Waters

PITTCON 2005
Orlando, Florida
28FEB – 03MAR

Acquity™
Ultra Performance LC

ACQUITY UPLC™ in the Chromatography Lab

Developing Methods

For Complete Confidence

Waters

What is UPLC™?

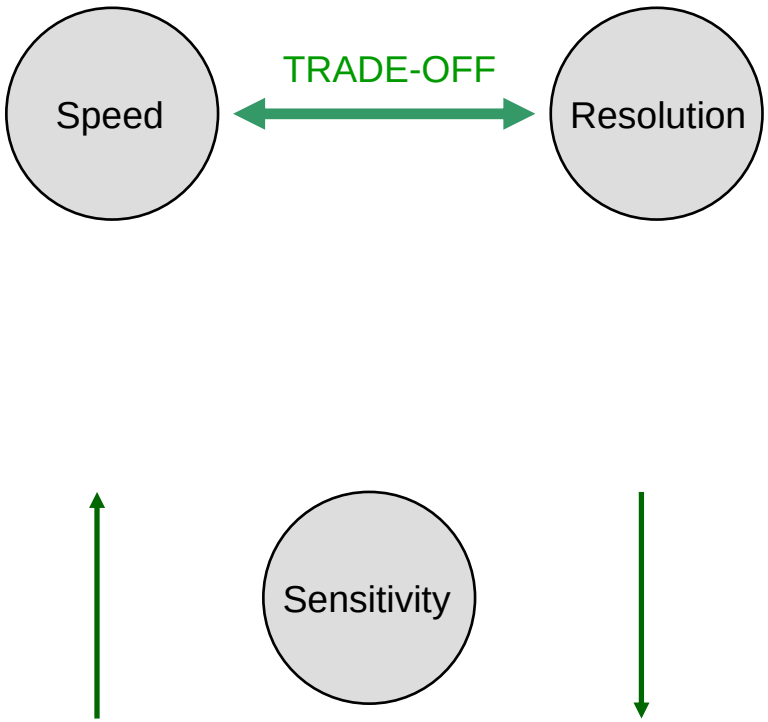
Ultra **P**erformance **L**iquid **C**hromatography

- UPLC™ is Particle Size Driven
- Requires a system which can sustain very high pressure to attain the linear velocities required to achieve the resolution enabled by small particles, and maintain that resolution through to detection

Speed, Resolution, Sensitivity

Balancing priorities

HPLC



UPLC™ Separations

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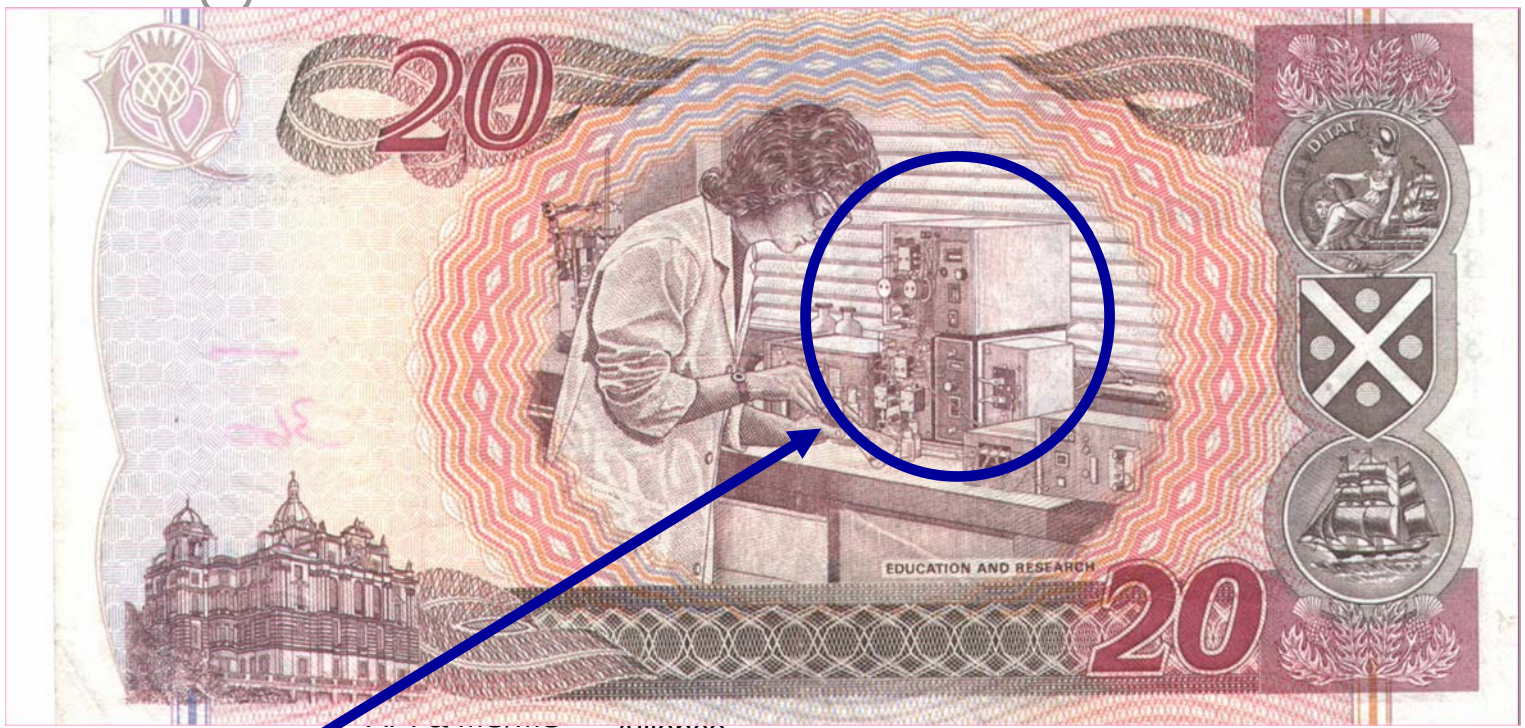
Technology Landscape

1960-2003



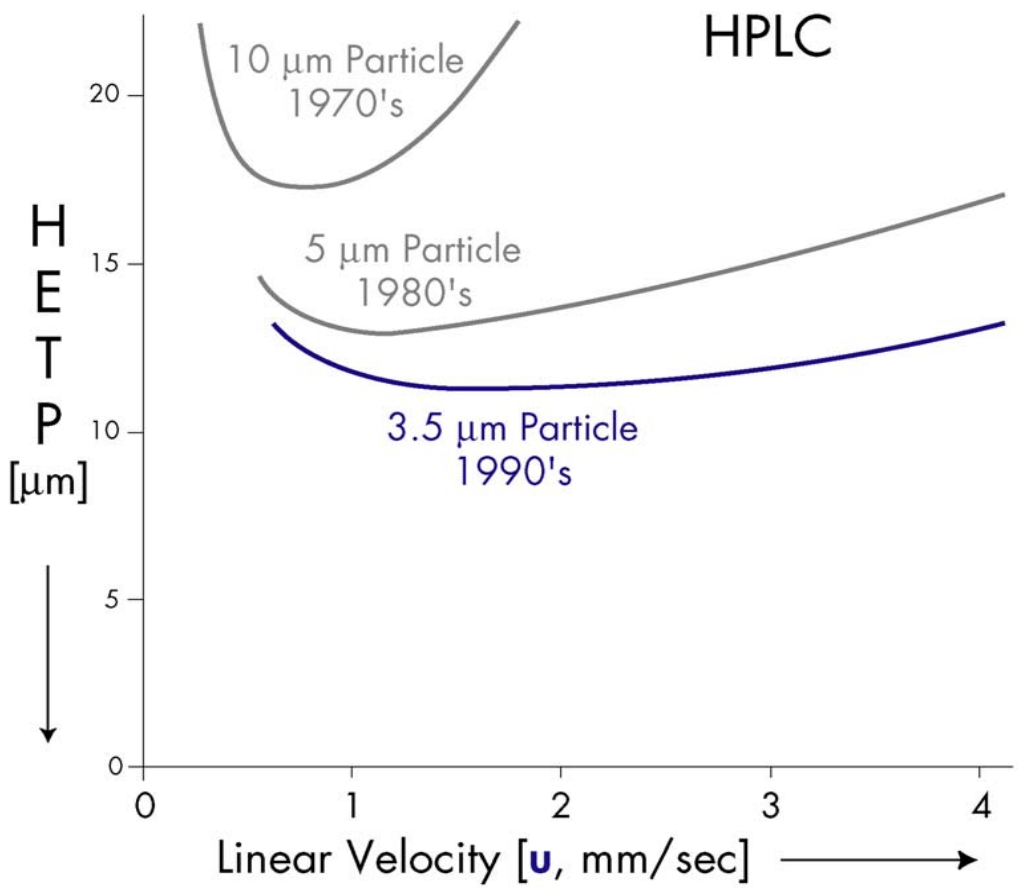
1985-87
Advances in laboratory networking

Alliance

1999
XTerra

Smaller Particles

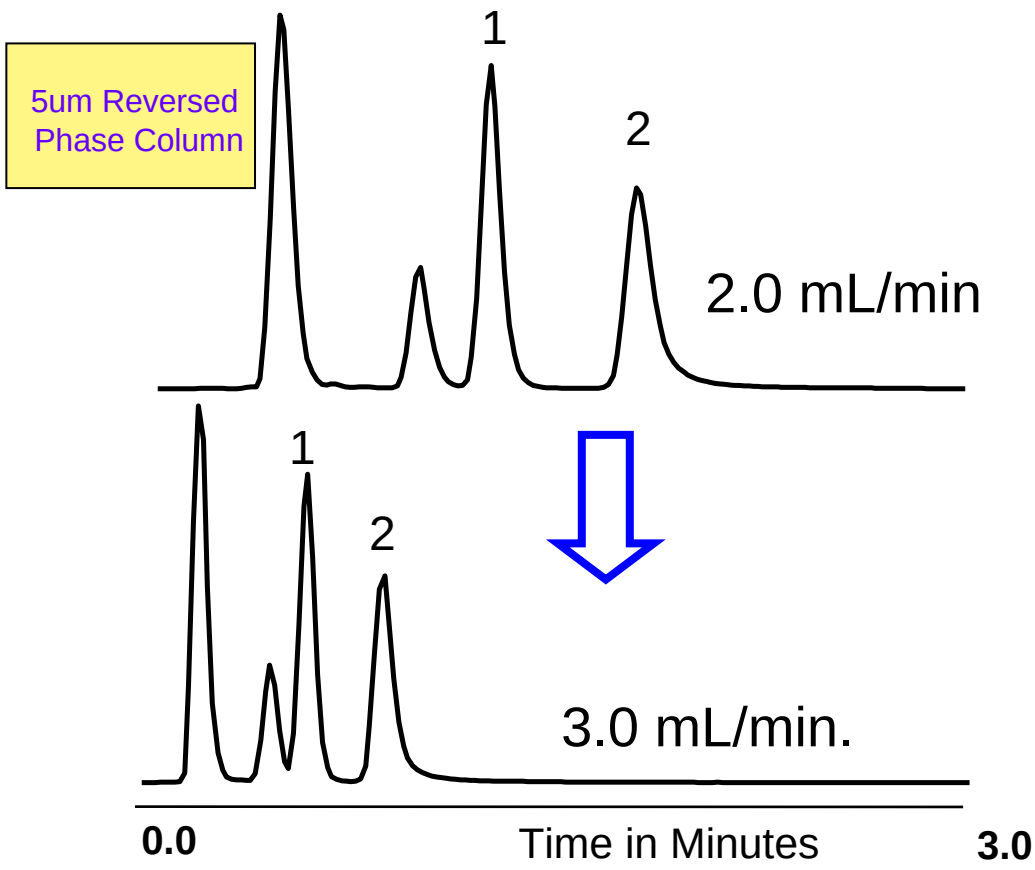
The enabler of productivity



- Smaller particles provide:
  - increased efficiency
  - maintain efficiency over a wider linear velocity
  - ability for both added resolution and increased speed of separation
- Particles are central to the quality of the separation

Compressed Chromatography

Compromised resolution



***** 50 mm column

* Higher Flow Rates

Peak	Rs	RT %RSD	Area %RSD
1	--	0.4	0.1
2	3.3	0.3	0.3

Peak	Rs	RT %RSD	Area %RSD
1	--	0.8	0.3
2	2.3	0.6	0.4

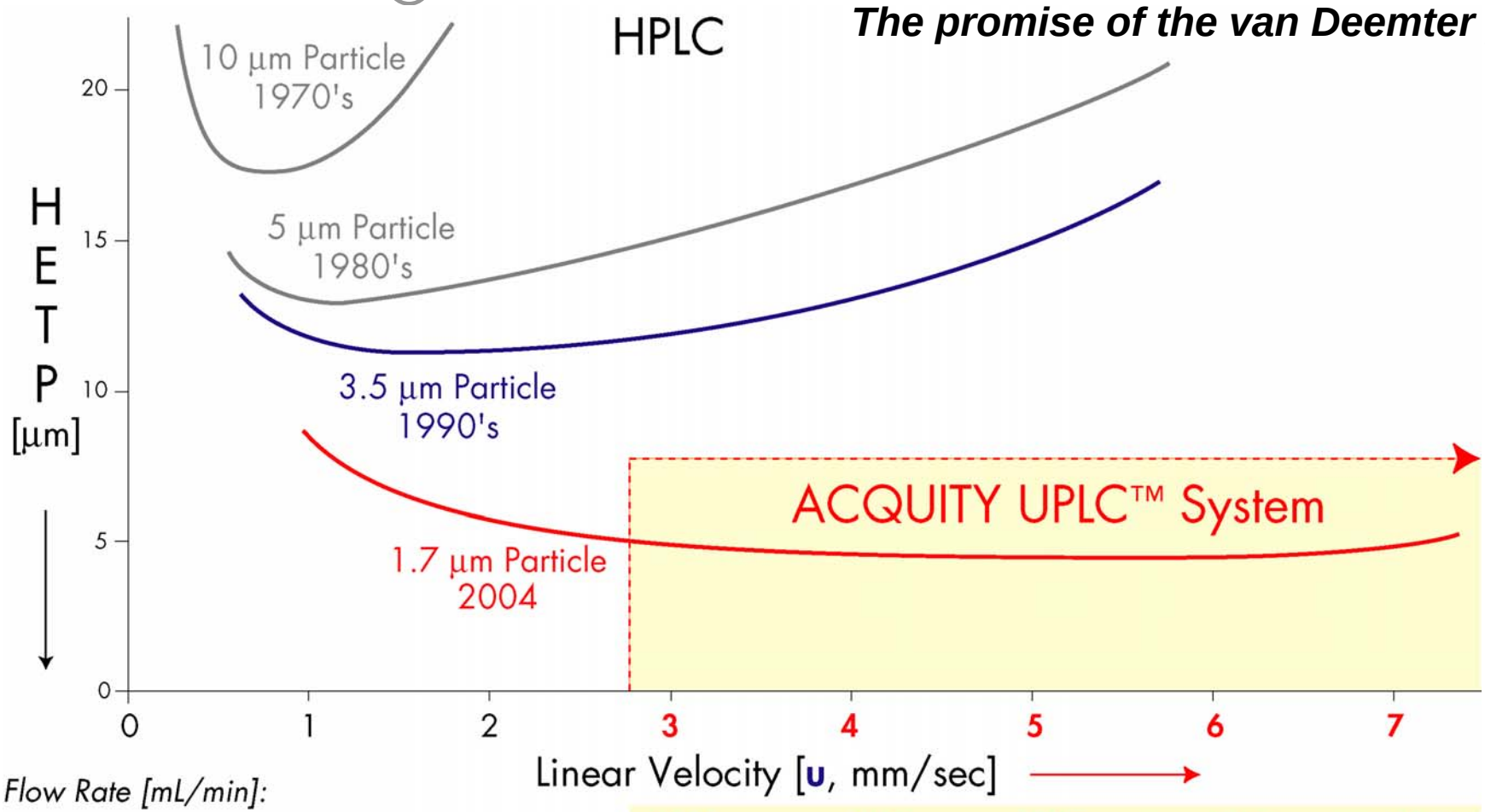
Fails Rs Goal of 3 Limitation

Run time is reduced, but required resolution Is lost!

Smaller Particles

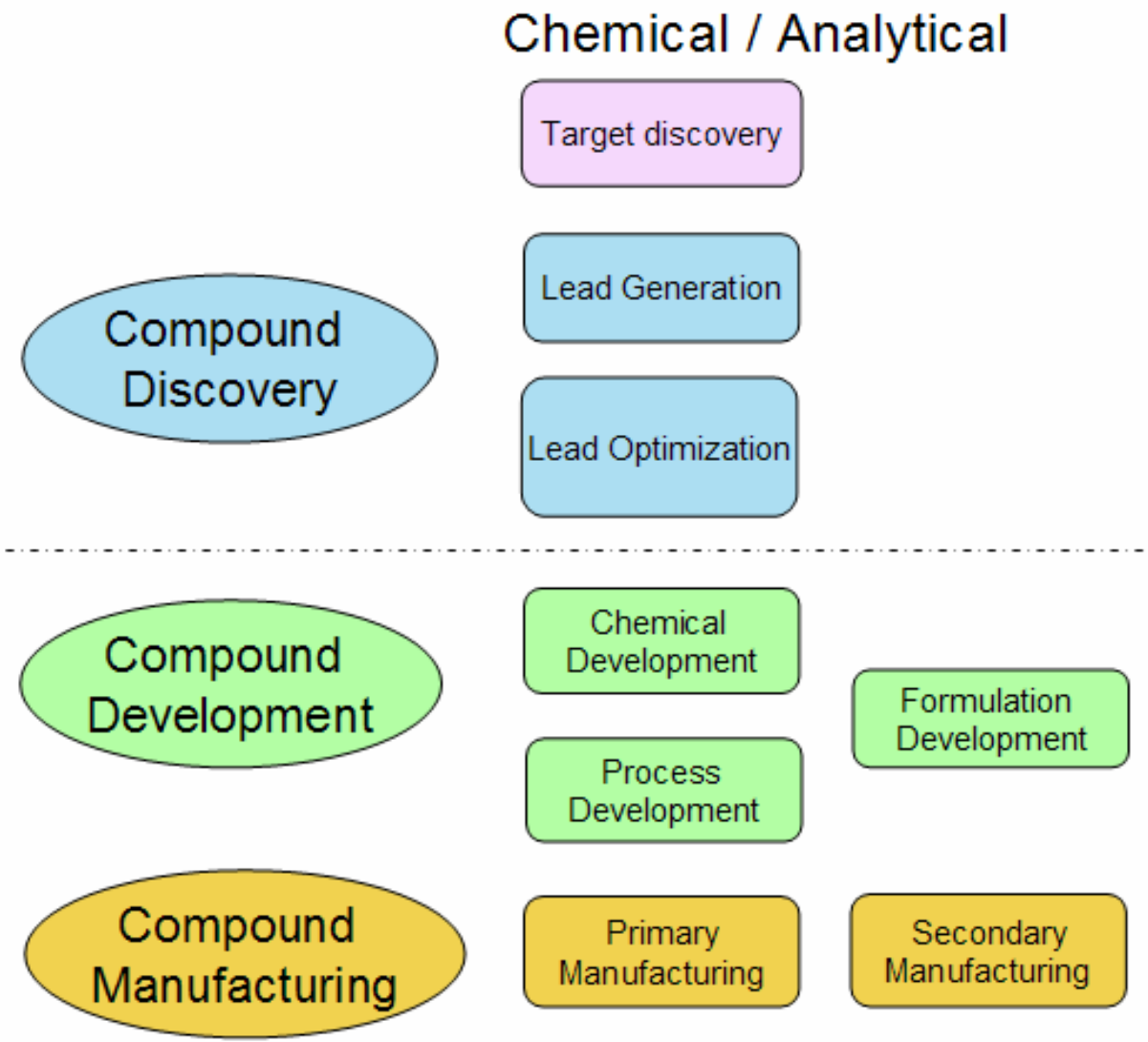
The enabler of productivity

The promise of the van Deemter plot



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*

Where are Methods Developed?



General Approach to Method Development

- Sample Information
- Define Goals
- Determine Instrument Requirements
- Choose A Method
  - Column, solvents
    - Preliminary runs
    - Determine best separation conditions
- Optimize Conditions
- Confirm
- Validate

Sample Information

- How complex is the Sample
  - Unknown
  - “Single” component
    - Compound analysis
    - API analysis
  - Mixtures - Acids, Bases, Neutrals
    - Multiple API’s or compounds
    - Degradents
    - Additives
    - Stabilizers
    - Other impurities
      - From the manufacturing process
      - Excipients

Method Goals and Considerations

- Method Goals
  - Sensitivity
  - Speed
  - Resolution
    - “Sensitivity”
  - Robustness
    - Reliability
  - Ruggedness
    - Reproducibility
- Method Considerations
  - UV
  - Mass Spec

Potential Method Requirements

- Some requirements to consider
  - Development
    - Impurity analysis
      - Sensitivity & Resolution
    - Product ID / Purity
      - Speed & Throughput
    - Forced Degradation
      - Sensitivity & Resolution
    - Stability Indicating
      - Sensitivity & Resolution
    - Cleaning Validation
      - Sensitivity & Resolution
  - Final QC / Lot Release
    - The financial impact of the methods

Instrumentation Requirements

- Analytical LC Instrumentation
  - Accuracy
  - Precision
  - Sensitivity
  - Reproducibility
  - Ease of use

UPLC™ Technology Enablers

- High pressure fluidic modules
  - Holistic design, low dwell volume
- Reduced cycle time autosampler with minimum carryover
- High speed detectors; optical and mass
  - Low dispersion UV detector cell
- Software designed for system integration
- Comprehensive diagnostic suite
- Increased Productivity
  - Higher peak capacity, faster chromatography
  - Higher laboratory throughput
  - Same chromatography principles

Acquity™
Ultra Performance LC

UPLC Method Development

- Different from HPLC Method Development?
  - An extension of everything you already know from HPLC
- Can we take advantage of the ACQUITY UPLC™ System's:
  - Theoretical Speed advantage?
  - Theoretical Sensitivity advantage?
  - Theoretical Resolution advantage?
- Generic Gradient?
  - Sample dependent, but
    - Starting with a 4 minute and a 7 minute 0% to 100% Organic linear gradient will typically generate enough data to calibrate the separation.

Method Development

- Probably the most difficult chromatographic task
- Typically the area where the most experienced chromatographers are found

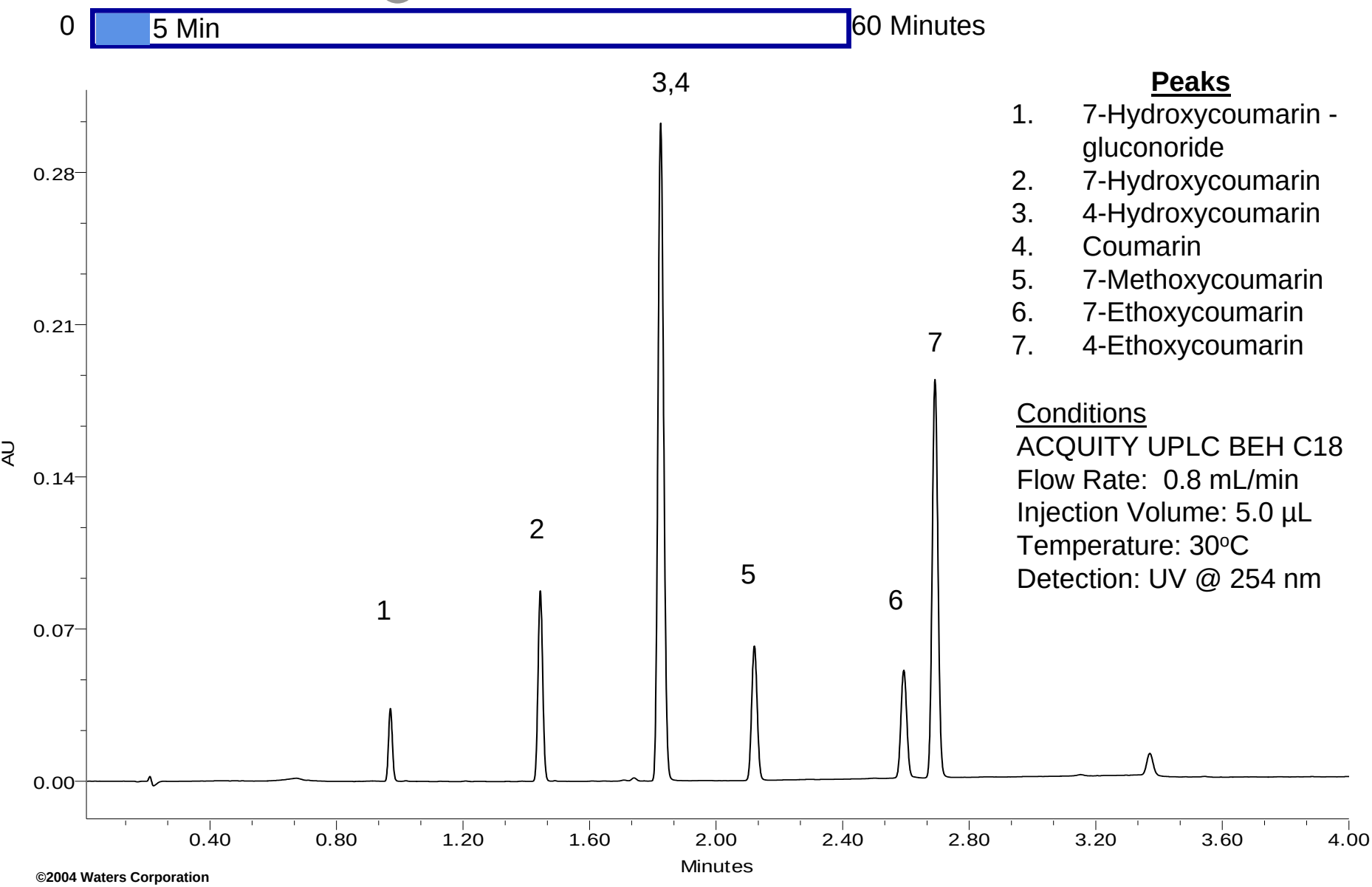
"Success consists of going from failure to failure without loss of enthusiasm."

- Winston Churchill

Related Compound Analysis

- Method Development process for 7 related compounds (coumarins)
- Analytical considerations
  - Speed
  - Resolution
  - Reproducibility
  - Robustness
  - Sensitivity
- Method development in less than an hour?

Related Compound Analysis
Generic Linear 0-60%B 4min



Peaks

1. 7-Hydroxycoumarin - gluconoride
2. 7-Hydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 7-Methoxycoumarin
6. 7-Ethoxycoumarin
7. 4-Ethoxycoumarin

Conditions

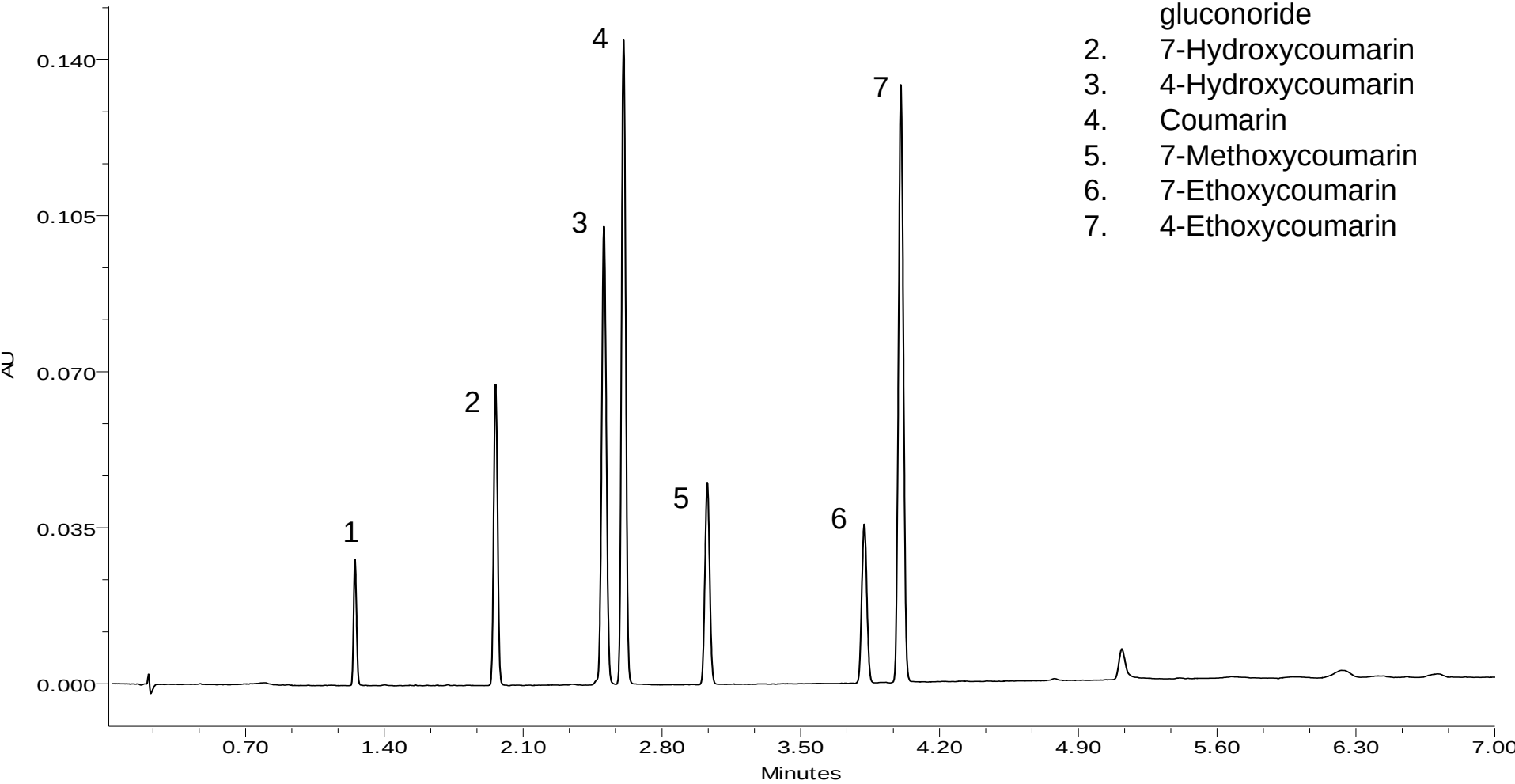
ACQUITY UPLC BEH C18
Flow Rate: 0.8 mL/min
Injection Volume: 5.0 µL
Temperature: 30°C
Detection: UV @ 254 nm

Related Compound Analysis
Generic Linear 0-60%B 7min

14 Min — 60 Minutes

Peaks

1. 7-Hydroxycoumarin - gluconoride
2. 7-Hydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 7-Methoxycoumarin
6. 7-Ethoxycoumarin
7. 4-Ethoxycoumarin

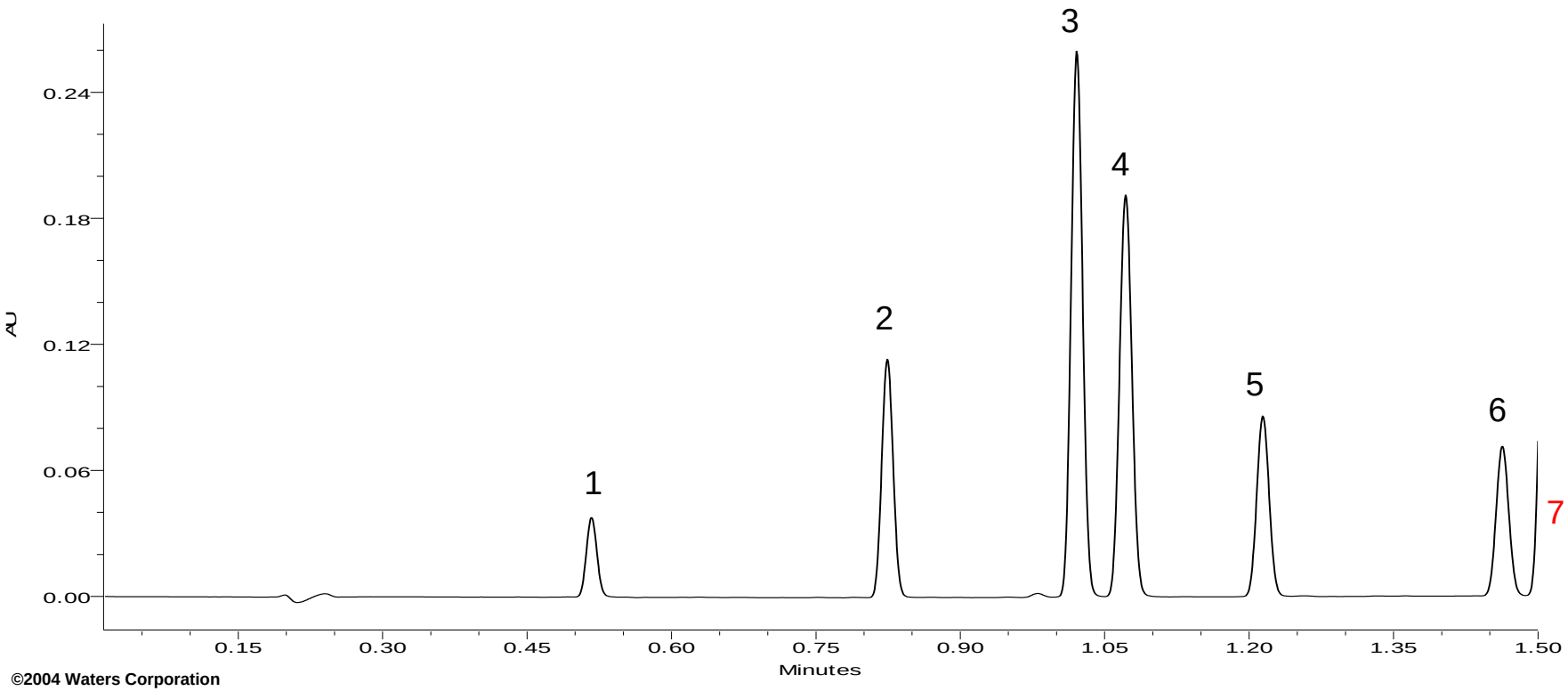


Related Compound Analysis
Linear 5-50%B 1.5min

16.25 Min — 60 Minutes

Peaks

1. 7-Hydroxycoumarin - gluconoride
2. 7-Hydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 7-Methoxycoumarin
6. 7-Ethoxycoumarin
7. 4-Ethoxycoumarin

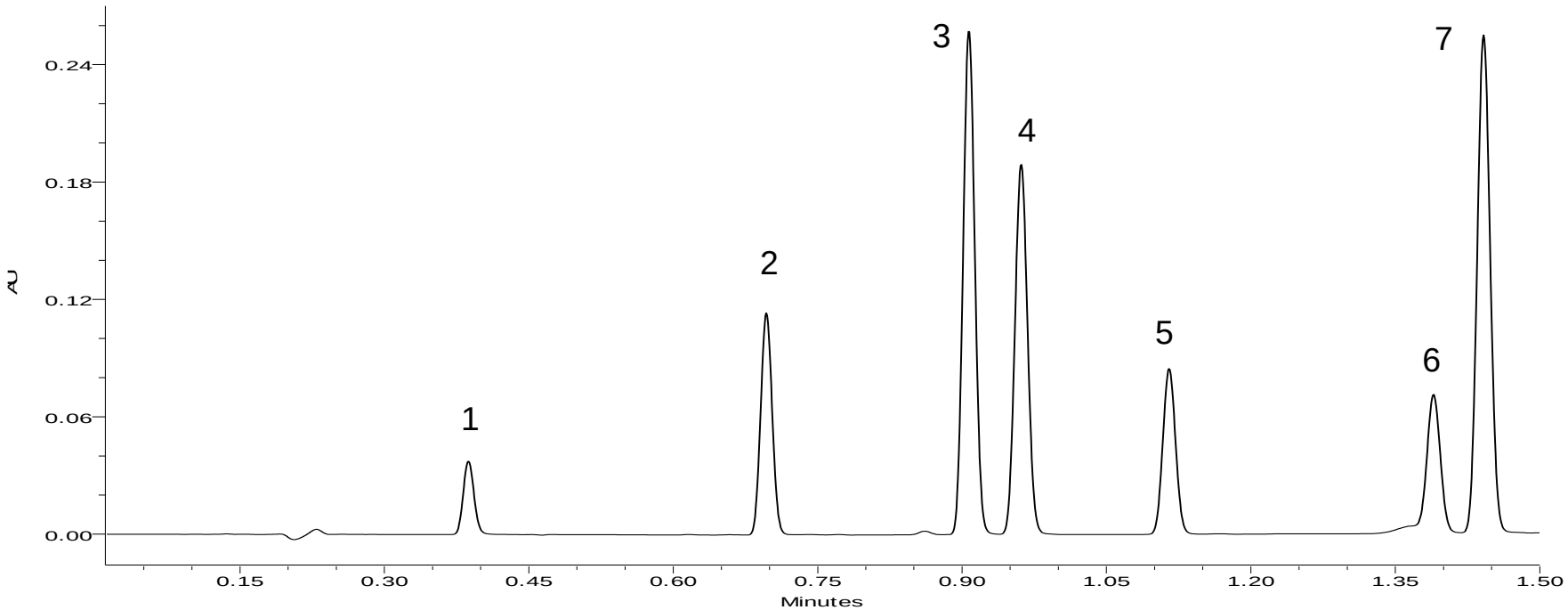


Related Compound Analysis
Linear 10-50%B 1.5 min

18.5 Min — 60 Minutes

Peaks

1. 7-Hydroxycoumarin - gluconoride
2. 7-Hydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 7-Methoxycoumarin
6. 7-Ethoxycoumarin
7. 4-Ethoxycoumarin

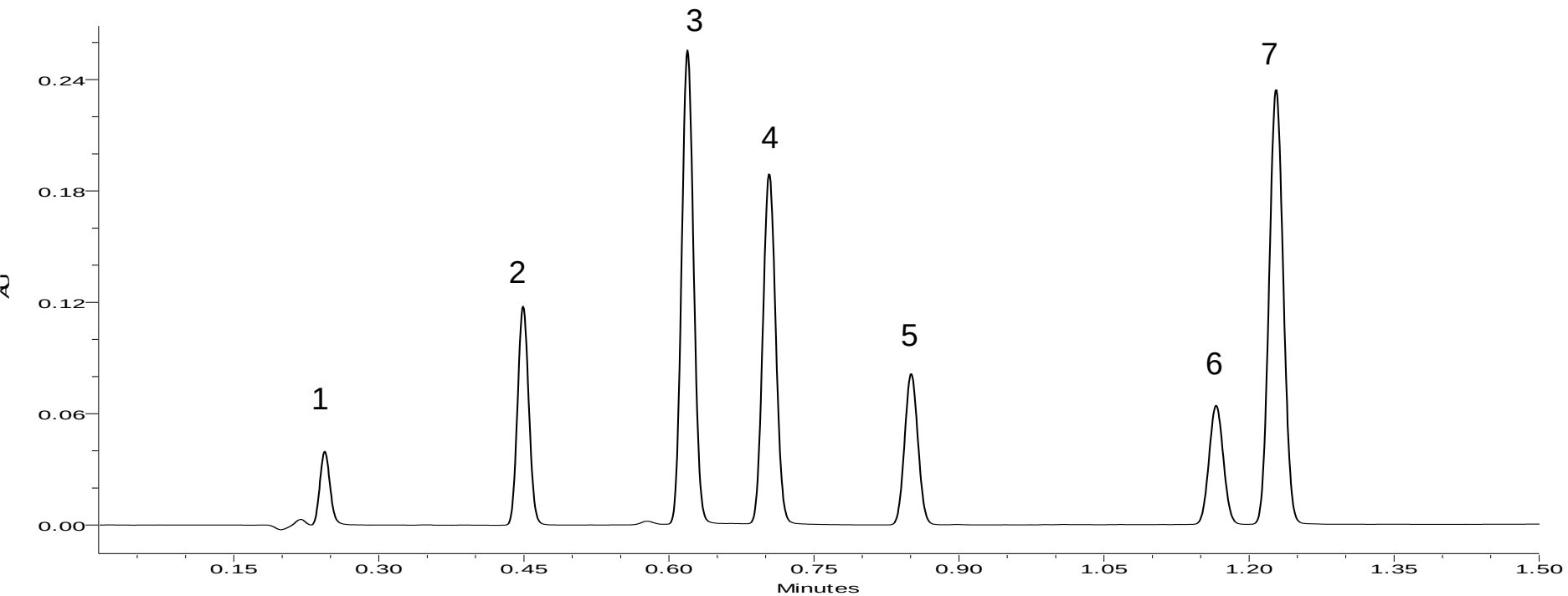


Related Compound Analysis
Linear 20-50%B 1.5min

20.75 Min 60 Minutes

Peaks

1. 7-Hydroxycoumarin - gluconoride
2. 7-Hydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 7-Methoxycoumarin
6. 7-Ethoxycoumarin
7. 4-Ethoxycoumarin



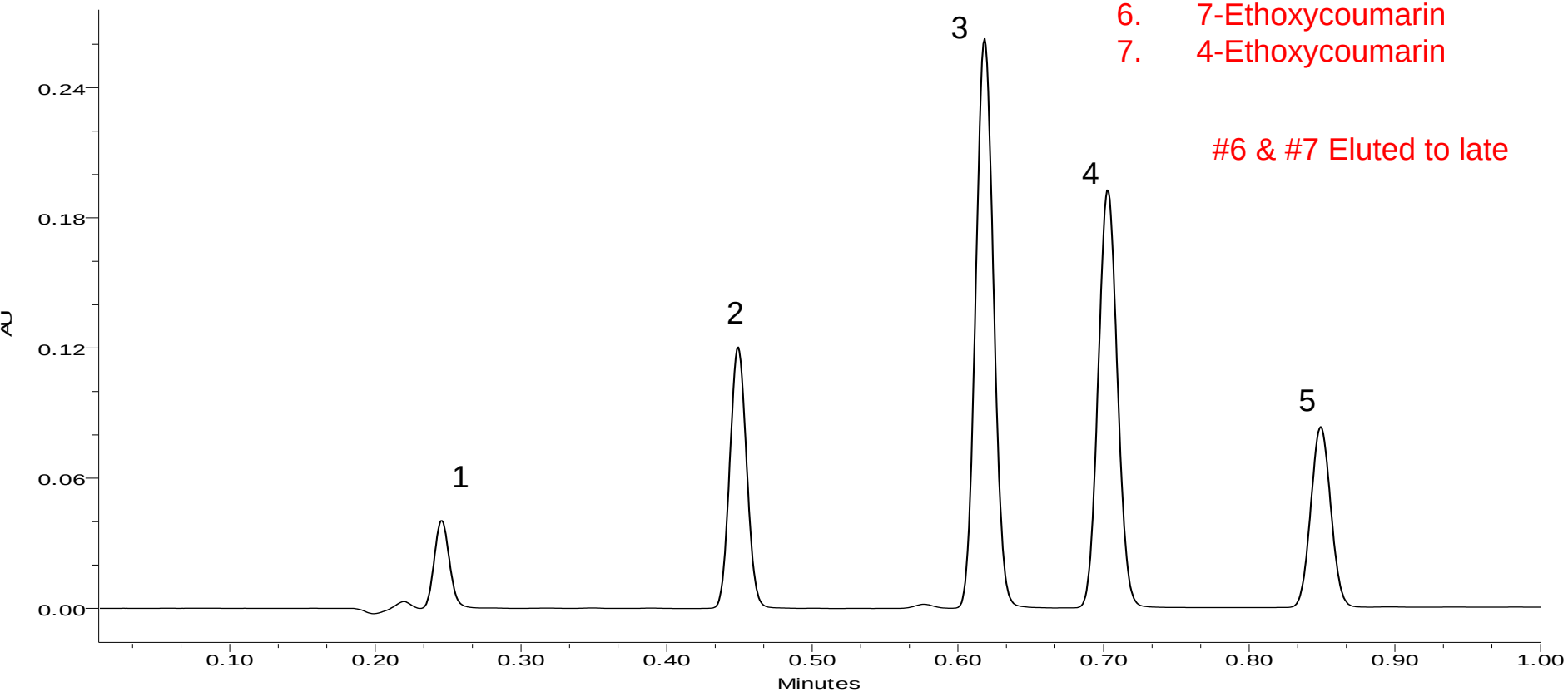
Related Compound Analysis
Linear 20-40%B 1.0min

22.5 Min — 60 Minutes

Peaks

1. 7-Hydroxycoumarin - gluconoride
2. 7-Hydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 7-Methoxycoumarin
6. 7-Ethoxycoumarin
7. 4-Ethoxycoumarin

#6 & #7 Eluted to late



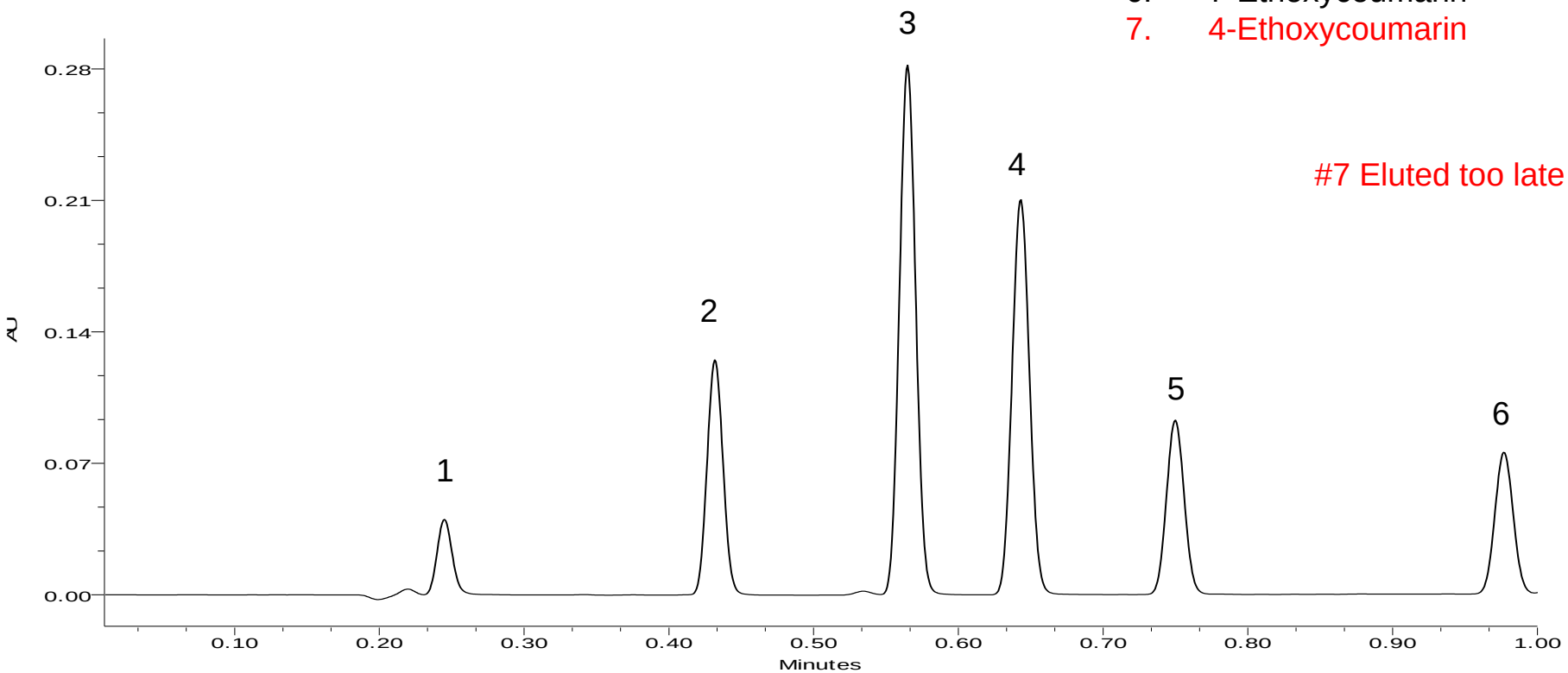
Related Compound Analysis
Linear 20-50%B 1.0min

24 Min 60 Minutes

Peaks

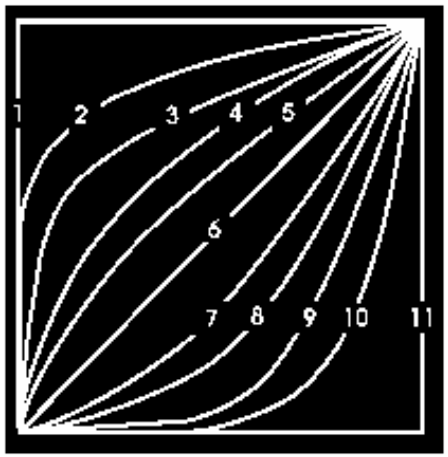
1. 7-Hydroxycoumarin - gluconoride
2. 7-Hydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 7-Methoxycoumarin
6. 7-Ethoxycoumarin
7. 4-Ethoxycoumarin

#7 Eluted too late



Related Compound Analysis
Curve 4 Gradient 20-40%B 1.0min

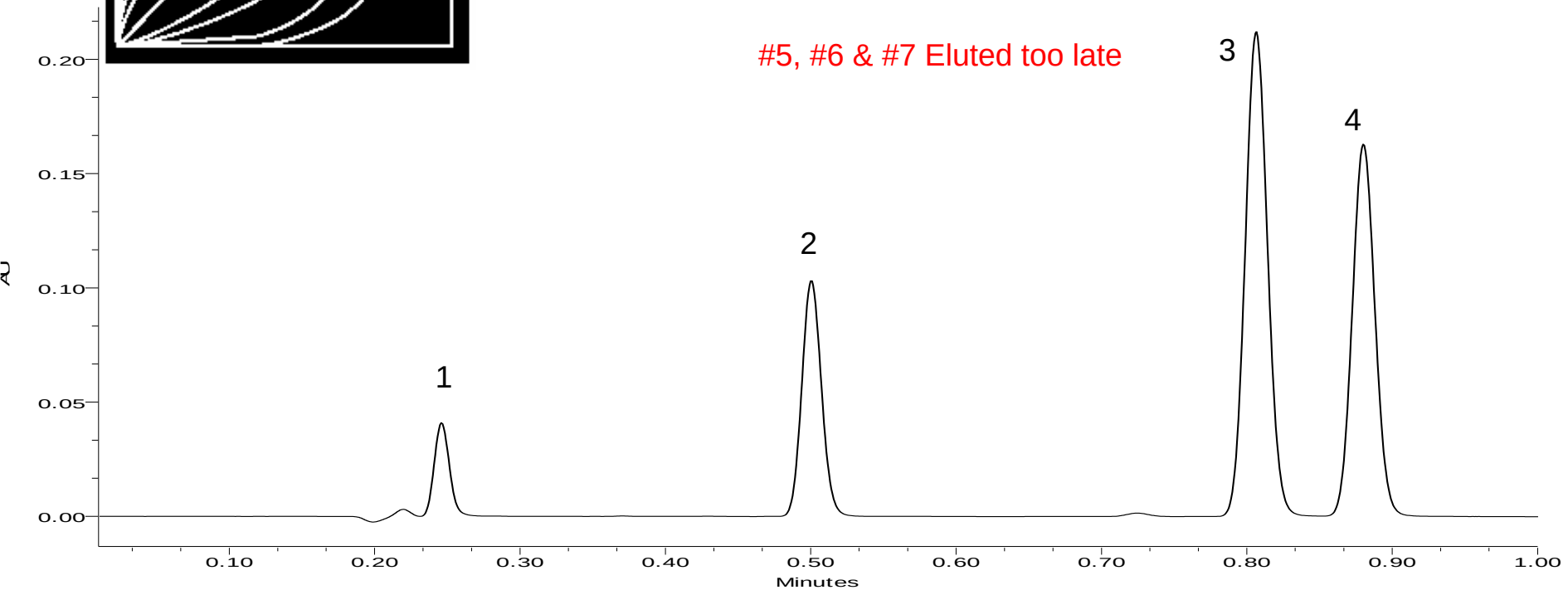
25.5 Min | 60 Minutes



Peaks

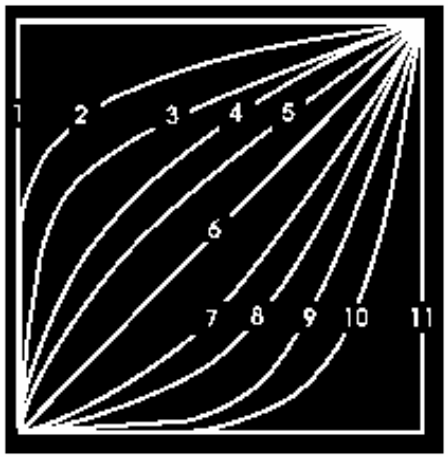
1. 7-Hydroxycoumarin - gluconoride
2. 7-Hydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 7-Methoxycoumarin
6. 7-Ethoxycoumarin
7. 4-Ethoxycoumarin

#5, #6 & #7 Eluted too late



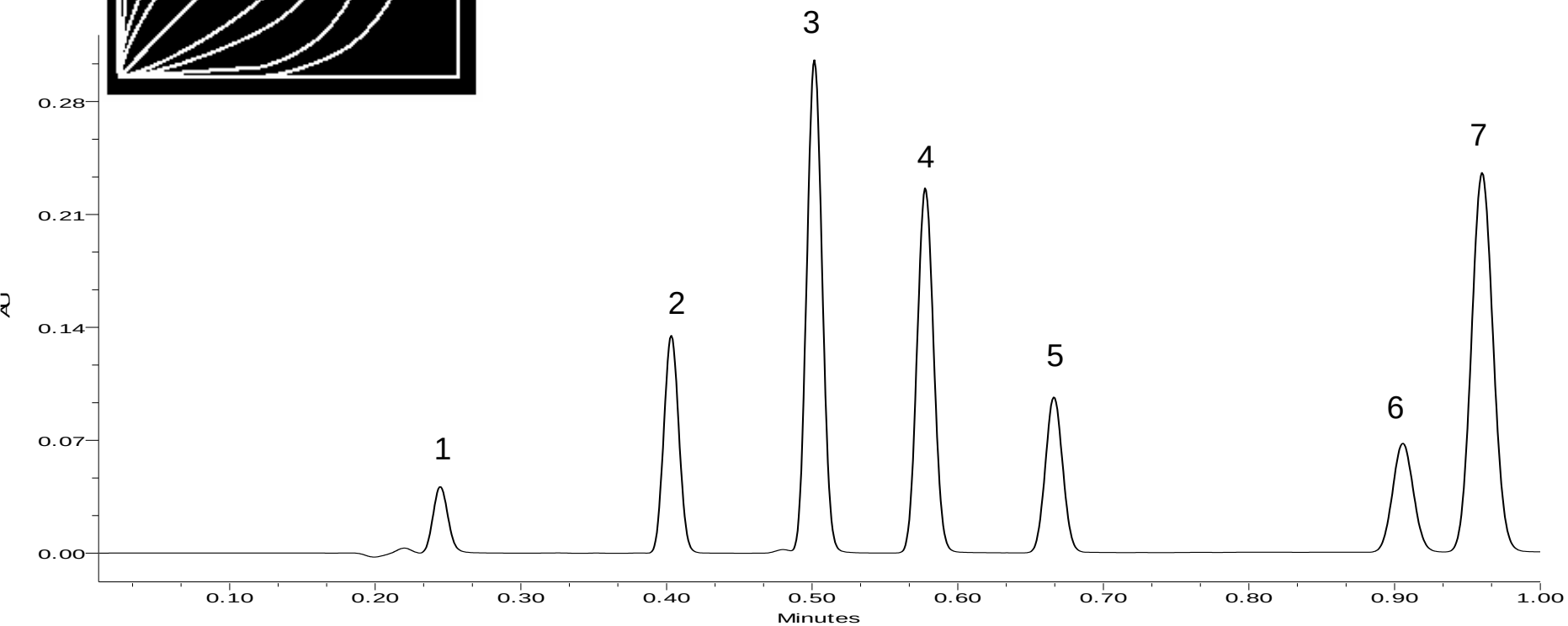
Related Compound Analysis
Curve 8 Gradient 20-40%B 1.0min

27 Min 60 Minutes



Peaks

1. 7-Hydroxycoumarin - gluconoride
2. 7-Hydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 7-Methoxycoumarin
6. 7-Ethoxycoumarin
7. 4-Ethoxycoumarin

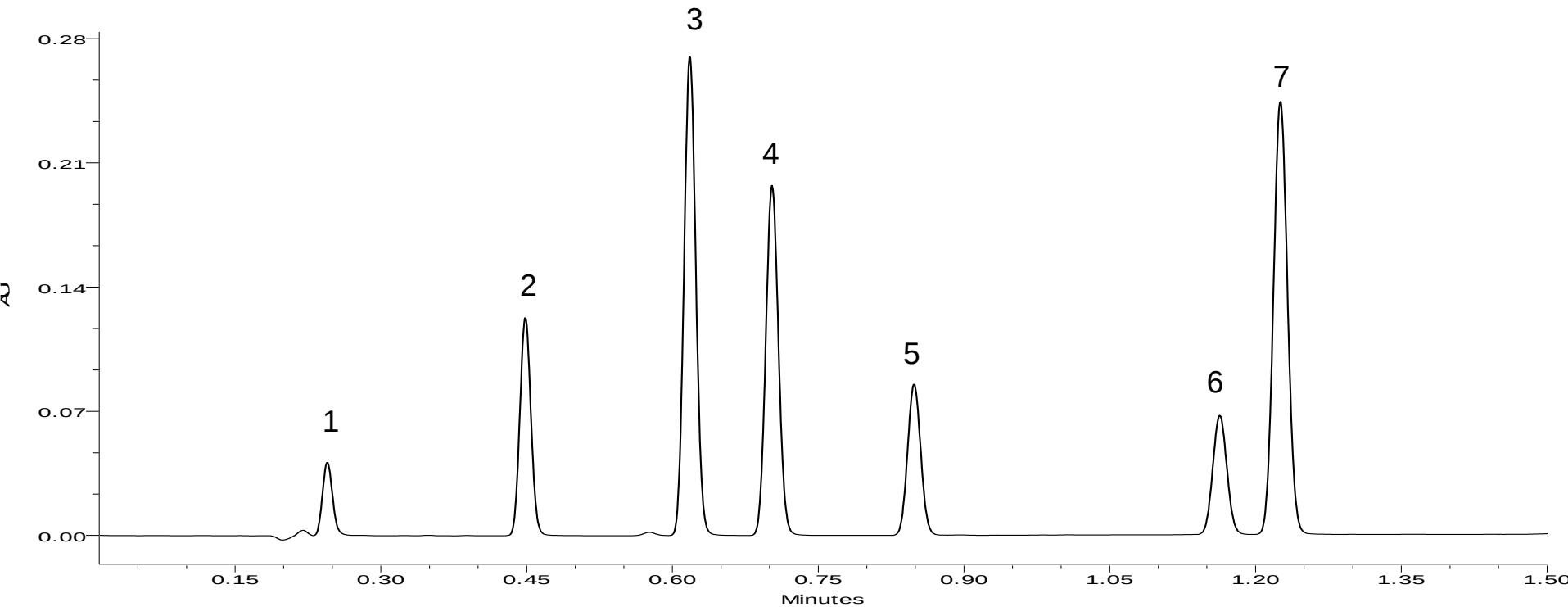


Related Compound Analysis
Final 20-40%B 1.0min with 0.5min hold

29 Min 60 Minutes

Peaks

1. 7-Hydroxycoumarin - gluconoride
2. 7-Hydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 7-Methoxycoumarin
6. 7-Ethoxycoumarin
7. 4-Ethoxycoumarin

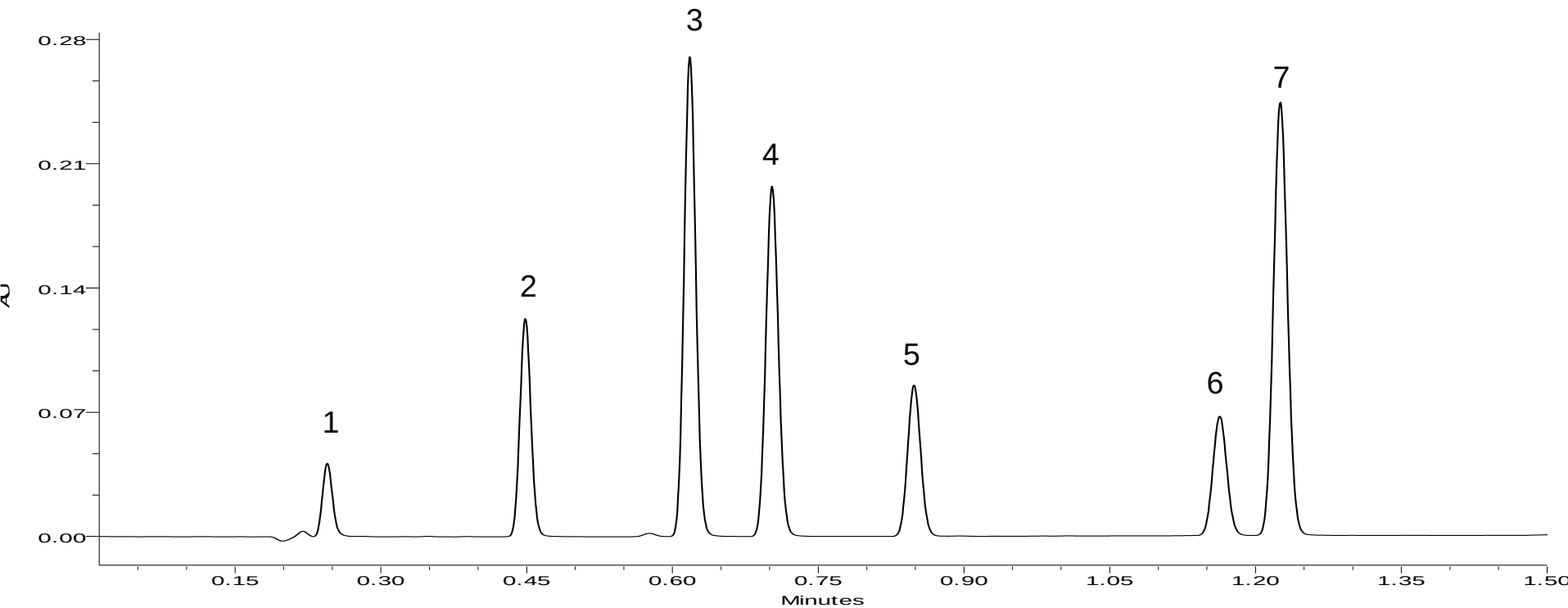


Related Compound Analysis
Confirm Final 20-40%B

31 Min — 60 Minutes

Peaks

1. 7-Hydroxycoumarin - gluconoride
2. 7-Hydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 7-Methoxycoumarin
6. 7-Ethoxycoumarin
7. 4-Ethoxycoumarin



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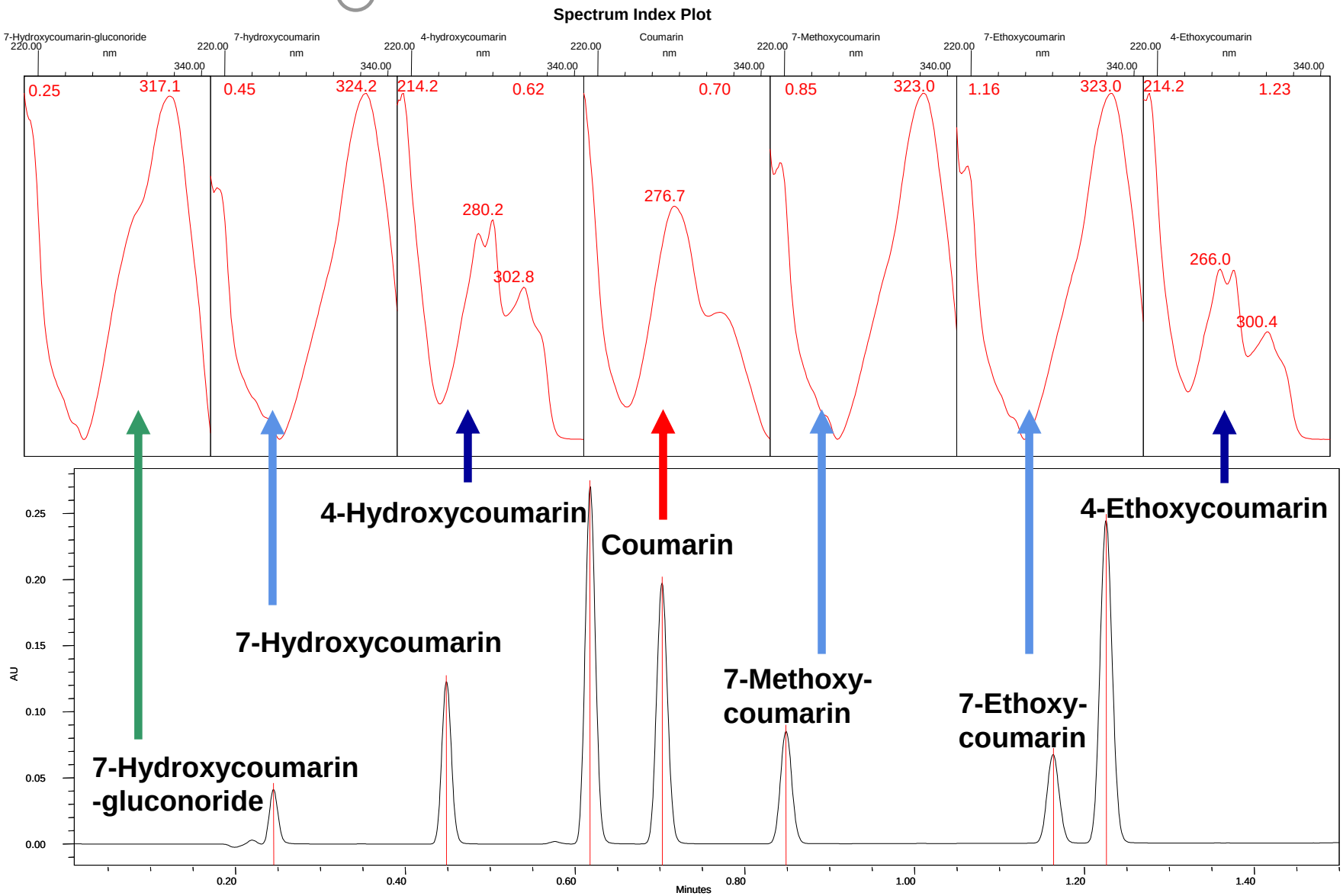
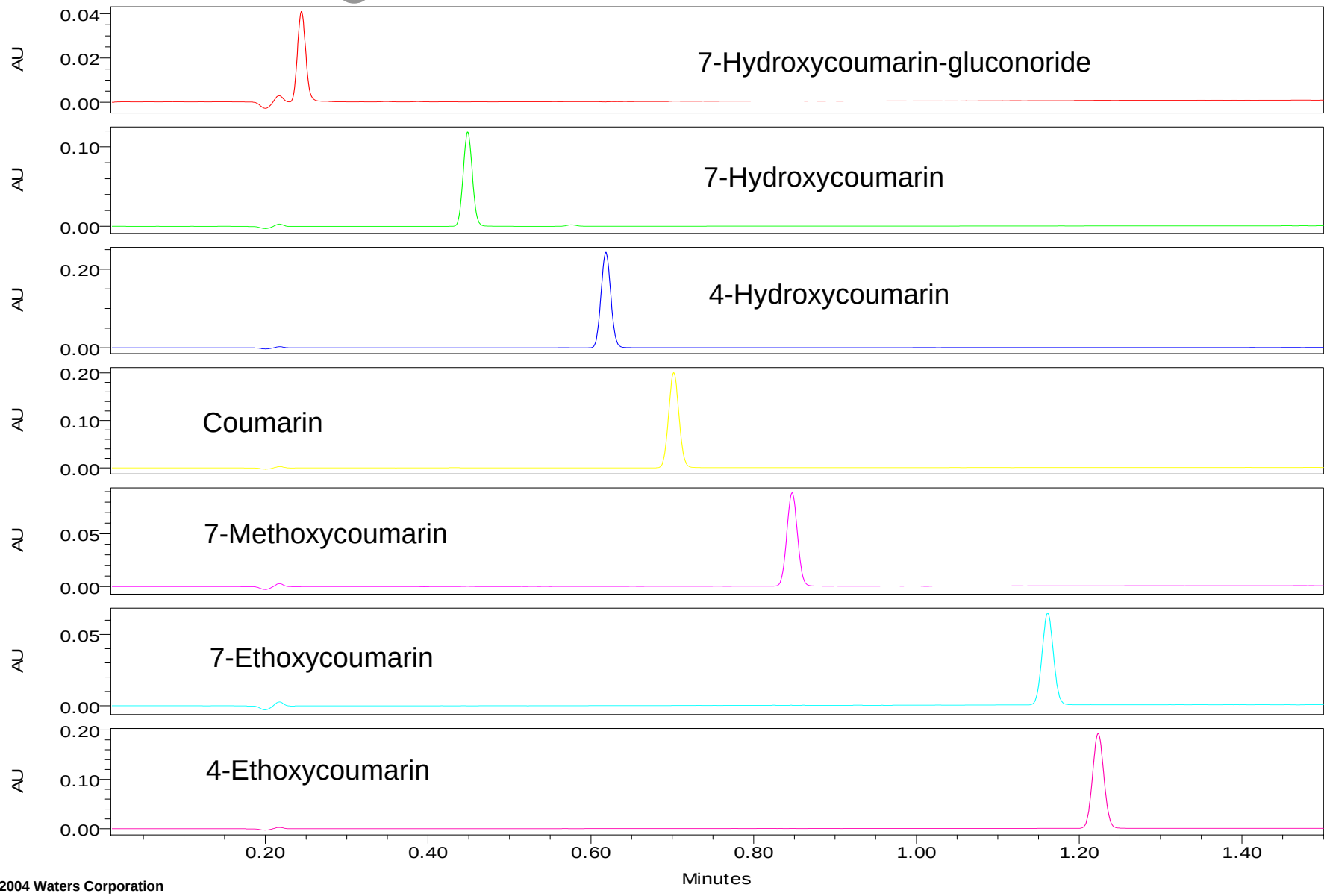
Spectral Index Plots
Coumarins

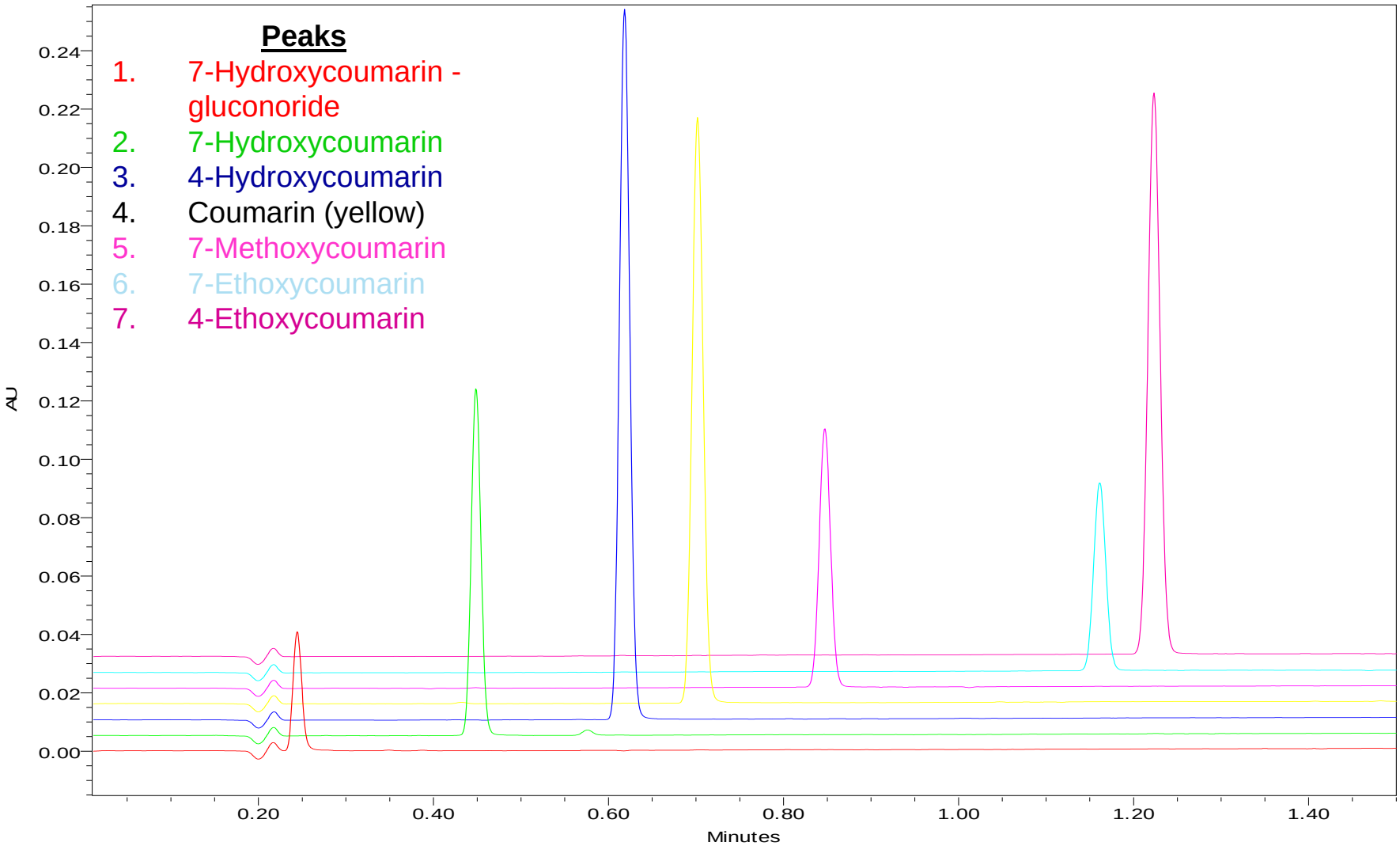


Standards Overlay
(Spectra used to build spectral library)



Related Compound Analysis
Standards Overlay

60 Minutes



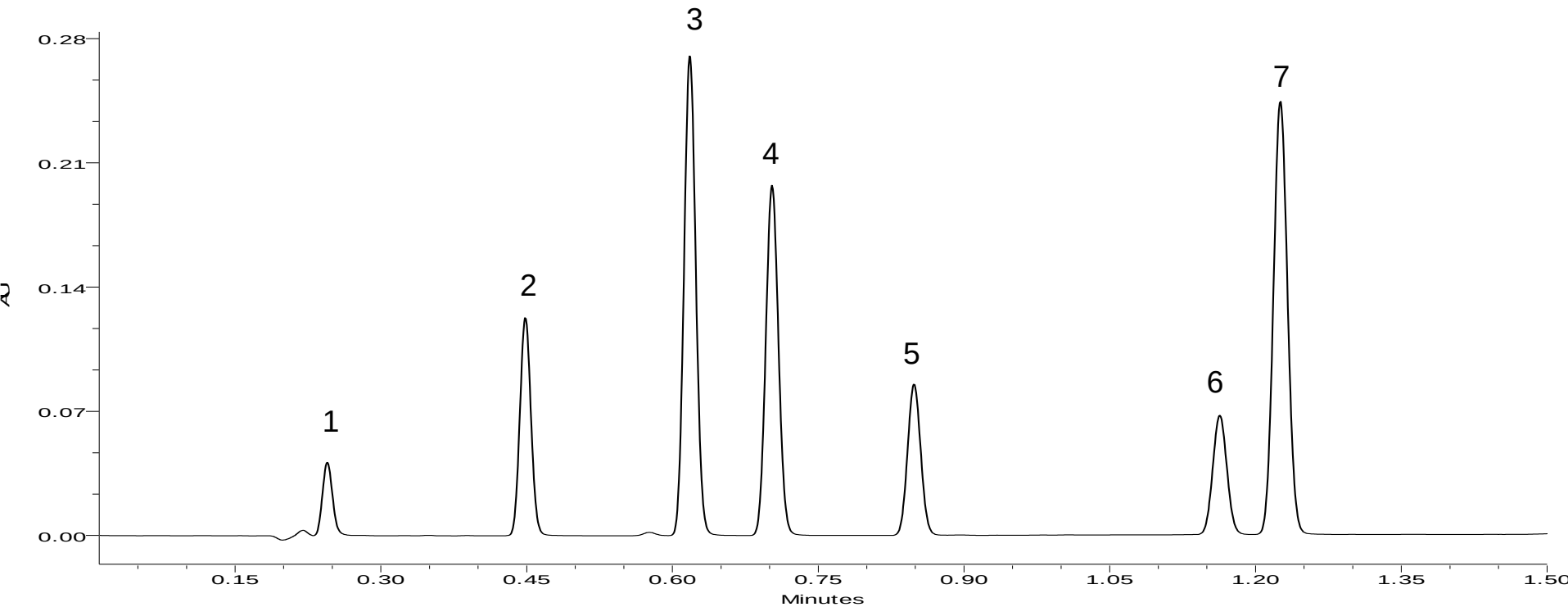
Related Compound Analysis
Duplicate Standards

60 Minutes

Total Development Time: 59 Minutes

Peaks

1. 7-Hydroxycoumarin - gluconoride
2. 7-Hydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 7-Methoxycoumarin
6. 7-Ethoxycoumarin
7. 4-Ethoxycoumarin



UPLC Method Development

- Selectivity
  - Complete resolution of all peaks
    - If a coelution exists, there is no “Sensitivity”
    - Must detect “everything”
- Chromatography parameters that may effect Selectivity
  - Column ligand chemistry
    - C_{18}, RP_{18}, C_8, Phenyl
  - Organic modifier
    - MeCN, MeOH, mixes…
  - pH and buffer type
  - Temperature
  - Linear Velocity?
- What if a single chemistry family and organic modifier could accomplish your Selectivity goals?

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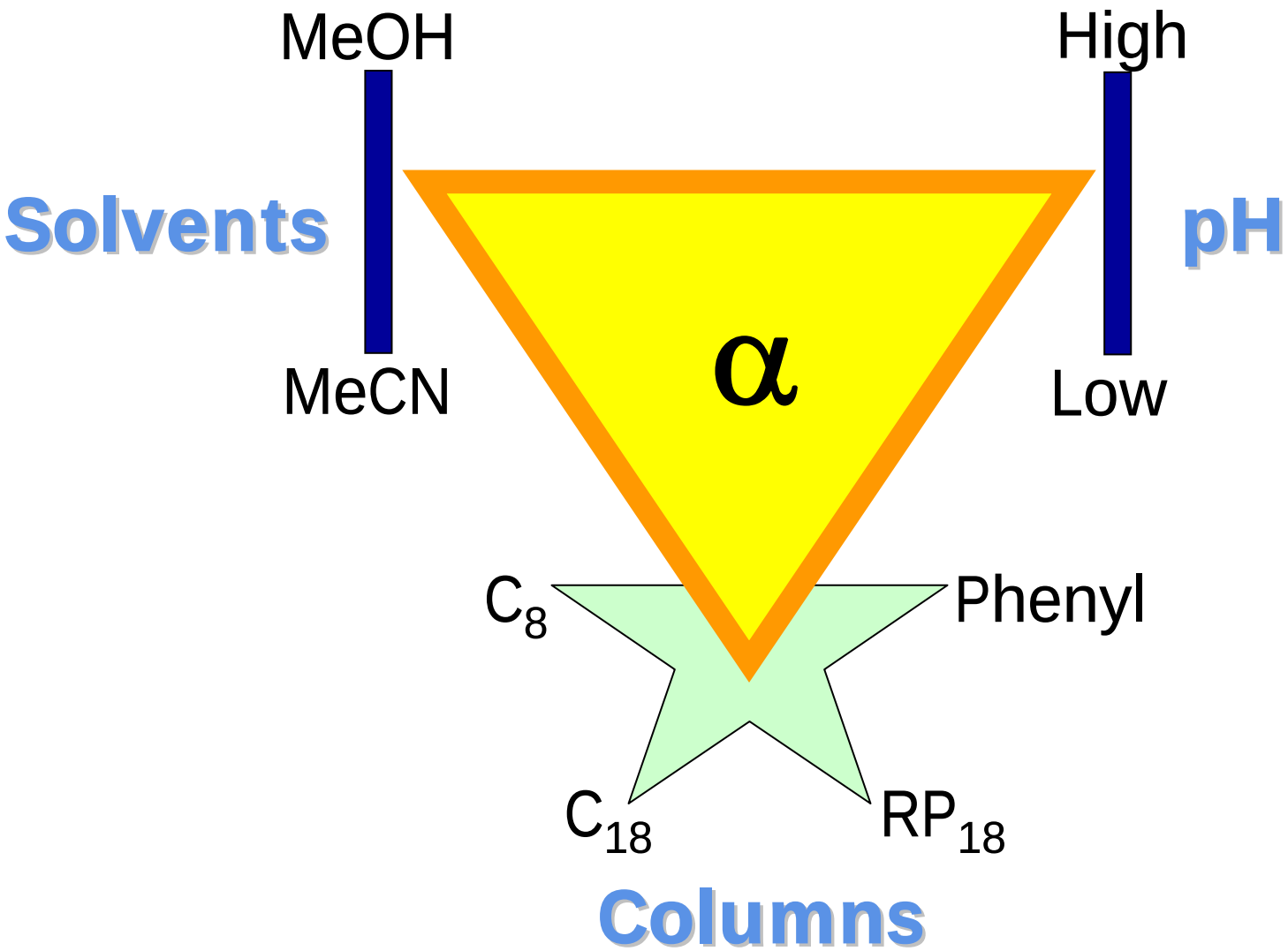
Selectivity Tools
Reversed-Phase ACQUITY UPLC™



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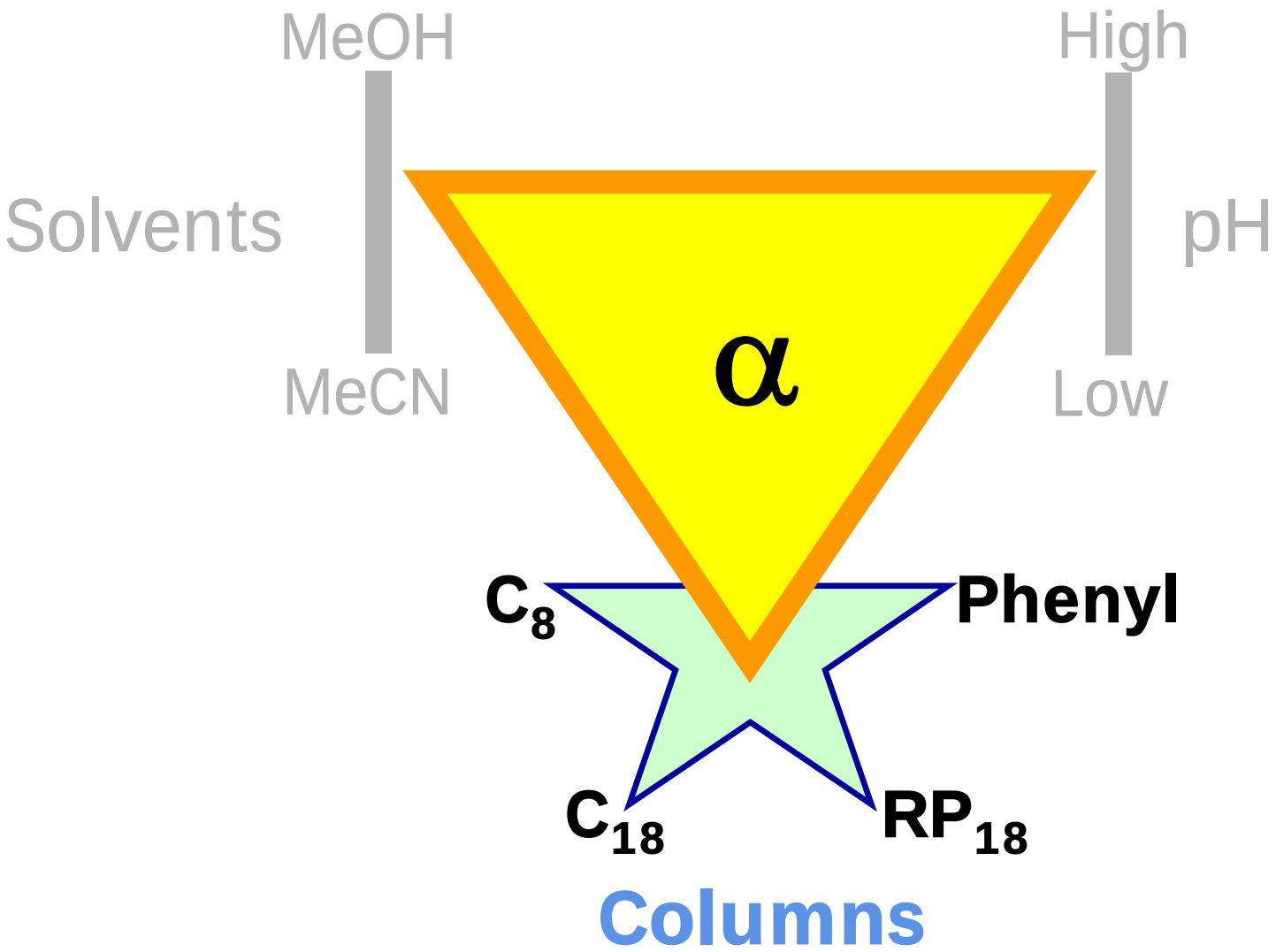
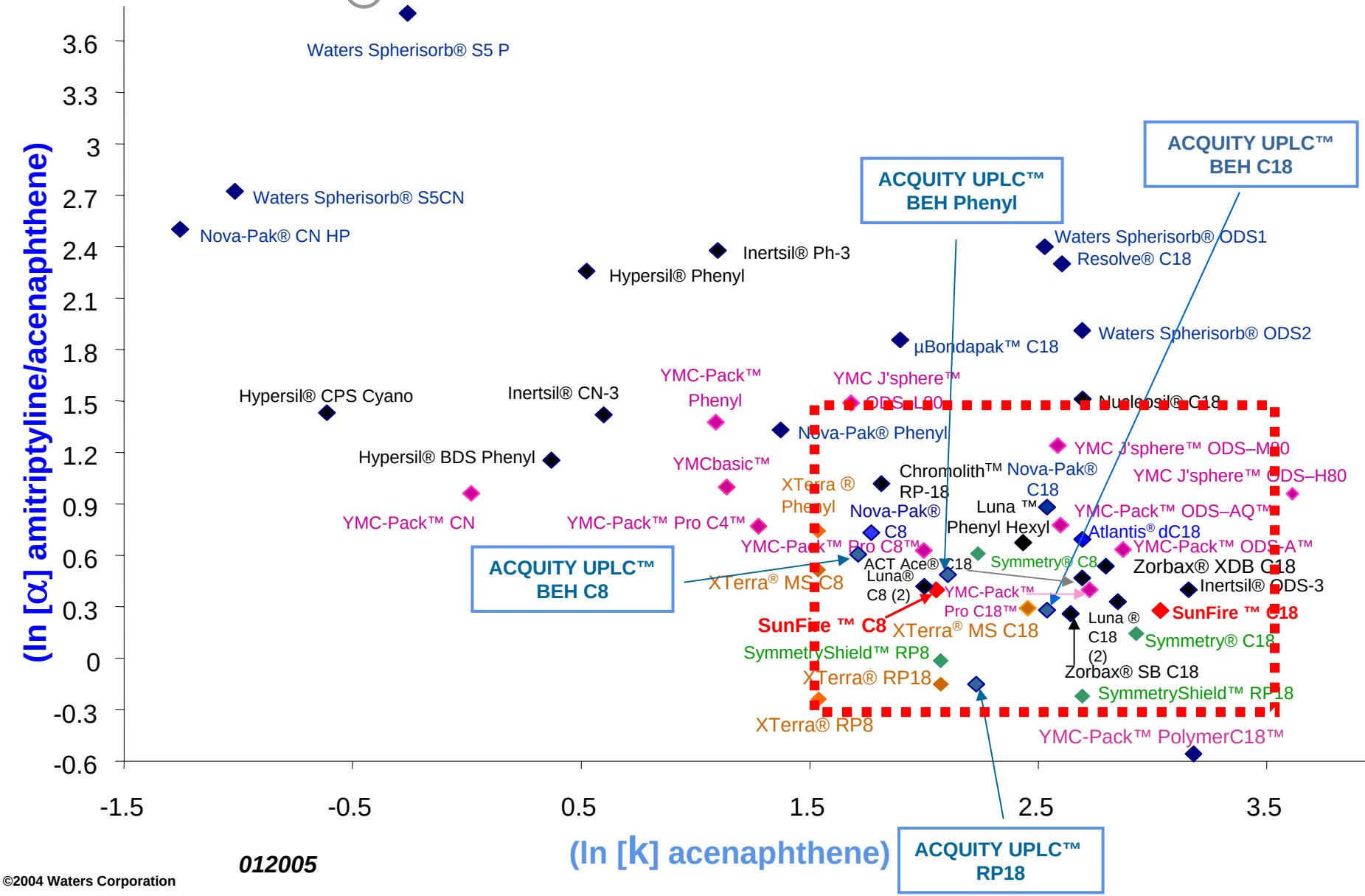
Selectivity Tools
Reversed-Phase ACQUITY UPLC™



Reversed Phase Columns
Selectivity Chart



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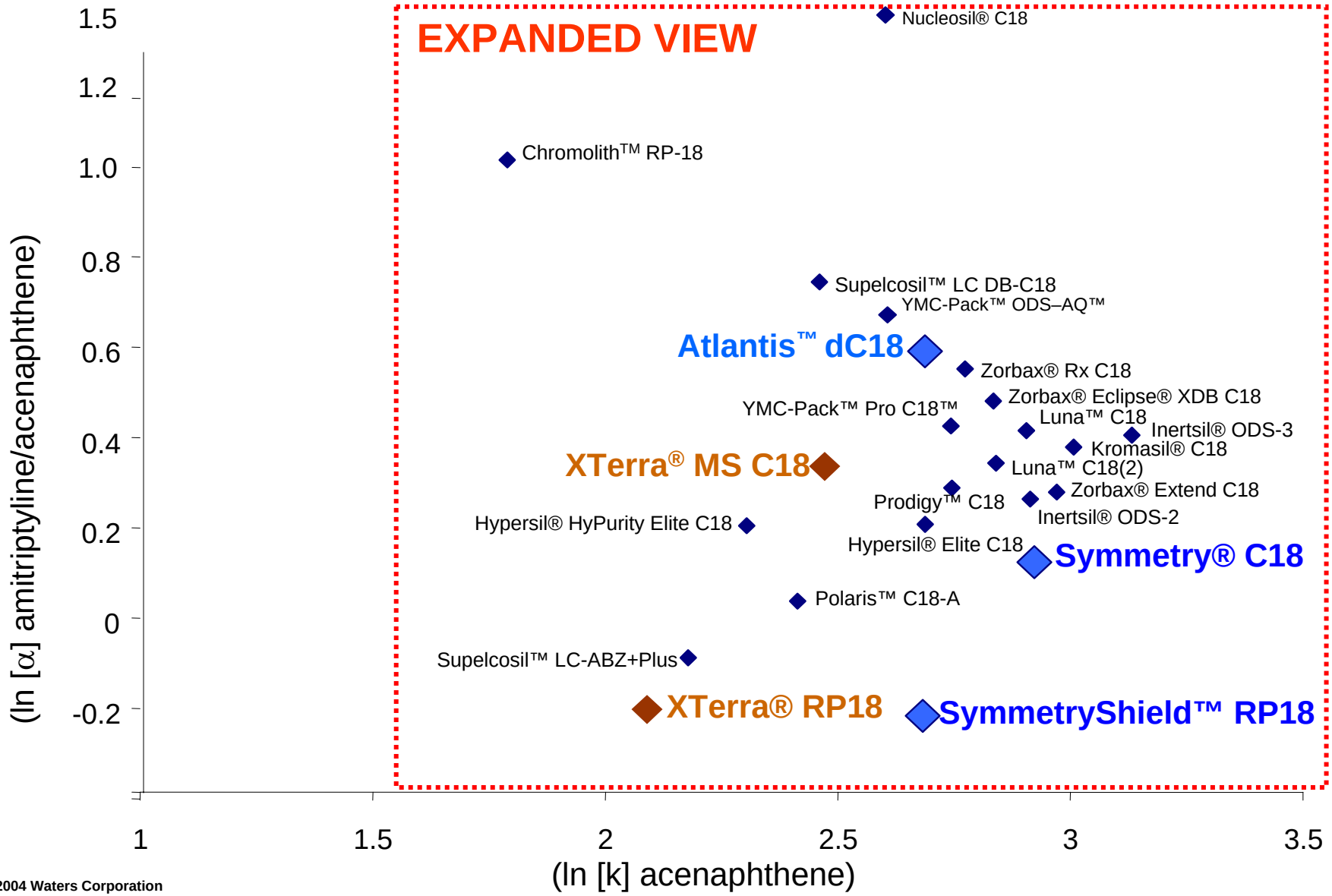
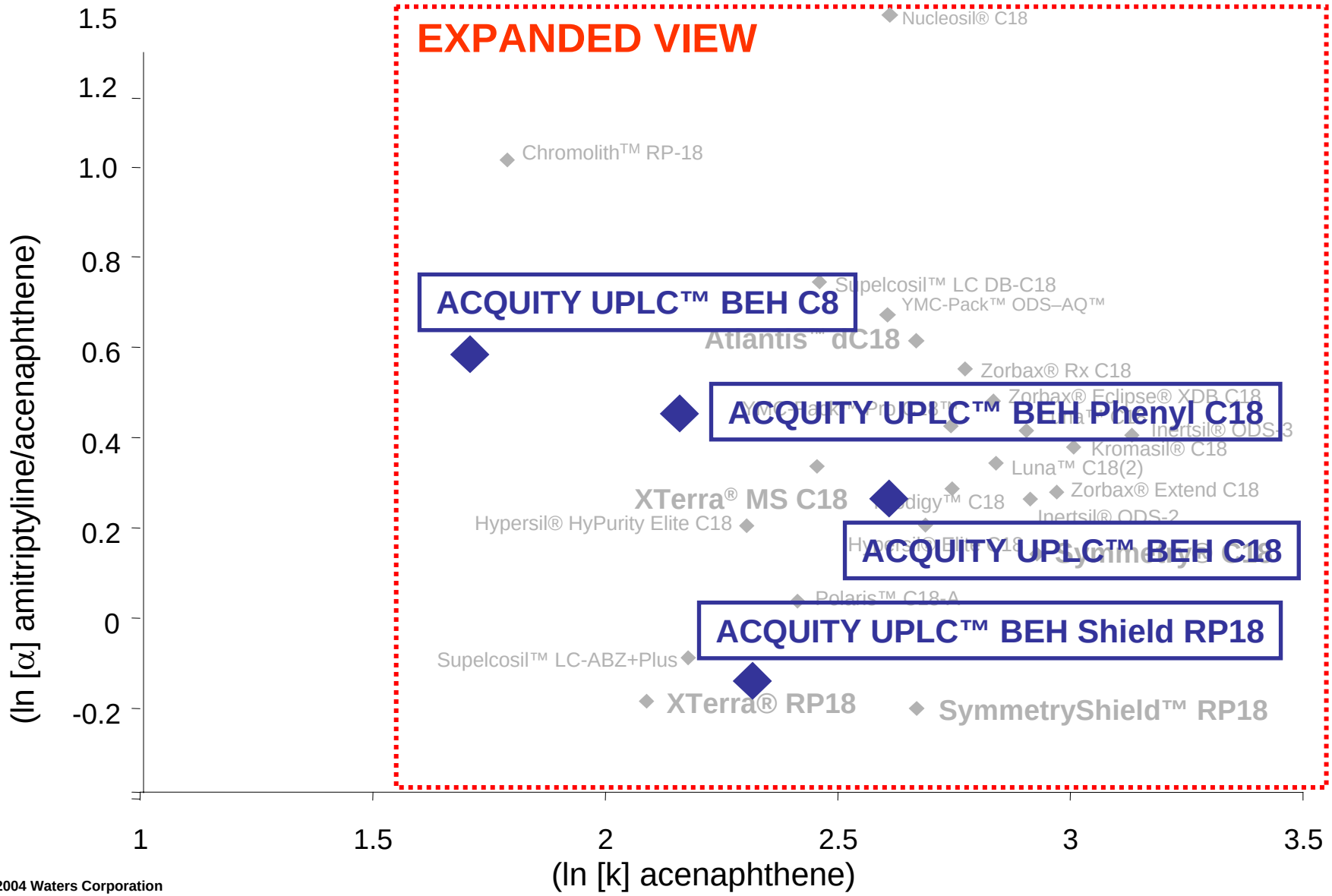
The Modern "C_{18} Zone"
Selectivity Chart



The Modern "C_{18} Zone"

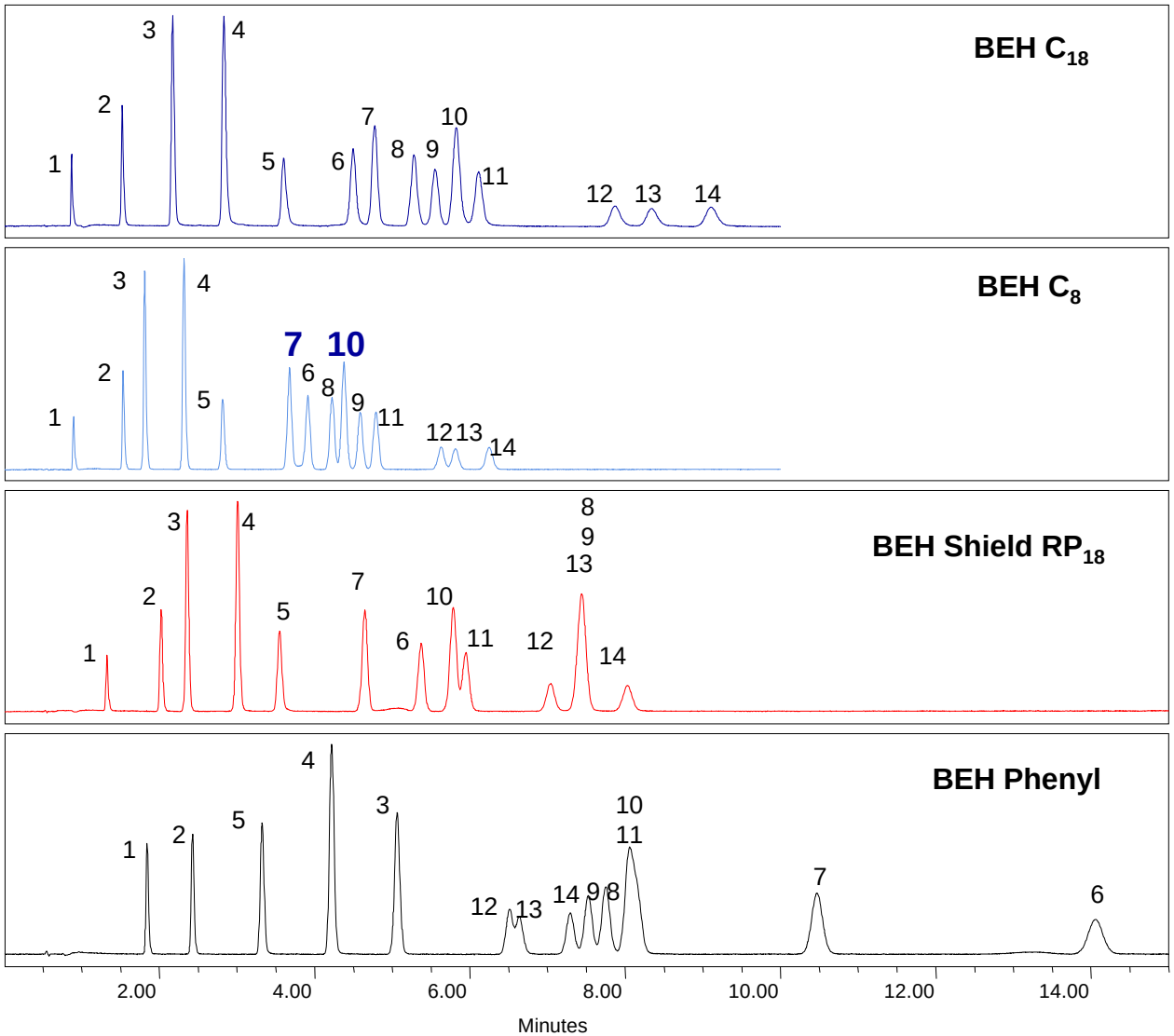
Greater Resolution through Efficiency



Column Ligand Selectivity
Explosives (from EPA Method 8330)

Chromatographic Conditions :
Flow Rate: 0.5 mL/min
Isocratic: 28% methanol
Injection Volume: 5.0 µL
Sample Concentration: 10 µg/mL

	Name
1	HMX
2	RDX
3	1,3,5-TNB
4	1,3-DNB
5	NB
6	Tetryl
7	TNT
8	2-Am-4,6 DNT
9	4-Am-2,6 DNT
10	2,4 DNT
11	2,6-DNT
12	2-NT
13	4-NT
14	3-NT

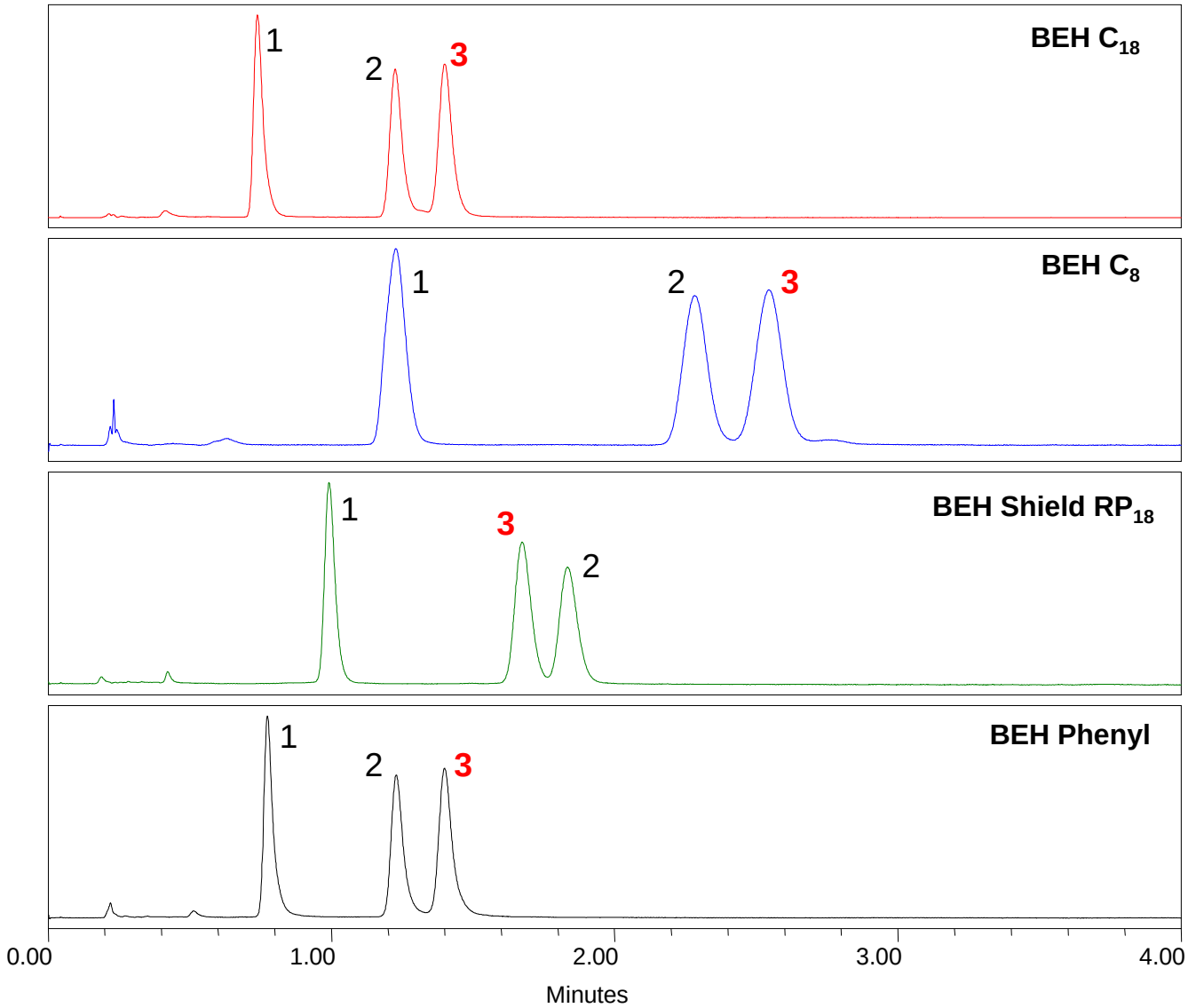


Note:
- **Elution order change between C_{18} and C_8 is not common**
- **Both C_{18} and C_8 provide sufficient resolution between all 14 analytes**

Flavanoids

Chromatographic Conditions
Flow Rate: 0.6 mL/min
Isocratic: 45% Methanol
Injection Volume: 5.0 µL
Sample Concentration: 17 µg/mL

Analyte
1. Quercetin
2. Kaempferol
3. Isorhamnetin



Caffeic Acid Derivatives in Echinacea Purpurea

Chromatographic Conditions :

Flow Rate: 0.5 mL/min

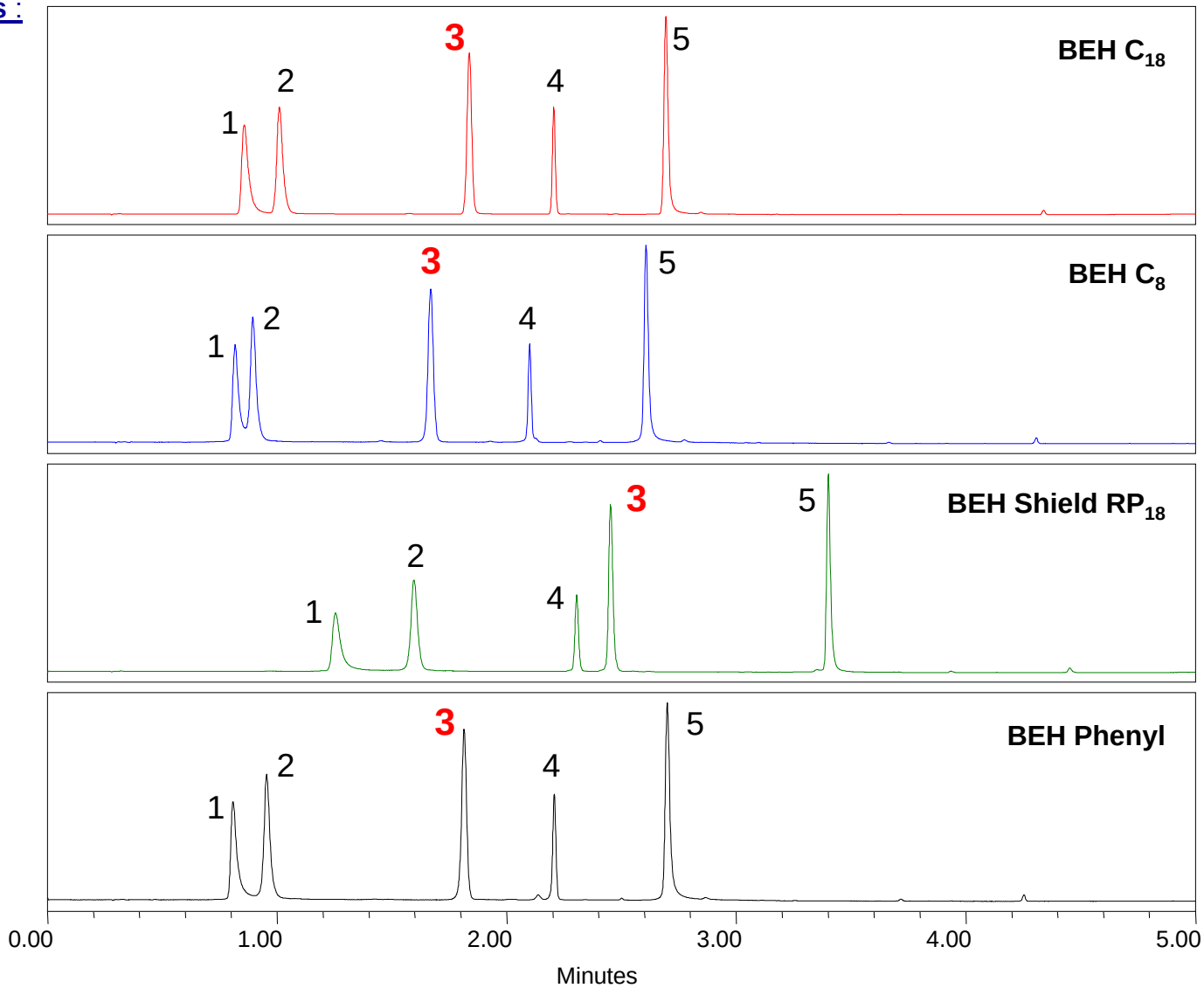
Gradient:

Time	Profile
0.0	8%B
0.1	8
4.45	50
4.86	90
5.0	8

Injection Volume: 1.0 µL

Sample Conc: 100 µg/mL

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



Non Steroidal Anti Inflammatory Drugs

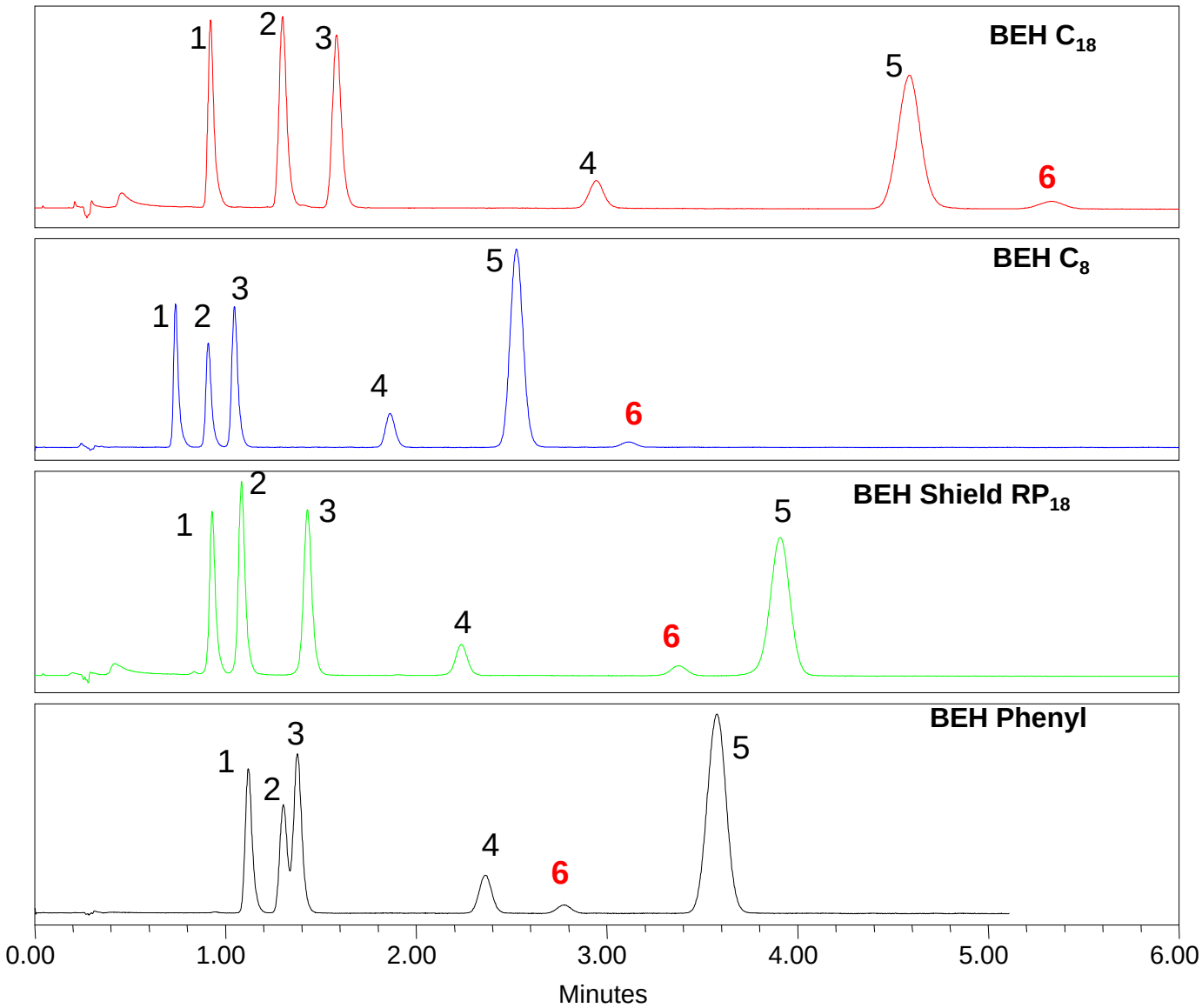
Chromatographic Conditions

Flow Rate: 0.5 mL/min

Isocratic: 50% Methanol

Injection Volume: 5.0 µL

Analyte	Conc. (µg/mL)
1. suprofen	4.2
2. tolmetin	8.3
3. naproxen	12.5
4. fenoprofen	25
5. diclofenac	25
6. ibuprofen	25



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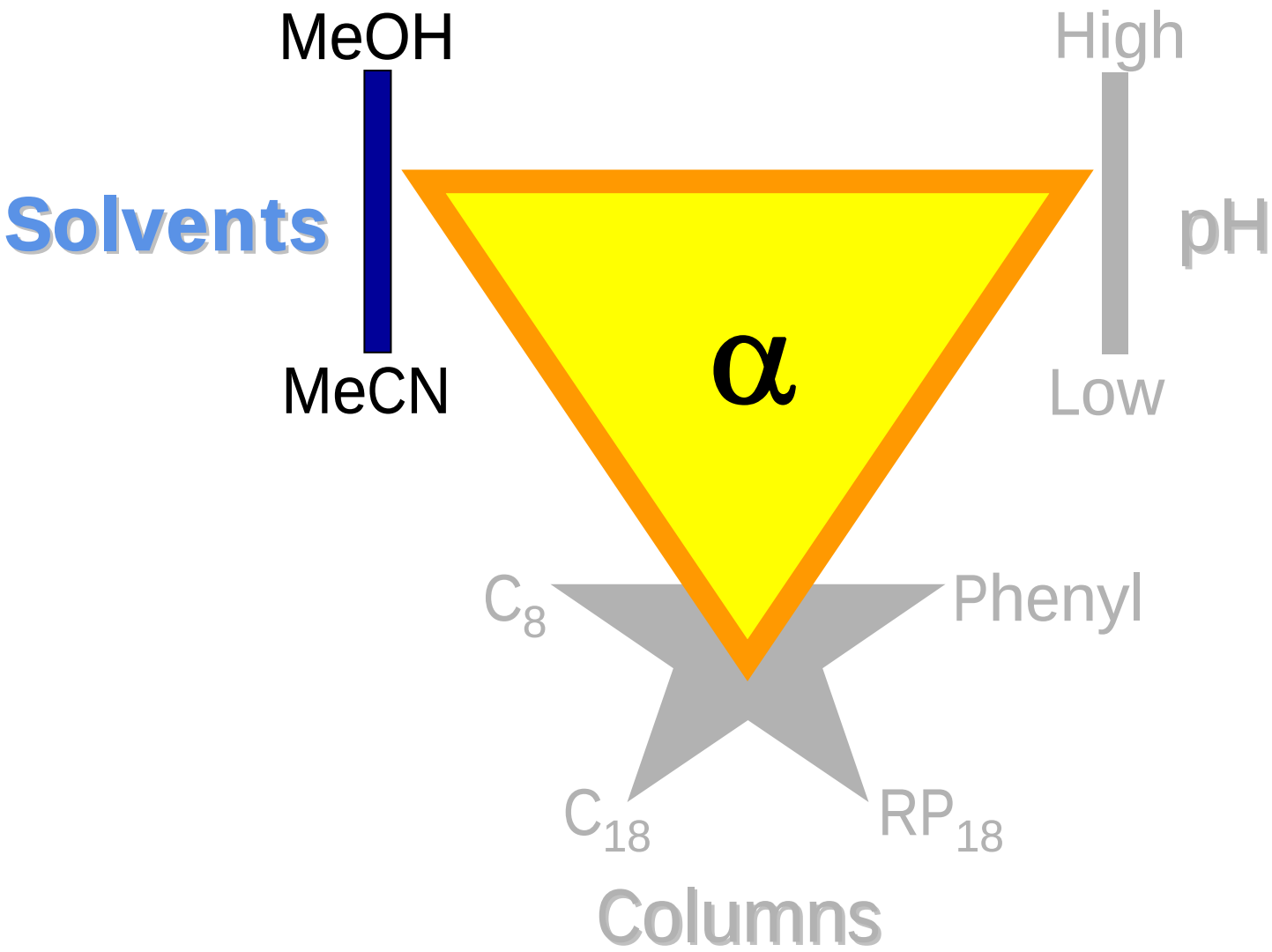
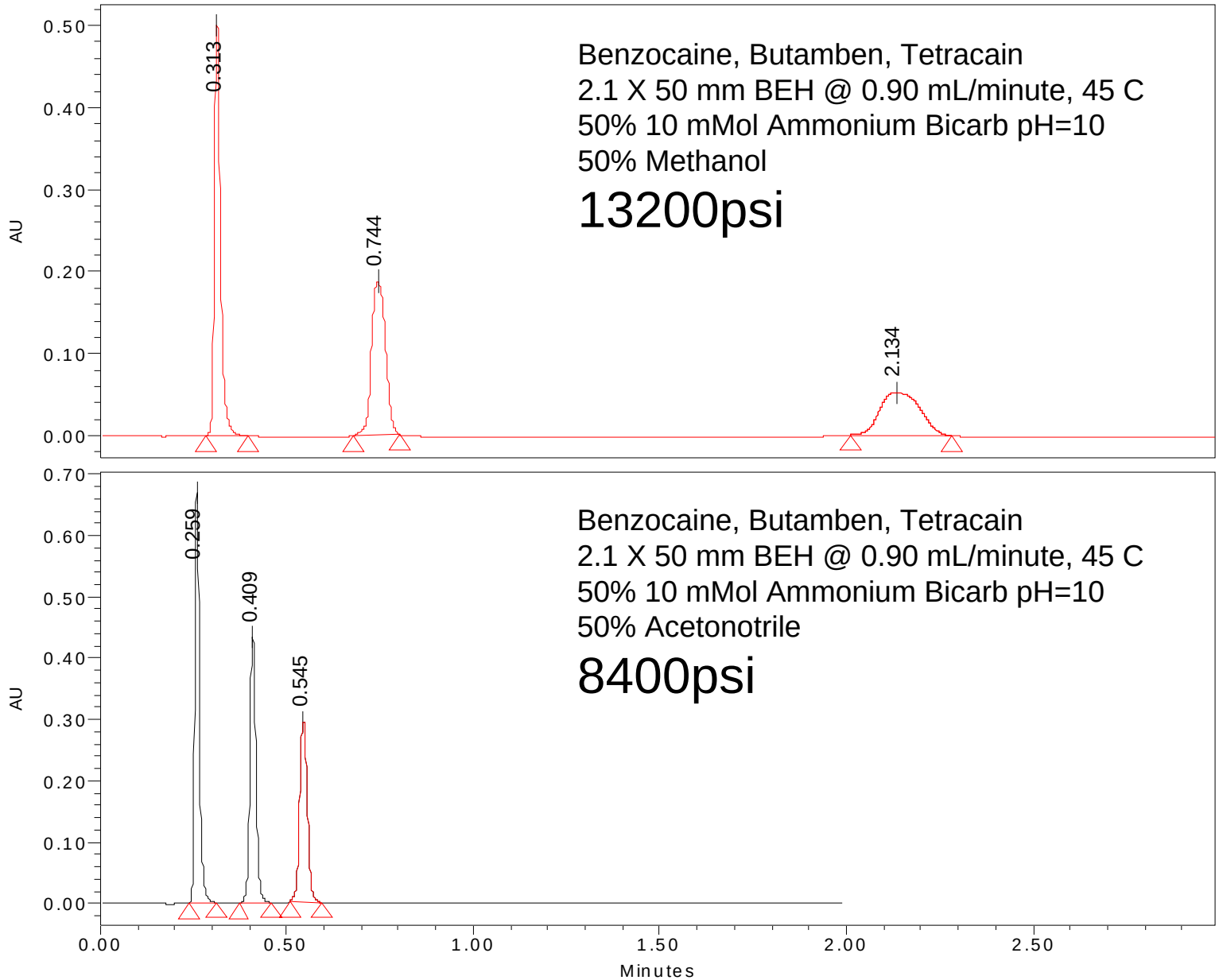
Selectivity Tools
Reversed-Phase ACQUITY UPLC™

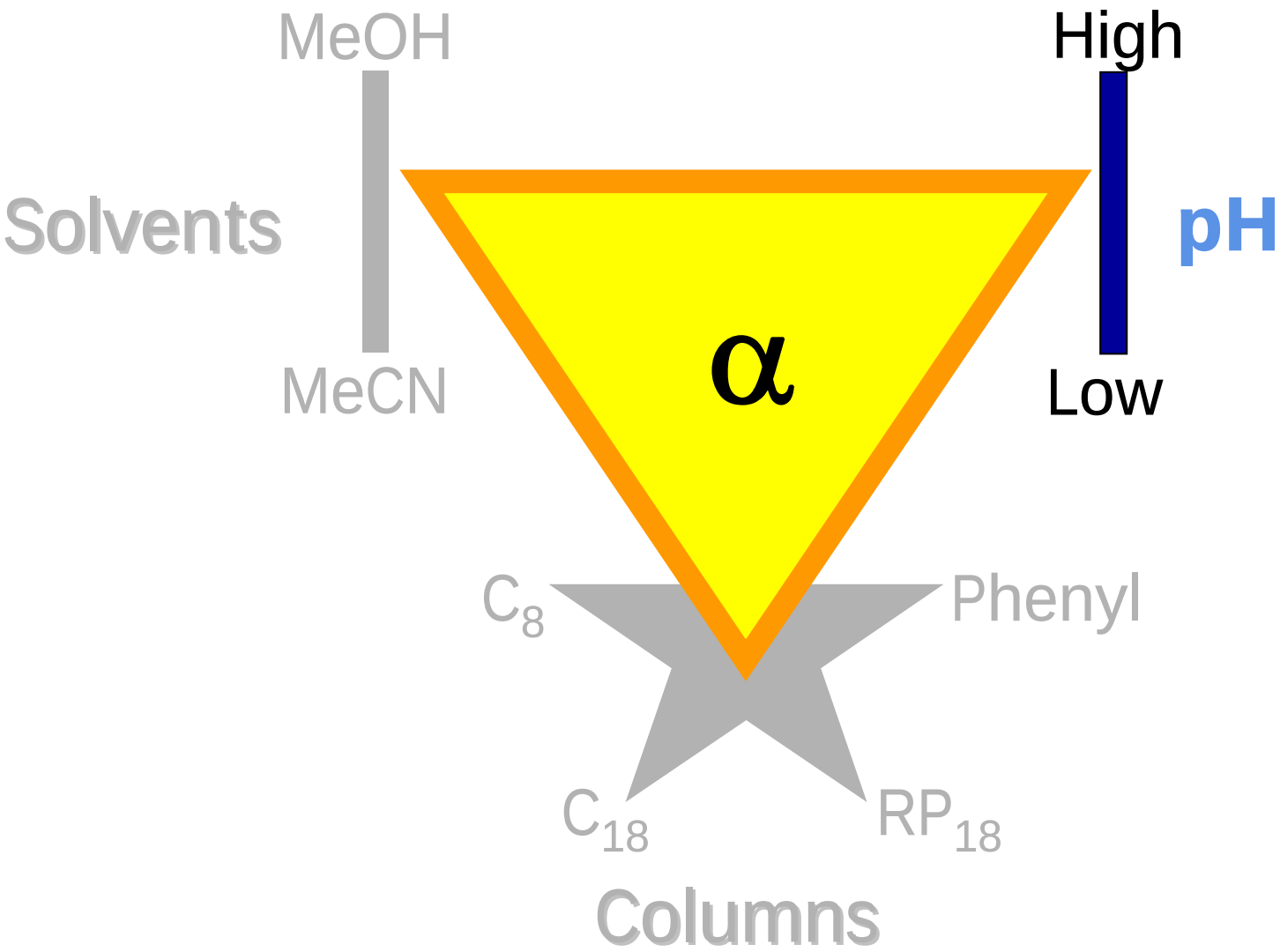


Organic Solvent as a Tool

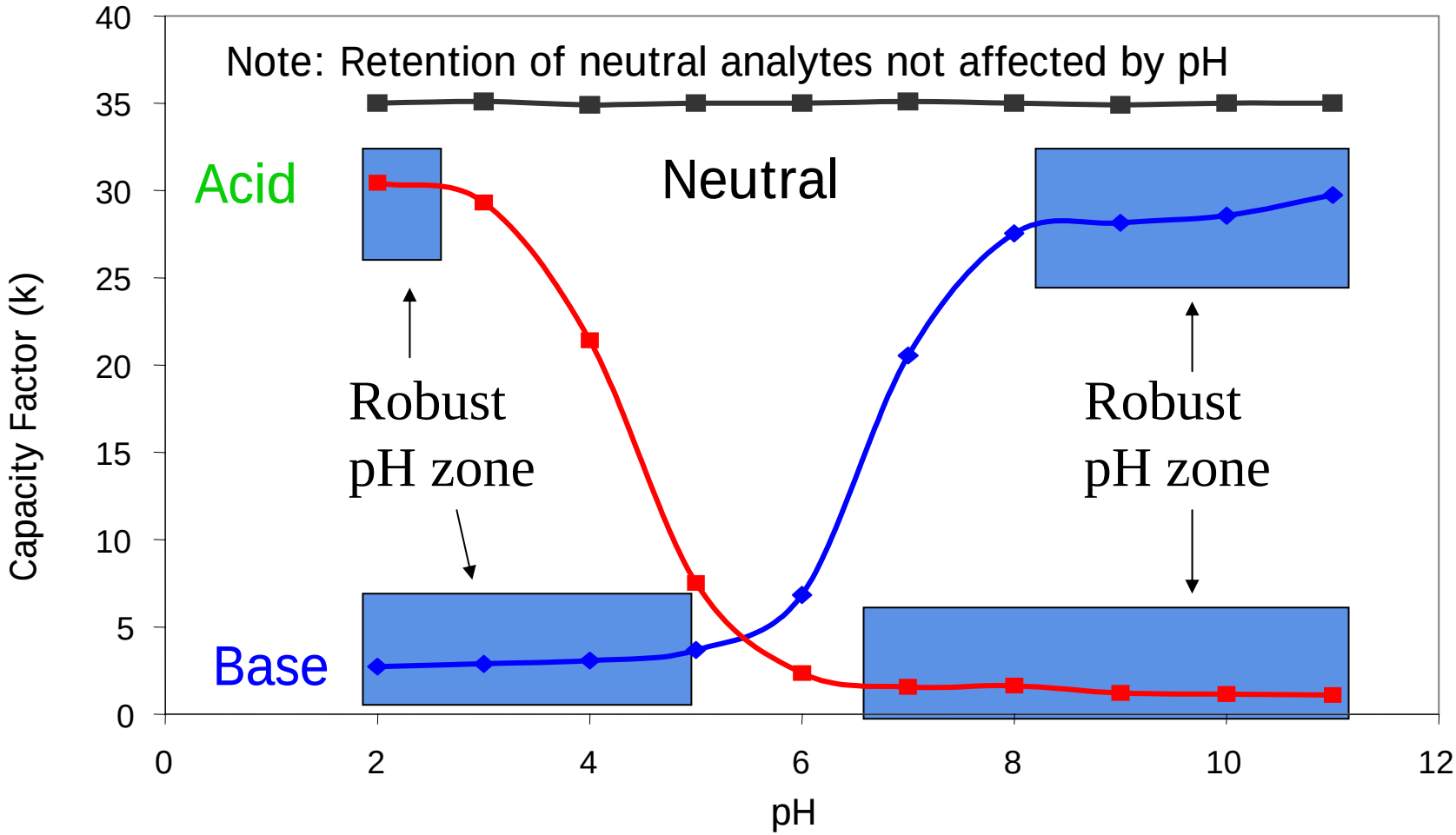


Waters

Selectivity Tools
Reversed-Phase ACQUITY UPLC™

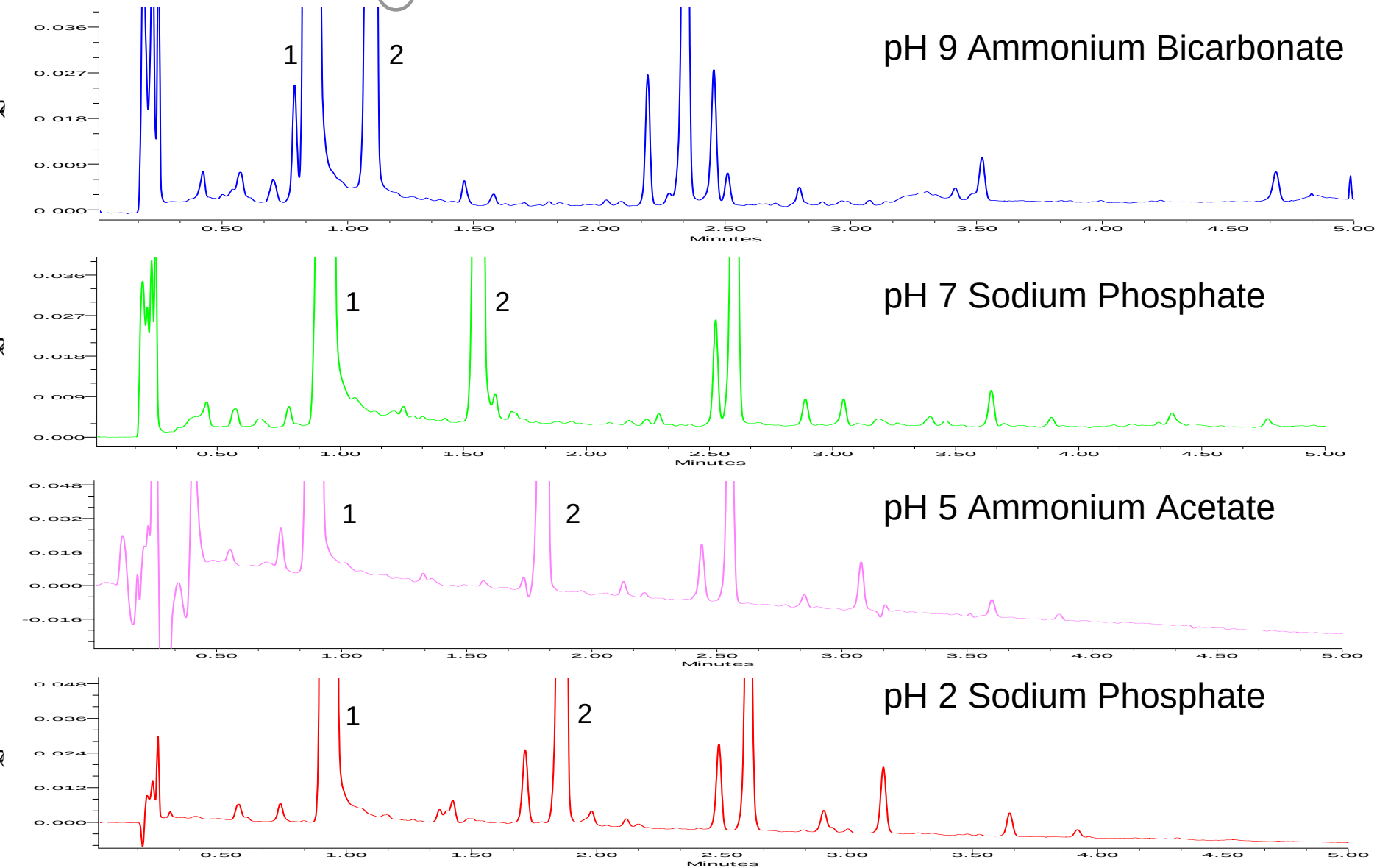


Behavior of Acidic, Basic and Neutral Compounds in RP Chromatography



Waters

pH as a Selectivity Tool
Hydrocodone (1) and APAP (2)

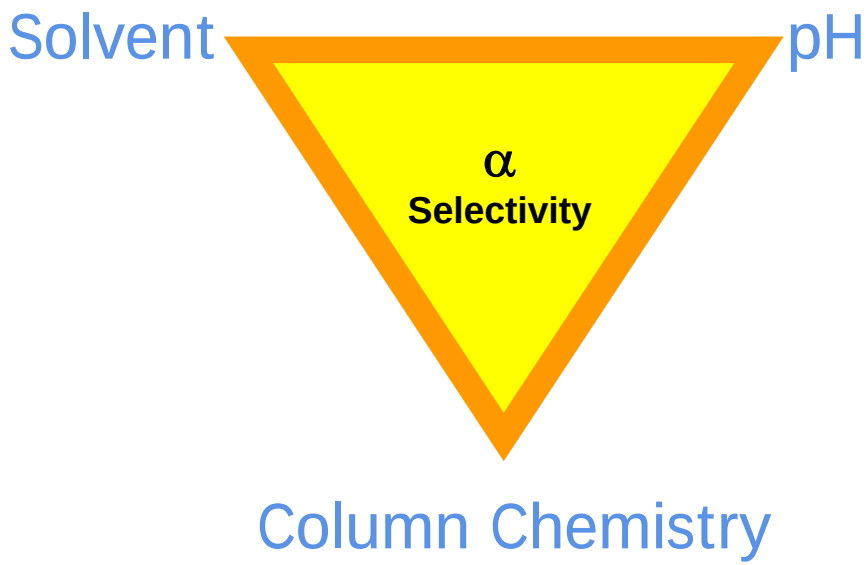


Change of Retention and Selectivity with pH

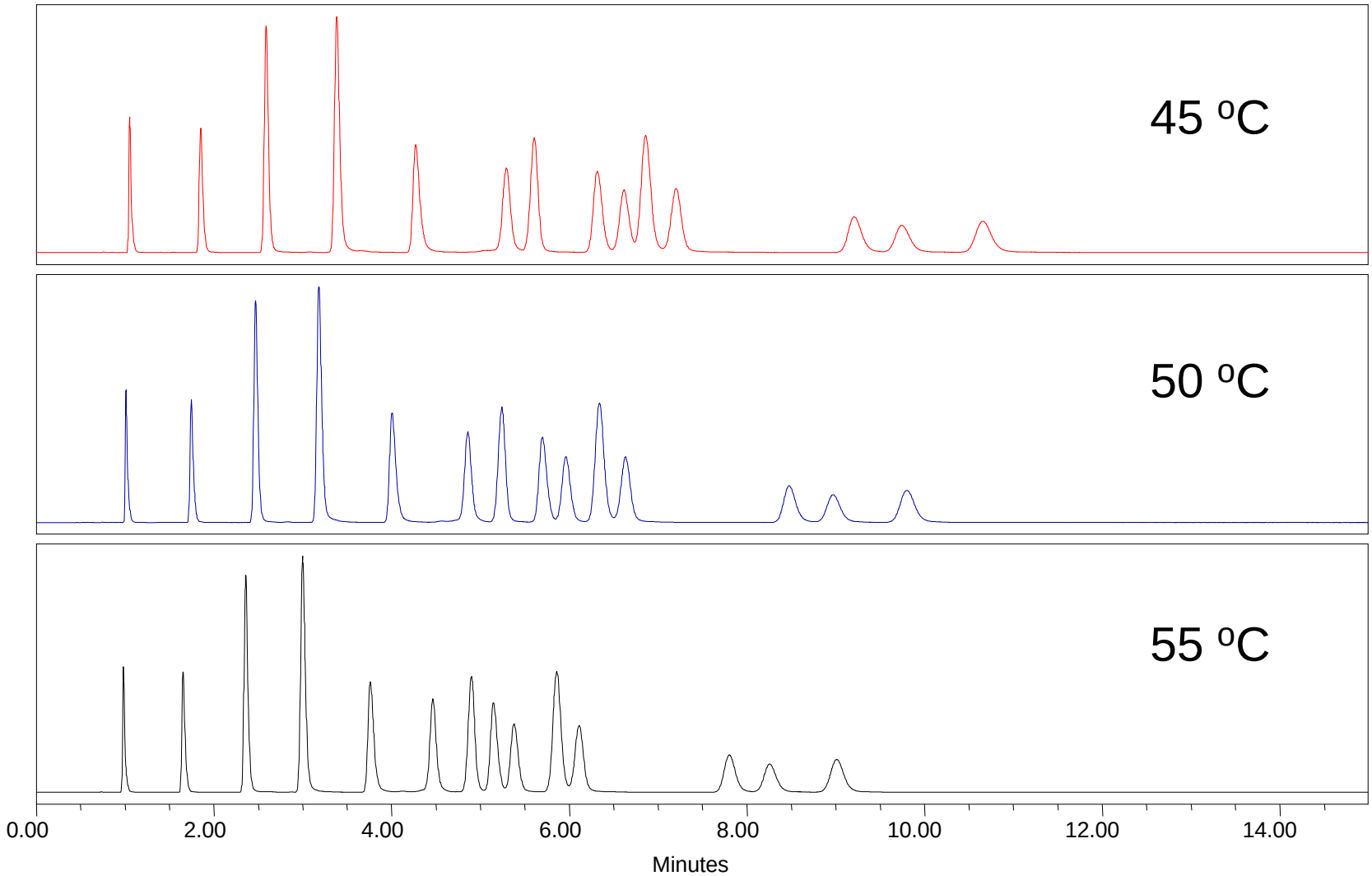
- 10 to 30 fold change of retention factor between ionized and non-ionized form of analyte
- Can have the most drastic changes in selectivity
- pH is a very useful tool in method development
- ACQUITY BEH™ Particle highly effective across a very broad pH range

Fine Tuning
Retention, Selectivity, and Run Time

- Temperature
- Linear Velocity



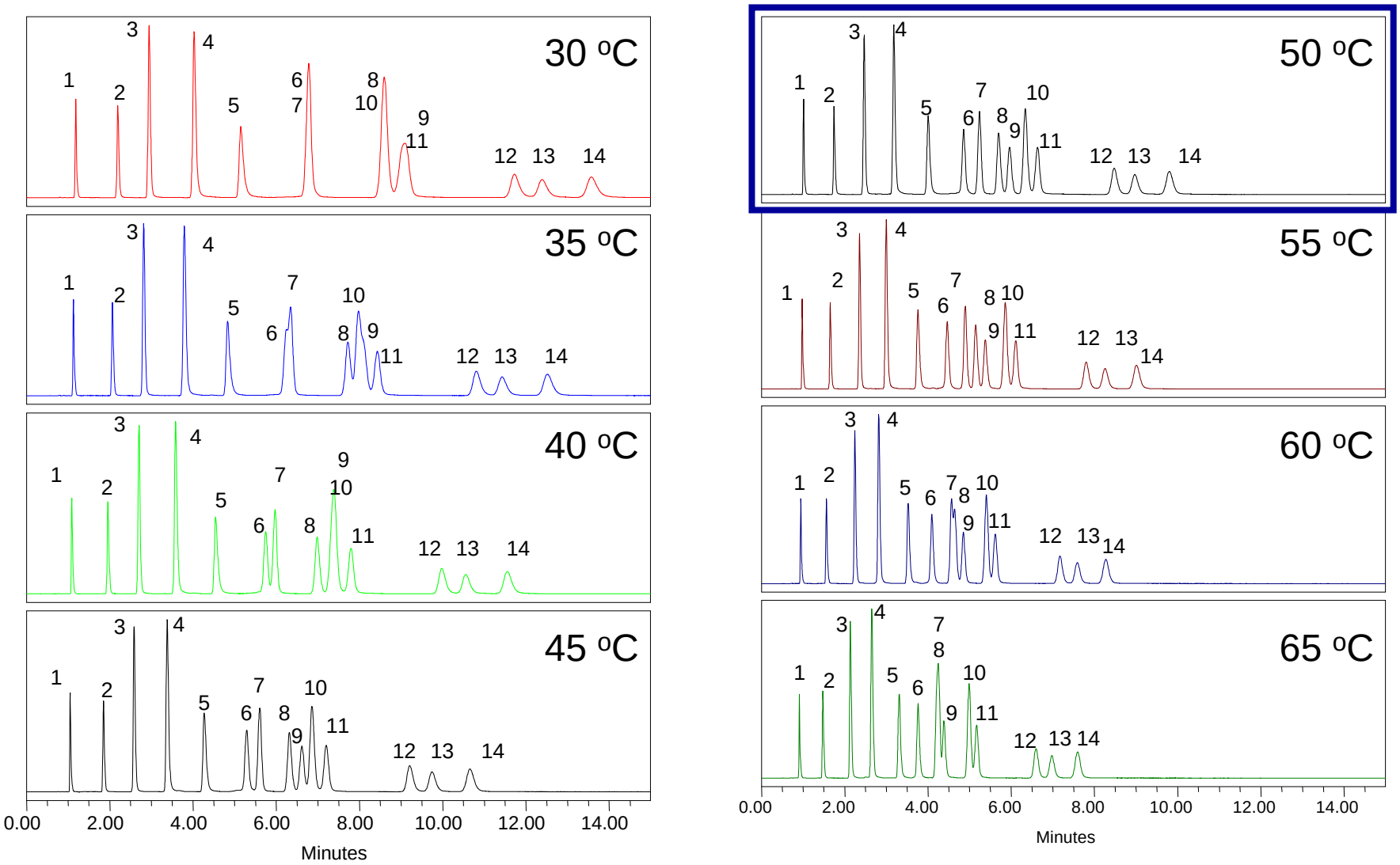
Method Robustness
Explosives (EPA Method 8330)



Note:

- **Analytes are very temperature sensitive, resolution effected in IPA**

Temperature as a Selectivity Tool:
ACQUITY UPLC™ BEH C_{18}

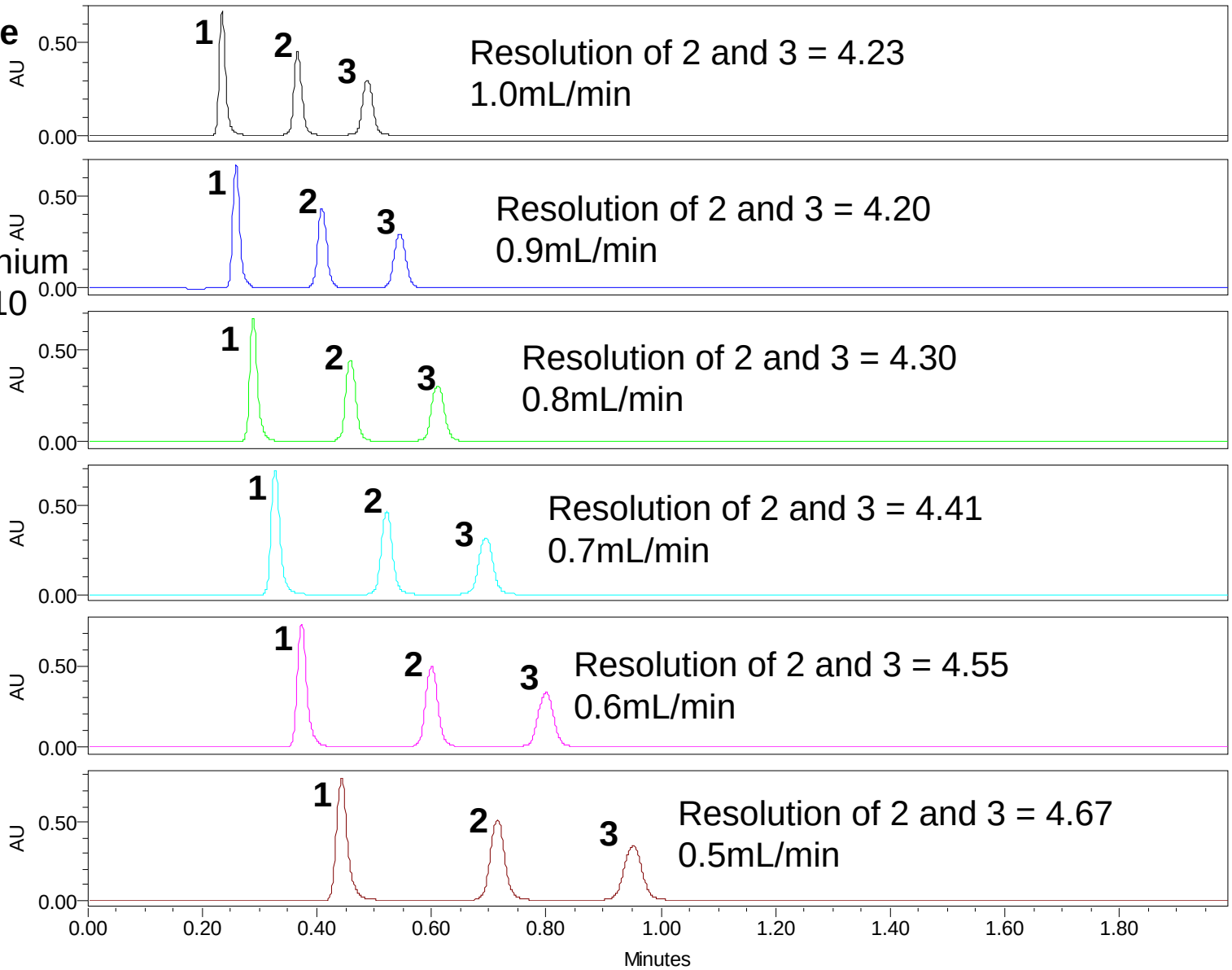


30% MeOH, 0.4 mL/min

Linear Velocity as a Tool

1. **Benzocaine**
2. **Butamben**
3. **Tetracain**

10 mM Ammonium Bicarb pH=10
50% AcN



Decreasing Stability Testing Costs by Increasing the Effectiveness of the Analytical Testing Process

American Pharmaceutical Outsourcing, JAN/FEB 2005

"Some (stability indicating) chromatographic runs may be complicated and/or have long run times due to using old … technology"

Paul Newton, Ph.D.

GlaxoSmithKline

Objective

To synergize the multiple USP methods including Triamterene, Hydrochlorothiazide, and related compounds into one comprehensive method

- Triamterene Capsules USP
  - Assay
- Hydrochlorothiazide Tablets USP
  - Assay
  - Related Compounds
- Triamterene and Hydrochlorothiazide Tablets USP
  - Assay
  - Related compounds
  - Dissolution assay
- Triamterene and Hydrochlorothiazide Capsules USP
  - Assay
  - Related compounds
- Triamterene (drug substance) USP
  - Limit of N-TAP
  - Assay

10 separate instances of instrument set-up!

Stability Indicating Method
Five USP Methods

Considerations

- Components of interest
  - Hydrochlorothiazide (**HCT**)
  - Triamterene (**TMT**)
  - Benzothiadiazine Related Compound A (**ACBS**)
    - Aka: 4-amino-6-chloro-1,3-benzenesulfanamide (HCT related)
  - 2,4,6-triamino-5-nitrosopyrimidine (**N-TAP**)
    - TMT related for drug substance
  - Methylbenzenesulfanamide (**MBS**)
    - Observed degradant on expired samples
    - Note: MBS not specifically mentioned in any of the USP methods.
- Requirements to meet
  - Tailing for Triamterene < 2.0
  - Resolution between HCT and TMT > 3.0
  - Resolution between HCT and ACBS >2.0

Waters

Stability Indicating Method
Five USP Methods

USP methods consist of:

- 3 mobile phases
  - All with different buffers
  - pH's vary from 3.0, 5.0, and 5.5
- 3 columns
  - L1, L10 (CN), and L11 (phenyl, with L7 guard column)
  - ID's and Lengths vary
- 4 flow rates
  - 1mL/min, 1.2 mL/min, 1.5mL/min and 2.0mL/min
- 2 injection volumes
  - 10uL and 20uL
- Run times vary from 8 minutes to 30 minutes

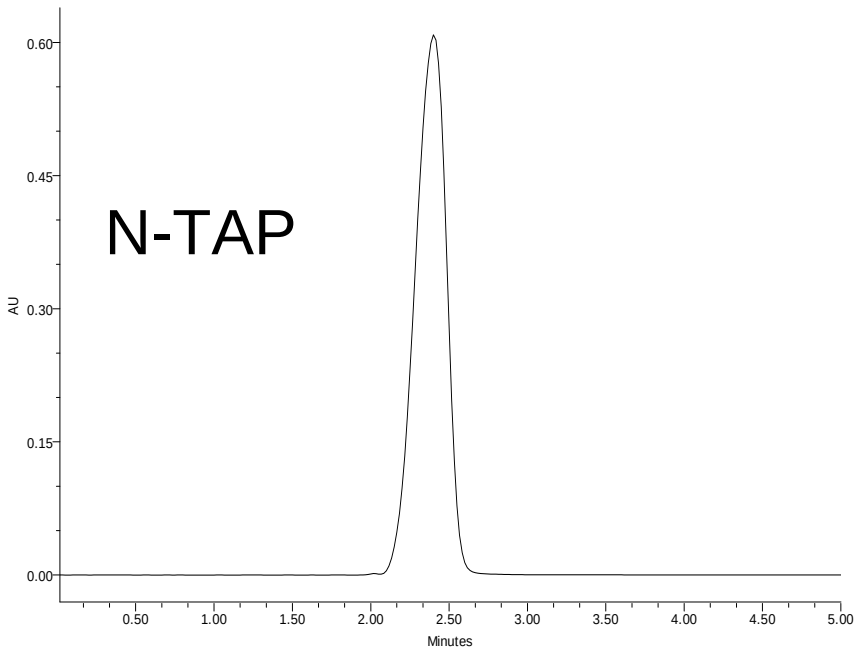
Goal

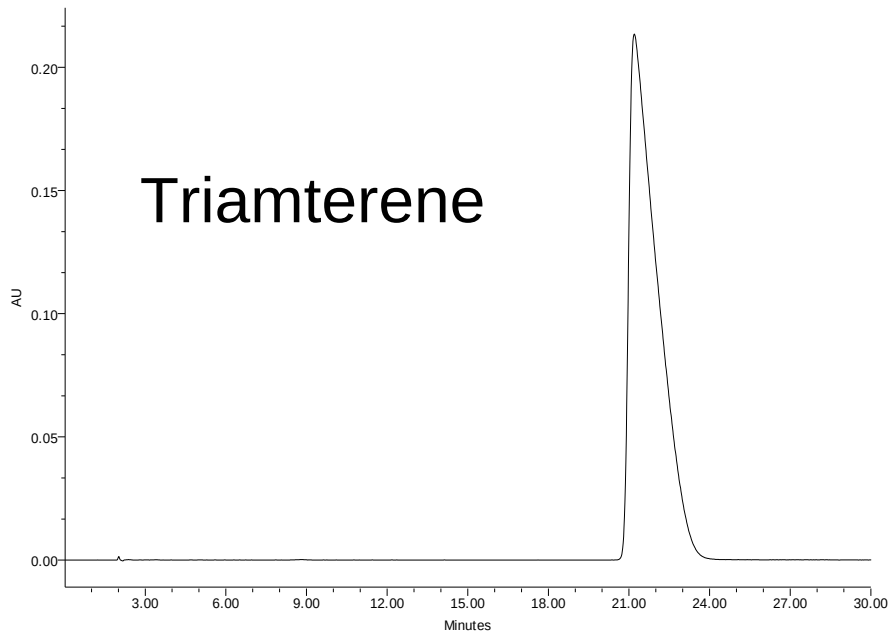
- Consider combining various standard and test solution methods for a "universal" application.

Triamterene USP

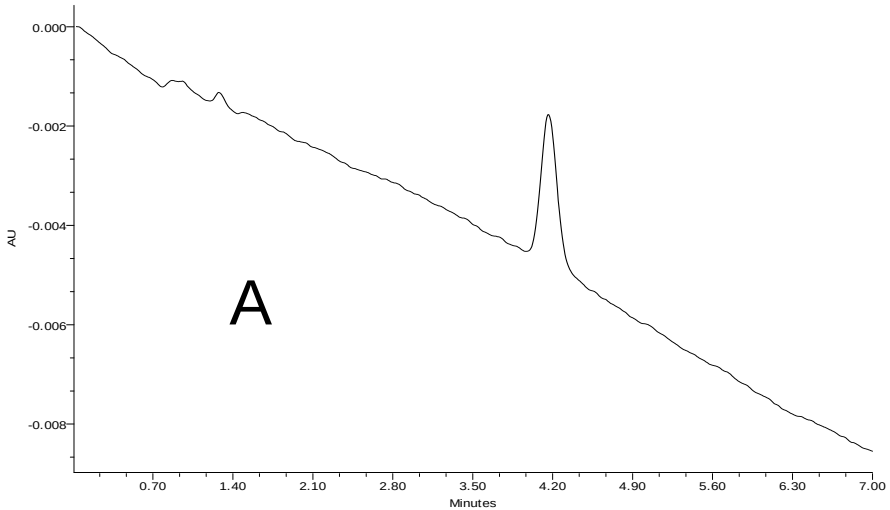
Challenges:

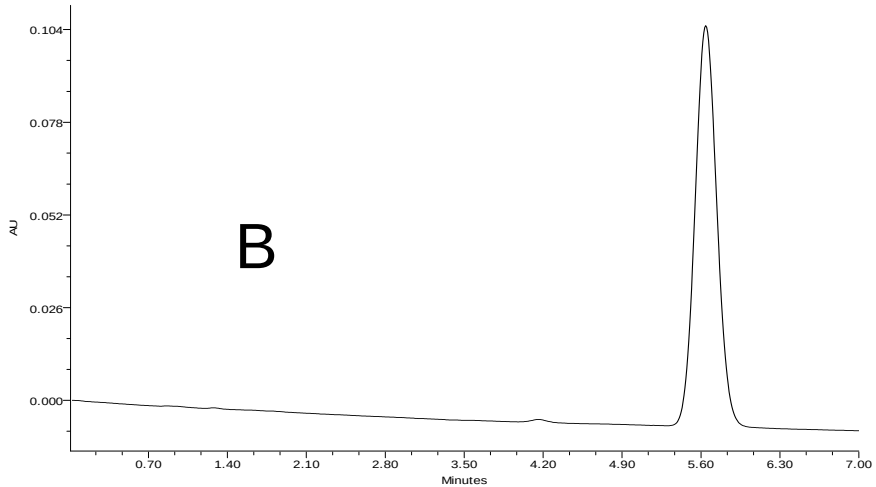
- Two separate injections
  - –N-TAP elutes close to void
  - –Tailing issues with TMT
- 30 minute run time for TMT

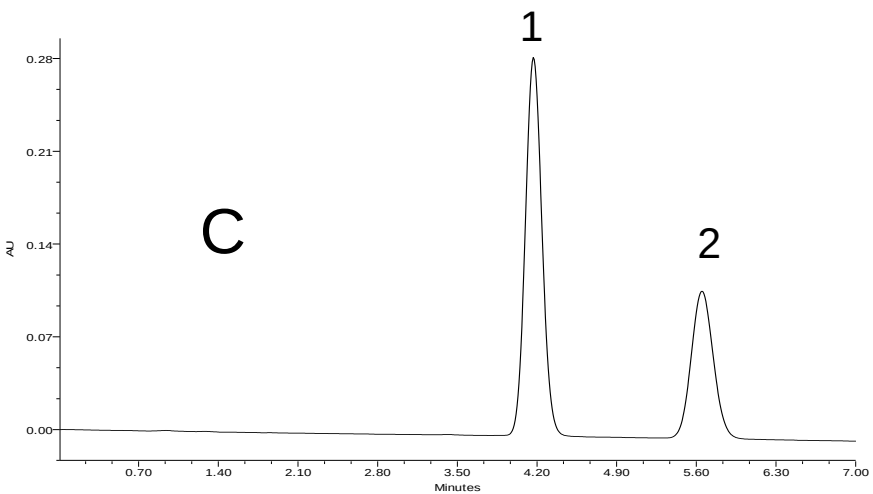




Hydrochlorothiazide Tablets USP





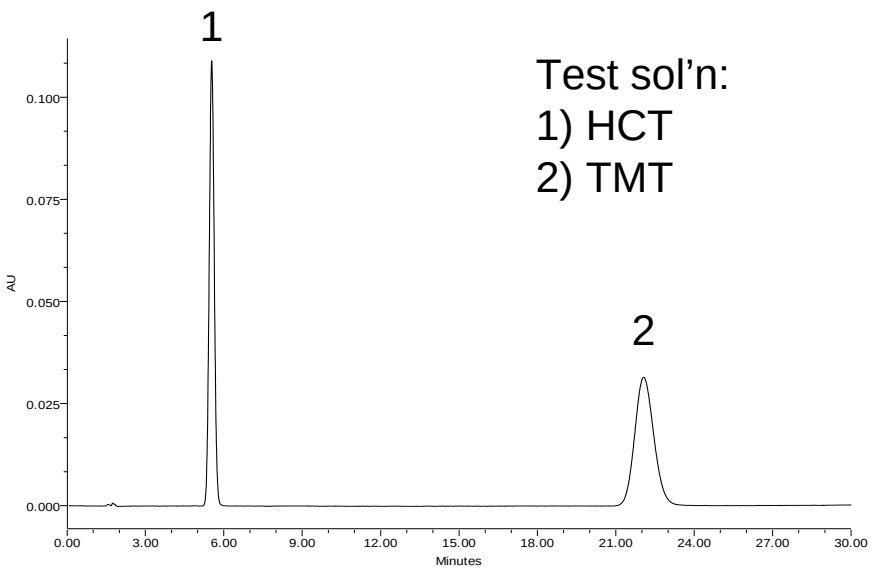


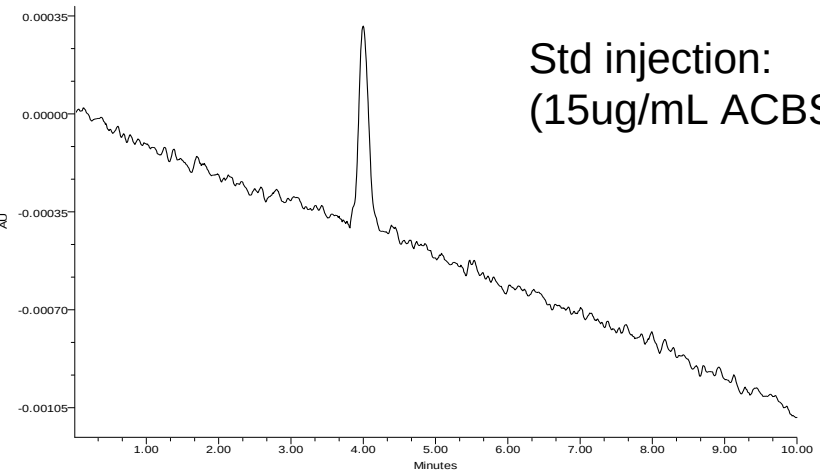
A – HCT related compound
B – HCT Standard sol'n
C – HCT Test sol'n
    1) ACBS
    2) HCT

*Assay and related compounds use same mobile phase and test solution

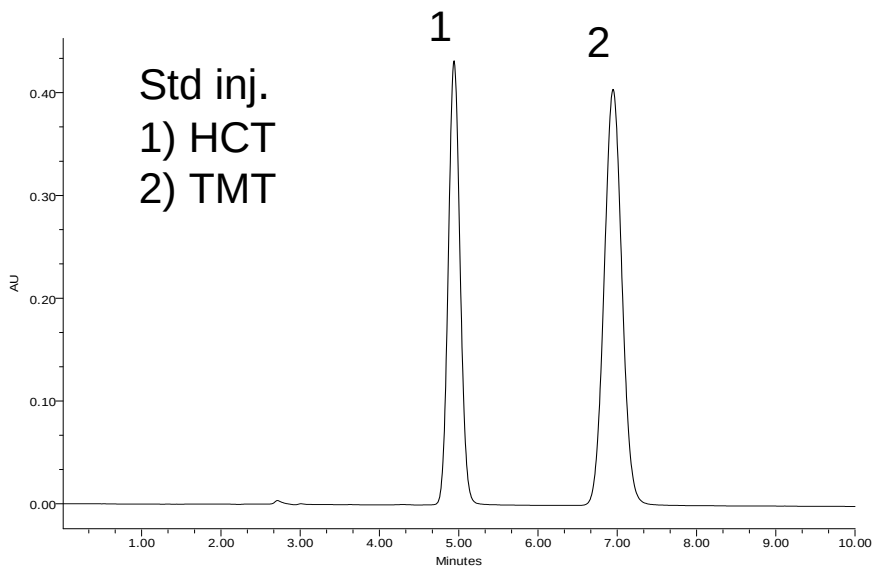
Hydrochlorothiazide and Triamterene
USP for Tablets and USP for Capsules (2x)

Related compound (Section1)





Assay (Section2)



Tablets and Capsules similarities:

1. Assays have same MP and Std
2. Rel subs. have same MP and Std
3. Tablets dissolution HPLC conditions are the same as assay procedure

Waters

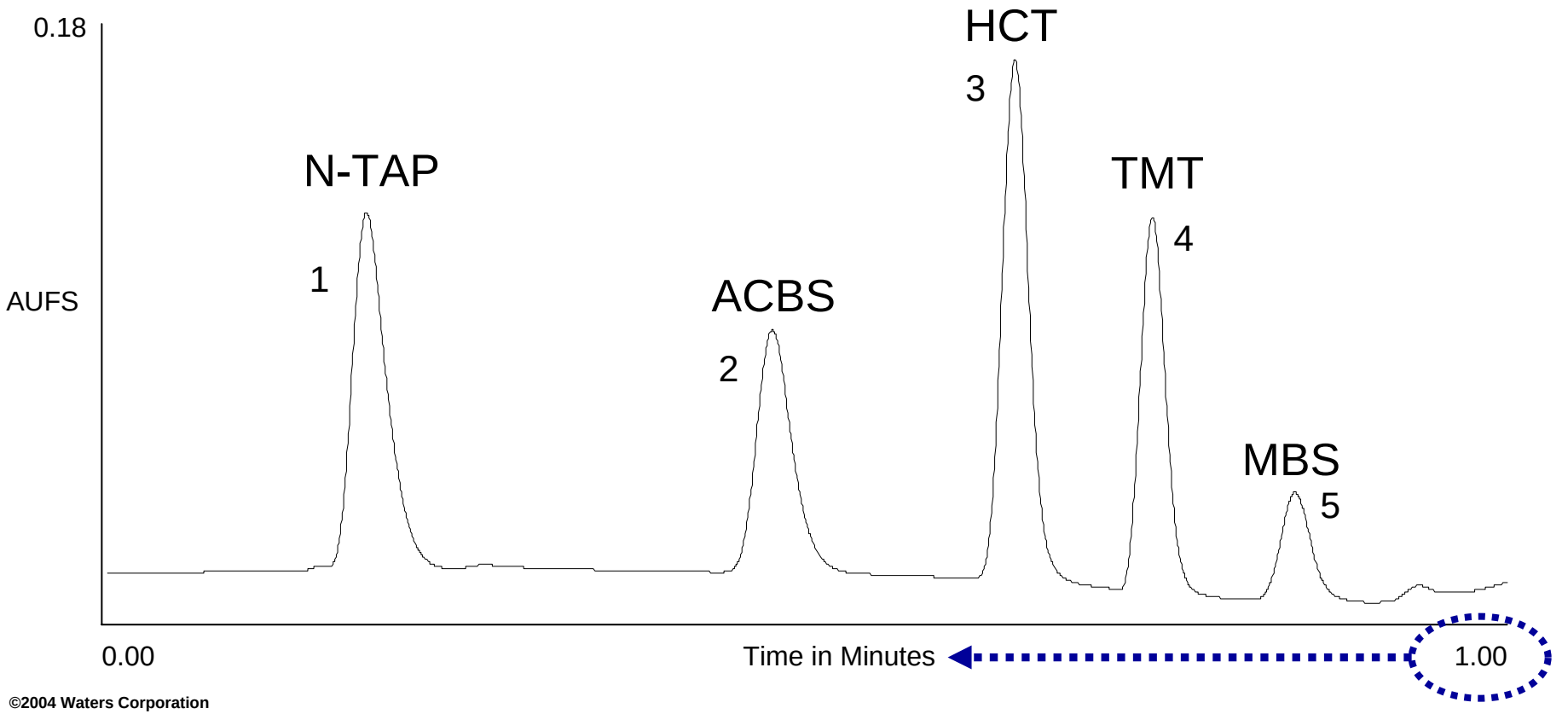
USP HPLC System
Stability Indicating Method

- 5 HPLC USP Stability Indicating Methods for Hydrochlorothiazide and Triamterene capsules and tablets, and their related compounds
  - 3 mobile phases
  - 3 columns
  - 4 wavelengths
  - 4 flow rates
  - 2 injection volumes
  - 11 Chromatograms
- Total Run time of just over 2.5 **hours**, total analysis time of 9.5 hours on 2 HPLC Systems
- System Suitability Time?
  - 2 additional days

ACQUITY UPLC™ System
Stability Indicating Method

Peak ID:
1: 5-nitroso-2,4,6-triaminopyrimidine
2: 4-amino-6-chloro-1,3-benzenesulfanamide
3: hydrochlorthiazide
4: triamterine
5: methylbenzenesulfanamide
0.1 mg/mL each

UPLC Conditions:
A=10mm Ammonium Formate, pH 4.0
B=ACN
45 sec gradient, 5-85 %B linear
2.1 x 30mm ACQUITY UPLC™ Column
30° C, 0.8 mL/min. UV 273nm, 40 pts/sec.



Waters

ACQUITY UPLC™ System
Stability Indicating Method

- Single ACQUITY UPLC™ Stability Indicating Method
  - 1 mobile phase
  - 1 column
  - 1 wavelength
  - 1 flow rate
  - 1 injection volume
- Total Run time of 60 ***seconds***, total analysis time of 15 minutes
- **35.5 times** more productivity on a single ACQUITY UPLC™ System
- System Suitability Time?
  - Less than 10 minutes

UPLC Method Development

- Different from HPLC Method Development?
  - An extension of everything you already know from HPLC
- Can we take advantage of the ACQUITY UPLC™ System's:
  - Theoretical Speed advantage?
  - Theoretical Sensitivity advantage?
  - Theoretical Resolution advantage?
- Generic Gradient?
  - Sample dependent, but
    - Starting with a 4 minute and a 7 minute 0% to 100% Organic linear gradient will typically generate enough data to calibrate the separation.

Conclusion

- "More Resolution *Faster*"
  - ACQUITY UPLC™ technology enables you to consistently get your products to market faster
    - Speed
      - Methods developed faster, more analyses per unit time with no loss of information, more product released faster to market
    - Method simplification
      - Potential elimination of complex organic modifiers and solvent systems, enhanced method Robustness, methods developed faster
    - Reproducibility
      - Sharper peaks, increased resolution, and positive identification means fewer out of specification (OOS) events
    - Reliability
      - Less downtime, more continuous operation, more throughput
    - Service and Support
      - Automated Method Validation, IOP/Q products, operator training, and regulatory compliance

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THANK YOU

craig_dobbs@waters.com
508.482.2578