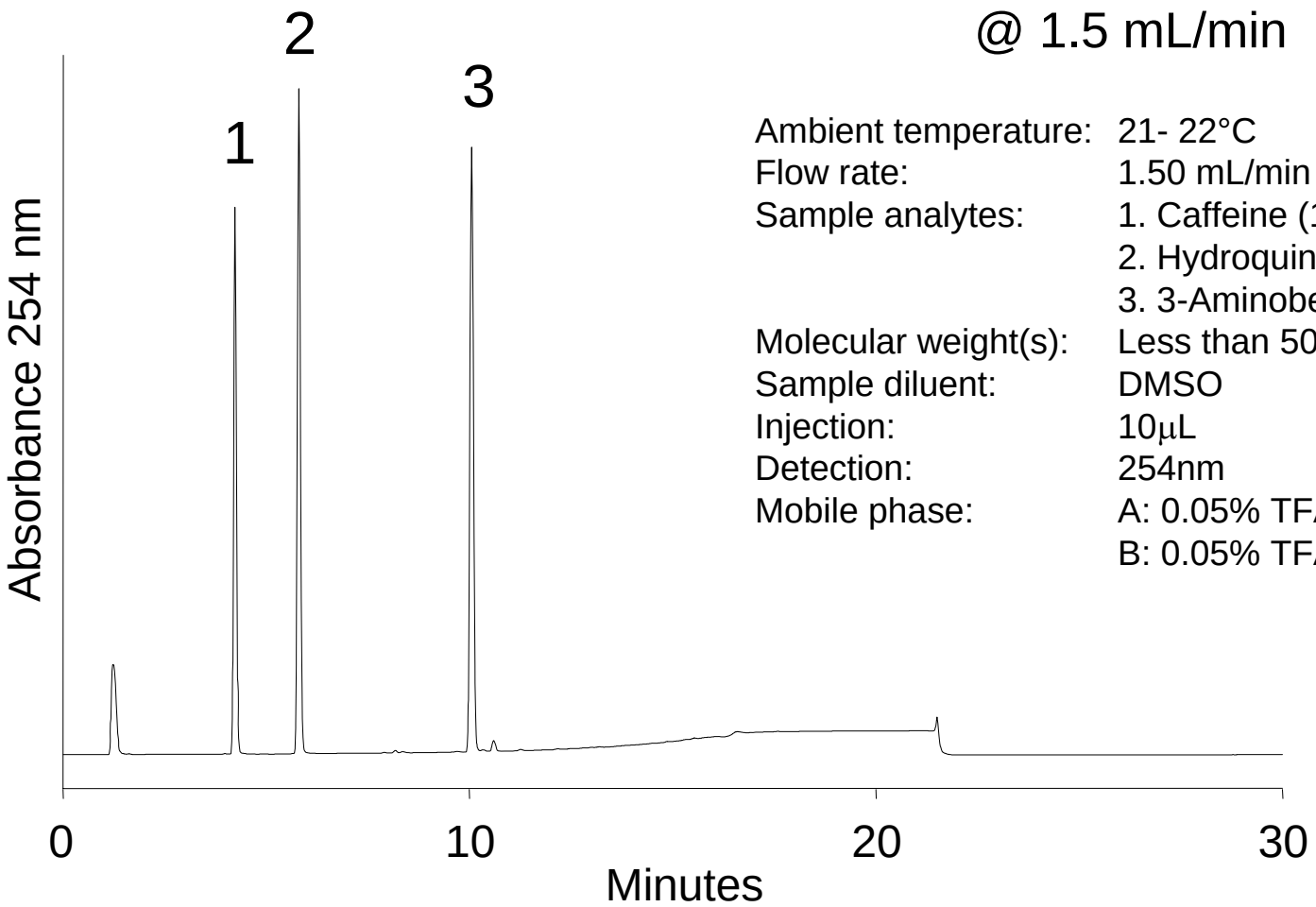
Method Migration Example

Original HPLC Method

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Objective: Maintain resolution while increasing speed

Resolution (1,2) = 12
Resolution (2,3) = 28



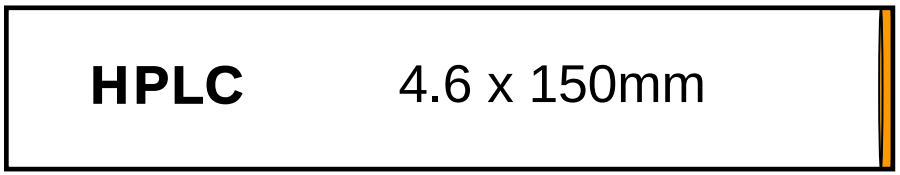
Original Column: 4.6 x 150 mm, 5 μm
@ 1.5 mL/min

Ambient temperature:	21- 22°C
Flow rate:	1.50 mL/min
Sample analytes:	1. Caffeine (100mg/mL), 2. Hydroquinidine (33mg/mL), 3. 3-Aminobenzophenone (39mg/mL)
Molecular weight(s):	Less than 500
Sample diluent:	DMSO
Injection:	10μL
Detection:	254nm
Mobile phase:	A: 0.05% TFA in water B: 0.05% TFA in acetonitrile

Method Migration
Column Comparison: Injection Volumes

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

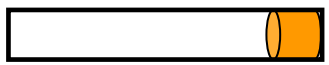
HPLC 4.6 x 150mm



20μL injection/2.49mL = 0.8%

2.1 x 50mm

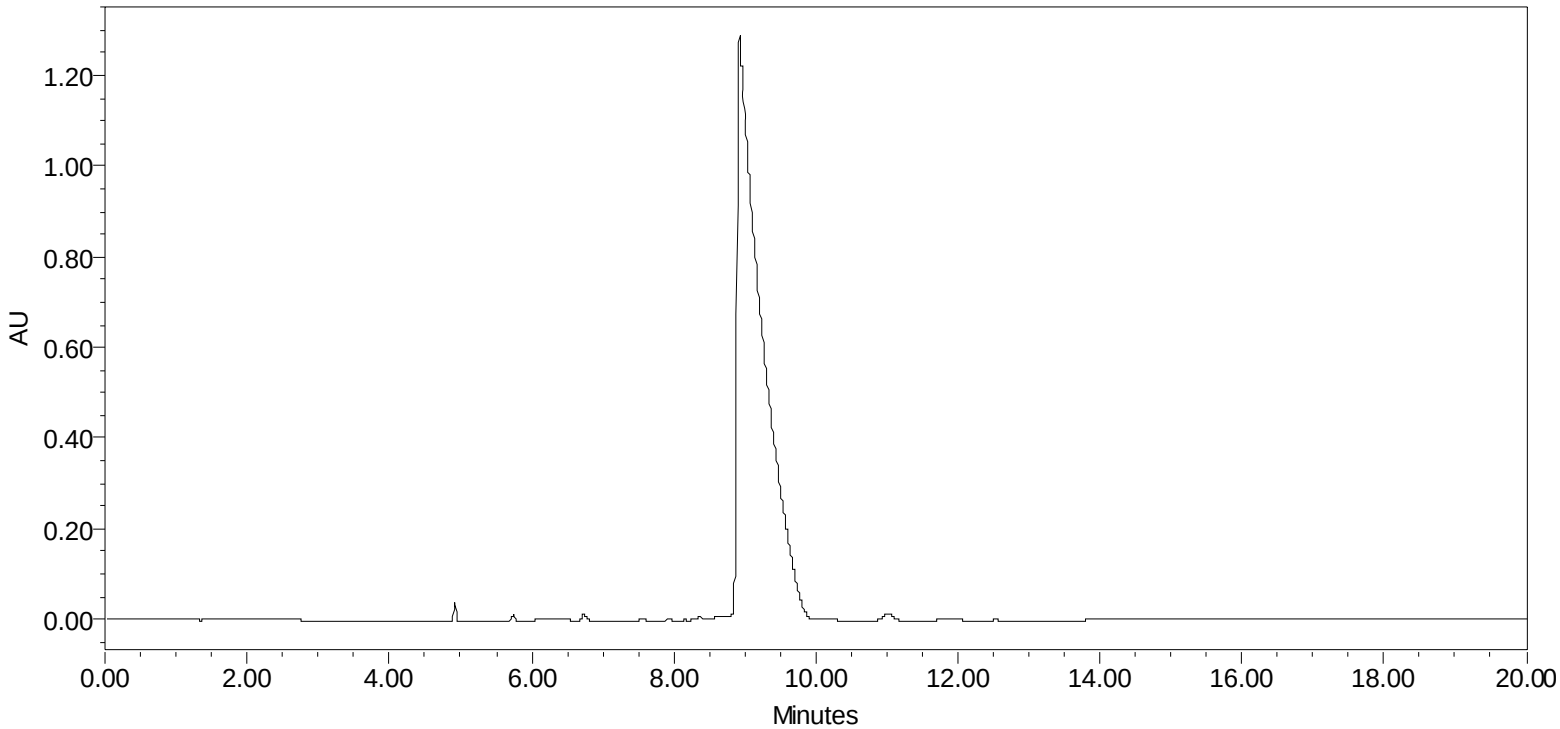
UPLC



20μL injection/0.19mL = 11%

Sample volume too large for smaller column volume

Column transfer from 4.6mm to 2.1mm i.d.
No injection volume scaling



Method Migration Example
Injection Volume Considerations

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Geometrically scale injection volume to volume of column
- Capacity proportional to surface area and internal solvent volume
- Suggested minimum injection volume on the instrument is 0.5 – 1 µL
  - —If calculated volume too small for injection, dilute 5 - 10x with initial strength mobile phase
  - —Typically 5 µL maximum injection on 2.1 x 50 mm

Method Migration
Calculate Injection Volume

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Target injection volume =

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

Scaling a **10µL** injection on 4.6 x 150mm to 2.1 x 50mm

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

$$\mathbf{10\mu L} \times \frac{0.19}{2.49} = \mathbf{10\mu L} \times 0.076$$

$$= \mathbf{0.8\mu L}$$

Method Migration Example
Flow Rate

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- For Migration:
  - First, ***adjust flow rate*** proportional to column diameter squared for constant linear velocity (geometrically scaled)
  - Second, ***adjust Gradient Table*** to maintain the same number of column volumes of solvent through the target column
  - Finally, ***adjust*** flow rate (linear velocity) ***for smaller particle***
    - Analyte molecular weight must be considered

Scaling a **1.5mL/min** flow rate on 4.6x150mm to 2.1x50mm*

$$\text{Target Flow Rate} = \text{Original Flow Rate} \times \frac{\pi \times r^2 \text{ of Target}}{\pi \times r^2 \text{ of Original}}$$

This reduces to:

$$\text{Target Flow Rate} = \text{Original Flow Rate} \times \frac{d^2_{\text{Target}}}{d^2_{\text{Original}}}$$

So:

$$1.5\text{mL/min.} \times \frac{2.1^2}{4.6^2} = 0.31\text{mL/min.}$$

*Note: this assumes same particle size

Method Migration Example

Gradient Profile

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Express gradient duration in percent change per column volume (cv) 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 chosen flow rate

Original Gradient Profile

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

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

Gradient Segments

Express as Column Volumes

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

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

Gradient Volume = Flow Rate x Time = 1.5mL/min x 15min = 22.5mL

Column Volume = π x r^2 x L = 3.14 x 0.23^2 x 15.0 = 2.49mL

$$\text{Gradient Duration } \mathbf{(cv)} = \frac{\text{Gradient Volume}}{\text{Column Volume}}$$

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

Original Gradient Profile for Scaling

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

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**

Scaling Gradient Step Time
Maintain Duration (cv)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Original Step 2: 15 min @ 1.5 mL/min with duration of **9.03cv**

Calculate Target Step 2: (keeping duration @ **9.03cv**)

Column Volume = π x r2 x L = 3.14 x 0.105^2 x 5.0 = 0.17mL

Gradient Step Volume = Duration (cv) x Target Column Volume

= 9.03cv x 0.17mL = 1.54mL

Gradient Step Time = Gradient Step Volume/Flow Rate

= 1.54mL / 0.31 mL/min =

5 min

Scaled Gradient Profile
2.1x50mm Column

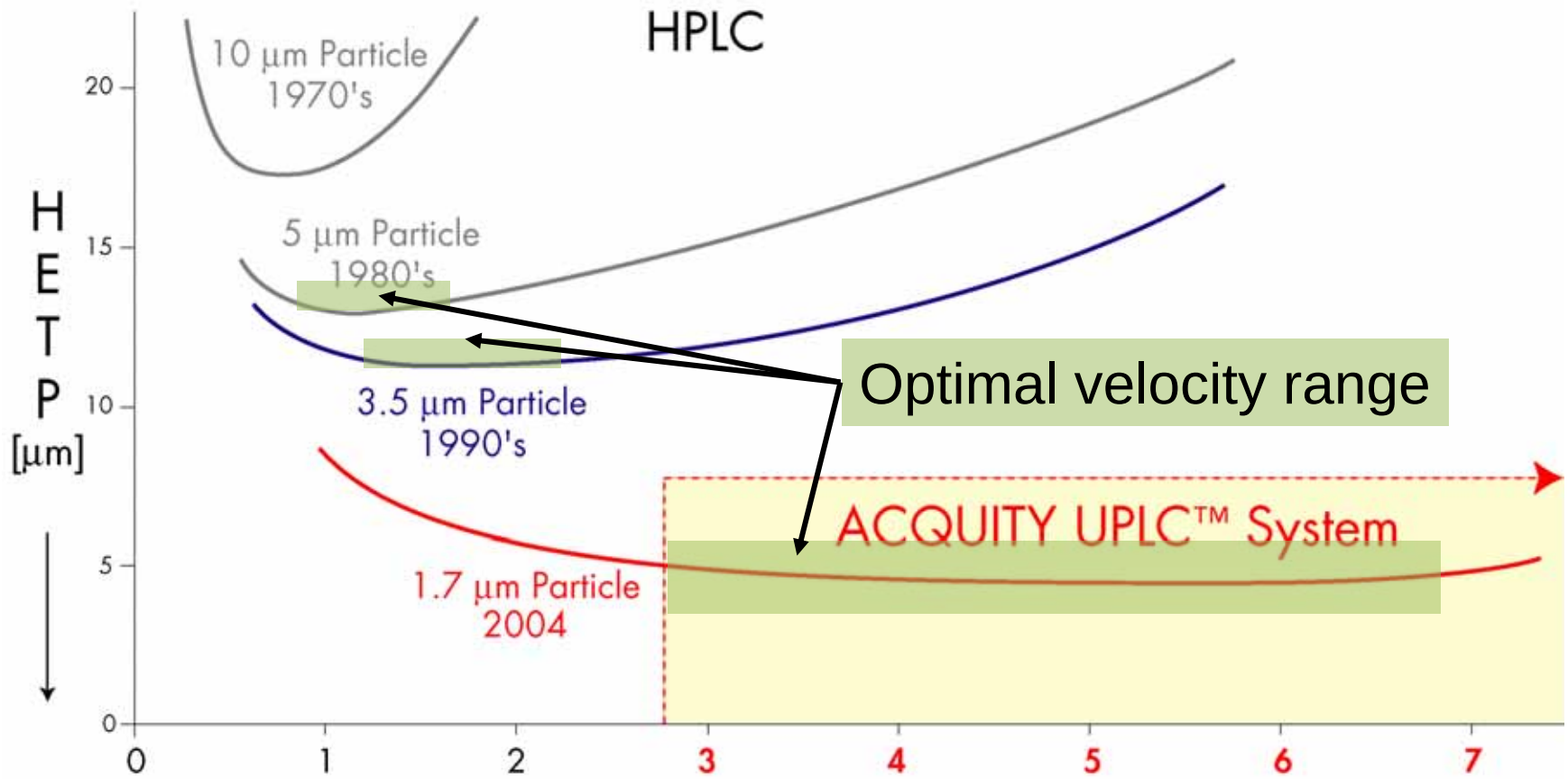
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Adjust time for same number of column volumes per gradient segment

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**	**0.31**	**95**	**5**	**1**	**3.33**	**6.02**

Smaller Particles
The Enabler of Productivity

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Flow Rate [mL/min]:							
ID = 1.0 mm	0.04	0.07	0.10	0.13	0.17	0.20	0.24
ID = 2.1 mm	0.15	0.3	0.45	0.6	0.75	0.9	1.05
ID = 4.6 mm	*0.7*	*1.4*	*2.1*	*2.8*	*3.5*	*4.2*	*4.9*

Estimate Optimum Flow Rate

UPLC®

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Consider 1.7µm target particle (2.1mm ID column)
- Assume temperature and viscosity transferred
- Adjust flow rate based on van Deemter curve and approximate molecular weight
  - ~0.6 mL/min for smaller molecules
    - average 500 dalton (molecular weight) molecules
  - ~0.1 mL/min for larger molecules because diffusion is slower
    - e.g., ~2,000 dalton peptides

Scaling for UPLC® Flow Rate

Step Time to Maintain Duration (cv)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Original Step 2: 15 min.@ 1.5 mL/min with Duration of **9.03cv**

Calculate Target Step 2: (keeping duration @ **9.03cv**)

Target Column Volume (2.1 x 50) = 0.17mL

Gradient Step Volume = Duration (cv) x Target Column Volume

= 9.03cv x 0.17mL = 1.54mL

Gradient Step Time = Gradient Step Volume / UPLC™ Flow Rate

= 1.54mL / 0.60 mL/min. =

2.6min

Optimized Gradient Profile

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Select UPLC® flow rate and adjust time to maintain same number of column volumes per segment

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	**2.61**	**0.6**	**5**	**95**	**6**	**2.61**	**9.03**
3	**3.48**	**0.6**	**5**	**95**	**1**	**0.87**	**3.01**
4	**5.22**	**0.6**	**95**	**5**	**1**	**1.74**	**6.02**

Review steps

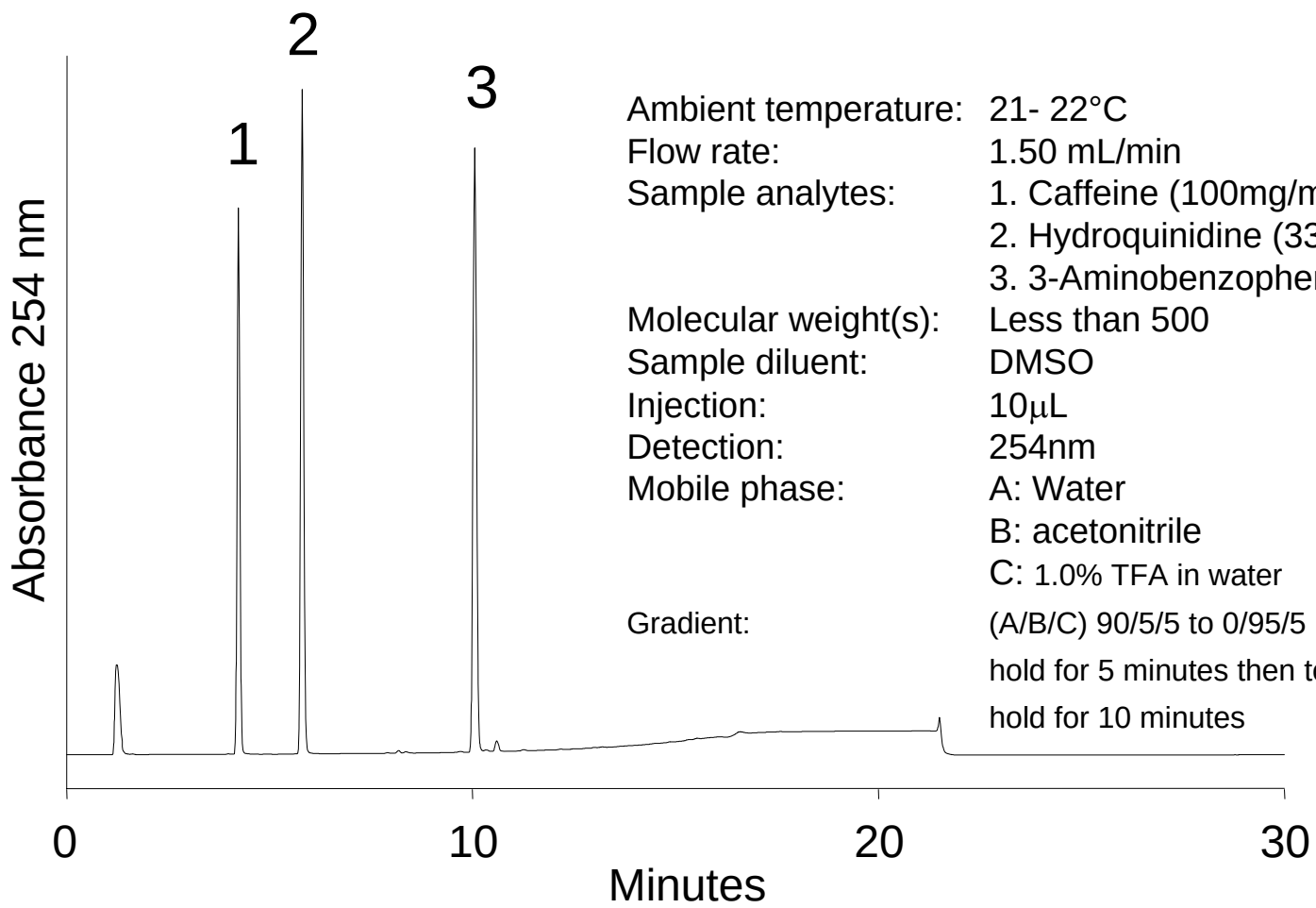
- Refer to current chromatography
  - —Observe system volumes, solvents and detection technique
  - —Define objectives/room for improvement
- Select column dimensions – scale flow for linear velocity
  - —50 mm length for speed
  - —100mm length for complex samples/resolution
- Scale injection volume to column dimensions
- Use a gradient and flow scaled from current method
  - —Adjust gradient to accommodate system differences, column differences and particle size differences
- Use a gradient and flow rate scaled for UPLC®

Method Conversion:
Original HPLC Method

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Resolution (1,2) = 12
Resolution (2,3) = 28

Original Column: 4.6 x 150 mm, 5 μm
@ 1.5 mL/min, 30 minute cycle time



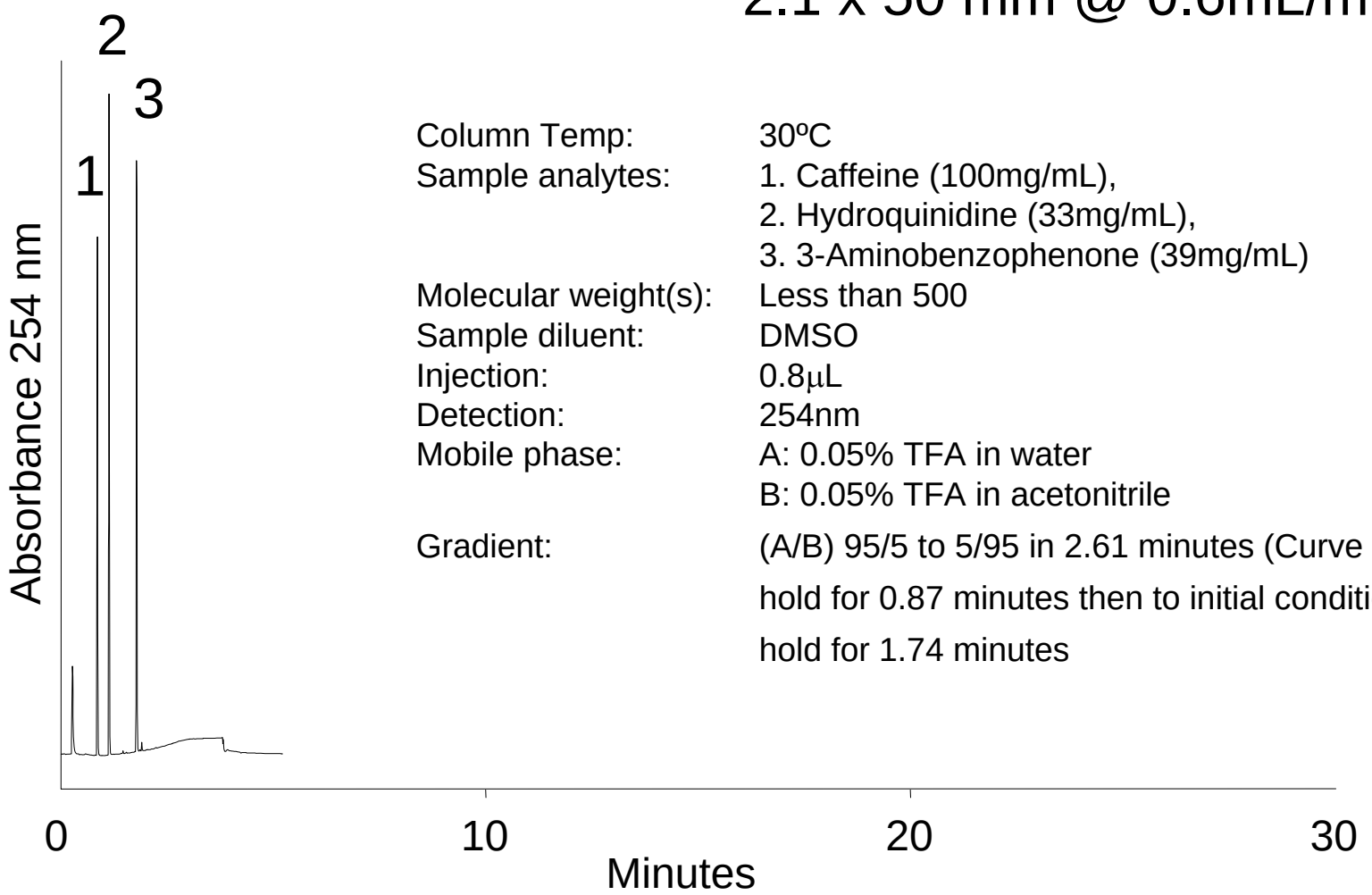
Ambient temperature:	21- 22°C
Flow rate:	1.50 mL/min
Sample analytes:	1. Caffeine (100mg/mL), 2. Hydroquinidine (33mg/mL), 3. 3-Aminobenzophenone (39mg/mL)
Molecular weight(s):	Less than 500
Sample diluent:	DMSO
Injection:	10μL
Detection:	254nm
Mobile phase:	A: Water B: acetonitrile C: 1.0% TFA in water
Gradient:	(A/B/C) 90/5/5 to 0/95/5 in 15 minutes (Curve 6) hold for 5 minutes then to initial conditions (curve 11) hold for 10 minutes

Method Conversion
Scaled UPLC®

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Resolution 1,2 = 11
Resolution 2,3 = 26

ACQUITY UPLC BEH C18, 1.7 µm,
2.1 x 50 mm @ 0.6mL/min



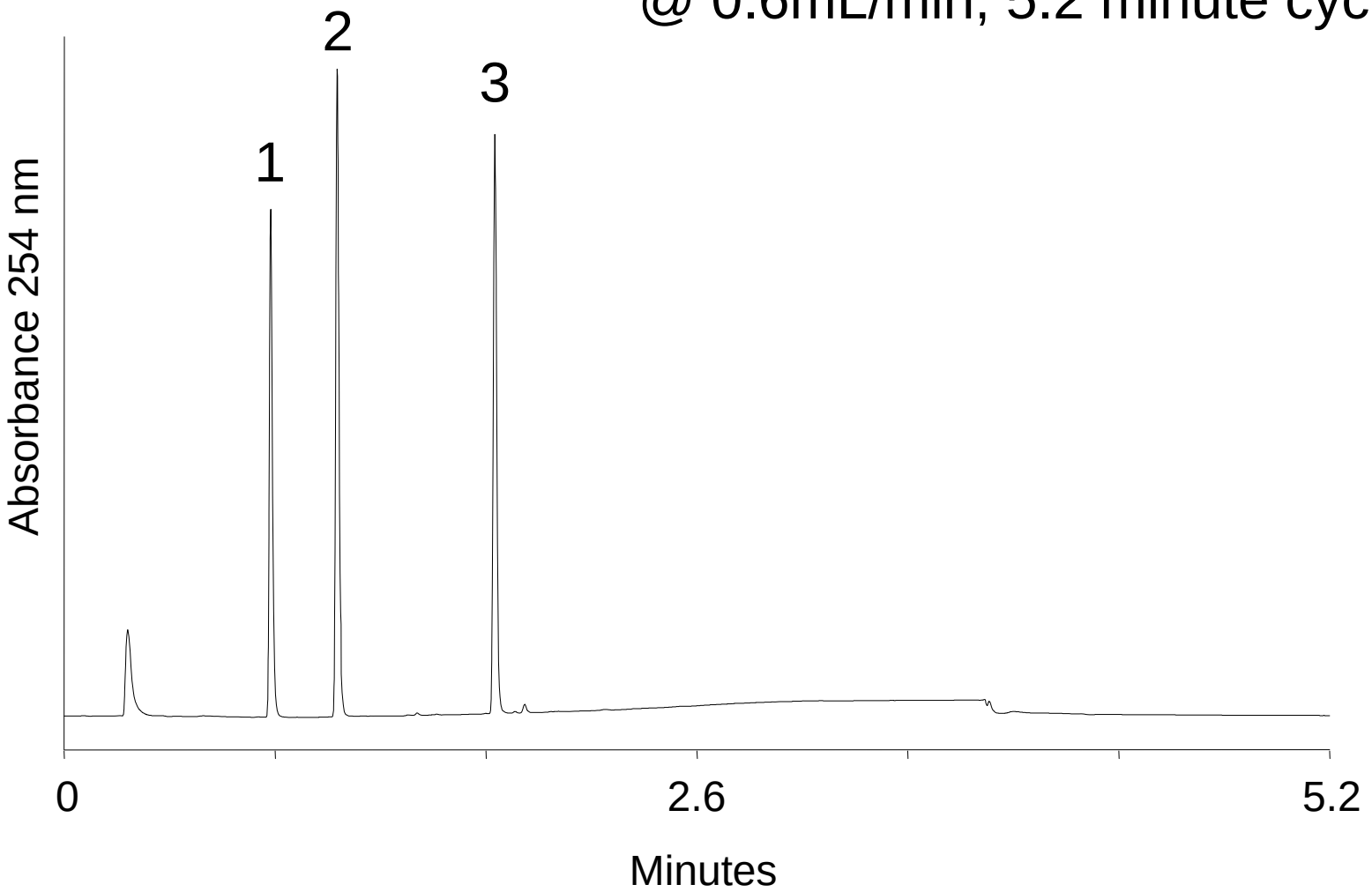
Column Temp:	30ºC
Sample analytes:	1. Caffeine (100mg/mL), 2. Hydroquinidine (33mg/mL), 3. 3-Aminobenzophenone (39mg/mL)
Molecular weight(s):	Less than 500
Sample diluent:	DMSO
Injection:	0.8µL
Detection:	254nm
Mobile phase:	A: 0.05% TFA in water B: 0.05% TFA in acetonitrile
Gradient:	(A/B) 95/5 to 5/95 in 2.61 minutes (Curve 6) hold for 0.87 minutes then to initial conditions (curve 11) hold for 1.74 minutes

Method Conversion
Scaled UPLC® Magnified

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Resolution 1,2 = 11
Resolution 2,3 = 26

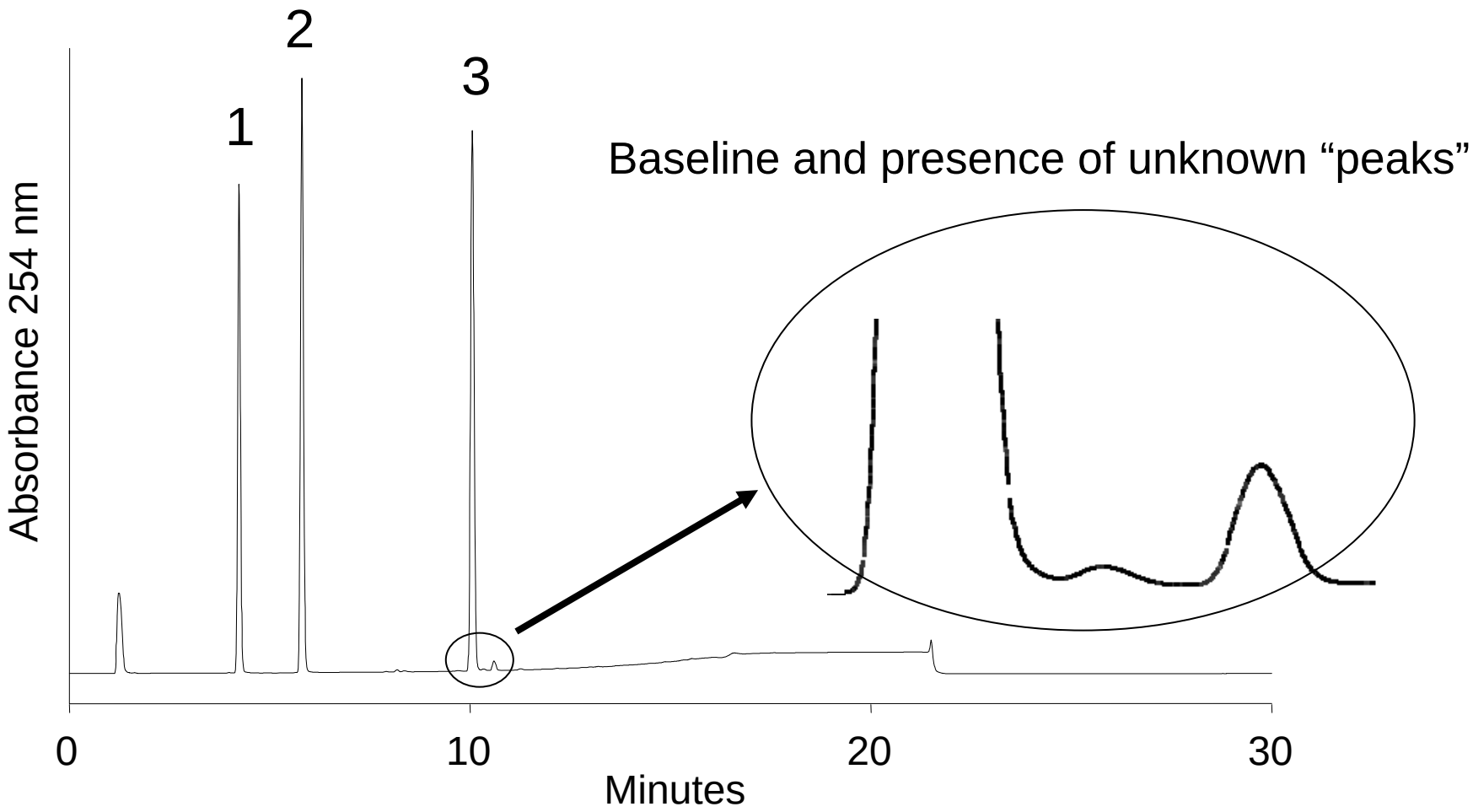
UPLC™: 2.1 x 50 mm column, 1.7µm
@ 0.6mL/min, 5.2 minute cycle time



Original HPLC Method
Critical resolution

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

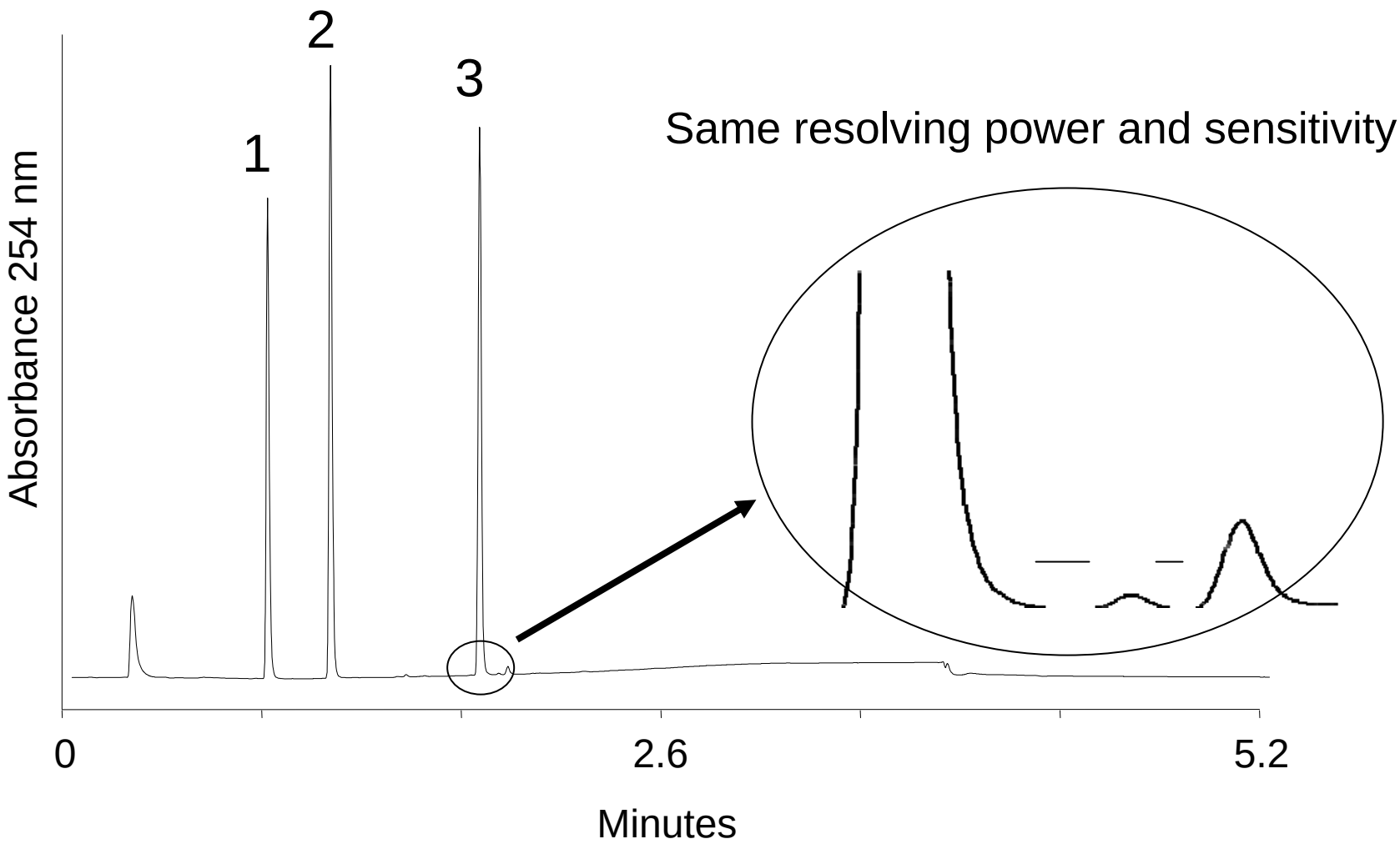
Resolution 1,2=12
Resolution 2,3=28



UPLC® Magnified View
Critical resolution

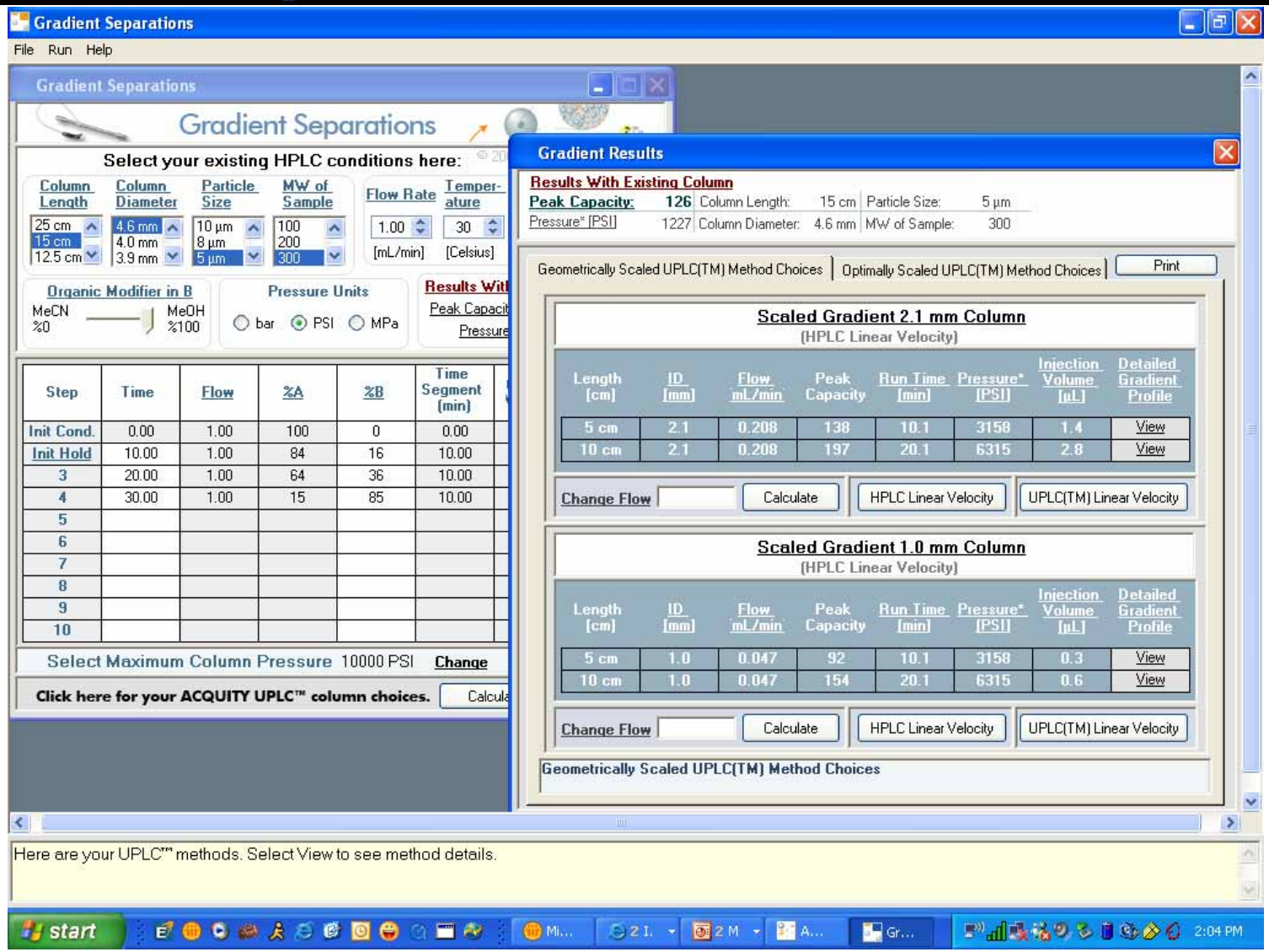
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Resolution 1,2=11
Resolution 2,3=26



ACQUITY UPLC® Calculator

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



Method Conversion

Significantly Different Column Chemistry

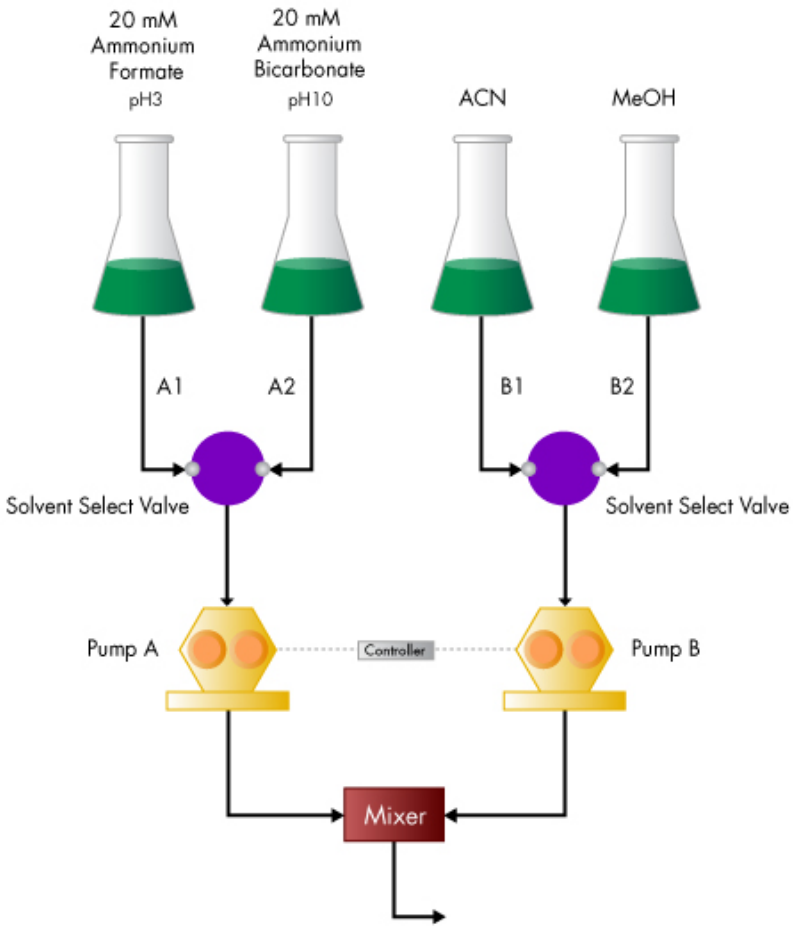
Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

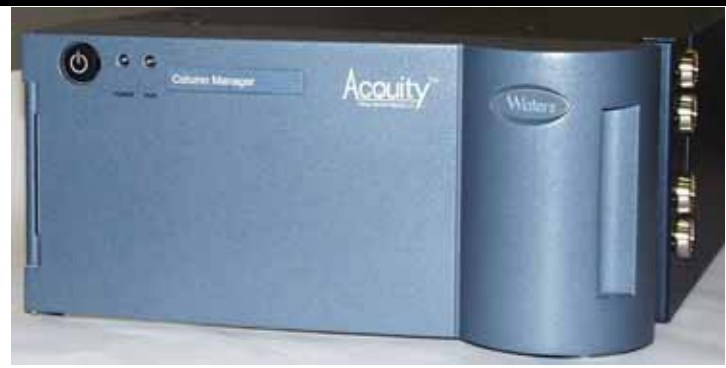
At what point does method conversion become method development?

- When column chemistries are different, try a modified method development approach
- Proposed Strategy 1:
  - —Understand general column selectivity differences
  - —Follow chemistry and system scaling protocols
  - —Generic gradients : 10 to 90 % organic solvent
  - —Run a short gradient (3 min) and a long gradient (6 min)
  - —Optimization tools (simulation software) are useful
- Proposed Strategy 2:
  - —Follow method development protocols

Automated Method Development

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™





Automated Method Development

- ACQUITY UPLC Column Manager, 4 column selection device
- ACQUITY UPLC Binary Solvent Manager, solvent select valves

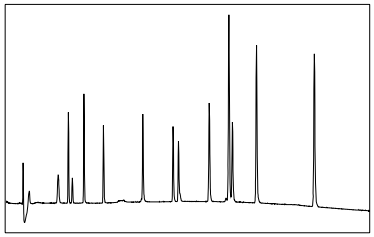
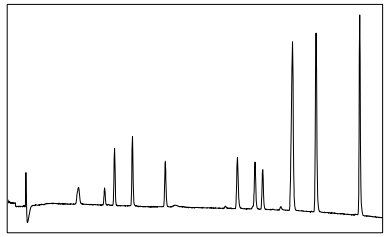
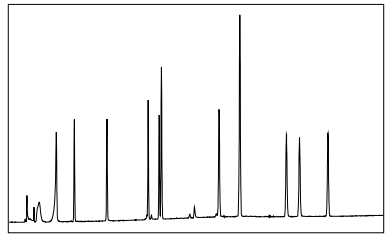
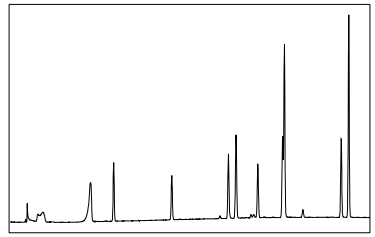
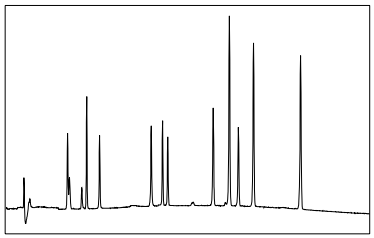
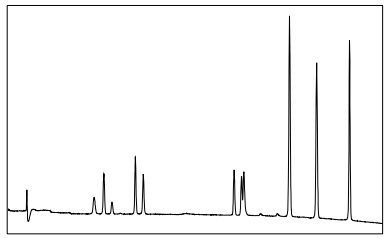
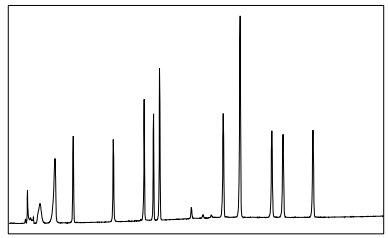
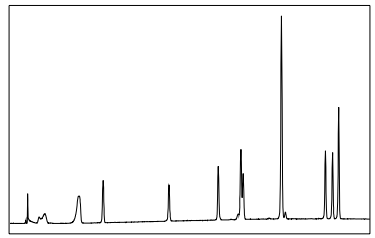
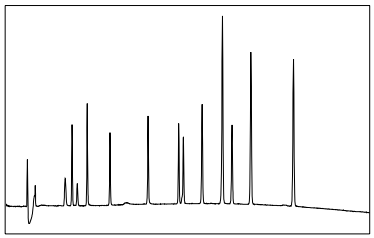
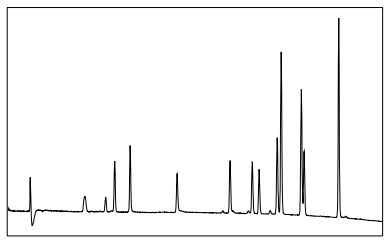
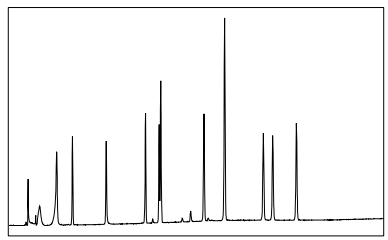
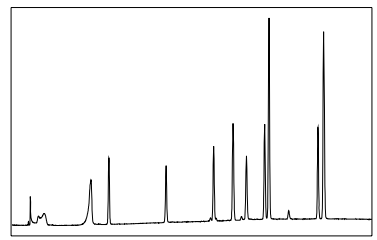
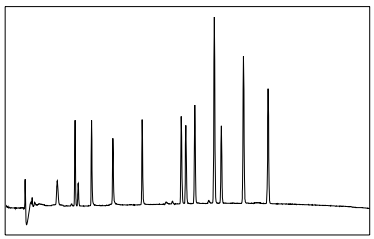
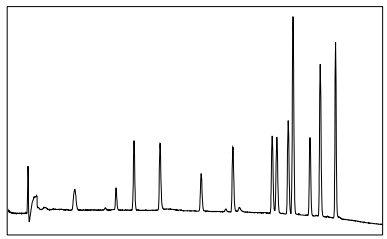
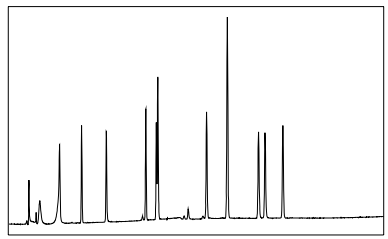
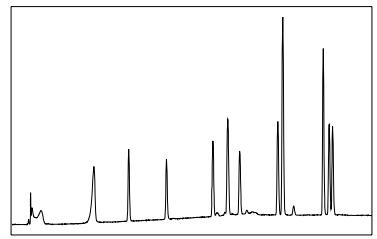
UPLC® Systematic Screening

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Four ACQUITY UPLC Chemistries 2.1 x 50 mm, 1.7 µm:
  - —ACQUITY UPLC™ BEH C_{18}
  - —ACQUITY UPLC™ BEH Shield RP_{18}
  - —ACQUITY UPLC™ BEH C_8
  - —ACQUITY UPLC™ BEH Phenyl
- Solvents:
  - —Acetonitrile
  - —Methanol
- Buffers:
  - —pH 3 ammonium formate
  - —pH 10 ammonium bicarbonate

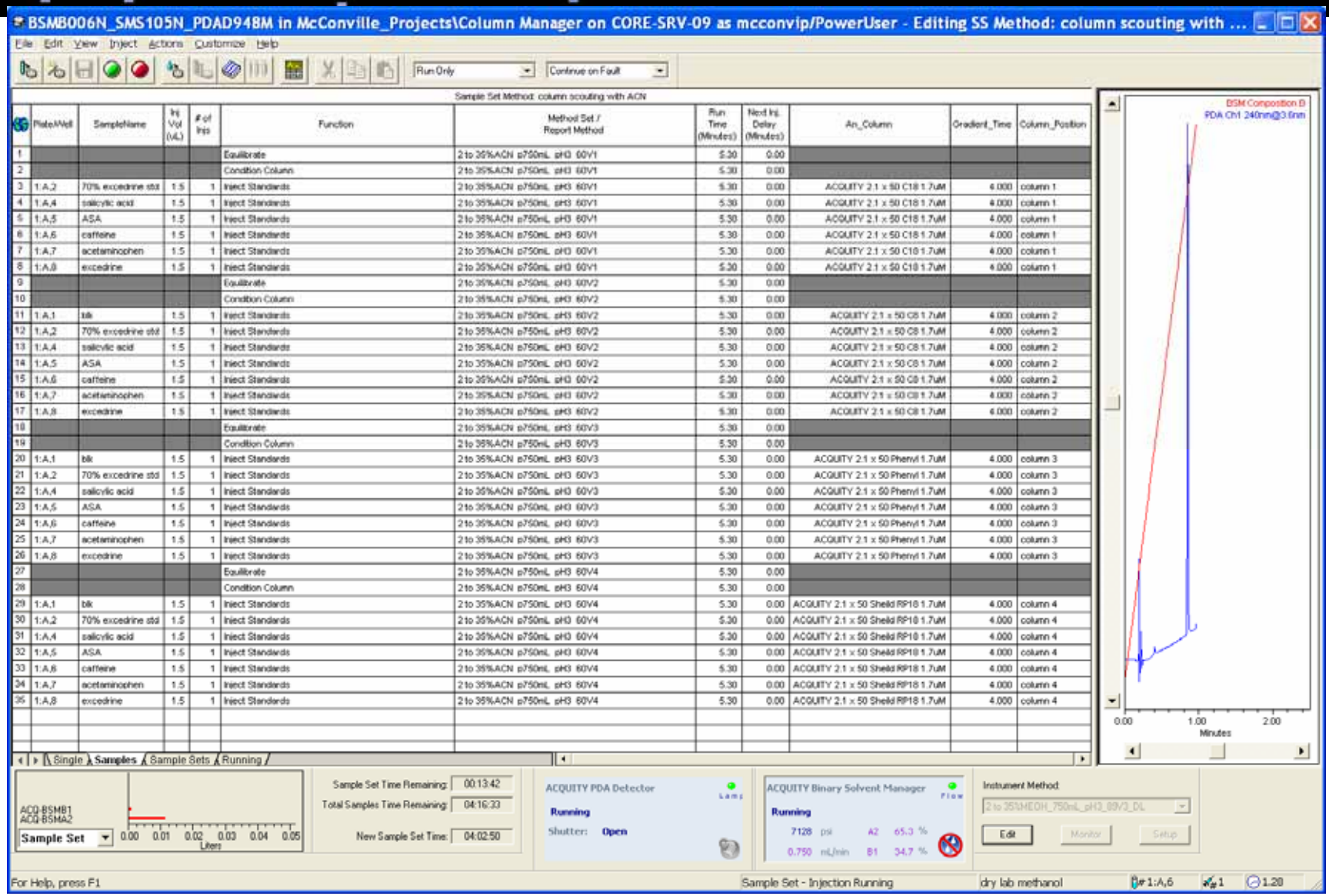
Experimental Matrix

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

	pH 3, Acetonitrile	pH 3, Methanol	pH 10, Acetonitrile	pH 10, Methanol
C18				
Shield RP18				
C8				
Phenyl				

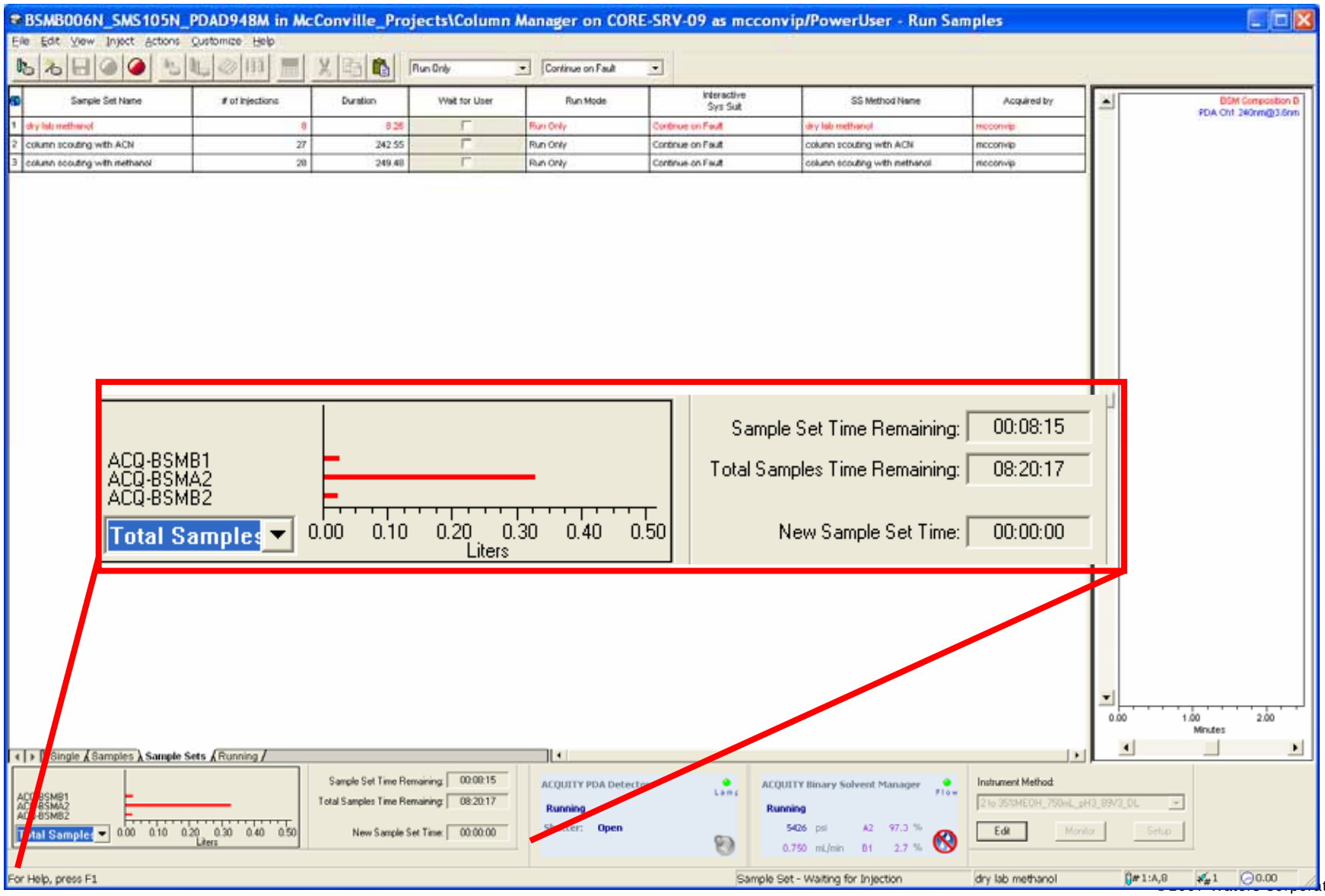
Automated Column and Solvent Scouting

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



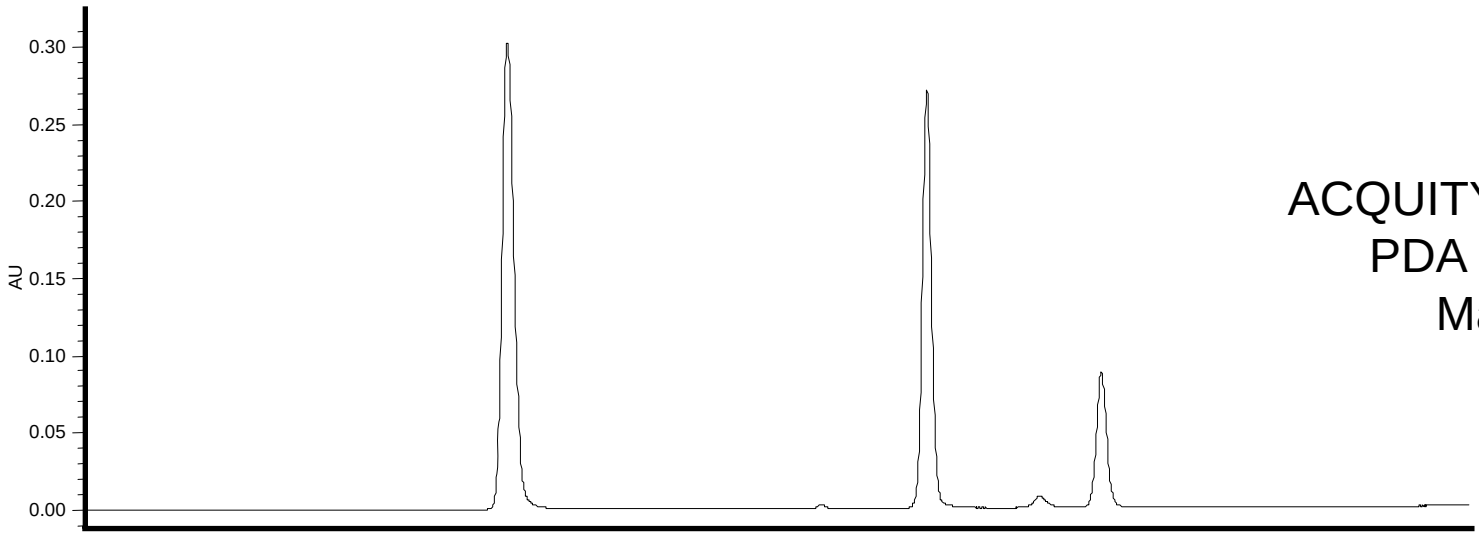
Total Time and Solvents Calculated

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

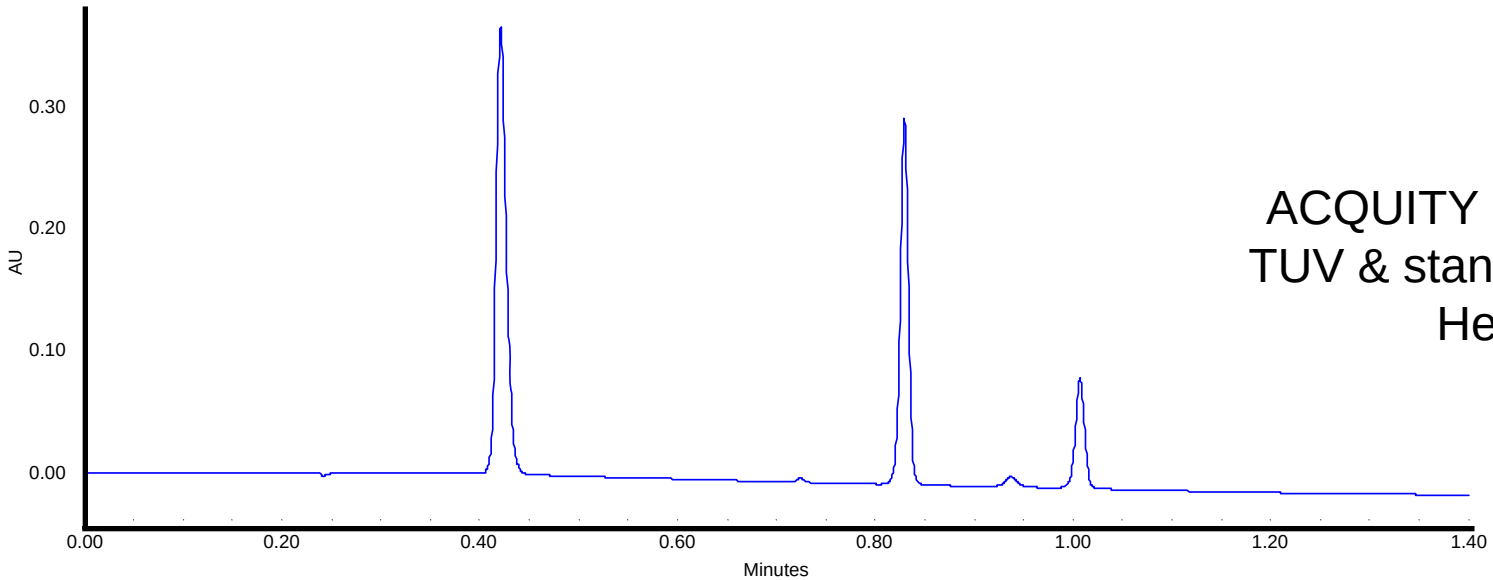


Can I transfer the method to other systems that have a standard column heater?

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



ACQUITY UPLC® with PDA & Column Manager



ACQUITY UPLC® with TUV & standard Column Heater

Develop Methods Faster with UPLC®:
Time Savings

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

UPLC Methods Development Protocol
2.1 x 50 mm, 1.7 µm

pH 3 / acetonitrile	**Time**
Flow ramp	5 min
Column conditioning (2 blank gradients)	11 min
Sample injection (2 replicates)	11 min
pH 3 / methanol	
Flow ramp	5 min
Column conditioning (2 blank gradients)	11 min
Sample injection (2 replicates)	11 min
Column purge	6 min
pH 10 / acetonitrile	
Flow ramp	5 min
Column conditioning (2 blank gradients)	11 min
Sample injection (2 replicates)	12 min
pH 10 / methanol	
Flow ramp	5 min
Column conditioning (2 blank gradients)	11 min
Sample injection (2 replicates)	11 min
Column purge	6 min
	120 min

SCREENING TIME — **2 Hours / column x 4 columns**

TOTAL SCREENING TIME — **8 HOURS**

EQUIVALENT HPLC Methods Development Protocol
4.6 x 150 mm, 5 µm

pH 3 / acetonitrile	**Time**
Flow ramp	5 min
Column conditioning (2 blank gradients)	80 min
Sample injection (2 replicates)	80 min
pH 3 / methanol	
Flow ramp	5 min
Column conditioning (2 blank gradients)	80 min
Sample injection (2 replicates)	80 min
Column purge	35 min
pH 10 / acetonitrile	
Flow ramp	5 min
Column conditioning (2 blank gradients)	80 min
Sample injection (2 replicates)	80 min
pH 10 / methanol	
Flow ramp	5 min
Column conditioning (2 blank gradients)	80 min
Sample injection (2 replicates)	80 min
Column purge	35 min
	730 min

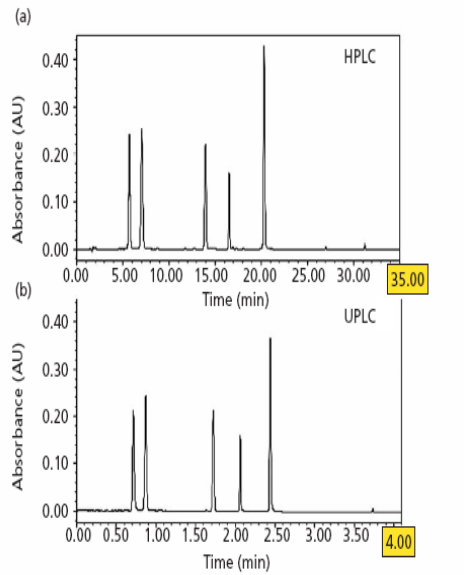
SCREENING TIME — **12.2 Hours / column x 4 columns**

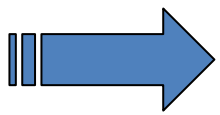
TOTAL SCREENING TIME — **48.8 HOURS**

Conclusion

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Strategies and Tools For HPLC to UPLC® Method Migration
  - Column chemistry and dimensions
  - Instrument considerations
  - Proper scaling
  - Optimization
  - Redevelopment
  - Method migration/conversion calculator





Acknowledgements

- Diane Diehl
- Eric Grumbach
- Jeff Mazzeo
- Uwe Neue
- Michael Jones
- Andy Aubin
- Craig Dobbs

And:

Inform 2007

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

- Waters premier Laboratory Informatics symposium where users can network with colleagues, share experiences, and gain valuable insights into how to best deploy and use Waters Informatics suite of solutions
  - May 7-10, 2007
  - Miami Marriott Biscayne Bay
- Three-day event with optional tutorials
  - Monday: (2) ½ Day Tutorial Session
  - Tuesday – Thursday: Symposium
- Download the agenda and register at:
  - Waters booth - 3641
  - www.inform2007.com

Fill out a seminar evaluation form and enter the raffle to win a Gold Package registration* for Inform 2007 which includes all tutorials and workshops - a $1595 value

One drawing daily. Winner will be notified.

* Prize covers conference registration only

