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UPLC® Method Development and Validation

Rev. 2

Challenges of Method Development

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- Methods are developed throughout the drug development process
  - Samples vary in complexity
  - Redundancy across organization
- Method development is costly and time-consuming
  - Desire to streamline processes to bring products to market faster
  - Faster chromatographic methods will improve profitability

UPLC Technology Can Streamline Method Development

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UPLC Technology enables faster method development

Methods can be developed in a single work day!

- Systematic scouting protocol involving pH, organic and column chemistry
- High-resolution sub-2 µm column technology enables high resolution separations, faster
- Automated column and mobile phase selection

Outline

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- Approaches Towards Method Development
- Selectivity and Retention Tools
  - Stationary Phase and Particle Substrate
  - Solvent
  - pH
- Method Development Strategy
- Applications
- Conclusions

Approaches Towards Method Development

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- Literature search, colleague, speculation?
- Stepwise iterative procedure
  - Next step experimental design based on results from previous experiment
- Systematic scouting
  - Evaluate pH, organic solvent and stationary phase
  - Select best combination of those three
  - Fine tuning/optimization (temperature, gradient slope)

Desirable Information for Method Development

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- Sample solubility
- Number of analytes
  - How many peaks of interest are you trying to separate?
- Chemical structure(s)
- Functional groups; how the analytes differ
  - Ionizable species? How will pH influence chromatography?
- Detection
  - Type of detection that is required or possible?
- Concentration range and quantitative requirements
- Sample matrix effects

Outline

- Approaches Towards Method Development
- Selectivity and Retention Tools
  - Stationary Phase and Particle Substrate
  - Solvent
  - pH
- Method Development Strategy
- Applications
- Conclusions

Selectivity Tools

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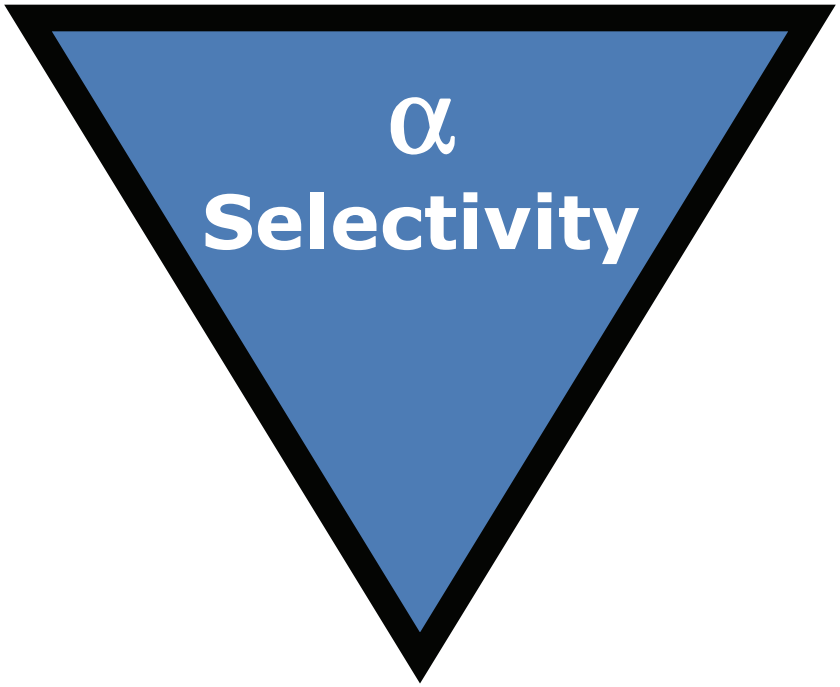
Solvent **pH**



Column Chemistry
-Ligand
-Base particle

Improving Resolution with Complementary Selectivities

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$$Rs = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k}{k + 1}$$

Physical

Maximized in UPLC Separations by:

- Ultra-low dispersion system
- Small (< 2 µm) particles
- Higher pressure capability
- Well-designed columns

Chemical

Maximized in UPLC Separations by:

- Range of chemistries
- Multiple particle substrates
- Wide usable pH range (BEH)
- Higher retentivity (HSS)

Impact on Resolution	% Improvement
Double N	20-40%
Double k	15-20%
Double α	> 400%

Different Ligands: Different Selectivity

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- Changes in hydrophobicity
  - Longer alkyl chain will provide greater retention
- Changes in silanol activity
  - Affect peak asymmetry and influences secondary interactions
- Changes in hydrolytic stability
  - Longer column lifetimes with greater number of ligand attachment points to the particle surface
- Changes in ligand density
  - Influences sample loadability

BEH Chemistries of UPLC Technology

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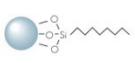
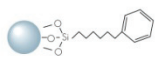
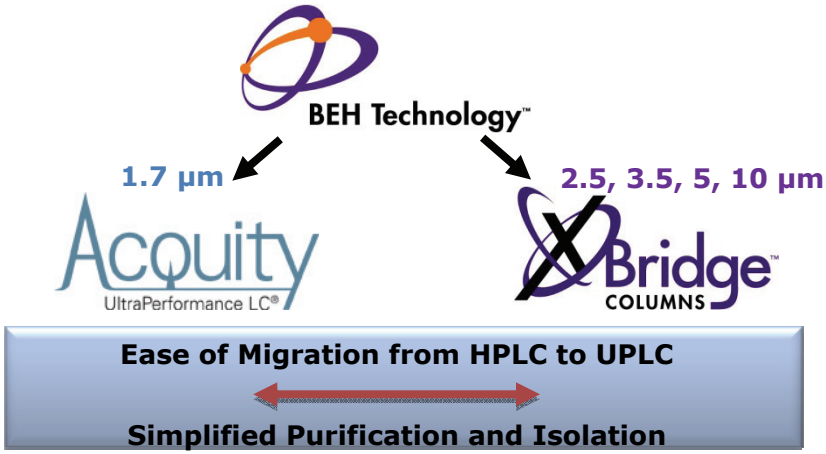
- **BEH C_{18}**
  - Trifunctionally Bonded C_{18}
  - First UPLC® column choice
  - Superior peak shape & efficiencies
- **BEH C_8**
  - Trifunctionally Bonded C_8
  - Wide pH range
- **BEH Shield RP18**
  - Monofunctionally bonded
  - Embedded carbamate group
  - Alternate selectivities
- **BEH Phenyl**
  - Trifunctionally Bonded C_6 (Hexyl) Phenyl
  - Unique combination of chemistry & particle
  - Wide pH range
- **BEH HILIC**
  - Unbonded, rugged BEH particle
  - HILIC for very polar bases

	BEH Particle				
Chemistry	C_{18} 	C_8 	Shield RP18 	Phenyl 	HILIC 
Ligand Type	Trifunctional C_{18}	Trifunctional C_8	Monofunctional Embedded Polar Group	Trifunctional C_6 Phenyl	—
Ligand Density*	3.1 µmol/m²	3.2 µmol/m²	3.3 µmol/m²	3.0 µmol/m²	—
Carbon Load*	18%	13%	17%	15%	—
Endcap Style	Proprietary	Proprietary	TMS	Proprietary	—
pH Range	1-12	1-12	2-11	1-12	1-8

** Expected or Approximate Values*



HSS Chemistries of UPLC Technology

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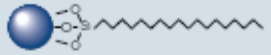
- **HSS T3**
  - **T3: Polar compound retention**
  - **Aqueous-compatible C_{18} chemistry**
  - **Designed for maximum retentivity**
- **HSS C_{18}**
  - **High coverage, trifunctionally bonded C_{18} chemistry**
  - **Universal, high performance C_{18} chemistry**
  - **Proprietary endcapping for superior peak shape**
  - **Silica particle performance**
- **HSS C_{18} SB**
  - **SB: Selectivity for Bases**
  - **Non-endcapped: optimum silanophilic selectivities**

	HSS Particle		
Chemistry	C_{18}	C_{18} SB	T3
			
Ligand Type	Trifunctional C_{18}	Trifunctional C_{18}	Trifunctional C_{18}
Ligand Density*	3.2 µmol/m²	1.6 µmol/m²	1.6 µmol/m²
Carbon Load*	15%	8%	11%
Endcap Style	Proprietary	None	Proprietary
pH Range	1-8	2-8	2-8

Challenges of Developing and Manufacturing UPLC Particles

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- Developing UPLC particles requires:
  - R&D scientists capable of synthesizing, sizing and evaluating prototype sub-2 µm chromatographic media
  - Cutting-edge synthesis facilities capable of reliably and reproducibly producing commercial quantities of bulk materials

- UPLC particle requirements:
  - Pressure tolerance
  - Proper morphology
  - High efficiency/mass transfer
  - Advanced bonding and endcapping processes

Additional requirements as compared to HPLC particles

Two Rugged, Efficient UPLC Particles: van Deemter Curves (<500 MW)

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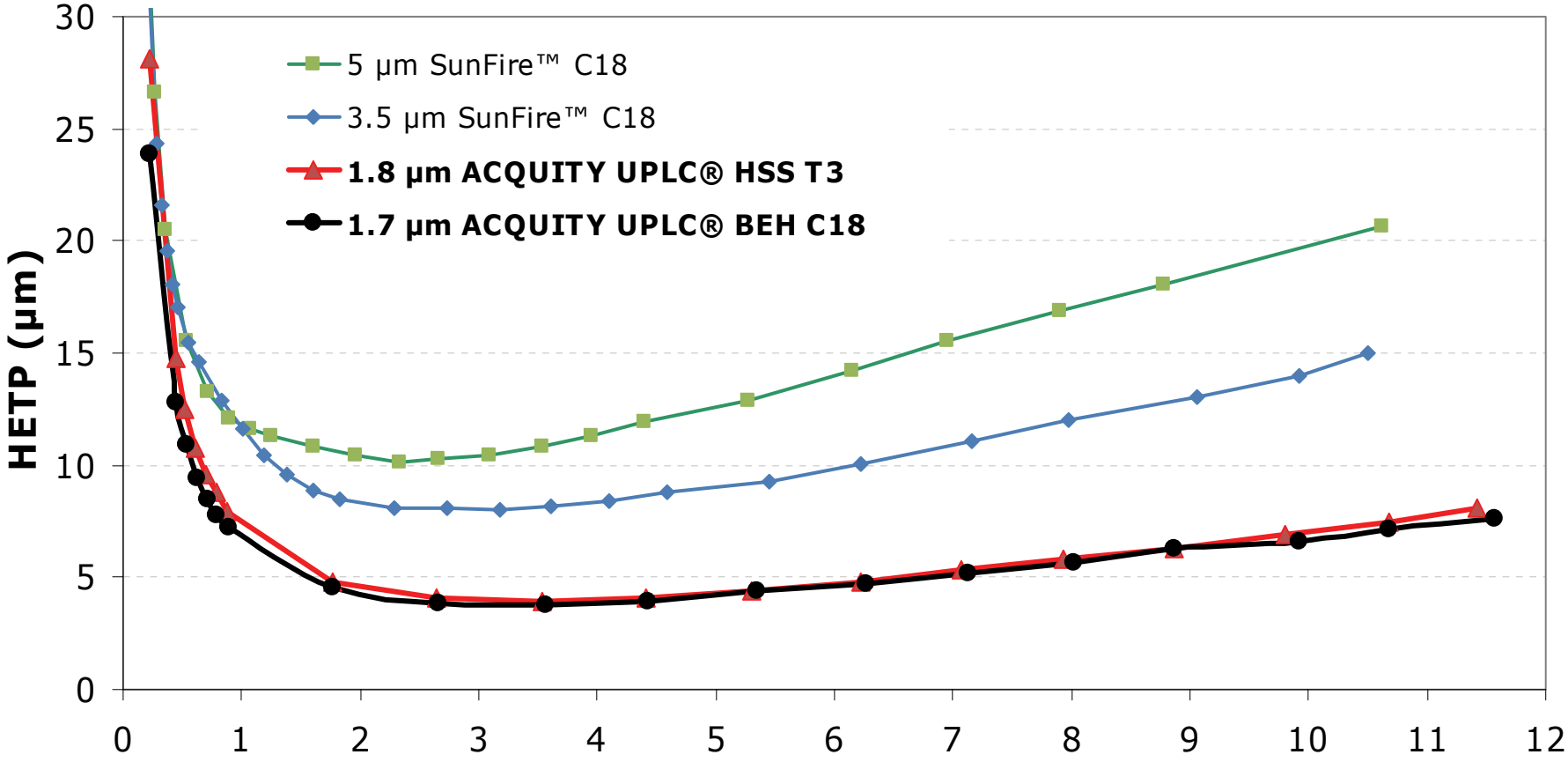

Flow rate (mL/min)											
1.0 mm ID	0.04	0.07	**0.11**	**0.14**	**0.18**	**0.21**	**0.25**	**0.28**	**0.32**	**0.35**	**0.39**	**0.42**
2.1 mm ID	0.15	0.30	**0.45**	**0.60**	**0.75**	**0.90**	**1.05**	**1.20**	**1.35**	**1.50**	**1.65**	**1.80**
4.6 mm ID	0.70	1.40	2.10	2.80	3.50	4.20	4.90	5.60	6.30	7.00	7.70	8.40

UPLC Particles and Chemistries Summary

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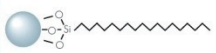
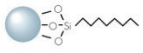
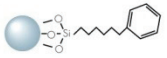
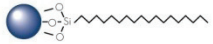
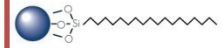
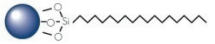
	BEH Particle					HSS Particle		
Chemistry	C_{18} 	C_8 	Shield RP18 	Phenyl 	HILIC 	T3 	C_{18} 	C_{18} SB 
Ligand Type	Trifunctional C_{18}	Trifunctional C_8	Monofunctional Embedded Polar Group	Trifunctional C_6 Phenyl	—	Trifunctional C_{18}	Trifunctional C_{18}	Trifunctional C_{18}
Ligand Density*	3.1 µmol/m²	3.2 µmol/m²	3.3 µmol/m²	3.0 µmol/m²	—	1.6 µmol/m²	3.2 µmol/m²	1.6 µmol/m²
Carbon Load*	18%	13%	17%	15%	—	11%	15%	8%
Endcap Style	Proprietary	Proprietary	TMS	Proprietary	—	Proprietary	Proprietary	None
pH Range	1-12	1-12	2-11	1-12	1-8	2-8	1-8	2-8

** Expected or Approximate Values*

Launch Date	Mar 2004	Mar 2005	Mar 2005	Mar 2005	Dec 2005	Sep 2006	Jun 2007	Jan 2008

- Used in following method development protocol

ACQUITY UPLC Column Selection: Method Development Approach

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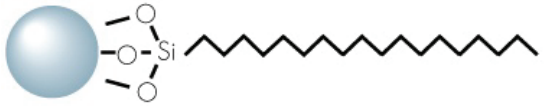
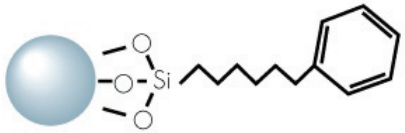
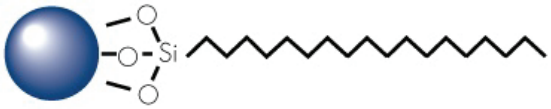


- **BEH C_{18}**
  - **Widest pH range for maximum selectivity**

- **BEH Shield RP18**
  - **Embedded polar group offers complementary selectivity vs. alkyl C_{18} chemistries**



- **BEH Phenyl**
  - **C_6 (Hexyl) Phenyl chemistry offers complementary selectivity to C_{18} and Shield RP18 chemistries, especially for analytes with aromatic rings**



- **HSS T3**
  - **Polar compound retention and aqueous mobile phase compatibility**

Selectivity Scouting Protocol

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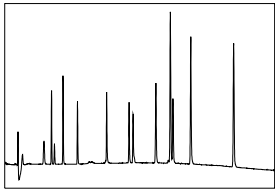
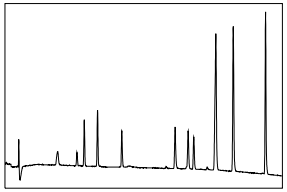
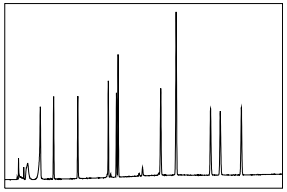
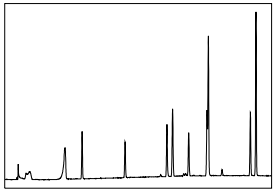
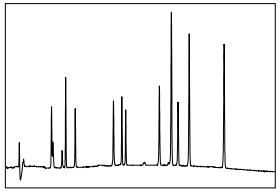
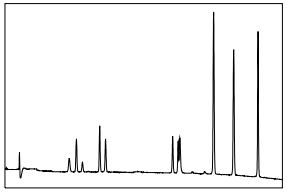
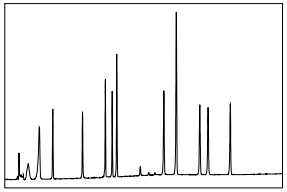
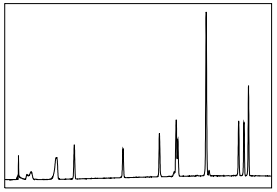
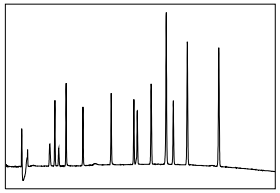
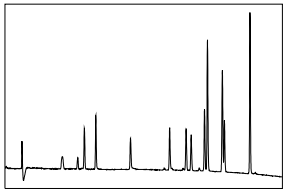
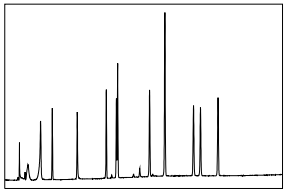
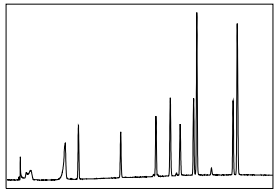
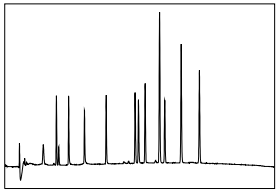
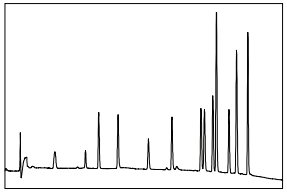
2.1 x 50 mm, <2 µm

	pH 3, ACN	pH 3, MeOH	pH 10, ACN	pH 10, MeOH
ACQUITY UPLC BEH C_{18}				
ACQUITY UPLC BEH Shield RP_{18}				
ACQUITY UPLC BEH Phenyl				
ACQUITY UPLC HSS T3				

Optimization

Selectivity Tools

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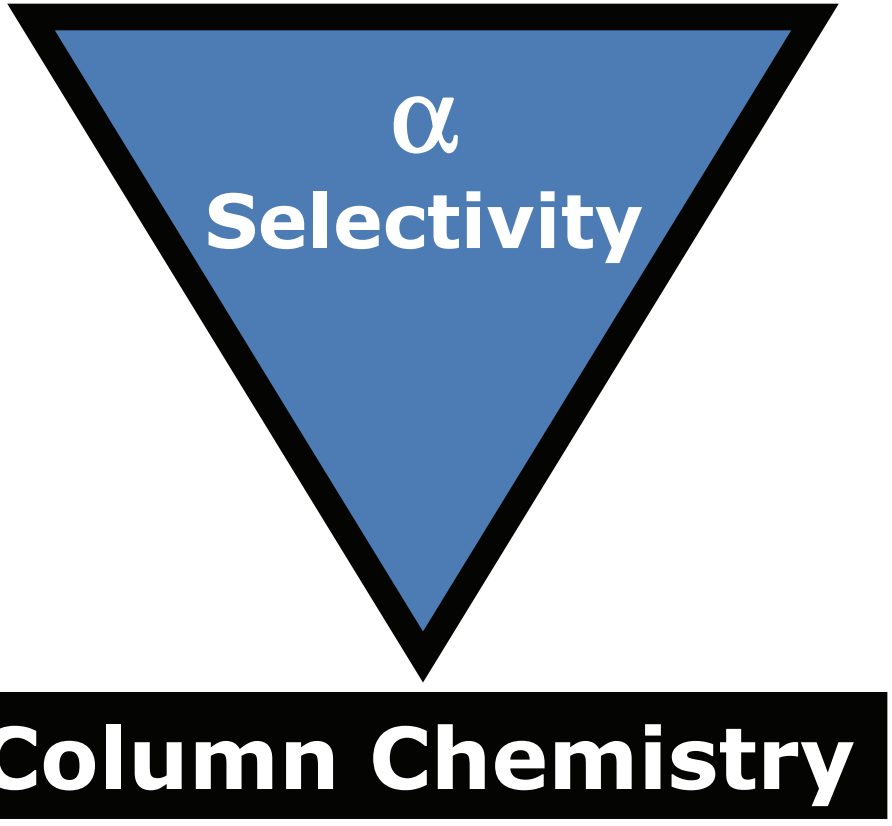
Solvent **pH**



Selectivity Observations: Chemical Structures

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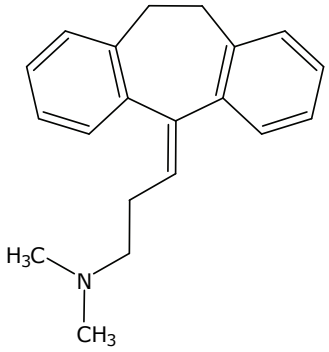
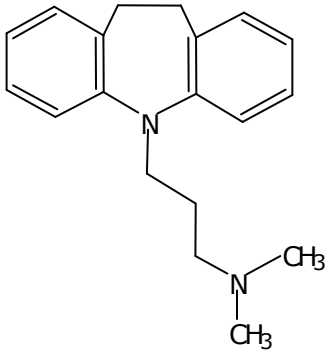
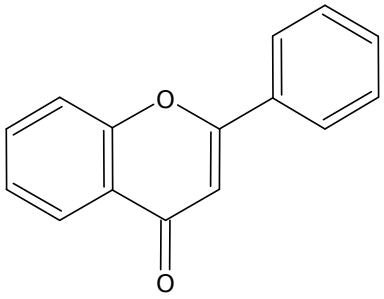


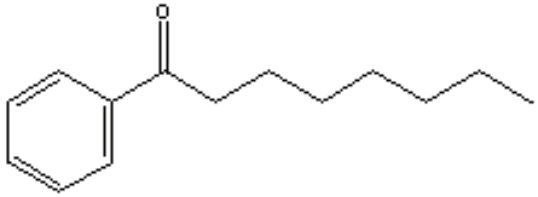
Amitriptyline (B)
m.w. 277.40



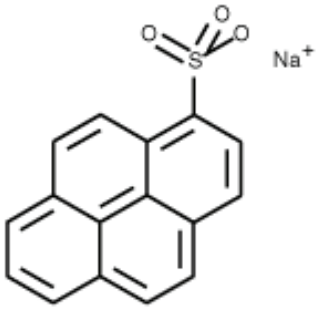
Imipramine (B)
m.w. 280.40



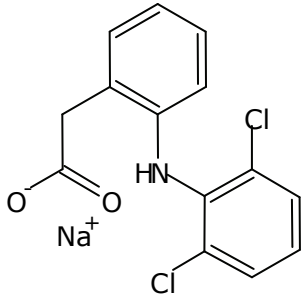
Flavone (N)
m.w. 222.24



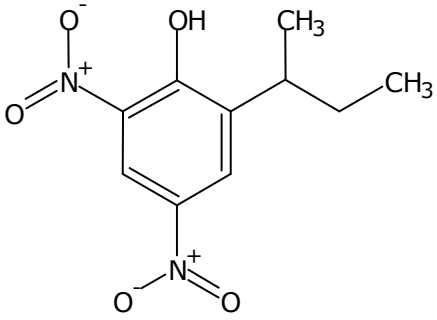
Octanophenone (N)
m.w. 204.31



1-Pyrenesulfonic acid (A)
m.w. 304.3



diclofenac (WA)
m.w. 318.13



Dinoseb (A)
m.w. 240.21

Stationary Phase Selectivity: Basic and Neutral Compounds

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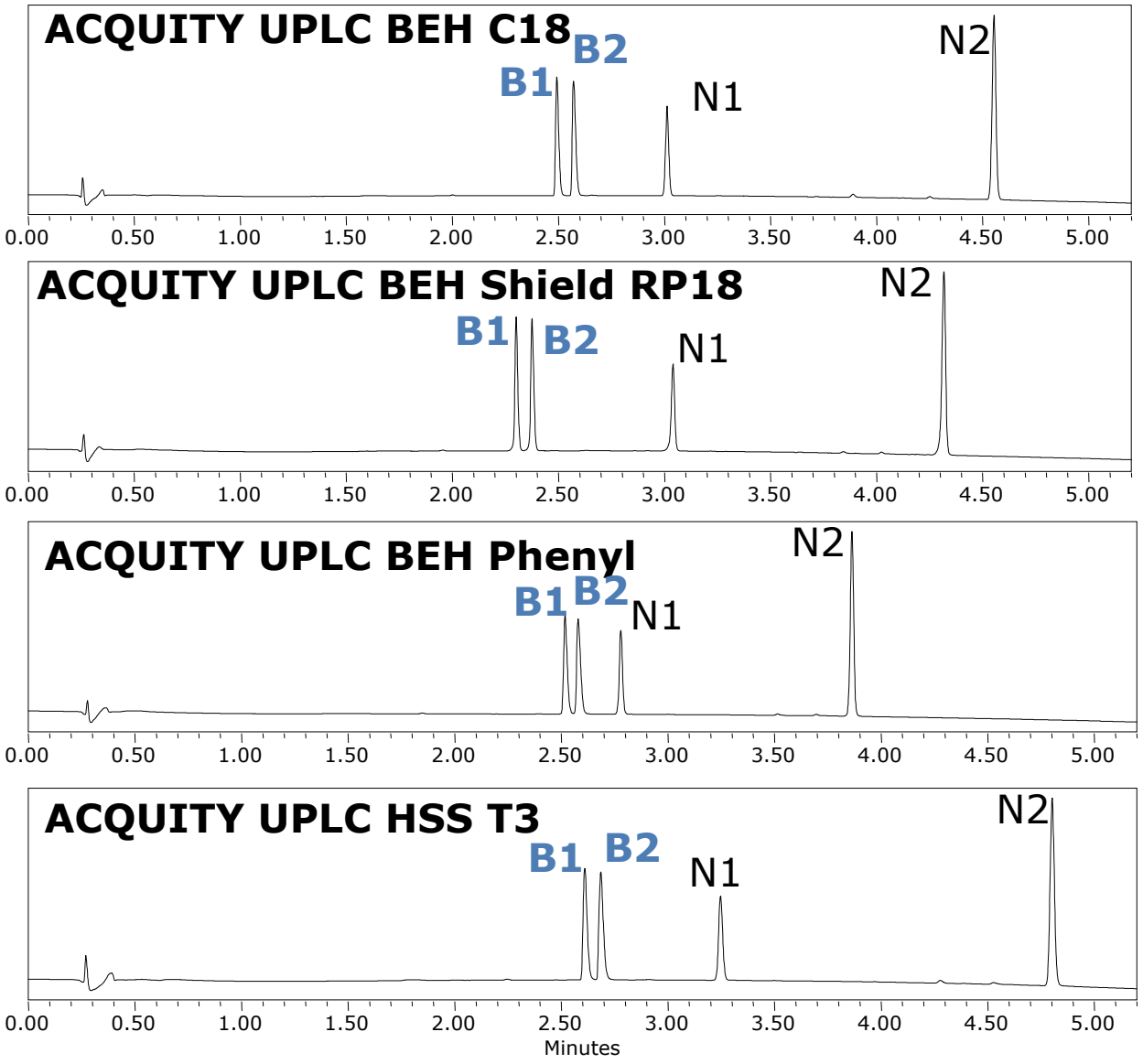

Acetonitrile pH 3.0

Test Probes:

B1 imipramine
B2 amitriptyline
N1 flavone
N2 octanophenone

Small differences in stationary phase selectivity with acetonitrile, low pH

Stationary Phase Selectivity: Acidic Compounds

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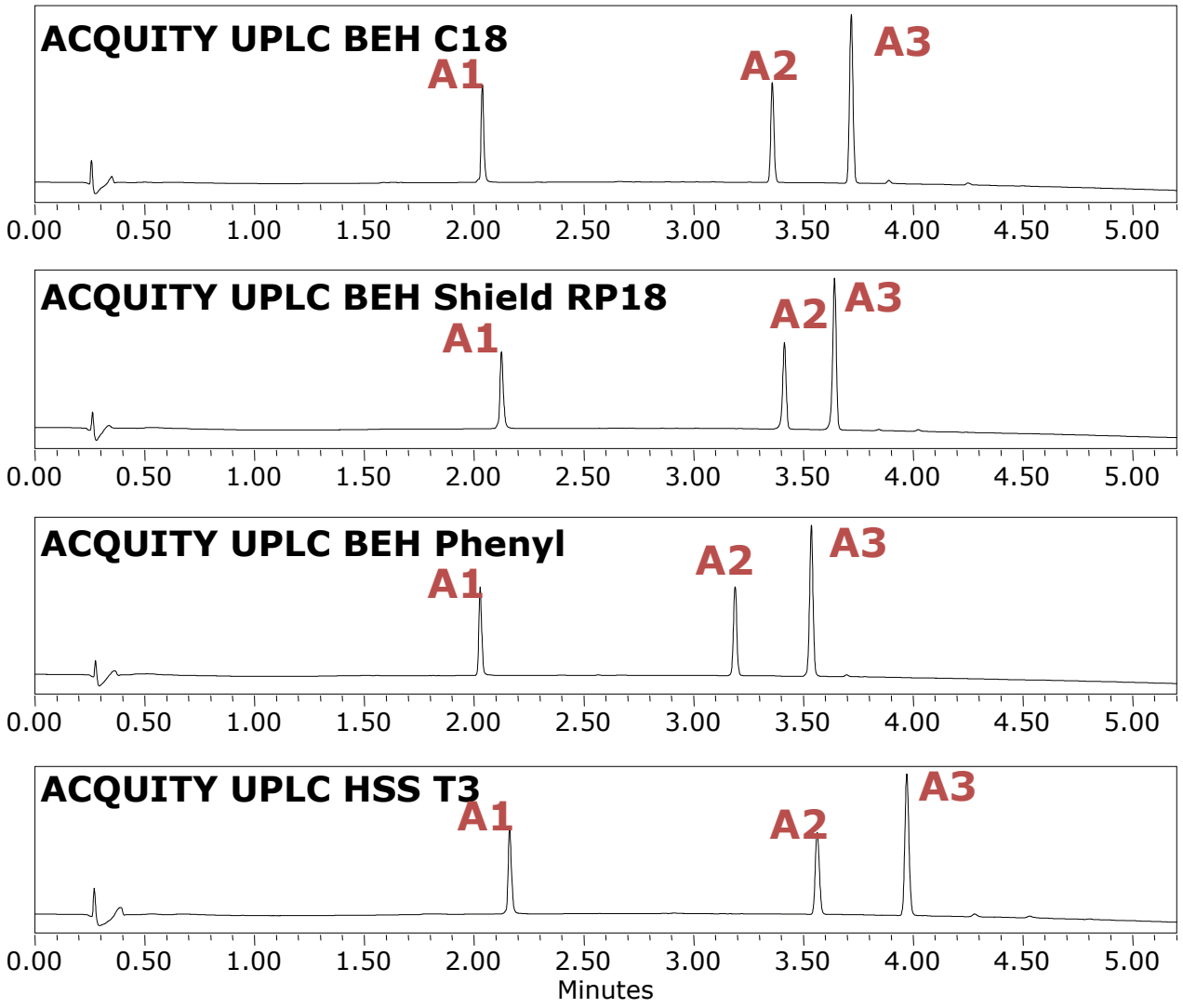

Acetonitrile pH 3.0

Test Probes:

A1 1-pyrenesulfonic acid
A2 diclofenac
A3 dinoseb

Small differences in stationary phase selectivity with acetonitrile, low pH

Selectivity Tools

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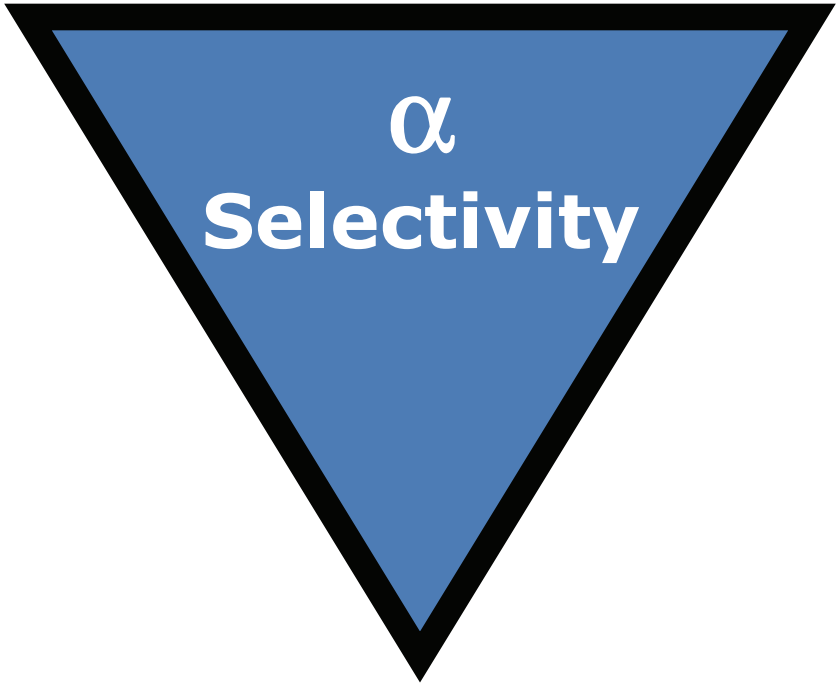
Solvent

pH



Column Chemistry

Solvent Properties

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- Methanol
  - Weaker eluent
  - H-bonding solvent
- Acetonitrile
  - Aprotic solvent
  - Stronger eluent
  - Lower viscosity

Solvent Selectivity: Basic and Neutral Compounds

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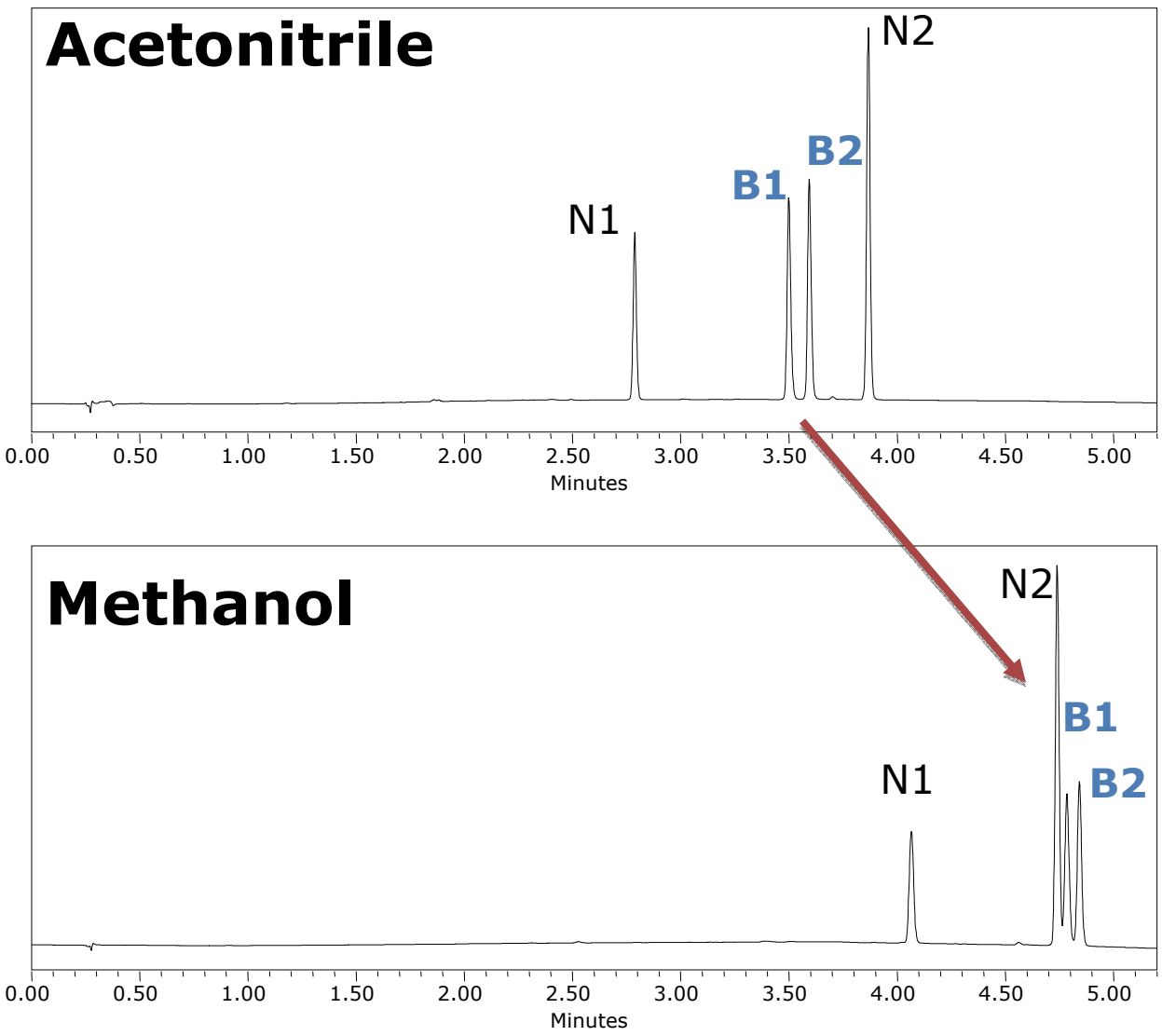
ACQUITY UPLC BEH Phenyl



Test Probes:

B1 imipramine
B2 amitriptyline
N1 flavone
N2 octanophenone

Methanol is weaker elution solvent, resulting in greater retention for all analytes

Greater retention of bases respective to neutral probes

Solvent Selectivity: Acidic Compounds

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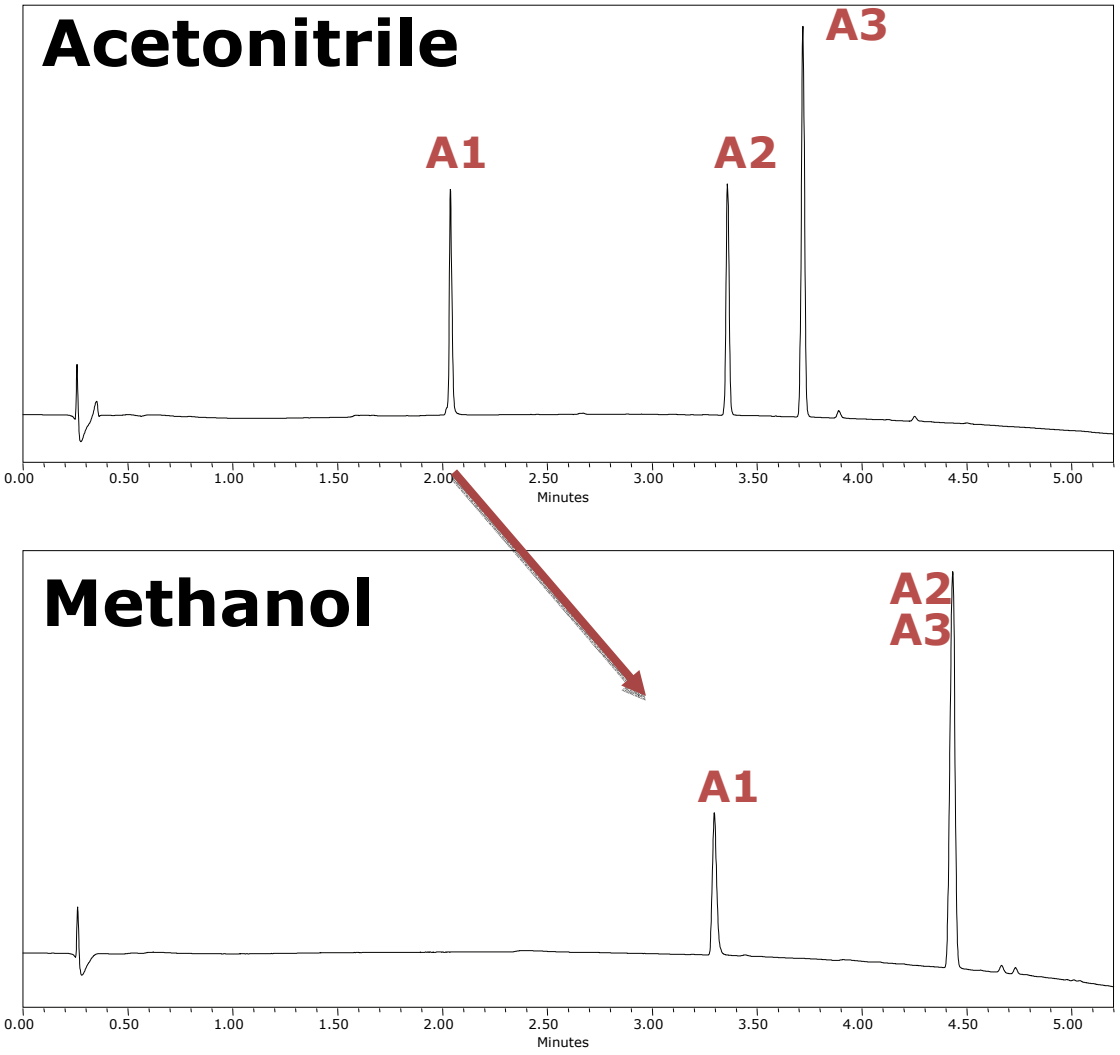
ACQUITY UPLC BEH C18



Test Probes:

A1 1-pyrenesulfonic acid
A2 diclofenac
A3 dinoseb

Methanol is weaker elution solvent resulting in greater retention for all analytes

Elution order change

Selectivity Tools

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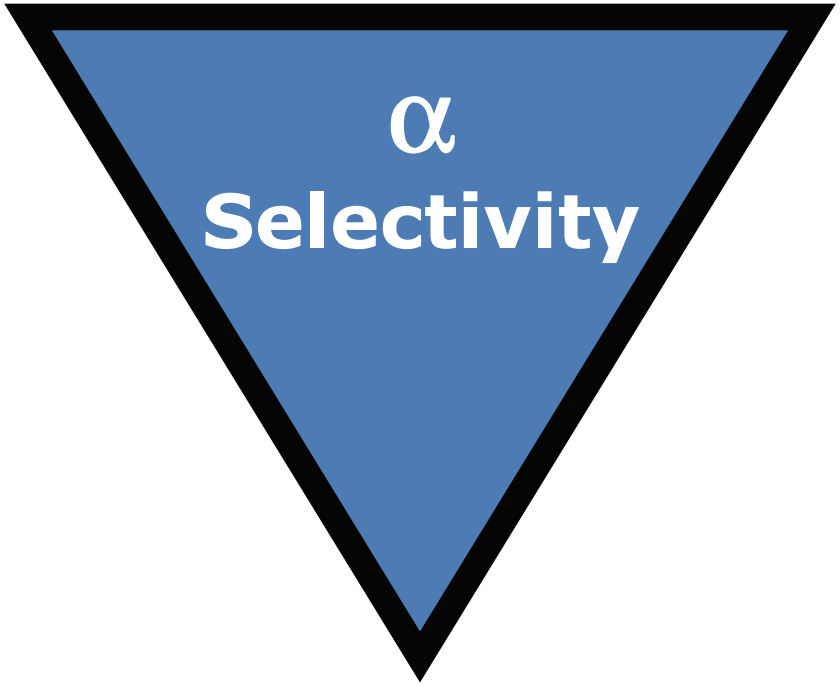
Solvent

pH



Column Chemistry

Effect of Mobile Phase pH

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- Affects only analytes with ionizable functional groups
  - Amines
  - Carboxylic acids
  - Phenols
- Some compounds contain one or more ionizable function
- Strongest selectivity effects can be caused by pH changes

Reversed-Phase Retention Map: The Importance of Mobile Phase pH

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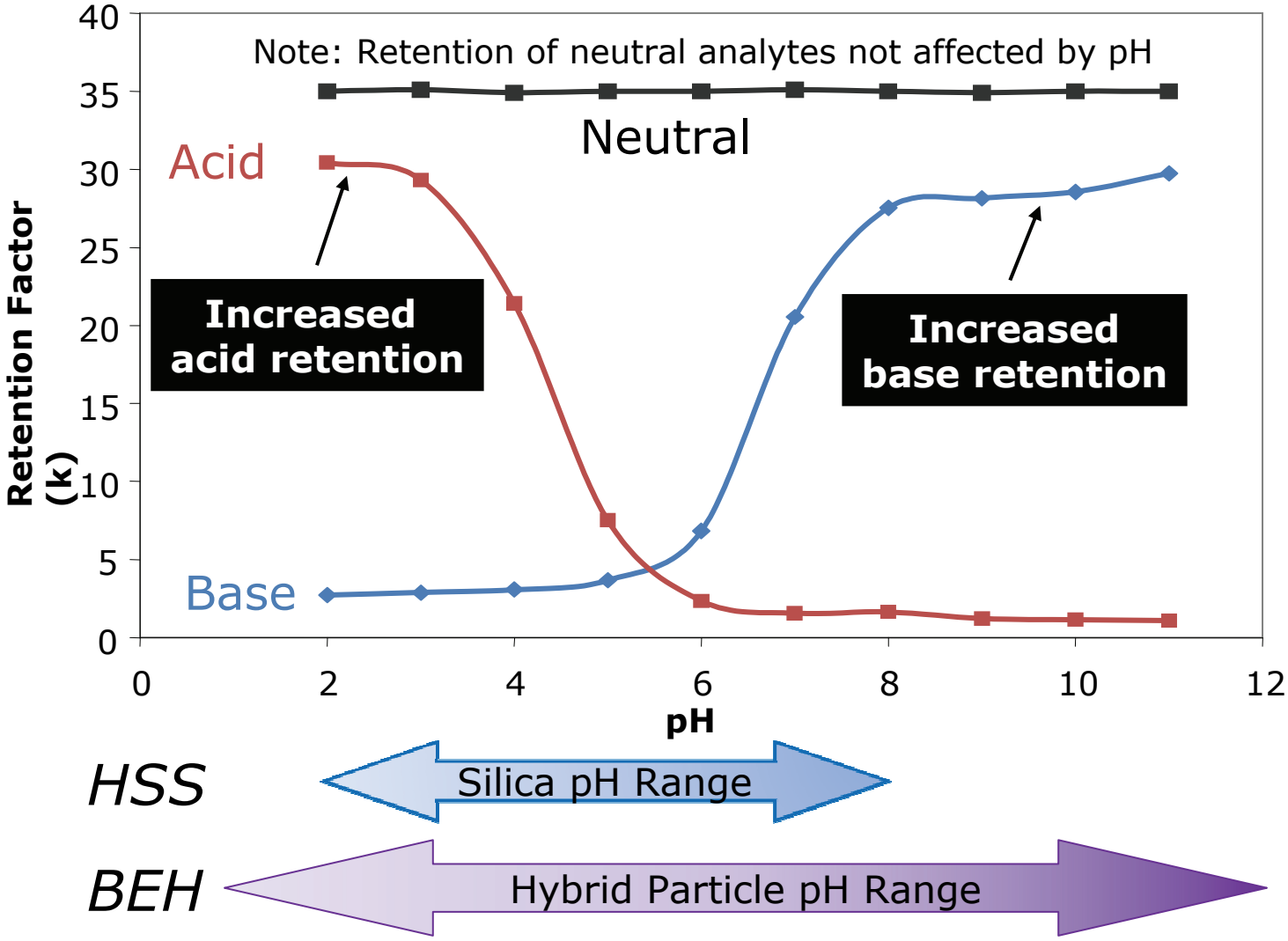

Neue et. al. American Laboratory 1999 (22) 36-39.

pH Selectivity: Basic and Neutral Compounds

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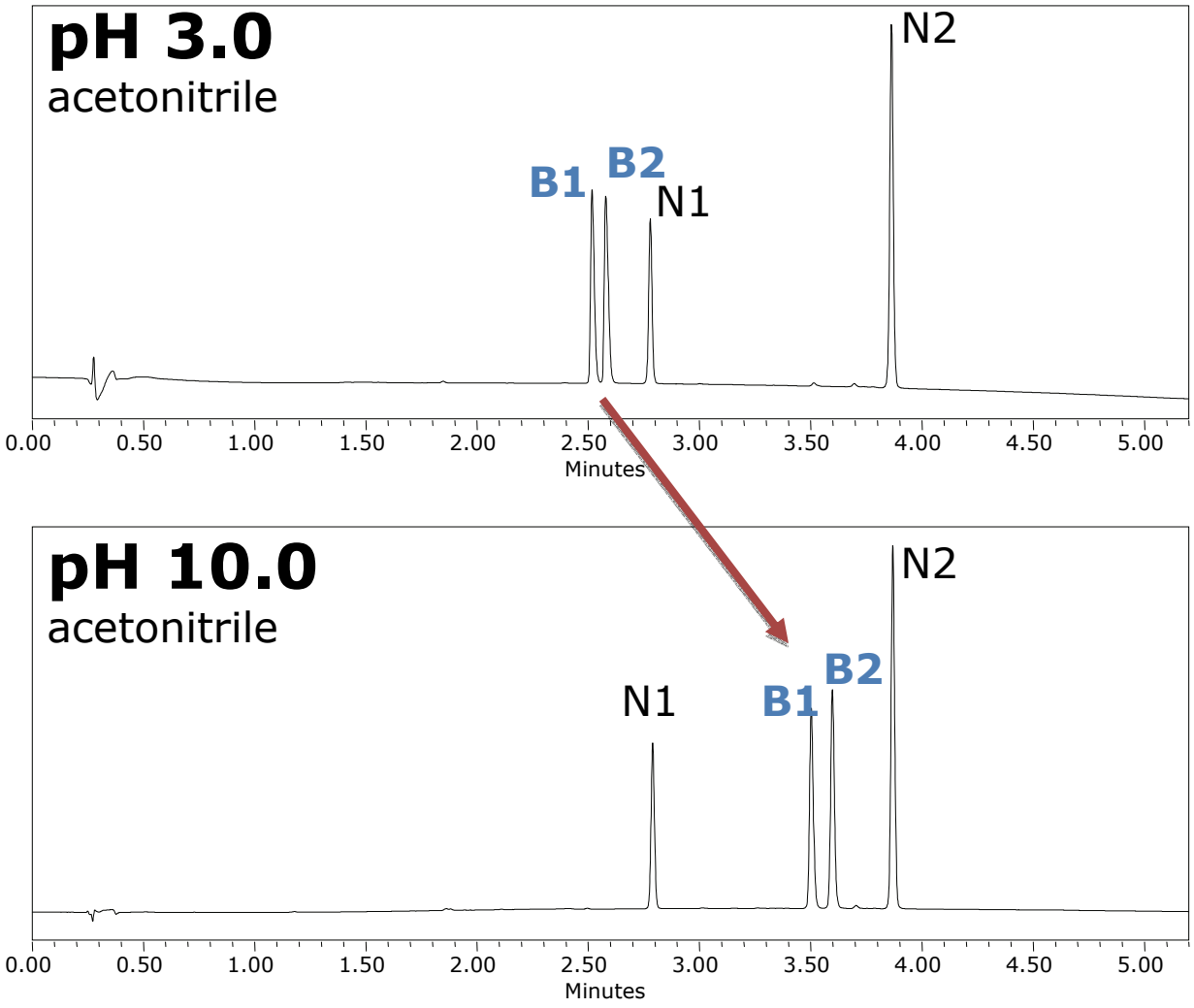
ACQUITY UPLC BEH Phenyl



Test Probes:
B1 imipramine
B2 amitriptyline
N1 flavone
N2 octanophenone

Neutrals unaffected by pH

At alkaline pH, bases are in their un-ionized form, resulting in greater retention

pH Selectivity: Acidic Compounds

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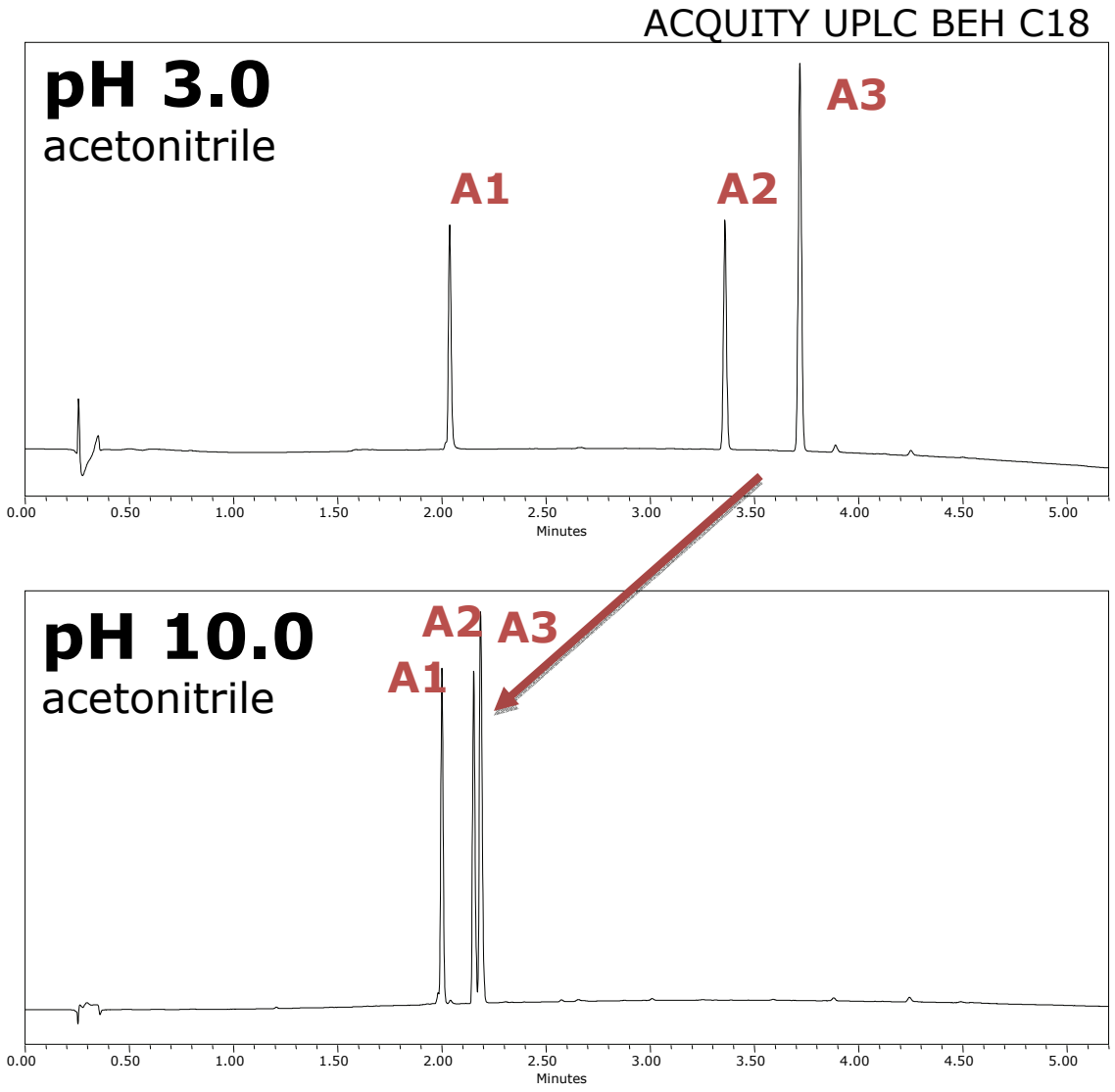

Test Probes:

A1 1-pyrenesulfonic acid
A2 diclofenac
A3 dinoseb

At acidic pH, acids are in their un-ionized form resulting in greater retention

Same elution order

Dramatic change in selectivity

Selectivity Tools: Combining Chemical Factors

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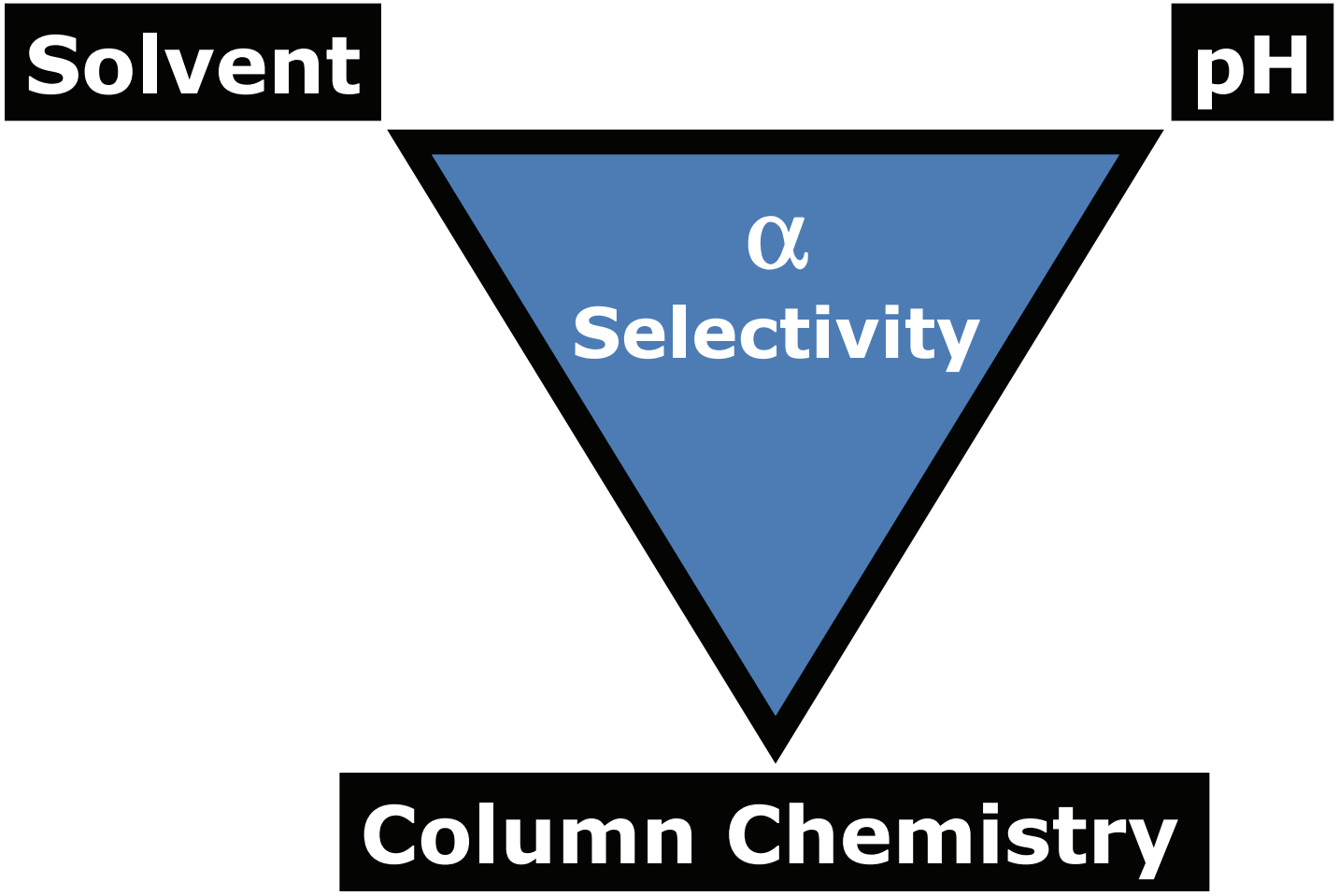

Maximizing Selectivity Differences: Basic and Neutral Compounds

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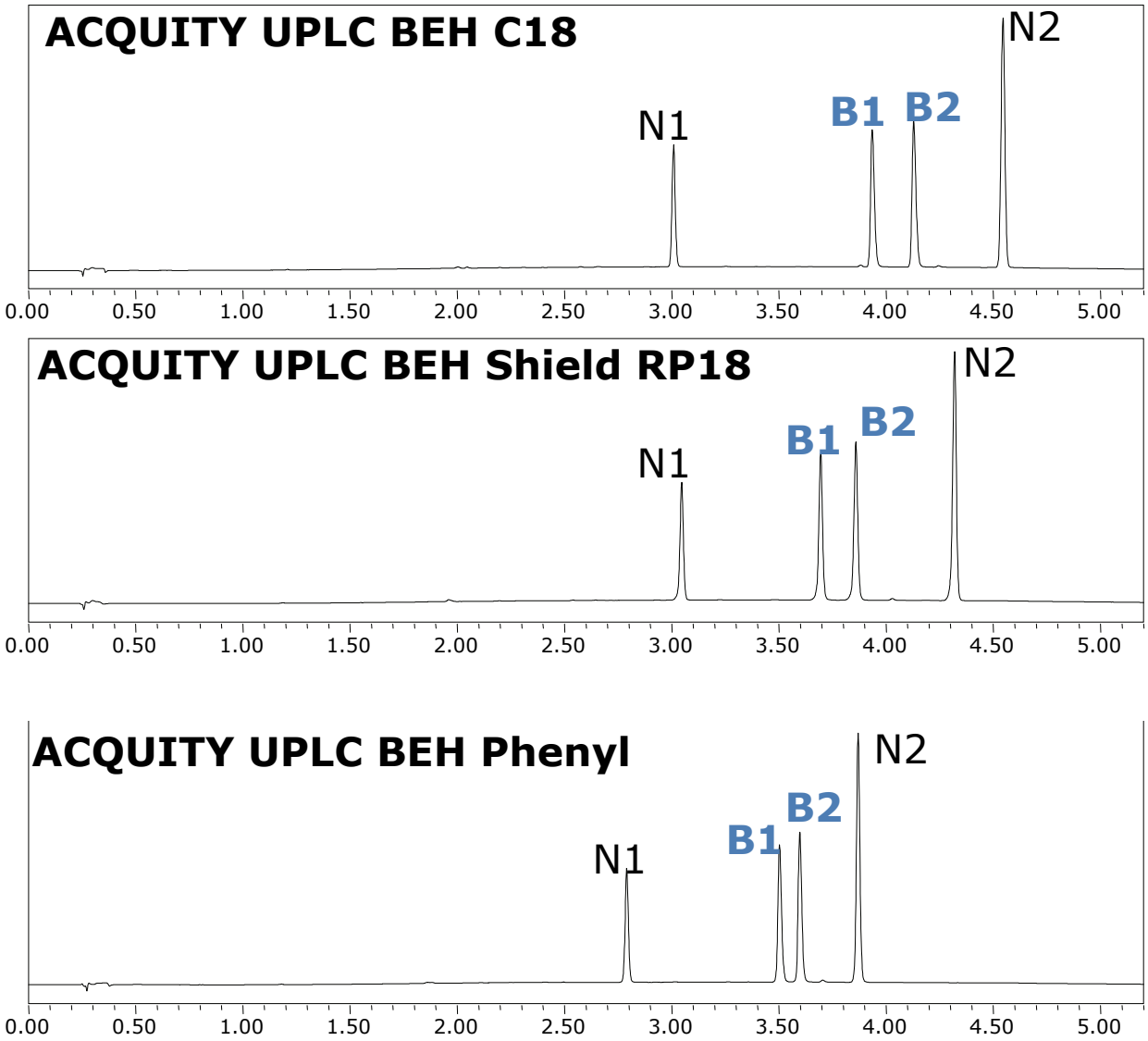

Acetonitrile, pH 10.0

Test Probes:
B1 imipramine
B2 amitriptyline
N1 flavone
N2 octanophenone

Small differences in stationary phase selectivity occur with un-ionized analytes and acetonitrile as organic modifier

Maximizing Selectivity Differences: Basic and Neutral Compounds

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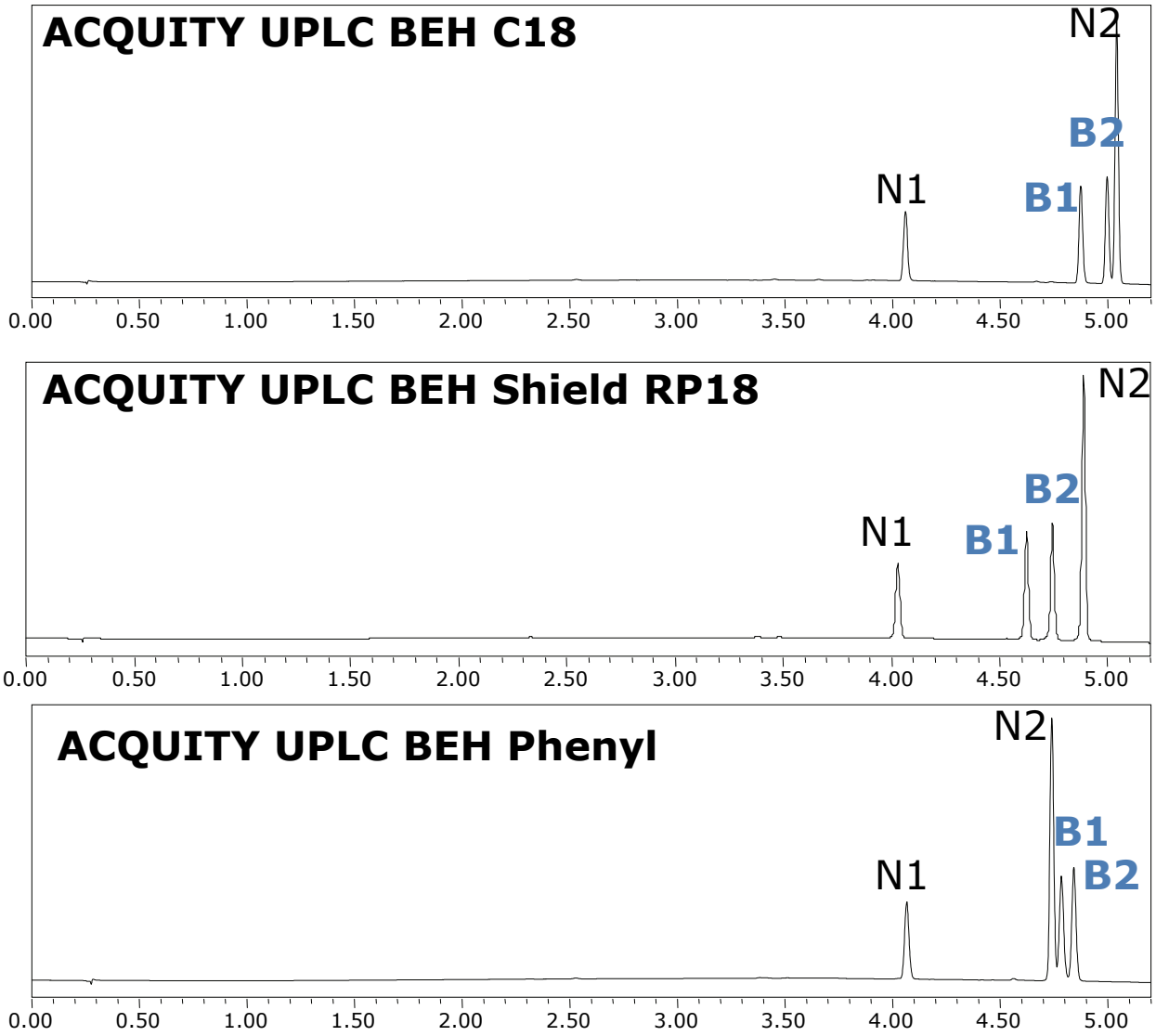

Methanol, pH 10.0

Test Probes:
B1 imipramine
B2 amitriptyline
N1 flavone
N2 octanophenone

Large differences in stationary phase selectivity with un-ionized analytes and methanol as organic modifier

Maximizing Selectivity Differences: Acidic Compounds

Waters
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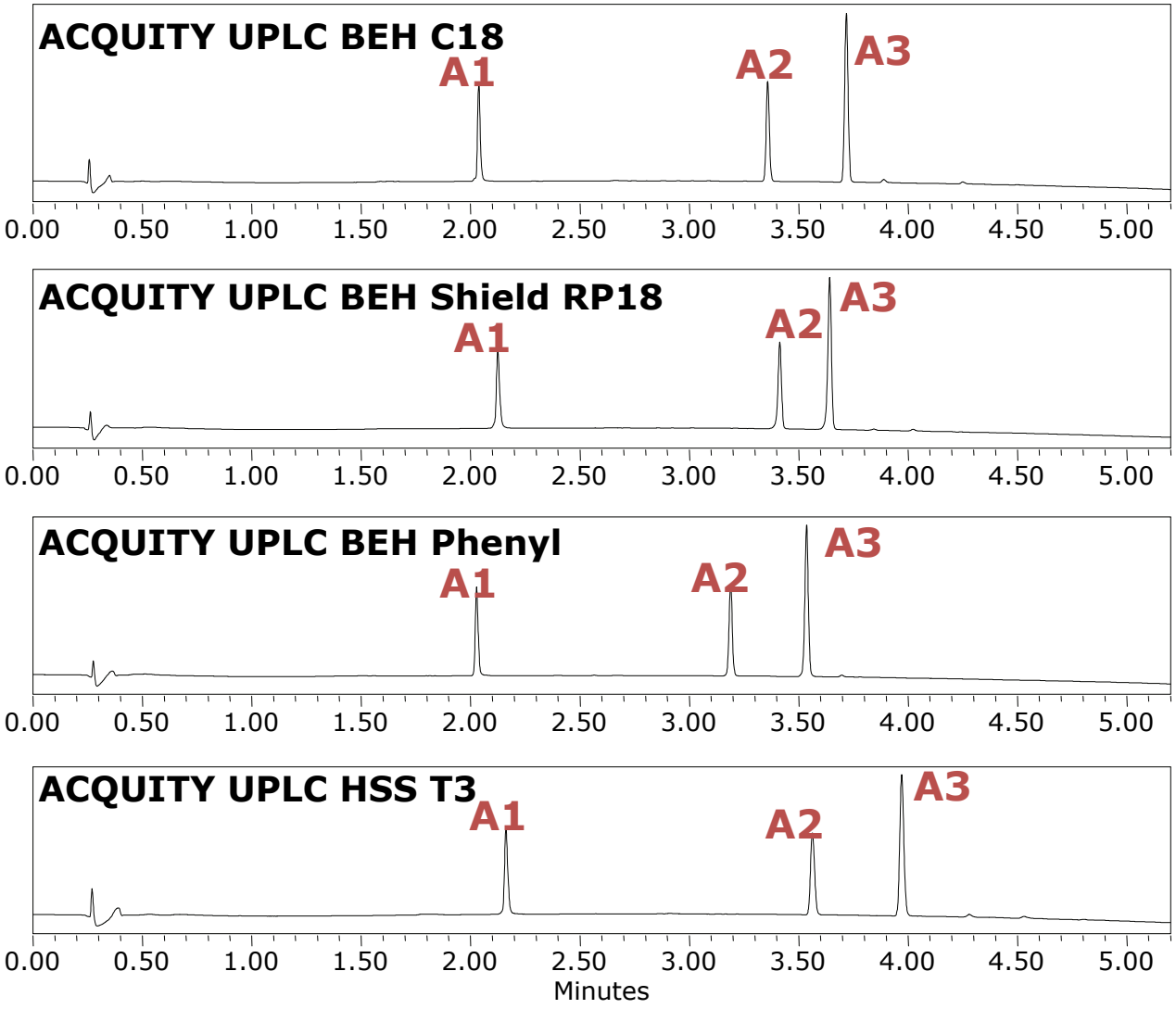

Acetonitrile, pH 3.0

Test Probes:

A1 1-pyrenesulfonic acid
A2 diclofenac
A3 dinoseb

Small differences in stationary phase selectivity with un-ionized analytes and acetonitrile as organic modifier

Maximizing Selectivity Differences: Acidic Compounds

Waters
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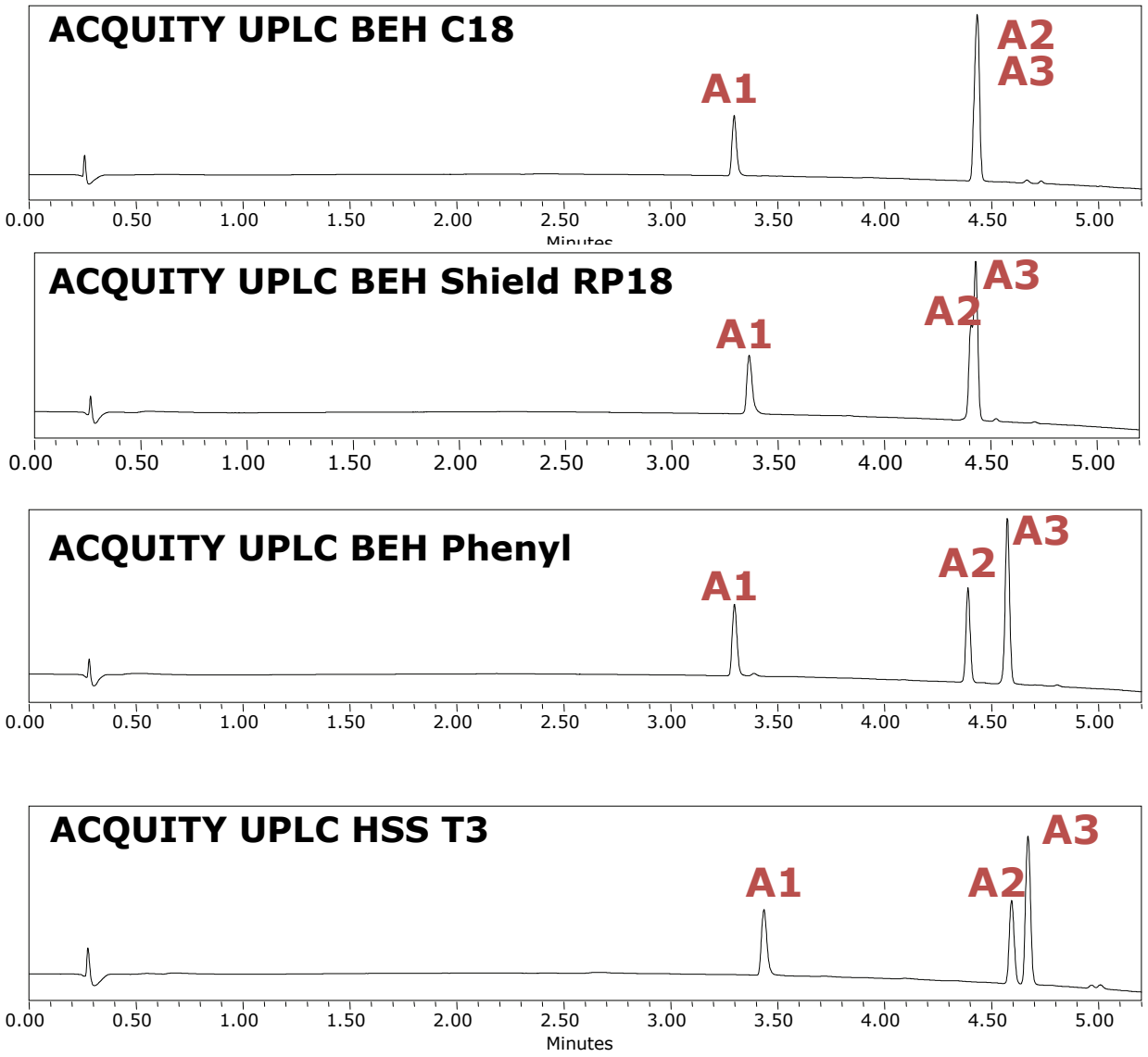

Methanol, pH 3.0

Test Probes:

A1 1-pyrenesulfonic acid
A2 diclofenac
A3 dinoseb

Large differences in stationary phase selectivity with un-ionized analytes and methanol as organic modifier

Selectivity Observations

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- Analytes in their un-ionized forms yield greater retention
- Methanol increases retention of all components compared to acetonitrile
- Large differences in selectivity are observed when change in pH alters charge state
- These four bonded phases yield similar results employing acetonitrile
- Largest selectivity differences between bonded phases occured with methanol as organic modifier and analytes in their un-ionized state

Selectivity Summary

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- Manipulation of parameters for method development remain the same, HPLC or UPLC separations
  - Acetonitrile and methanol
  - pH 3 and pH 10
- BEH particle technology enables the exploration of pH extremes in method development
  - Stability from pH 1 - 12
- Under the conditions and analytes of this study, large selectivity differences occurred between bonded phases with
  - Methanol as organic modifier
  - Analytes in their un-ionized state
- Evaluation of data from the complete screening matrix was essential to fully understand the analytes' chromatographic behavior

Why UPLC technology for method development?

Same Resolution and Selectivity with Increased Speed - Constant L/dp

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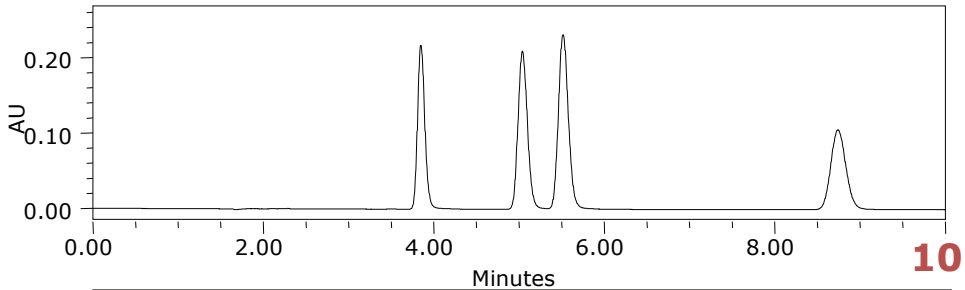
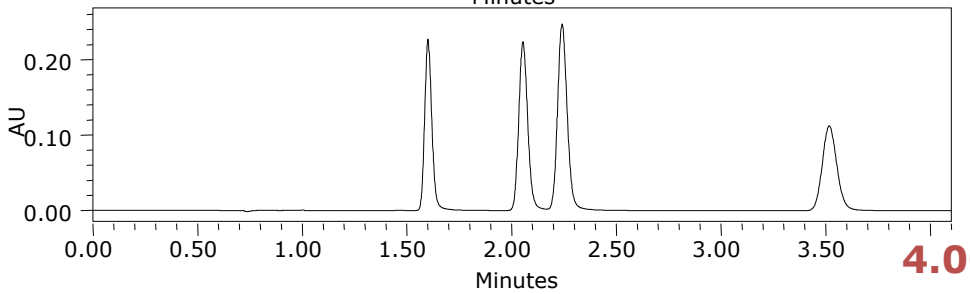
XBridge™ COLUMNS



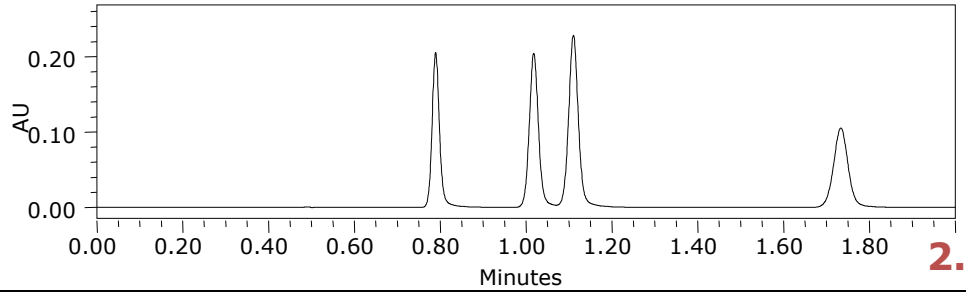
5 µm – 150 mm
F = 200 µL/min
Injection = 5.0 µL
Rs (2,3) = 2.28

10.00



3.5 µm – 100 mm
F = 300 µL/min
Injection = 3.3 µL
Rs (2,3) = 2.32

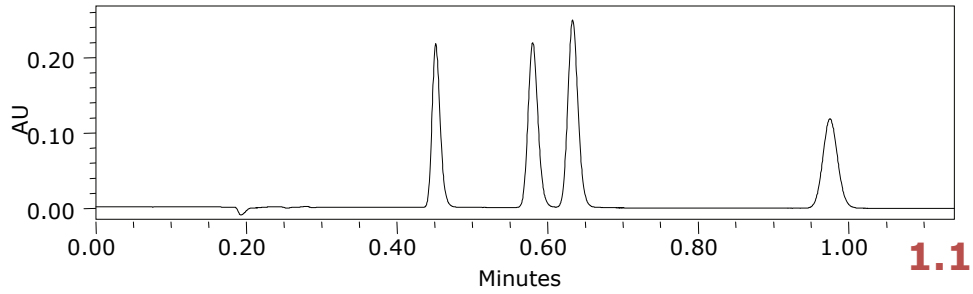
4.00



2.5 µm – 75 mm
F = 500 µL/min
Injection = 2.5 µL
Rs (2,3) = 2.34

2.00

Acquity UltraPerformance LC®



1.7 µm – 50 mm
F = 600 µL/min
Injection = 1.7 µL
Rs (2,3) = 2.29

1.10

Develop Methods Faster with UPLC Technology: Time Savings

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UPLC Gradient Conditions:
Column Dimensions: 2.1 x 50 mm, 1.7 µm
Flow Rate: 0.5 mL/min
Gradient:

Time (min)	Profile %A	%B	Curve
0.0	95	5	6
5.0	10	90	6

Peak Capacity (P_c) = 150

Equivalent HPLC Gradient Conditions:
Column Dimensions: 4.6 x 100 mm, 3.5 µm
Flow Rate: 1.4 mL/min
Gradient:

Time (min)	Profile %A	%B	Curve
0.0	95	5	6
15.0	10	90	6

Peak Capacity (P_c) = 150

$$P_c = 1 + \frac{t_g}{w}$$

Develop Methods Faster with UPLC: Time Savings versus 3.5 µm HPLC Column

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UPLC Method Development Protocol
2.1 x 50 mm, 1.7 µm, 0.5 mL/min

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

SCOUTING TIME **2 Hours/ Hybrid column x 3 columns**

1 Hour/ Silica column x 1 column

TOTAL SCOUTING TIME **7 HOURS**

EQUIVALENT HPLC Method Development Protocol, 3.5 µm
4.6 x 100 mm, **3.5 µm,** 1.4 mL/min

pH 3/ acetonitrile	**Time**
Flow ramp	5 min
Column conditioning (2 blank gradients)	37.7 min
Sample injection (2 replicates)	37.7 min
pH 3/ methanol	
Flow ramp	5 min
Column conditioning (2 blank gradients)	37.7 min
Sample injection (2 replicates)	37.7 min
Column purge	20.56 min
pH 10/ acetonitrile	
Flow ramp	5 min
Column conditioning (2 blank gradients)	37.7 min
Sample injection (2 replicates)	37.7 min
pH 10/ methanol	
Flow ramp	5 min
Column conditioning (2 blank gradients)	37.7 min
Sample injection (2 replicates)	37.7 min
Column purge	20.56 min
	362.72 min

SCOUTING TIME **6 Hours/ Hybrid column x 3 columns**

3 Hours/ Silica column x 1 column

TOTAL SCOUTING TIME **21 HOURS**

UPLC scouting **3X faster** than 3.5 µm HPLC

Outline

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- Approaches Towards Method Development
- Selectivity and Retention Tools
  - Stationary Phase
  - Solvent
  - pH
- Method Development Strategy
- Applications
- Conclusions

Automated Method Development and Validation

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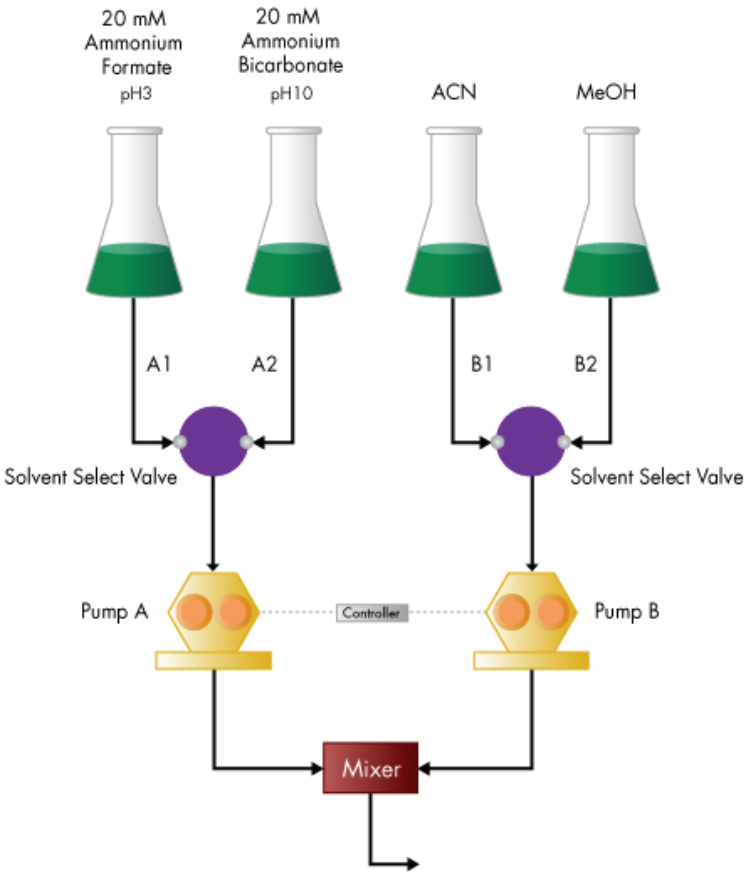

- Automated Method Development
  - ACQUITY UPLC Column Manager, 4-column selection device
    - Utilize 2.1 x 50 mm columns
  - ACQUITY UPLC Binary Solvent Manager, solvent select valves
    - Utilize pH 3 and pH 10 buffers
    - Utilize methanol and acetonitrile
  - Fast 5-minute gradient 5 – 90% B, Flow = 0.5 mL/min
- Automated Method Validation
  - Empower™ 2 Method Validation Manager, streamlines method validation process

Selectivity Scouting Protocol

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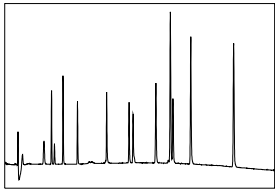
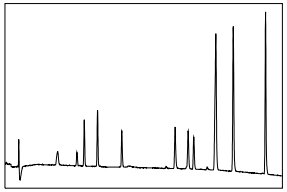
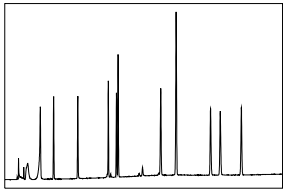
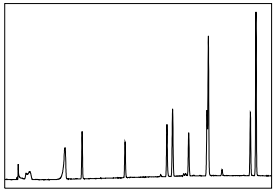
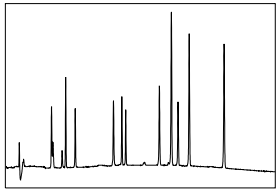
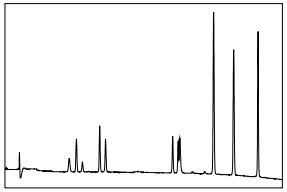
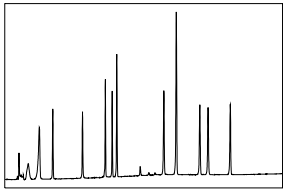
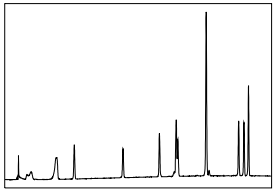
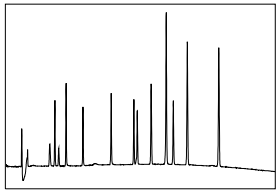
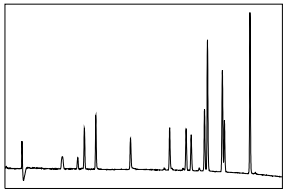
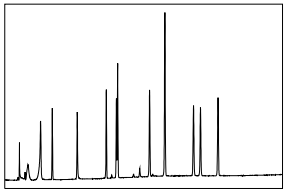
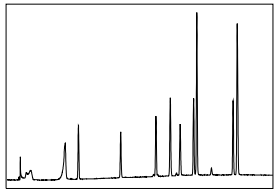
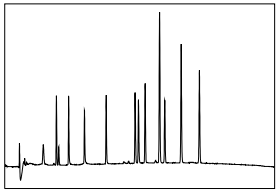
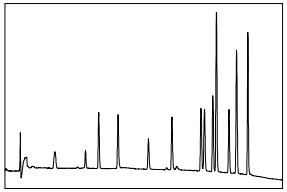
2.1 x 50 mm, <2 µm

	pH 3, ACN	pH 3, MeOH	pH 10, ACN	pH 10, MeOH
ACQUITY UPLC BEH C_{18}				
ACQUITY UPLC BEH Shield RP_{18}				
ACQUITY UPLC BEH Phenyl				
ACQUITY UPLC HSS T3				

Optimization

Outline

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- Approaches Towards Method Development
- Selectivity and Retention Tools
  - Stationary Phase
  - Solvent
  - pH
- Method Development Strategy
- Applications
  - Method Optimization
- Conclusions

Illustrating the Method Development Approach

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- As we go through the data for each separation, we should have some questions in mind to help select the best pH, organic solvent and column
  - Do I need resolution of every peak or simply my main component?
  - How much resolution do I need?
  - How much retention do I need and what is the retention profile?
  - What are the tailing factors?
  - Do I want a gradient or isocratic method?
- Step 1: Review data and select pH
- Step 2: Review data and select organic solvent and column
- Step 3: Optimize/fine-tune separation

Automated Method Development and Validation of Paroxetine and Related Compounds

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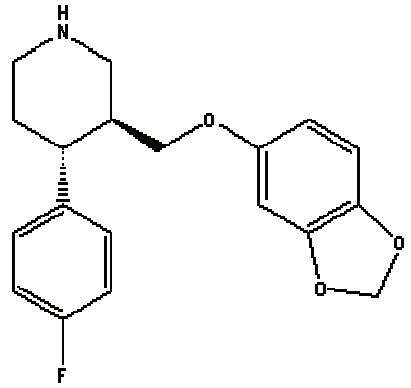
- Method Development
  - Use systematic screening protocol
  - Paroxetine (API) concentration: 0.2 mg/mL in 50:50 MeOH:H_2O
  - Related compounds at 10% concentration of API for easy identification during scouting
- Method Optimization
  - Related compounds at 0.1% concentration of API
    - Related compounds B, D, F and G
- Method Validation
  - Empower™ 2 Method Validation Manager



Paroxetine
m.w. 374.8

Stationary Phase Selectivity: Paroxetine

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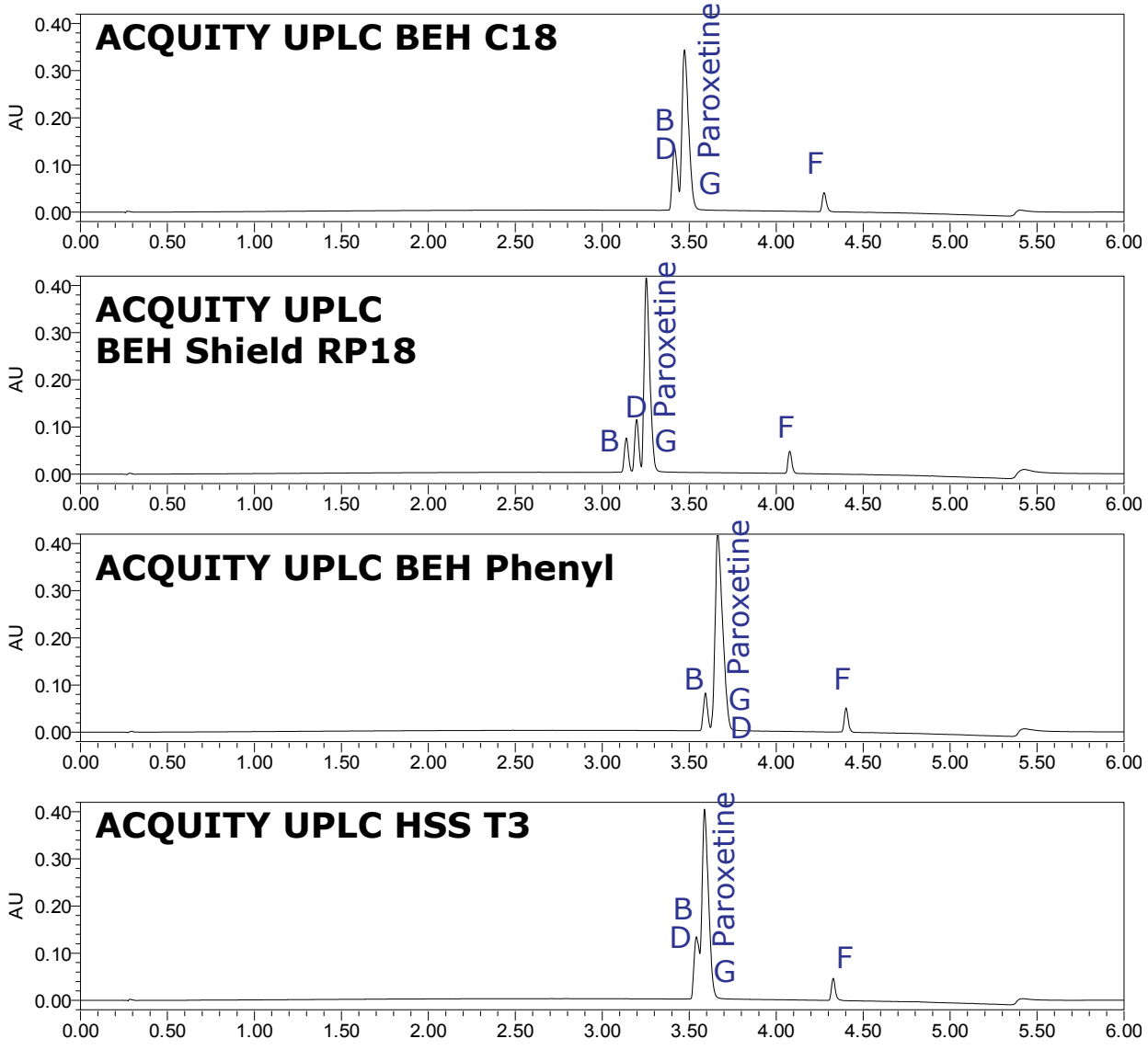

Methanol pH 3.0

Observation:

Poor resolution of paroxetine and related compounds (RC)

Action:

Investigate high pH

pH Selectivity: Paroxetine

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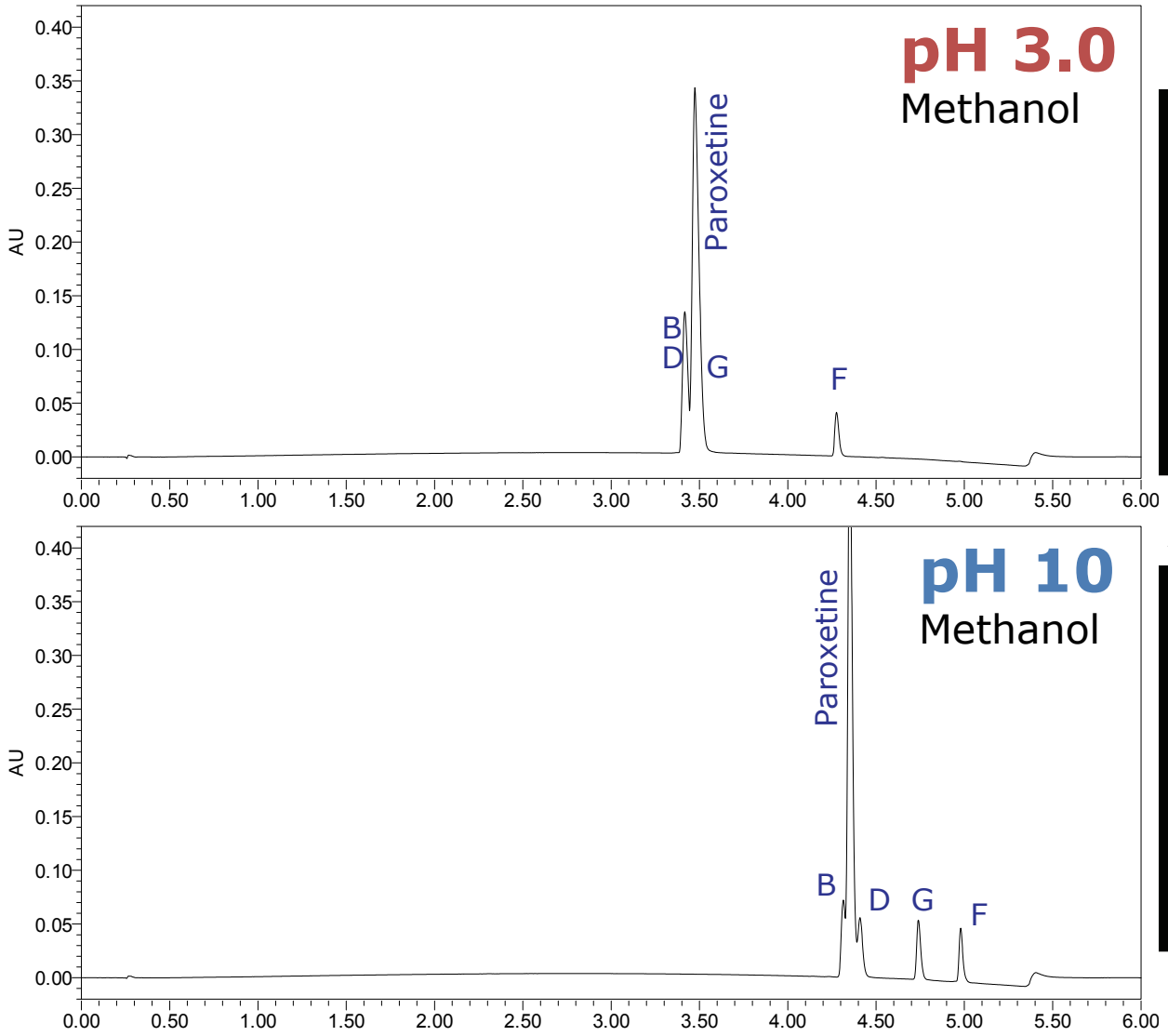

Observation:

Better retention and resolution of API from RC due to neutral charge state of analytes at alkaline pH

Actions:

Select pH 10 due to better separation

Compare stationary phase selectivity with pH 10 buffer

Stationary Phase Selectivity: Paroxetine

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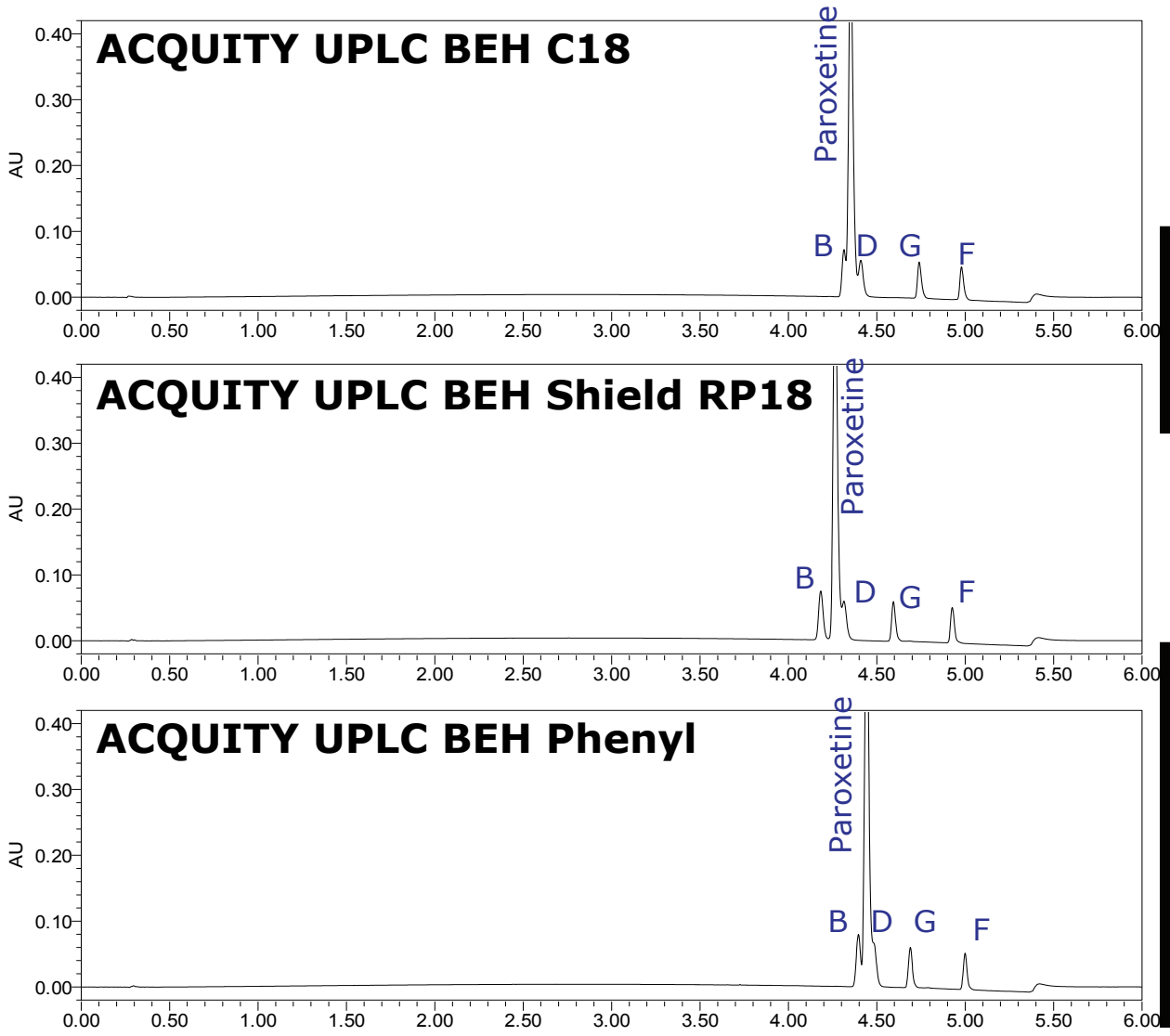

Methanol pH 10.0

Observation:

Any column may provide successful separation

Actions:

Select ACQUITY UPLC BEH C18

Compare selectivity between organic modifiers

Solvent Selectivity: Paroxetine

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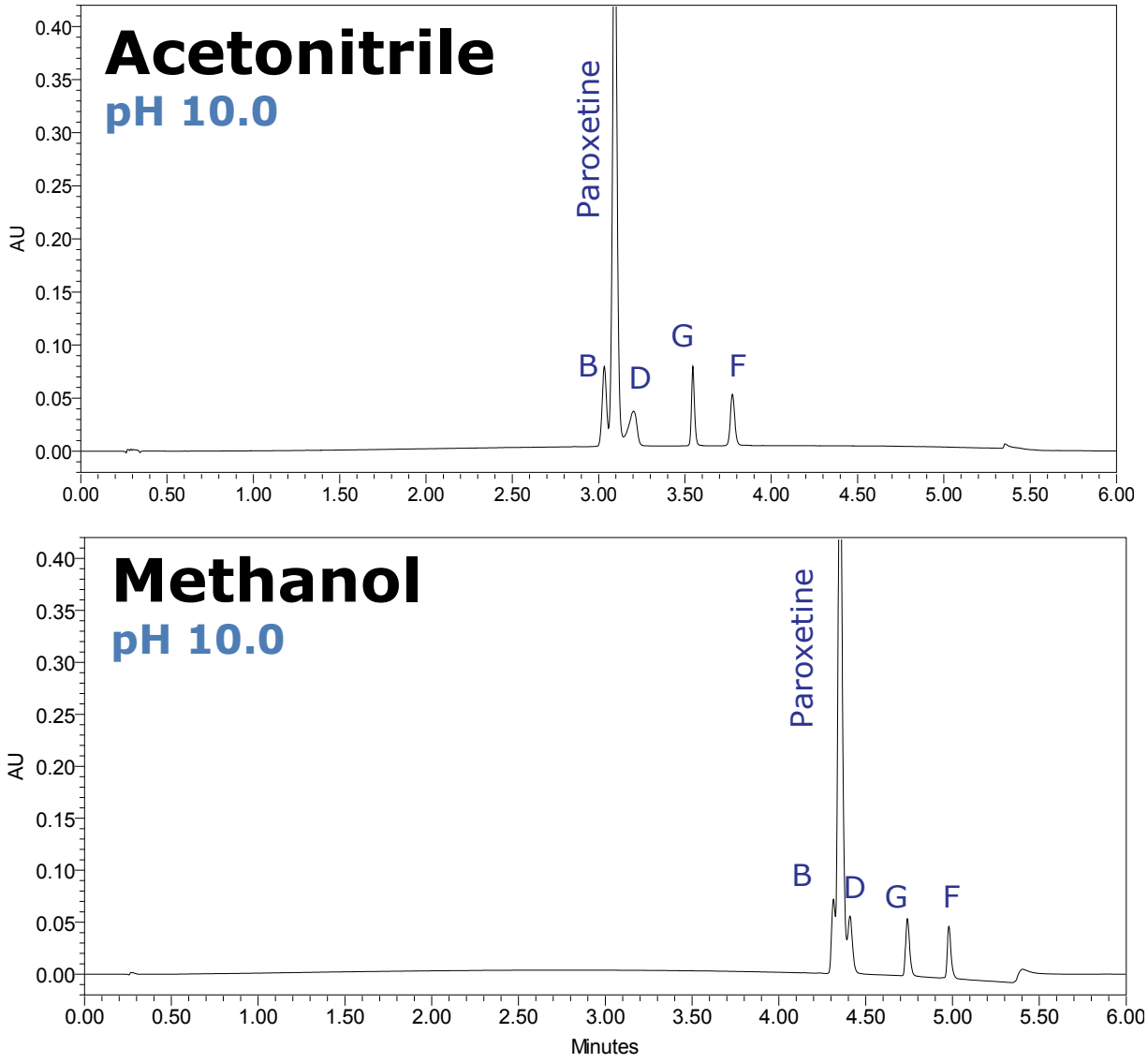

Observations:

Methanol is weaker elution solvent resulting in greater retention

Better resolution exhibited with acetonitrile as organic modifier

Actions:

Select acetonitrile as organic modifier

Optimize separation using appropriate concentration of RC

CM, ESG

Related Compounds at 0.1% Concentration of Paroxetine

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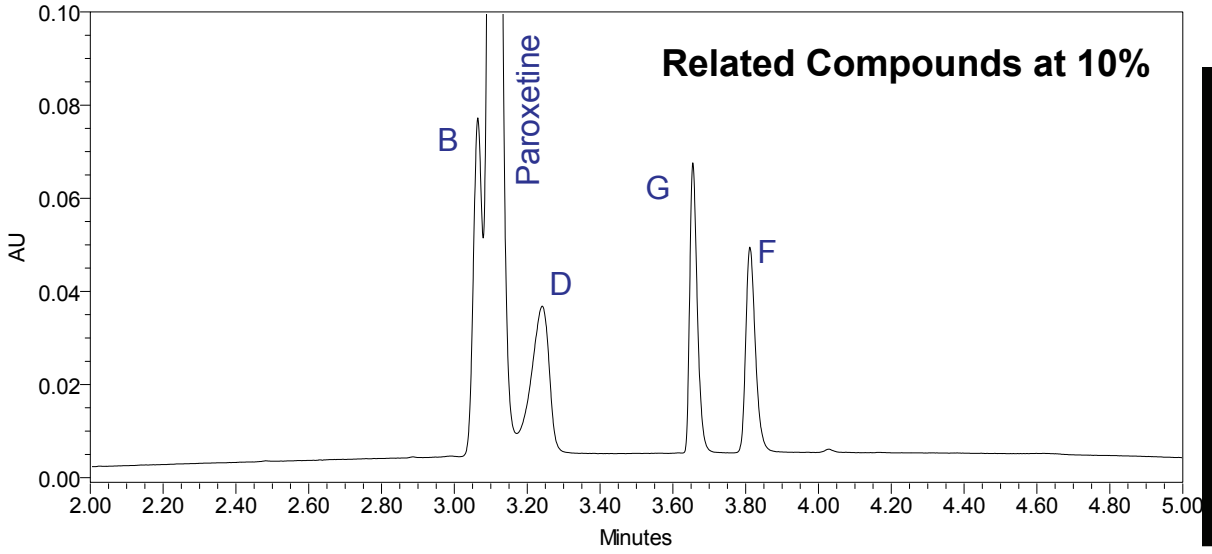

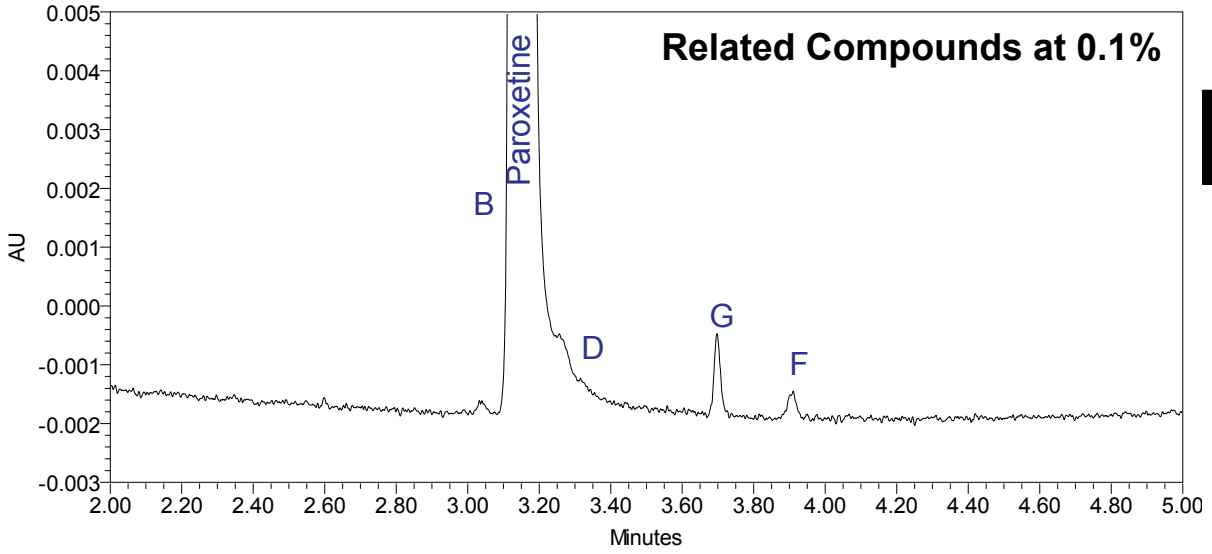

Observation:

Inadequate resolution among paroxetine and related compounds B and D due to disparate levels of concentration

Action:

Optimize Separation

Factors that Control Retentivity and Selectivity: Optimization Tools

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- Once the pH, mobile phase, and column are selected, the method can be fine-tuned and optimized
- Parameters for optimization
  - Gradient slope
  - Temperature

Method Optimization: Gradient Slope

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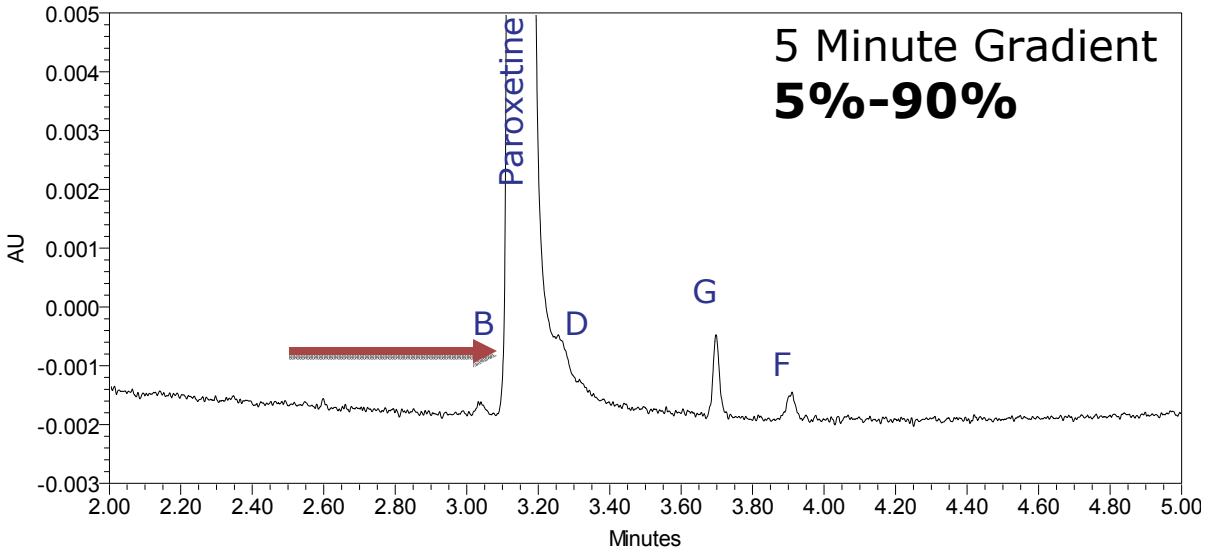
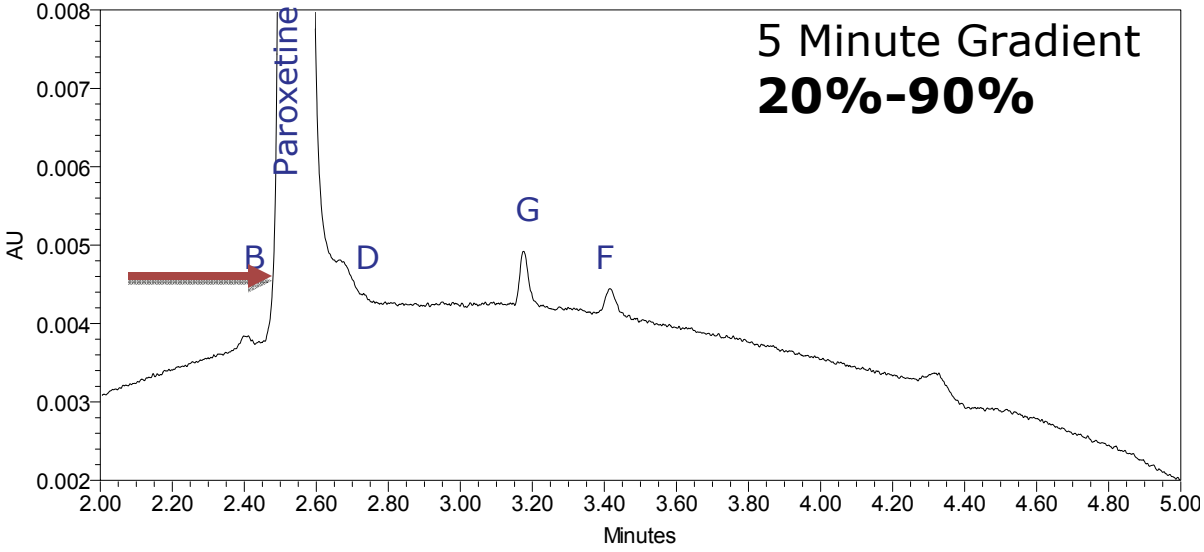
- Shallower gradient slope may improve resolution
  - Decreasing gradient slope will decrease sensitivity
- Steeper gradient slope may compress the peaks and often reduce the resolution
  - Increasing gradient slope will increase sensitivity
- Changing gradient slope is a balance between peak heights relative to resolution
- Changes in retentivity and selectivity

Method Optimization: Gradient Slope





Acetonitrile pH 10.0
30 °C

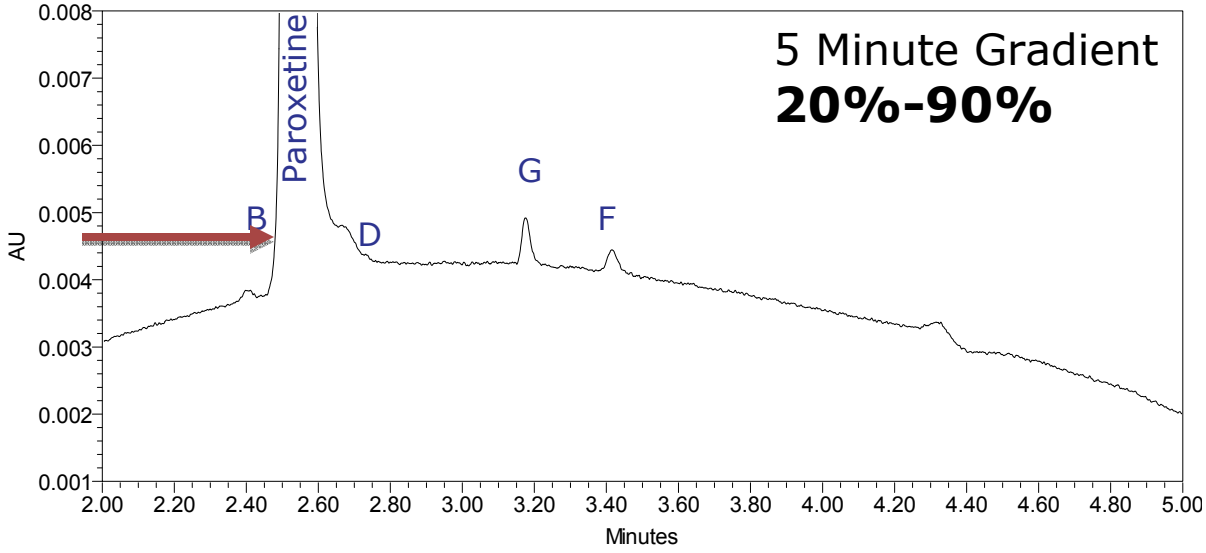
Observations:

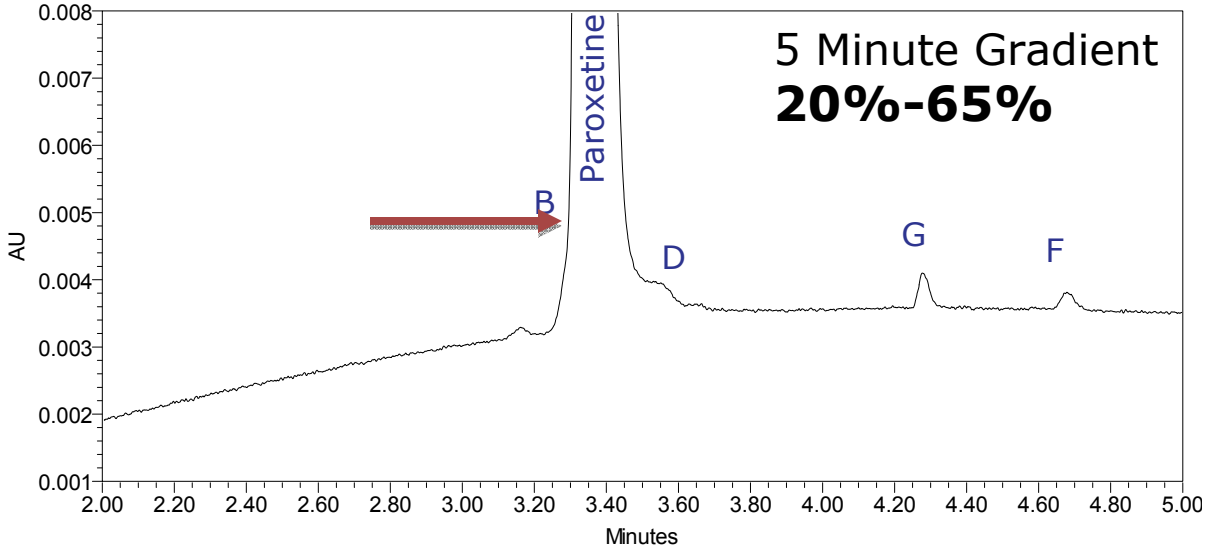
Marginal improvement in separation of RC from parent compound with shallow gradient slope

Action:

Alter gradient endpoint to produce shallower slope

Method Optimization: Gradient Slope





Acetonitrile pH 10.0
30 °C

Observations:

Resolution remains inadequate with shallow gradient slope

Action:

Investigate column temperature

CM, ESG

Method Optimization: Influence of Temperature

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- Reduced mobile phase viscosity
- Lower backpressure
  - If flow rate is held constant
- Improve analyte diffusivity
  - Higher optimal linear velocity
- Changes in retention and selectivity

Method Optimization: Column Temperature

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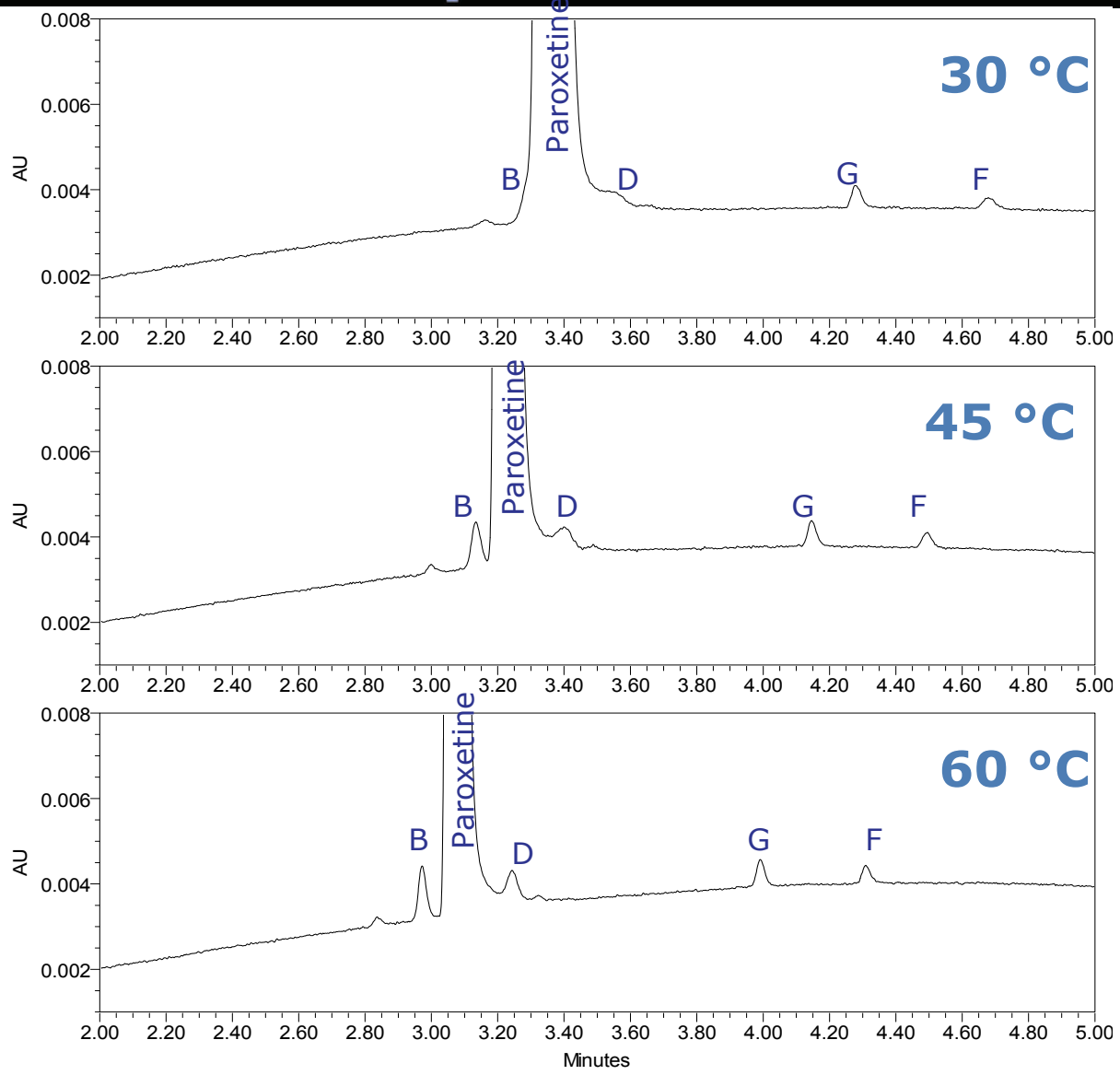

Acetonitrile pH 10.0

Observations:

Higher temperature improves separation of RC from paroxetine

Peak shape improves as temperature increases

Action:

Select 60 °C for best resolution and peak shape

Final Method: Paroxetine and Related Compounds at 0.1%

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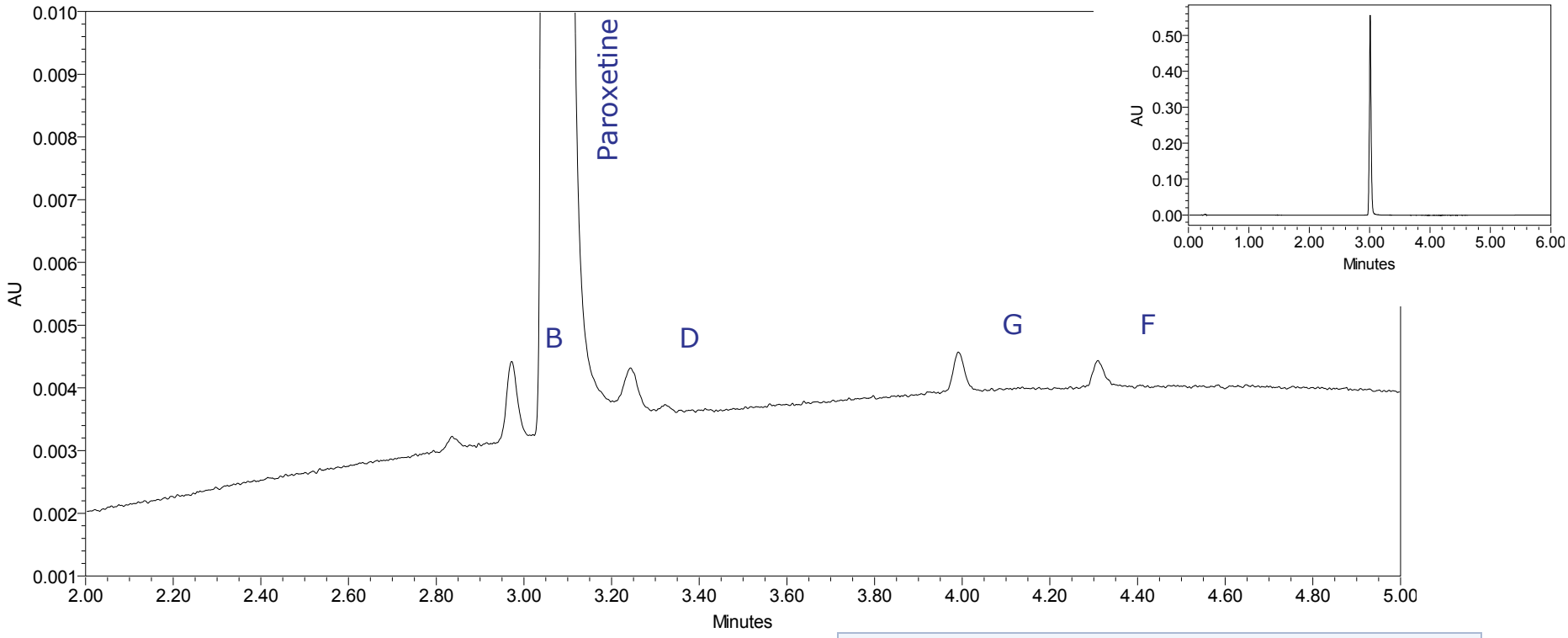

Compound	USP Rs
Related compound B	
Paroxetine	1.95
Related compound D	3.07
Related compound G	13.00
Related compound F	6.74

ACQUITY UPLC BEH C18
2.1 x 50 mm, 1.7 µm
20 mM ammonium bicarbonate, pH 10
60 °C
5 Min Gradient, 20%-65% acetonitrile
F = 0.5 mL/min
Inj. Vol. 4 µL
UV @ 295 nm

Analytical Method Validation

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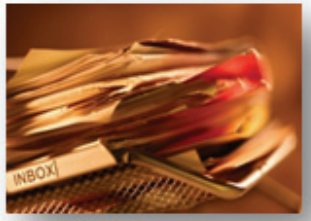
Create Sample Sequence

Data Acquisition & Processing

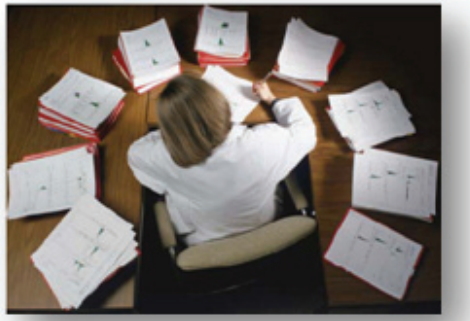
Calculation Statistical Results



Reports Compiled

Prepare Standards & Samples

Corporate Method Validation SOP



Data Management

Time-consuming, repetitive tasks consisting of several sequential steps

Analytical Method Validation with Empower 2 Method Validation Manager

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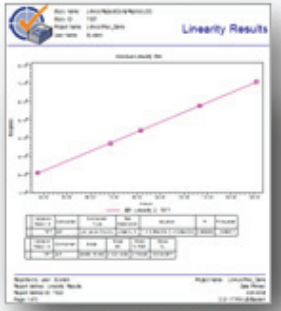
Create Sample Sequence

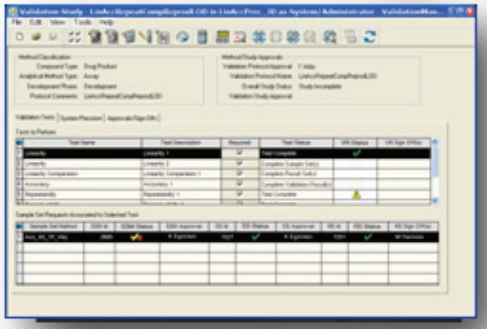
Data Acquisition & Processing

Calculation Statistical Results



Reports Compiled

Prepare Standards & Samples



Data Management

Faster and Easier Method Validation

Automated Method Validation Manager: Paroxetine Validation Results

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Parameter	Acceptance Criteria	Reported Value	Pass/Fail
Linearity	$R^2 \geq 0.995$ Residuals $\leq$ 2.0% RSD	0.9999 1.74% RSD	pass pass
Accuracy	80 – 120 %	97 – 102 %	pass
Intermediate Precision	Variance Component $\leq$ 10 %RSD Peak Area (Analyst, Instrument, Column)	Analyst 1.33% RSD Instrument 7.72% RSD Column 0.00% RSD	pass pass pass
LOD of impurities	Impurities 0.1% of active at 0.2 mg/mL	0.05% of active at 0.2 mg/mL (s/n 2.2 – 6.23)	pass
Method Robustness Peak Area	Variance Component $\leq$ 2 %RSD Peak Area (Buffer strength, Additive Conc., Column Temperature)	0.06 – 1.64% RSD	pass
Method Robustness Retention Time	Variance Component $\leq$ 5 %RSD Retention Time (Buffer strength, Additive Conc., Column Temperature, Flow Rate, Injection Volume)	0.00 – 2.57% RSD	pass

Method Development and Validation Timeline: Instrument Time

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UPLC Method Development and Validation Timeline

2.1 x 50 mm, 1.7 µm, 0.5 mL/min

Screening	**Time**
4 Columns, 2 Organics, 2 pHs	7.0 hours
Optimization	
Gradient Slope and Temperature	1.7 hours
Validation	
Accuracy, linearity, repeatability, Reproducibility, LOD/LOQ, Intermediate precision, robustness	21.1 hours

TOTAL TIME 29.8 HOURS

Conclusions

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- Achieve more resolution, faster by utilizing sub-2 µm UPLC columns
- Principles of method development remain the same
- Broad range of column selectivity to successfully develop methods efficiently
  - BEH C_{18}, BEH Shield RP18, BEH Phenyl and HSS T3
- UPLC Technology allows for efficient method development
  - Systematic scouting protocol
  - Automated column selection
  - High resolution sub 2 µm column technology
- UPLC Technology and Empower 2 software can significantly improve laboratory productivity and compliance

Acknowledgements

- Erin Chambers
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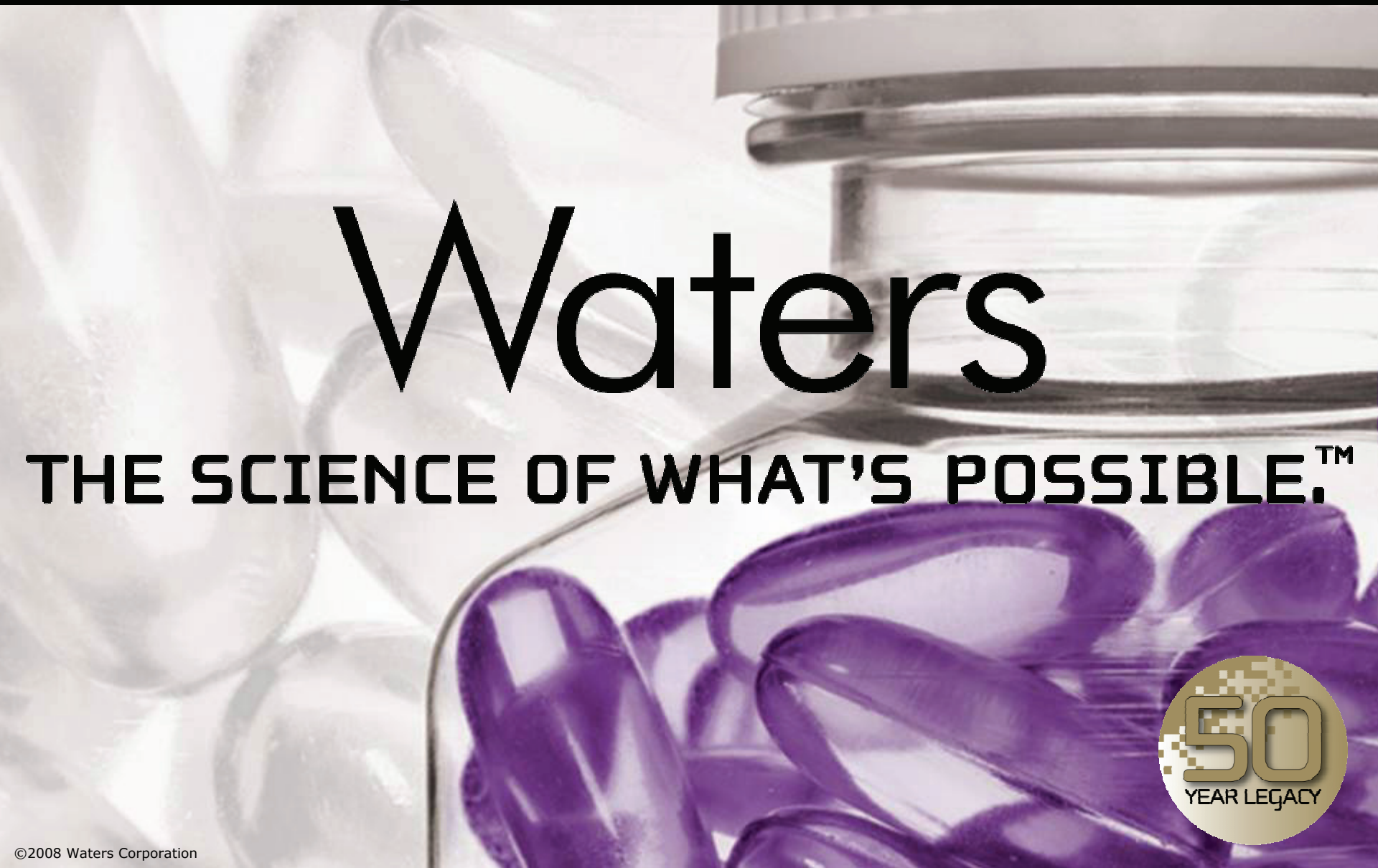
- Pat McDonald
- Christopher Messina
- Damian Morrison
- Tom Wheat



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50 YEAR LEGACY