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Transferring Yesterdays Methods to Tomorrows Technology: The Evolution from HPLC to UPLC™

Eric S. Grumbach

Thomas E. Wheat

Chuck Phoebe

Joe Arsenault

Jeffrey R. Mazzeo

Diane M. Diehl

PITTCON® 2005

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UPLC™ TECHNOLOGY

- 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 without compromise
- Suitable for chromatographic applications in general
  - Appropriate for developing new methods
  - Appropriate for improving existing methods

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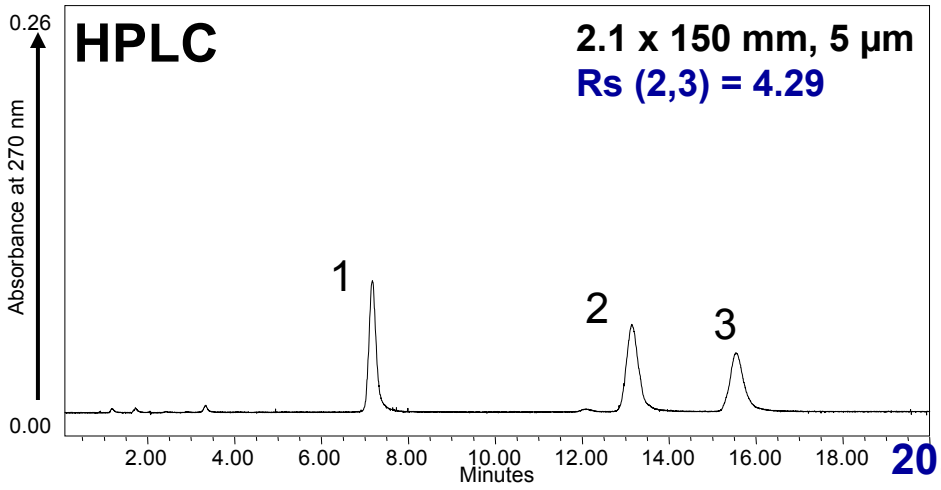
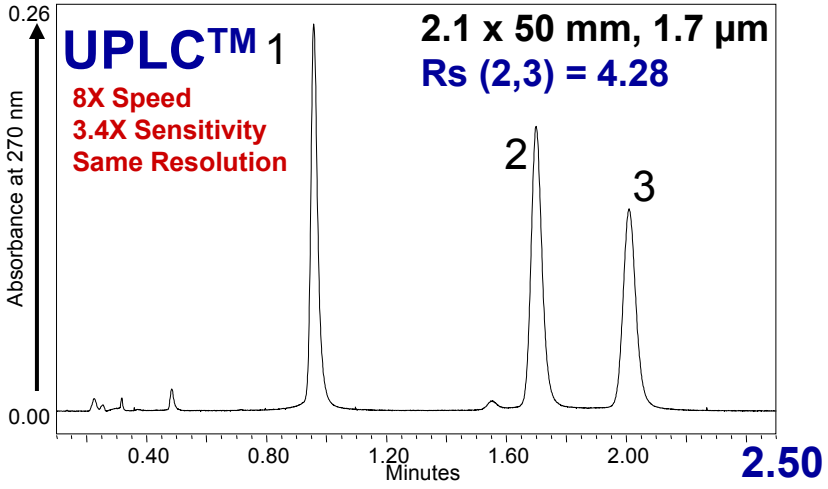
HPLC vs. UPLC™

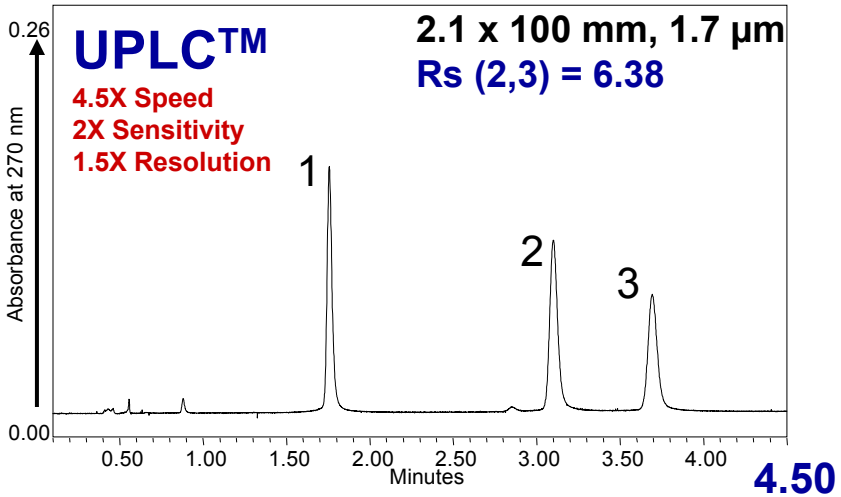
Speed, Sensitivity and Resolution



Faster, More Sensitive Methods

Faster, More Sensitive, Higher Resolution Methods





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What Does Ultra Performance LC™ Bring to the Chromatographic Laboratory?

Choices available:

- Increased speed and sensitivity with the same resolution
- Increased sensitivity and speed with resolution
- Increased resolution and sensitivity at the same speed

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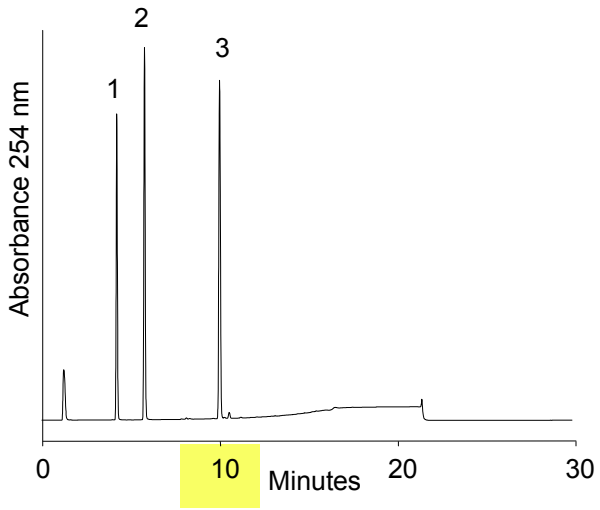
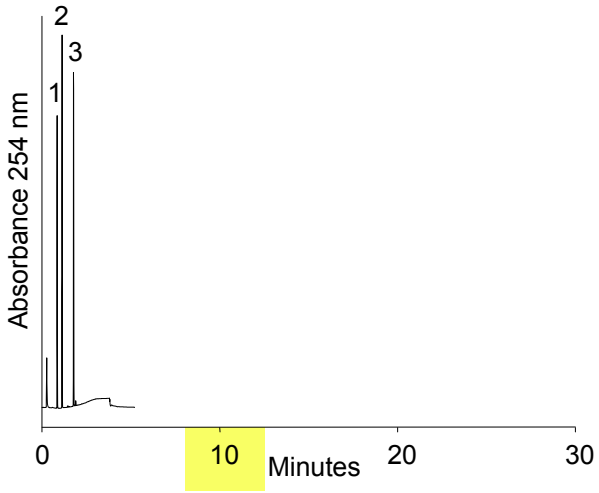
HPLC to Optimized UPLC™

An Example of a Successful Method Transfer

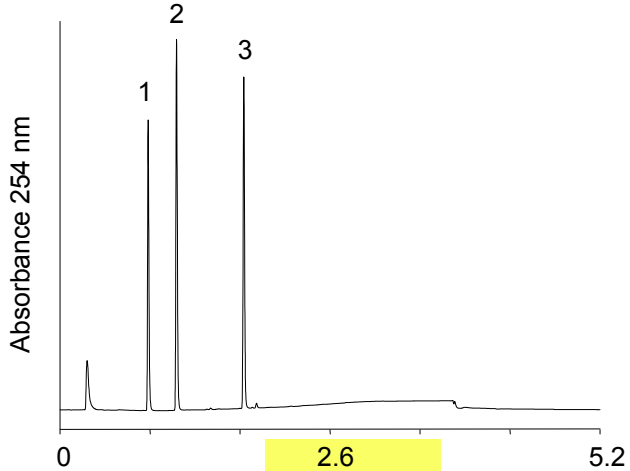


HPLC



Optimized UPLC™

Magnification of Optimized UPLC™



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Methods Transfer Considerations

- Classes of methods transfer
  - Replace a column brand with another
  - Replace an instrument with another
  - Replace both the instrument and the column
- The benefits of UPLC™ can only be realized with a new column on a new instrument
- As with any project to transfer a Liquid Chromatographic method, proper consideration of all the factors which control the separation process is essential for success

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UPLC™ Column Selection
Internal diameter and length

- **Internal diameter**
  - 2.1 mm or 1.0 mm

- **Length**
  - If primary goal is **speed**, choose 50 mm length
  - If primary goal is **resolution**, choose 100 mm length

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Ratio of Column Length to Particle Size

L/dp		RATIO		Typical Run Times
300mm / 10 µm	=	30,000	1970's	~ 30+ min.
150mm / 5µm	=	30,000	1980's	~10-15 min.
100mm / 3 µm	=	33,300	1990's	~ 5-10 min.
50mm / 1.7µm	=	29,500	2004	~1- 2 min.
100mm / 1.7µm	=	58,820	2x Maximum Resolution Capability	

If you keep the L/dp ratio the SAME for 2 columns, you will obtain the SAME Resolution. With smaller particle sizes in shorter columns, you will achieve the same separation, but in LESS TIME!

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UPLC™ Column Selection
Ligand Selection

Available Ligands:

- ACQUITY UPLC™ BEH C_{18}
  - Straight chain alkyl C_{18}
- ACQUITY UPLC™ BEH Shield RP_{18}
  - Embedded polar group (carbamate)
- ACQUITY UPLC™ BEH C_8
  - Straight chain alkyl C_8
- ACQUITY UPLC™ BEH Phenyl

Why Multiple Ligands?:

- Changes in hydrophobicity
- Changes in silanol activity
- Changes in hydrolytic stability
- Changes in ligand density
- Changes in selectivity

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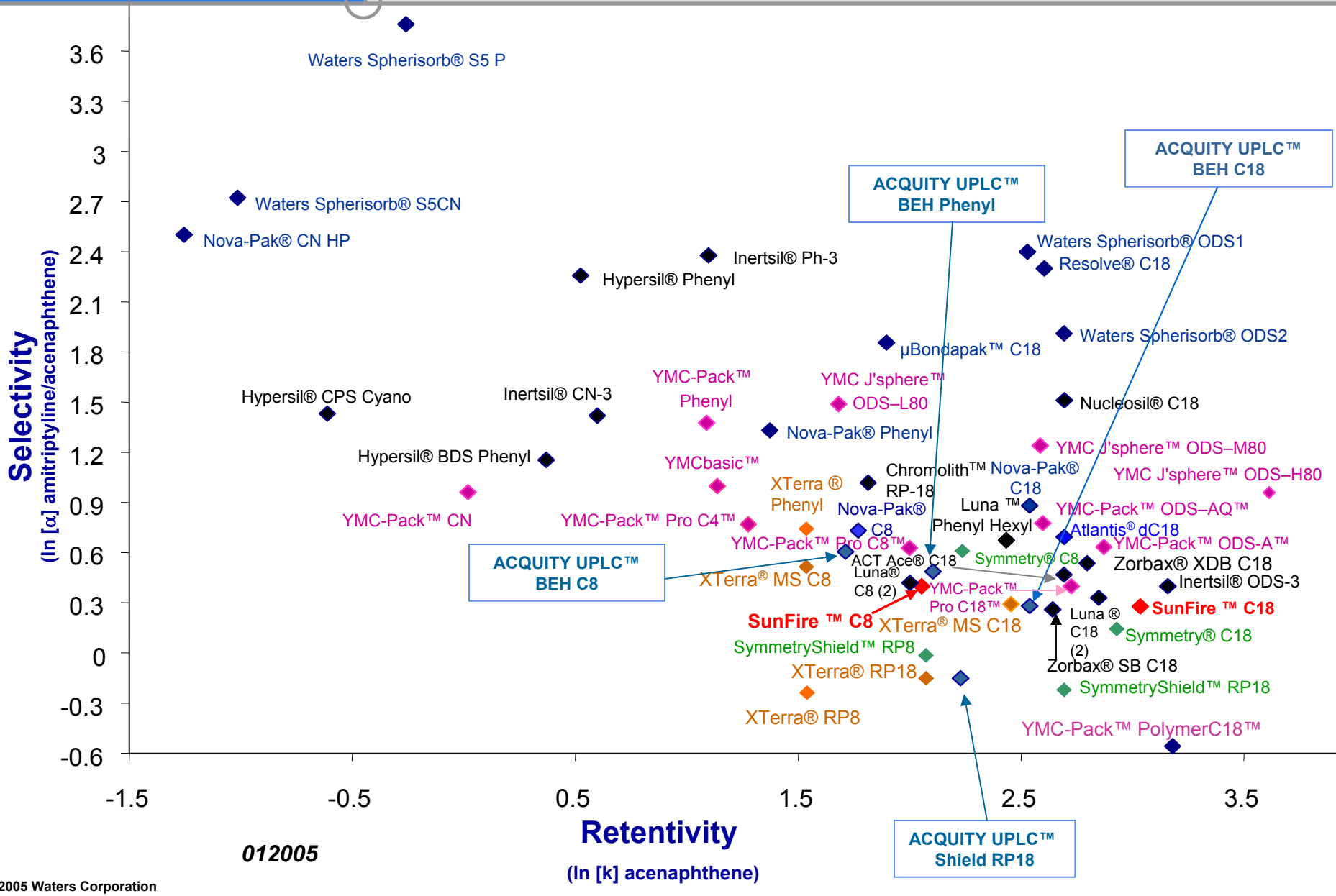
Reversed-Phase Column Selectivity Chart



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Method Transfer Consideration

- Solvent delivery
  - Scale flow rate appropriate to column dimensions
  - Scale linear velocity appropriate to particle size
  - Adjust all segments of method, including equilibration
- Sample injection
  - Scale injection volume
- Detection
  - Ensure proper data sampling rate
  - Ensure proper time constant

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Original Gradient Profile

4.6 x 150 mm, 5 µm HPLC Column

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

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Target Conditions – Gradient Profile

- Express gradient duration in % 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 UPLC™ column at the chosen flow rate.

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Express Gradient Segments (Steps) in Units of Column Volumes

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

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

Column Volume = π x r^2 x L = 3.14 x 2.3^2 x 150 = 2.49 mL

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

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

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Original Gradient Profile for Scaling

4.6 x 150 mm, 5 µm HPLC Column

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

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Estimate Optimum UPLC™ Flow Rate

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

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Scaling Gradient Step Time for UPLC™ Flow Rate - Maintain Duration (cv)

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) = 0.17 mL

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

= 9.03cv x 0.17 mL = 1.54 mL

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

= 1.54 mL / 0.60 mL/min. =

2.6 min.

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Optimized Gradient Profile

2.1 x 50 mm, 1.7 µm UPLC™ Column

Gradient Step	Time Since Injection	Flow Rate	% A	% B	Curve	Segment Duration (min)	Segment Duration (Col.Vol.)
Initial	0	**0.6**	95	5	*	0	0
Initial Hold	0	0.6	95	5	1	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**

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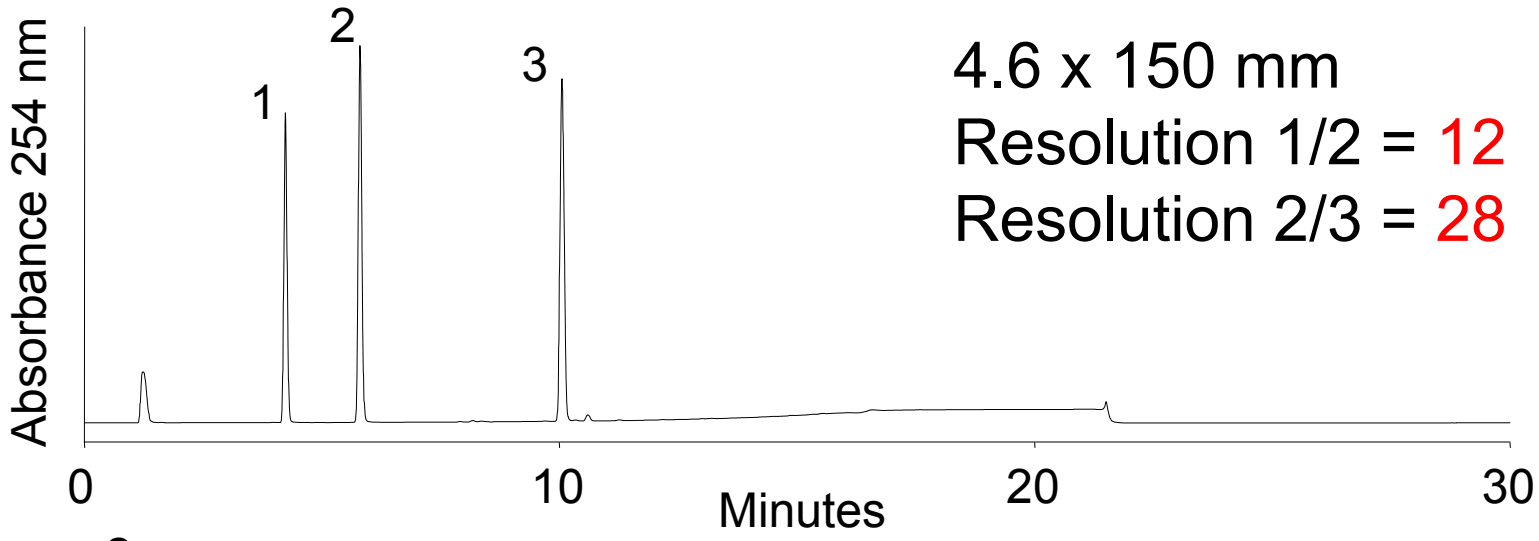
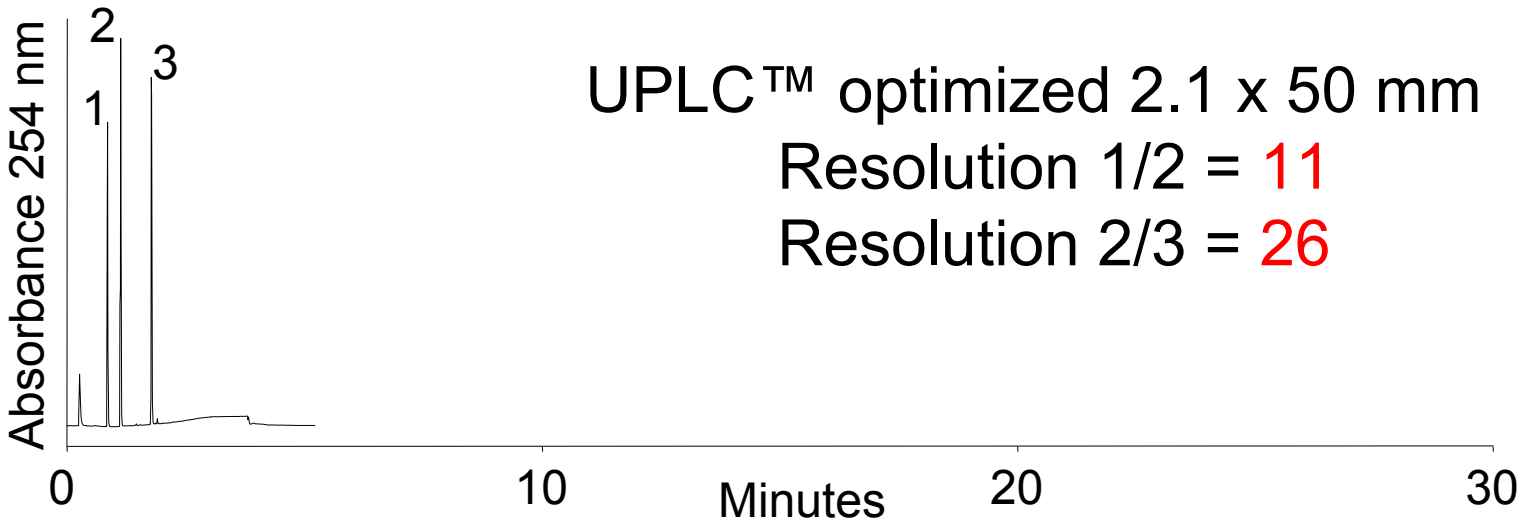
Comparing Results:
Does the new method meet resolution criteria?



4.6 x 150 mm
Resolution 1/2 = 12
Resolution 2/3 = 28



UPLC™ optimized 2.1 x 50 mm
Resolution 1/2 = 11
Resolution 2/3 = 26

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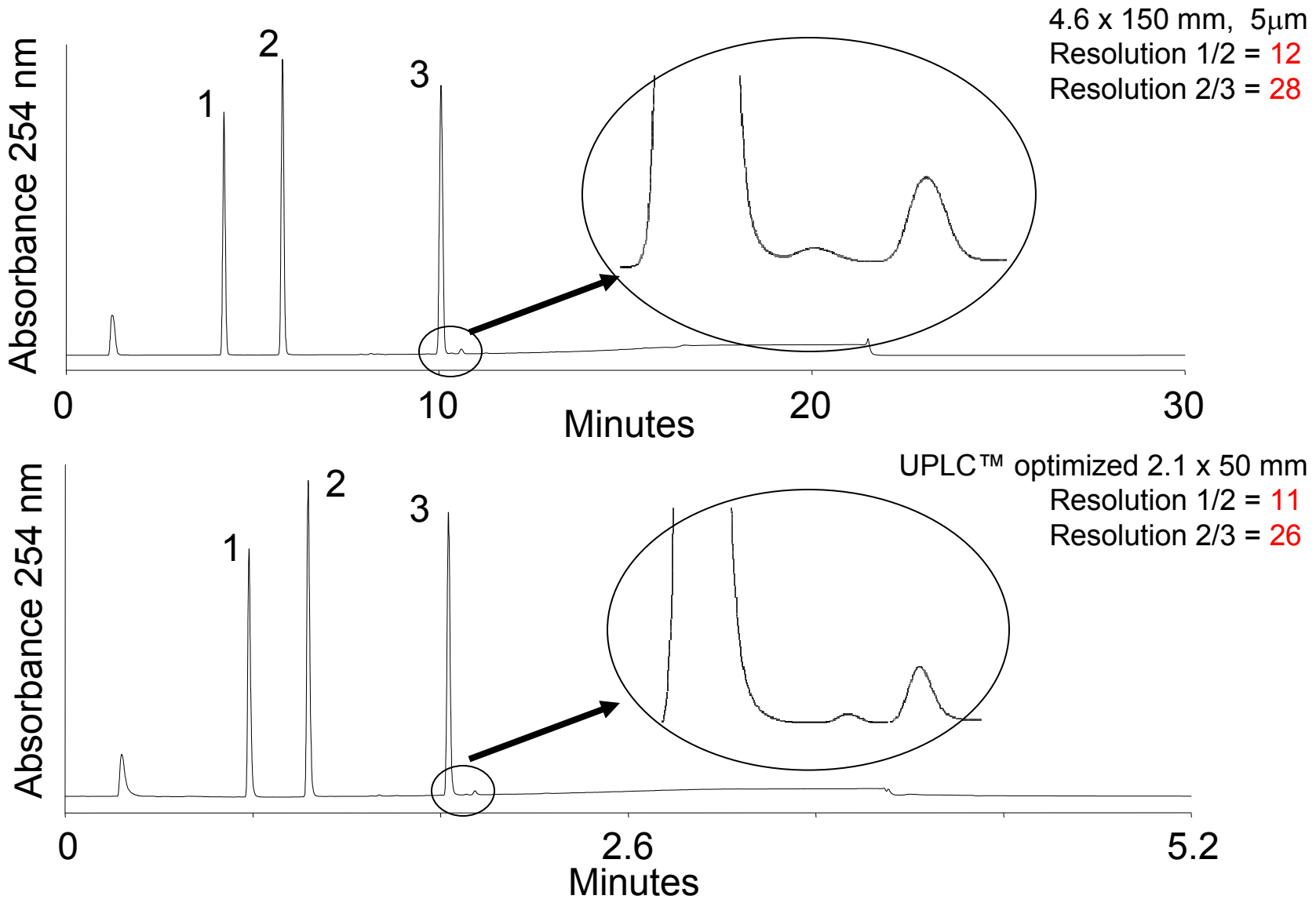
Comparing Results:
Does the new method meet resolution criteria?



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Original HPLC Method:
Caffeic Acid Derivatives in Echinacea Purpurea

Chromatographic Conditions :

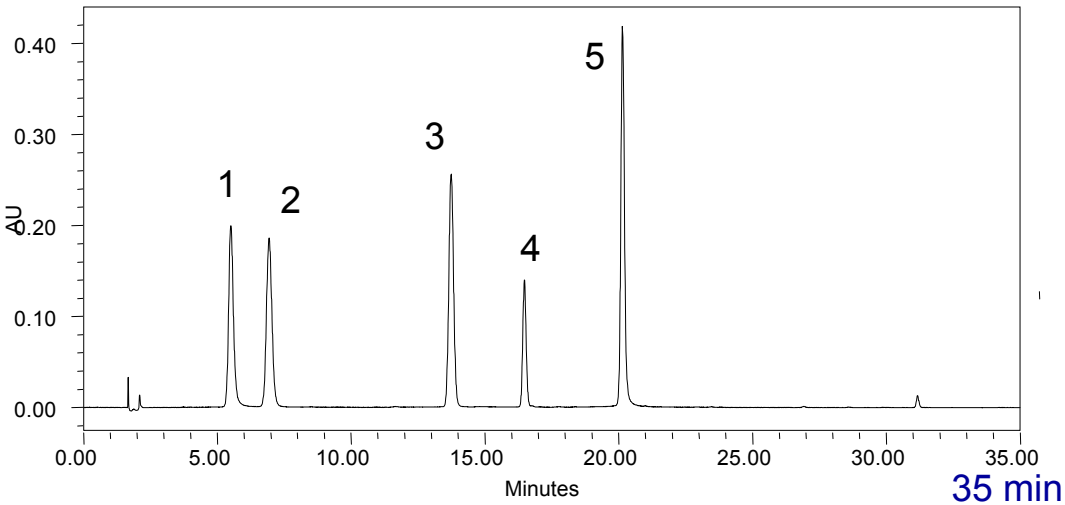
Columns: XTerra® MS C_{18} 4.6 x 150 mm, 5.0 µm
Mobile Phase A: 0.1% CF_3COOH in H_2O
Mobile Phase B: 0.08% CF_3COOH in ACN
Flow Rate: 1.0 mL/min
Gradient:

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

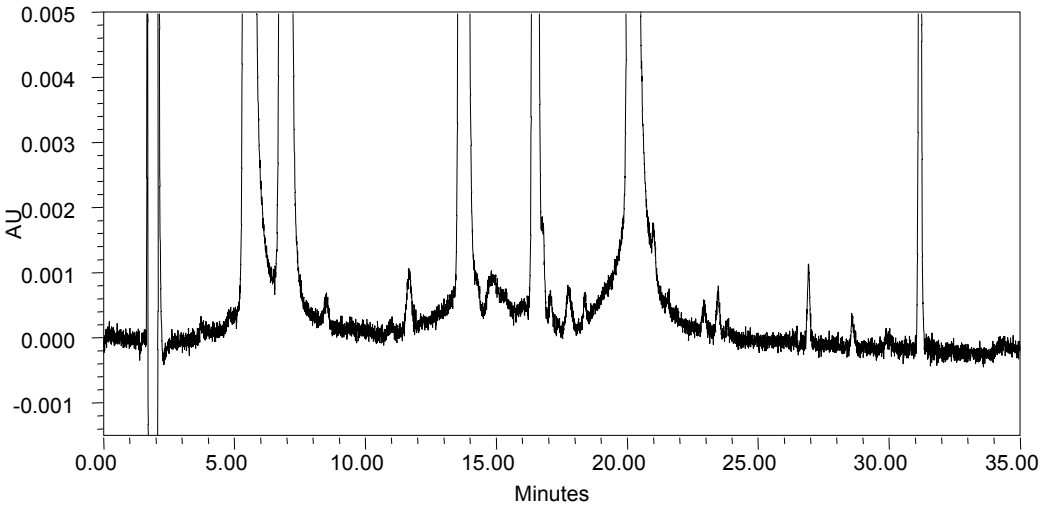
Injection Volume: 10.0 µL
Weak Needle Wash: 0.1% CF_3COOH in 8% ACN
Sample: Caffeic Acid Derivatives in Echinacea Purpurea
Sample Diluent: 50:50 H_2O: MeOH with 0.05% CF_3COOH
Sample Concentration: 100 µg/mL
Temperature: 40 °C
Detection: UV @ 330 nm
Sampling rate: 10 pts/sec
Time Constant: 0.1
Instrument: Alliance® 2695 Separations Module with 2996 PDA detector

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

E.G.



35 min.



Enhanced Baseline

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HPLC-to-UPLC™ Method Transfer:
Chromatographic Conditions

Original HPLC Method

Chromatographic Conditions :
Columns: XTerra® MS C_{18} 4.6 x 150 mm, 5.0 µm
Mobile Phase A: 0.1% CF_3COOH in H_2O
Mobile Phase B: 0.08% CF_3COOH in ACN
Flow Rate: 1.0 mL/min
Gradient:

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

Injection Volume: 10.0 µL
Sample: Caffeic Acid Derivatives in Echinacea Purpurea
Sample Diluent: 50:50 H_2O: MeOH with 0.05% CF_3COOH
Sample Concentration: 100 µg/mL
Temperature: 40 ºC
Detection: UV @ 330 nm
Sampling rate: 10 pts/sec
Time Constant: 0.1
Instrument: Alliance® 2695 Separations Module with 2996 PDA detector

Final UPLC™ Method

Chromatographic Conditions :
Columns: ACQUITY UPLC™ BEH C_{18} 2.1 x 50 mm, 1.7 µm
Mobile Phase A: 0.1% CF_3COOH in H_2O
Mobile Phase B: 0.08% CF_3COOH in ACN
Flow Rate: 0.5 mL/min
Gradient:

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

Injection Volume: 1.0 µL
Weak Needle Wash: 0.1% CF_3COOH in 8% ACN
Sample: Caffeic Acid Derivatives in Echinacea Purpurea
Sample Diluent: 50:50 H_2O: MeOH with 0.05% CF_3COOH
Sample Concentration: 100 µg/mL
Temperature: 40 ºC
Detection: UV @ 330 nm
Sampling rate: 40 pts/sec
Time Constant: 0.1
Instrument: Waters ACQUITY UPLC™, with TUV detector

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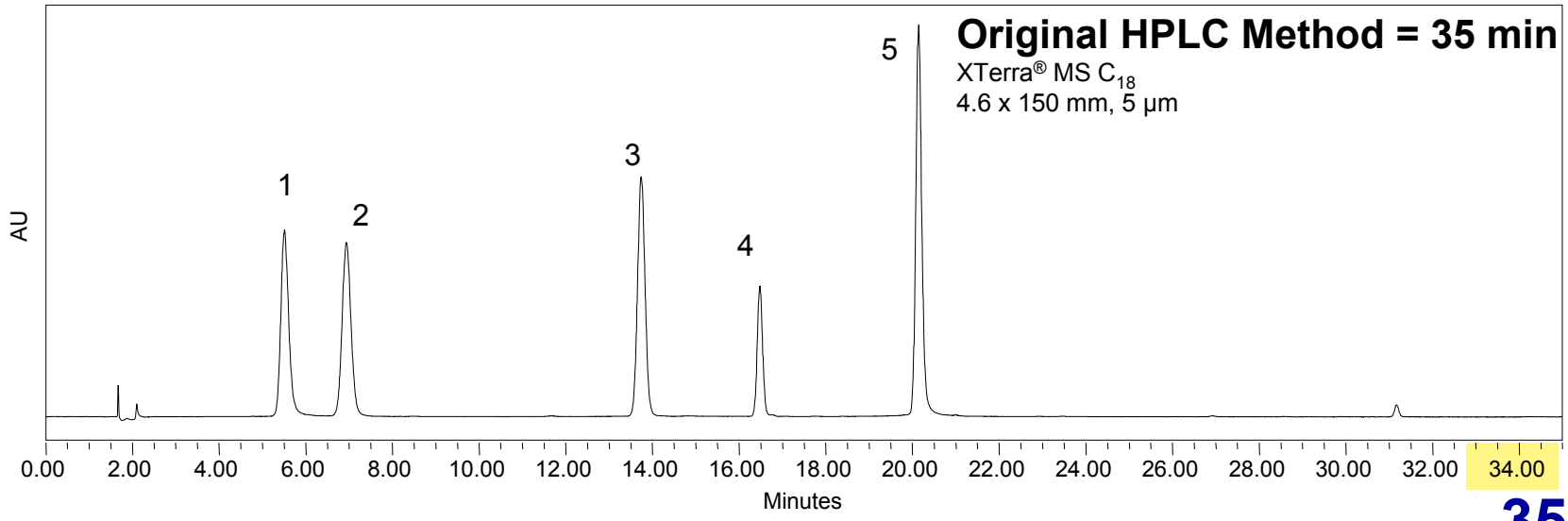
HPLC-to-UPLC™ Method Transfer:

Caffeic Acid Derivatives in Echinacea Purpurea



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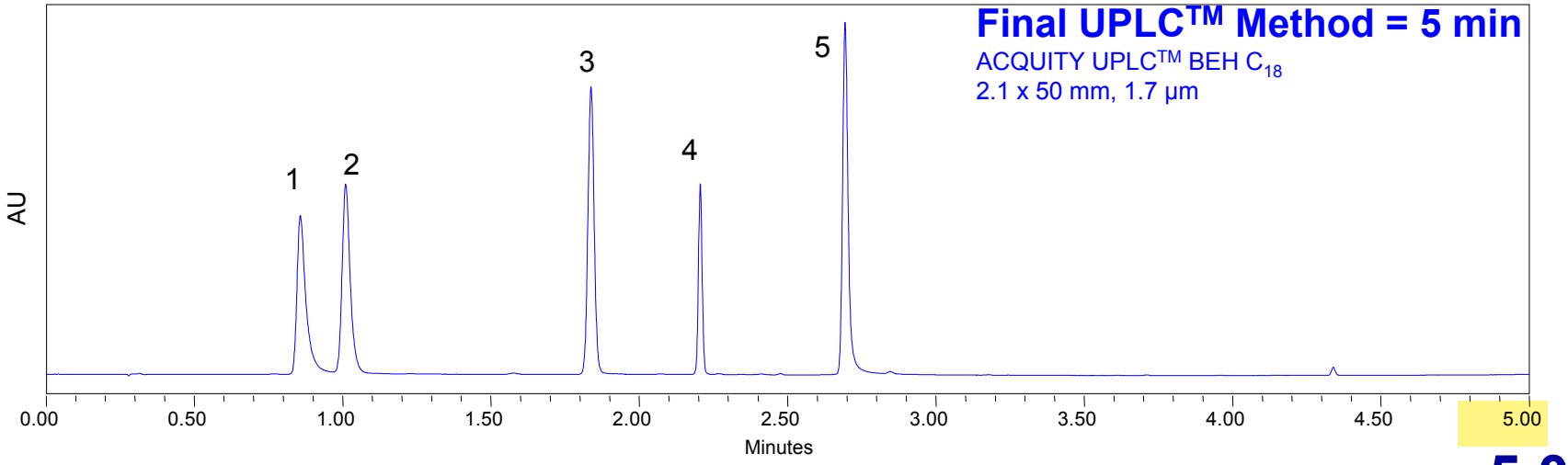

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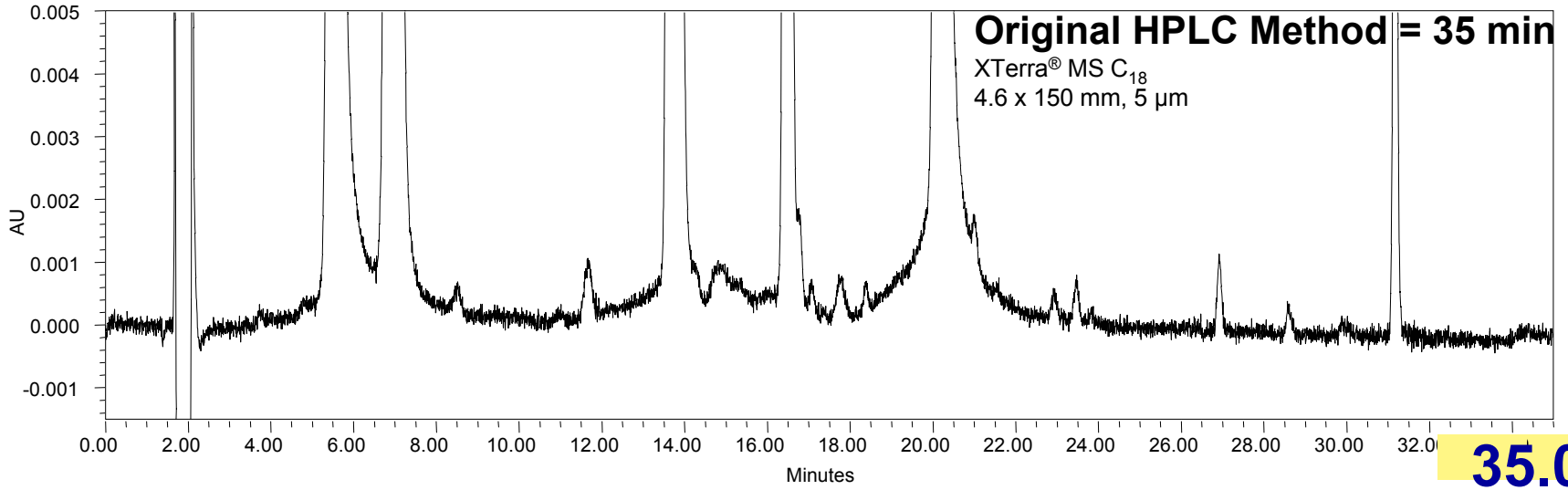
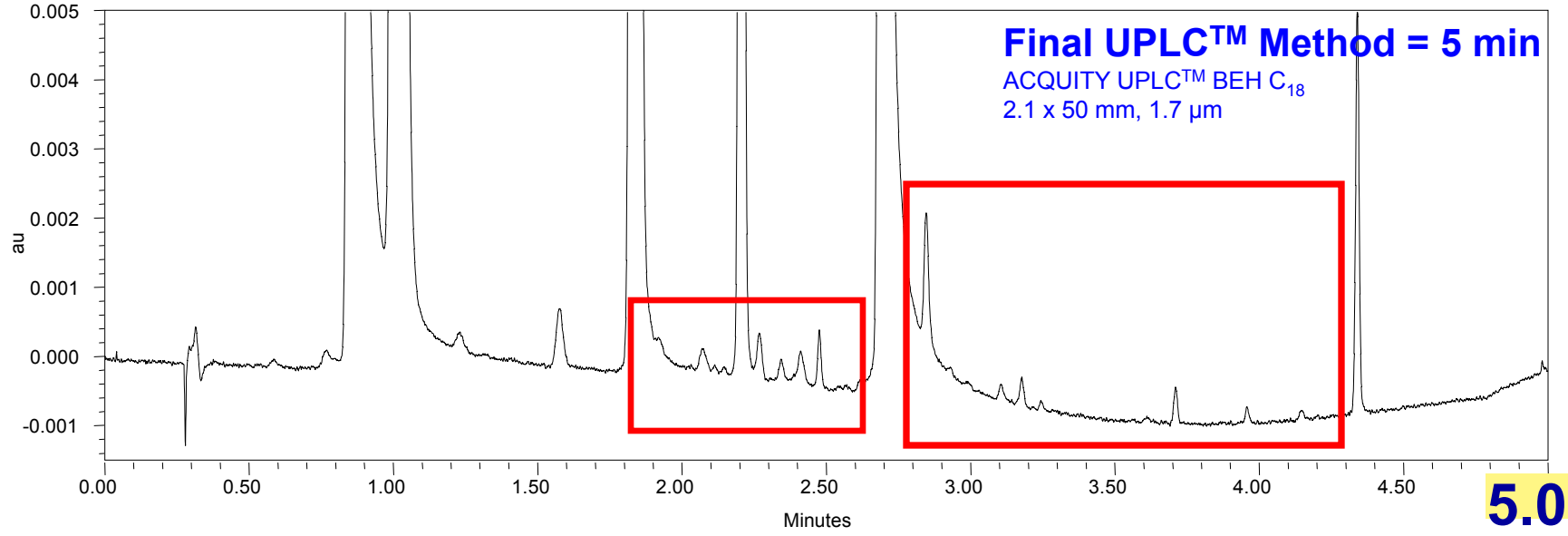
HPLC-to-UPLC™ Method Transfer:

Caffeic Acid Derivatives in Echinacea Purpurea





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Conclusions

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

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