Waters

Eric S. Grumbach

Thomas E. Wheat

Jeffrey R. Mazzeo

Diane M. Diehl

Increased Throughput and Sensitivity Obtained with UPLC™ Columns Packed with 1.7 µm Particles for High Resolution, Ultra Fast Stability-Indicating Method Development

PITTCON® 2005
Everything Science Under the Sun!
www.pittcon.org ⋆ February 27 – March 4, 2005 ⋆ Orlando, Florida

For Complete Confidence

Waters

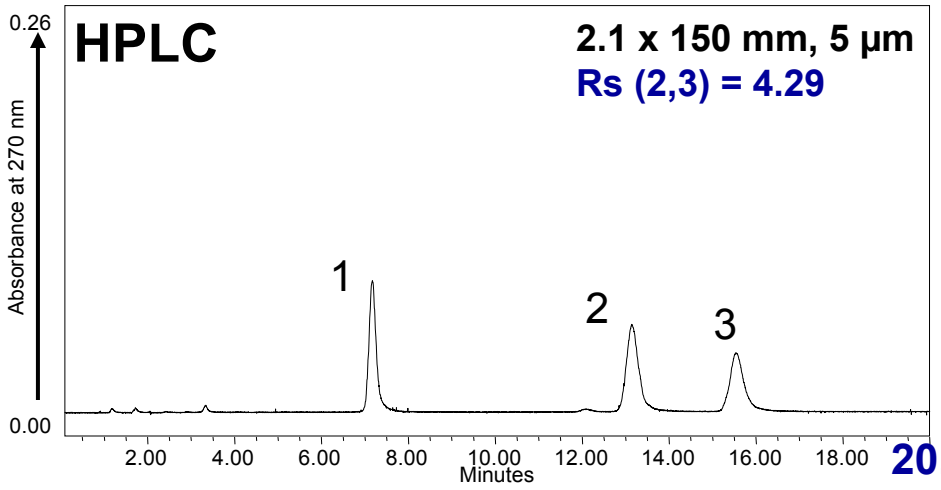
Introduction

- Pharmaceutical scientists must develop assay procedures that completely identify and measure all degradation products of an active pharmaceutical ingredient.
- Due to its ability to separate degradation products, excipients and process impurities from active ingredients, HPLC has become the analytical tool of choice for stability-indicating assays.
- However, there is always the requirement to achieve better resolution to ensure complete characterization of the degradants.
- At the same time, improvements in sensitivity to detect trace level components and improved sample throughput need to be addressed.
- These assays can benefit from utilizing sub-2 µm particulate columns to improve resolution for critical pairs or maintain existing resolution while improving sample throughput.
- In this study, we examine this approach, applying Ultra Performance LC™ (UPLC™) to the degradants of the antifungal terbinafine.

Waters

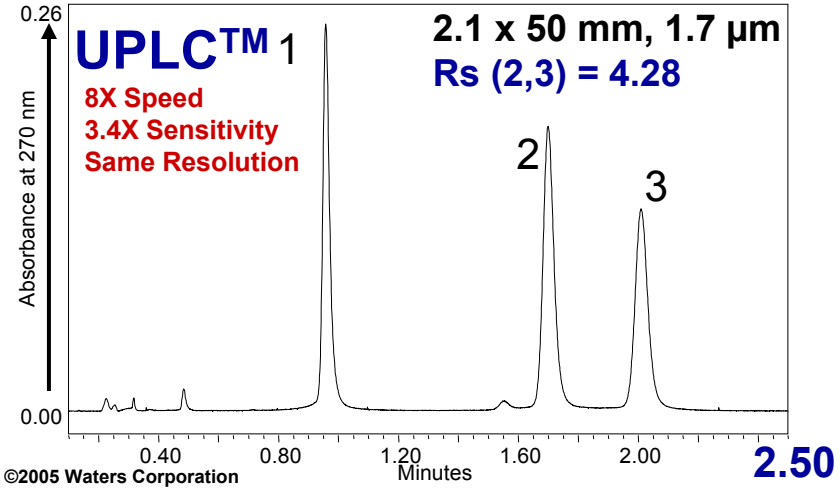
HPLC vs. UPLC™

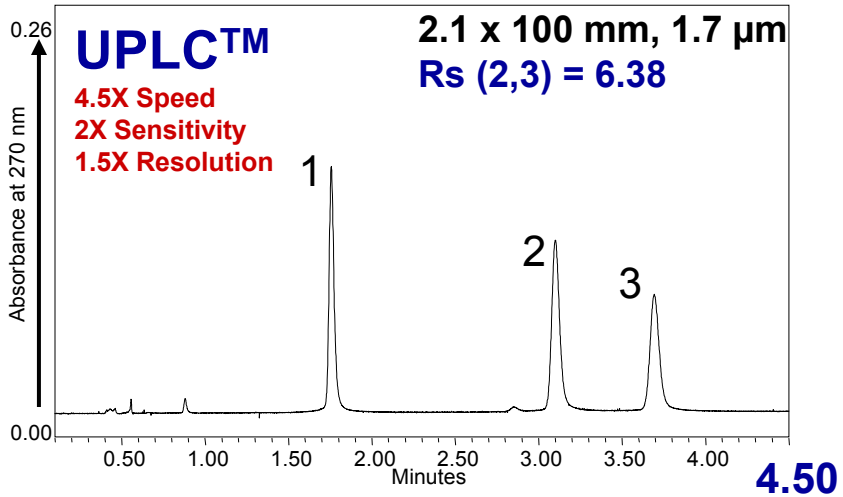
Speed, Sensitivity and Resolution



Faster, More Sensitive Methods

Faster, More Sensitive, Higher Resolution Methods





Waters

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

Waters

UPLC™ Technology Review

- 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
- Suitable for chromatographic applications in general
  - Appropriate for improving existing methods
  - Appropriate for developing new methods

Waters

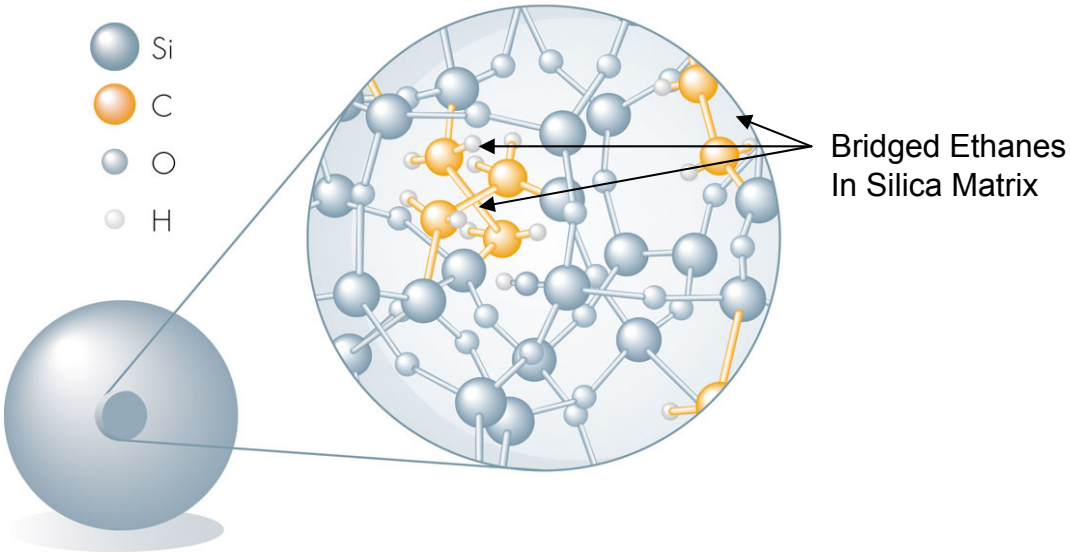
ACQUITY UPLC™
Innovation in column technology

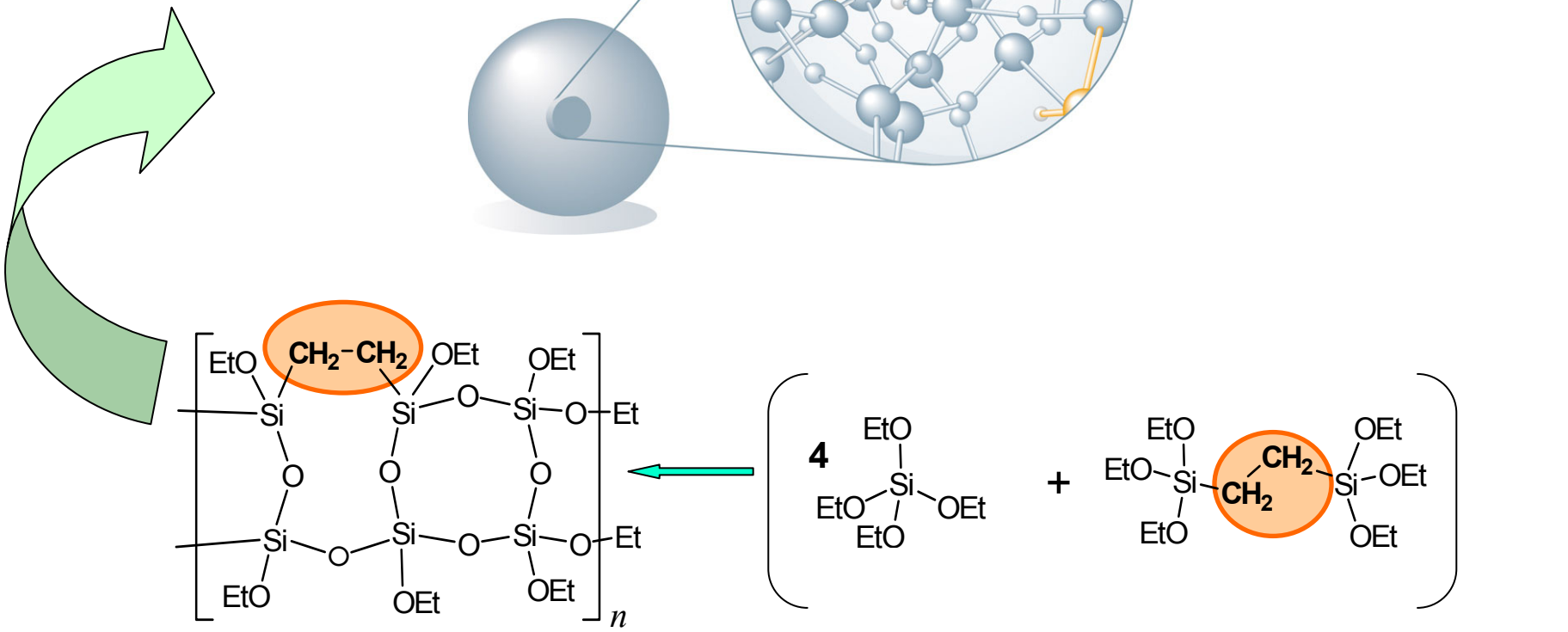
- Sub-2 µm particles
  - Porous for optimum loadability
  - Bridged hybrid particle* required for both high strength and outstanding chromatographic performance
  - Innovative sizing technology for narrow particle size distribution
- Column hardware
  - New frit technology* to retain particles
  - Fittings optimized for high pressure operation
- Packing technology
  - New column packing processes to optimize stability
- eCord™
  - New information chip to store column history

*Waters Patented Technology
No. 6,686,035 B2

Waters

Introducing 2nd Generation Hybrid: Bridged EthylSiloxane/Silica Hybrid Particles





Polyethoxysilane (BPEOS)

Tetraethoxysilane (TEOS)

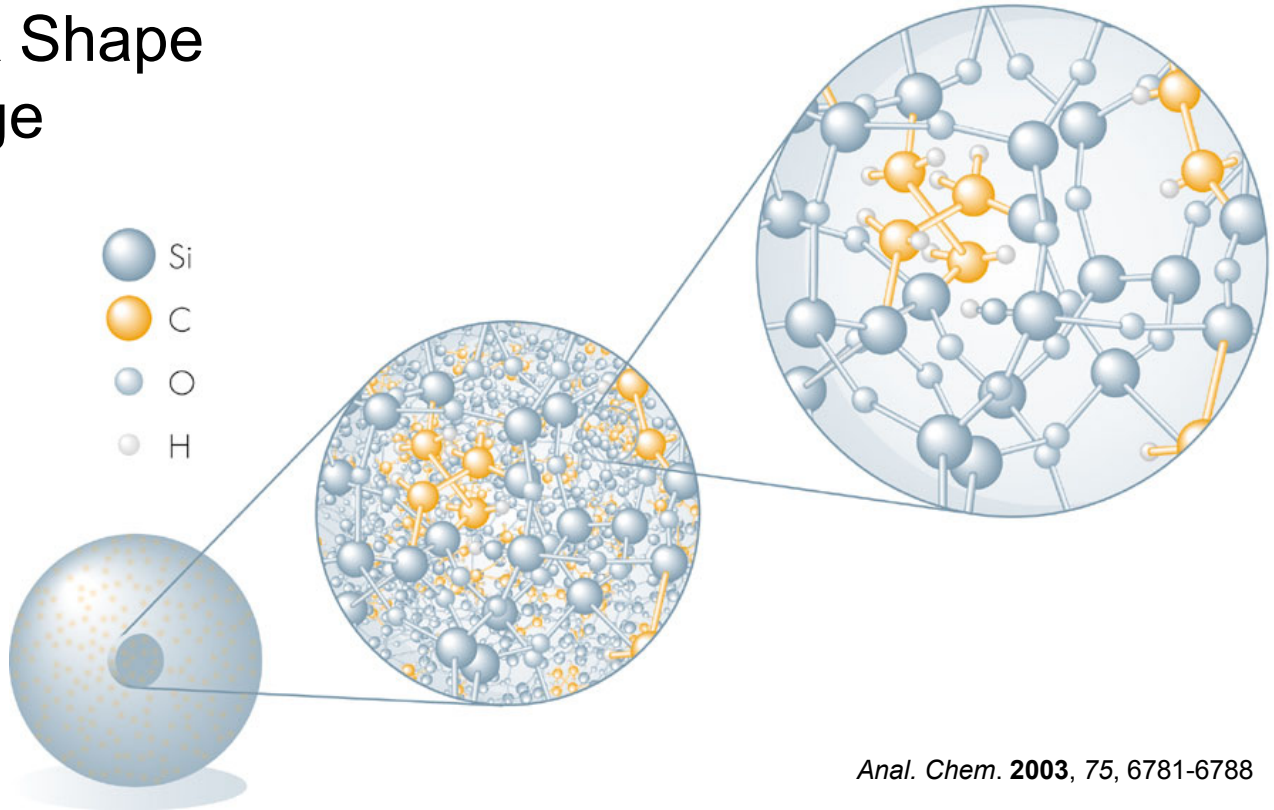
Bis(triethoxysilyl)ethane (BTEE)

Anal. Chem. **2003**, *75*, 6781-6788

New 2nd Generation Hybrid Bridged EthylSiloxane/Silica Hybrid Particles

1.7 µm BEH particle provides:

- Improved Strength
- Improved Efficiencies
- Improved Peak Shape
- Wider pH Range



Anal. Chem. **2003**, *75*, 6781-6788

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

Waters

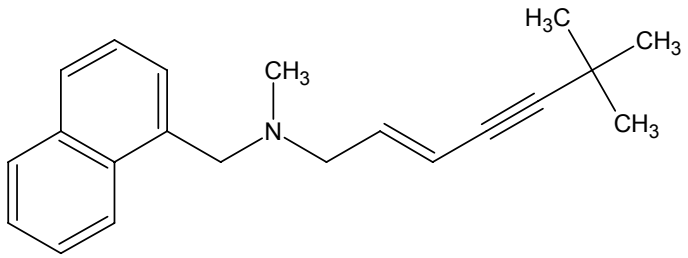
Instrument Considerations

- Specialized instrumentation was designed to meet the requirements of 1.7 µm packings.
- These requirements include:
  - low system volumes (<140 µL to minimize dispersion),
  - high pressure fluidic modules (up to 15,000 PSI),
  - high speed optical detectors (capable of 40 Hz) and
  - mass detectors (5000 Da/sec).
- The integration of both chemistry and instrumentation leads to the development of ultra-fast, sensitive, high resolution chromatographic methods.

Experimental Conditions

Sample Preparation:

- Terbinafine HCl was forcefully degraded with 8.0 N hydrochloric acid
- A 10 mg/mL solution was stirred in a 60 ^{o}C water bath for 60 minutes
- A 1 mL aliquot was then neutralized with sodium hydroxide.
- This solution was then diluted with 10 mL of water and 15 mL of acetonitrile for analysis on the UPLC™ system.



Terbinafine
mw = 291.43

Experimental Conditions

Instrument:

ACQUITY UPLC™ with TUV optical detector

Chromatographic Conditions:

Column: C_{18} 2.1 x 150 mm, 5.0 µm

Mobile Phase A: 20 mM ammonium bicarbonate pH 10.0

Mobile Phase B: acetonitrile

Flow Rate: 0.2 mL/min

Isocratic: 73% B

Injection Volume: 4.0 µL

Sample Diluent: 50 ACN with 10 mM NH_4HCO_3 pH 10

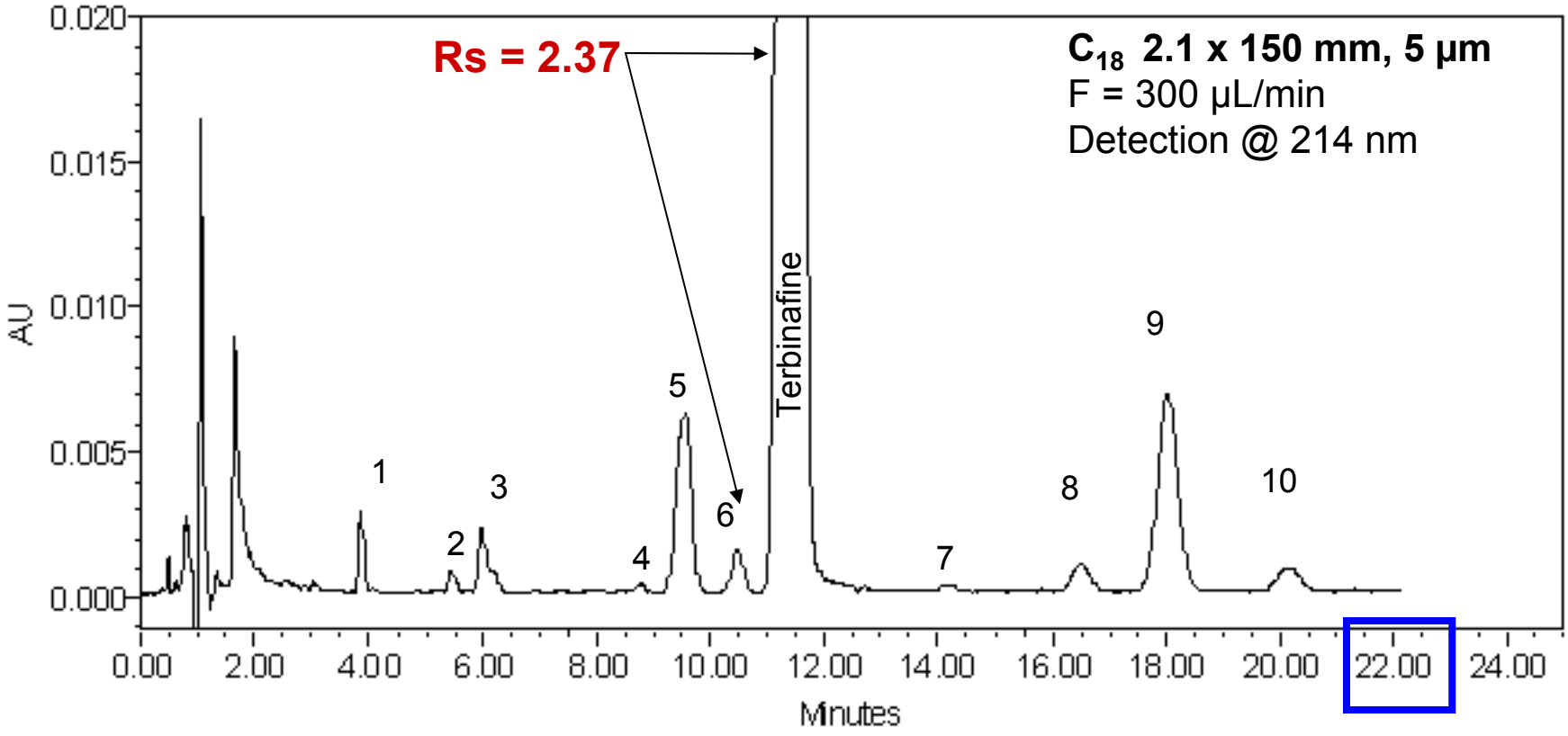
Temperature: 30 ^oC

TUV Optical Detector Settings:

Wavelength: 214 nm
Sampling Rate: 40 Hz
Time Constant: 0.1 seconds

Waters

Original Separation



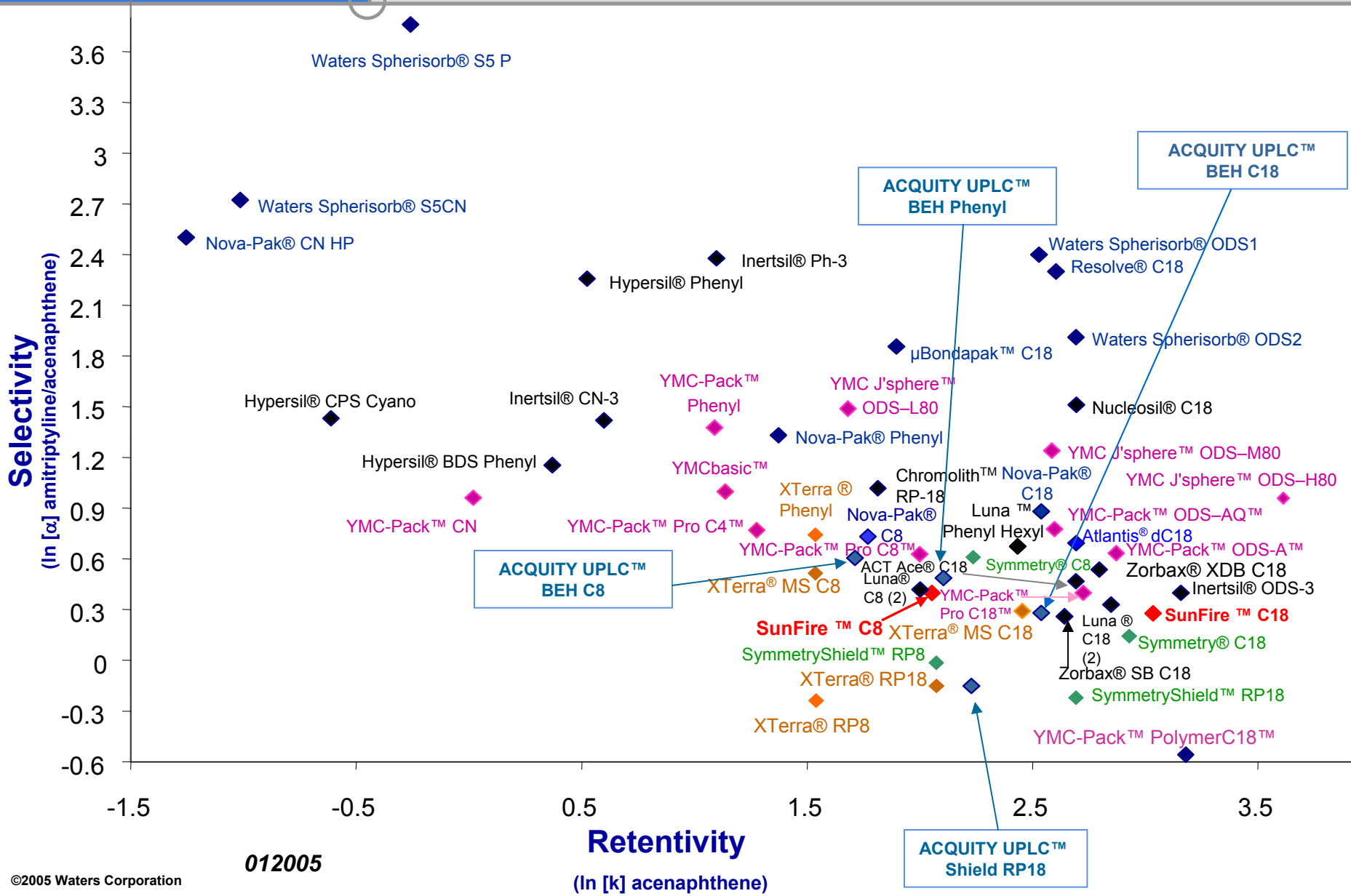
Implementing UPLC™

Choices available:

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

Waters

Reversed-Phase Column Selectivity Chart



012005

Waters

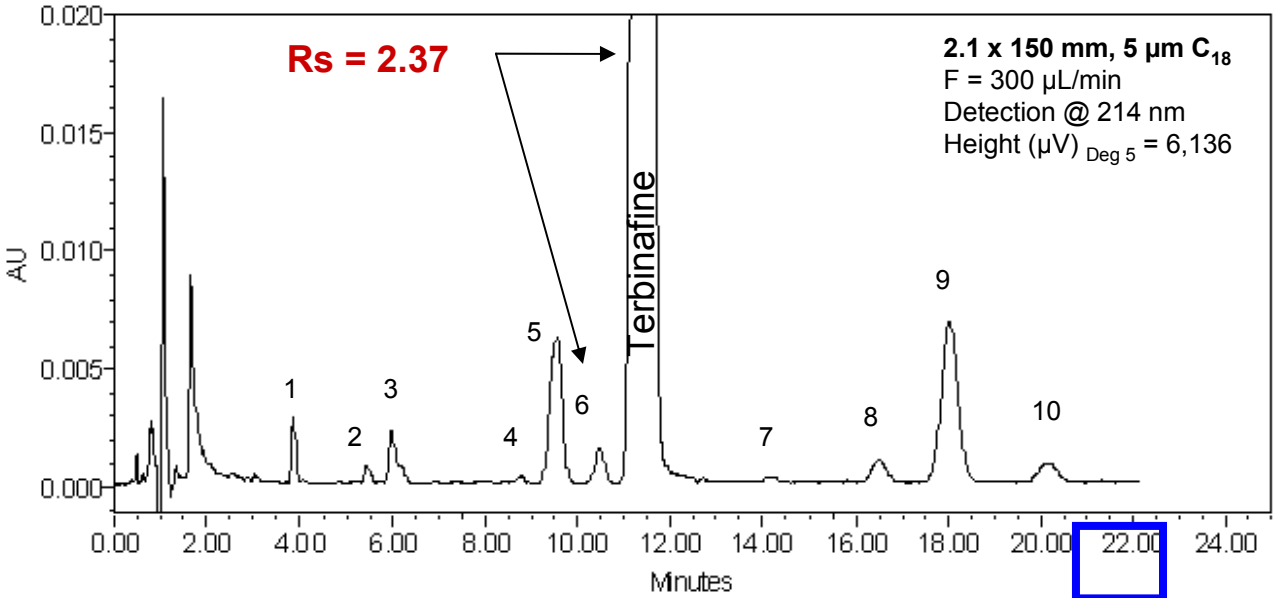
UPLC™ Column Selection

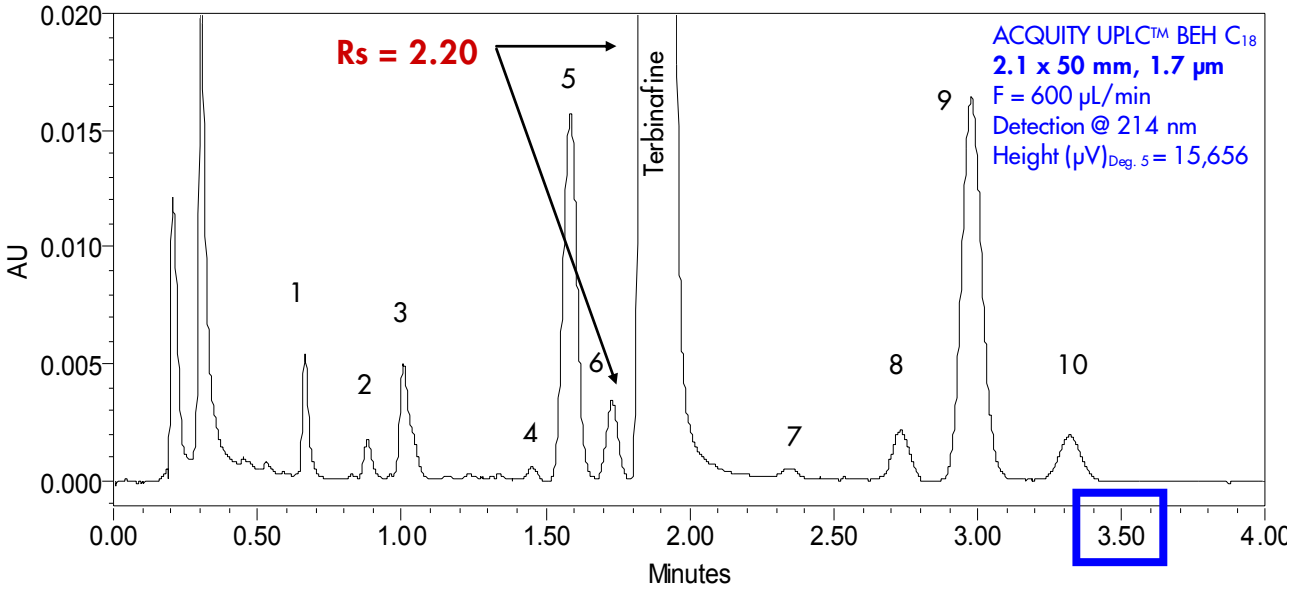
Ratio of Column Length to Particle Size

L/dp		RATIO
$\frac{150\text{mm}}{5\mu\text{m}}$	=	30,000
$\frac{50\text{mm}}{1.7\mu\text{m}}$	=	29,500
$\frac{100\text{mm}}{1.7\mu\text{m}}$	=	58,820

Waters

Improved Throughput and Sensitivity:
Stability-Indicating Assays





(F ↑ 2X, L ↓ 3X)

Speed ↑ 6X

Sensitivity ↑ 2.5X

Rs = 1X

Waters

Can the Separation be Further Improved?

- Greater sensitivity
  - Inject same volume onto a 1.0 mm i.d. column packed with 1.7 µm particles
- Greater resolution
  - Use a 100 mm column packed with 1.7 µm particles (L/dp = 58,820)

Waters

Can the Separation be Further Improved?

- Greater sensitivity
  - Inject same volume onto a 1.0 mm i.d. column packed with 1.7 µm particles
- Greater resolution
  - Use a 100 mm column packed with 1.7 µm particles (L/dp = 58,820)

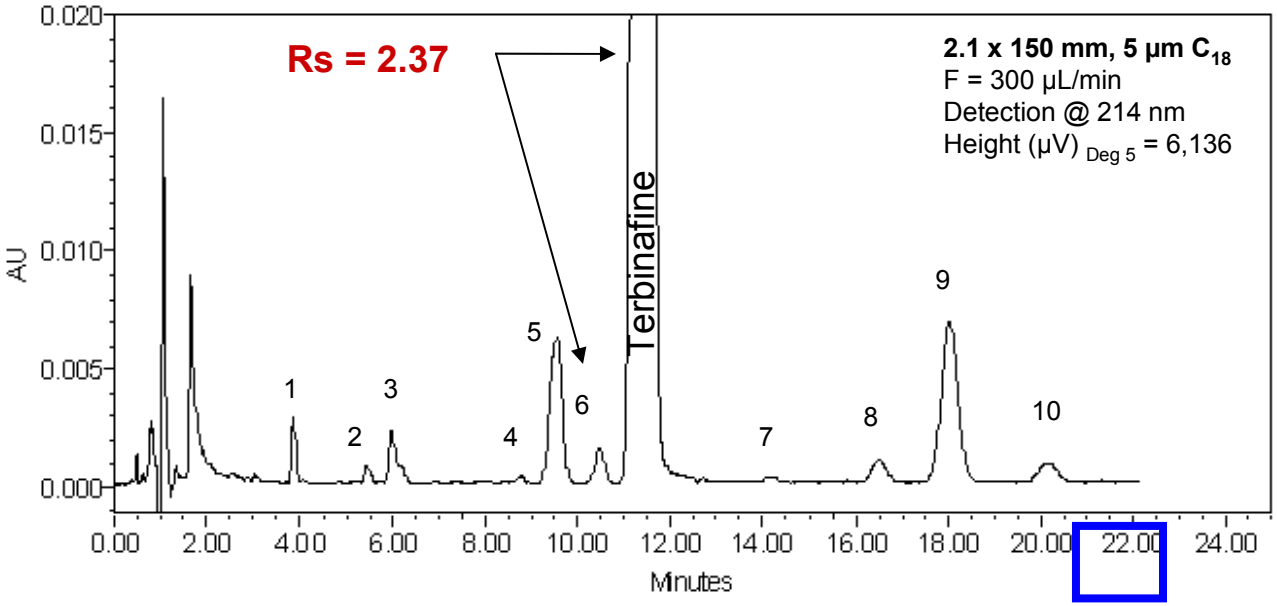
Implementing UPLC™

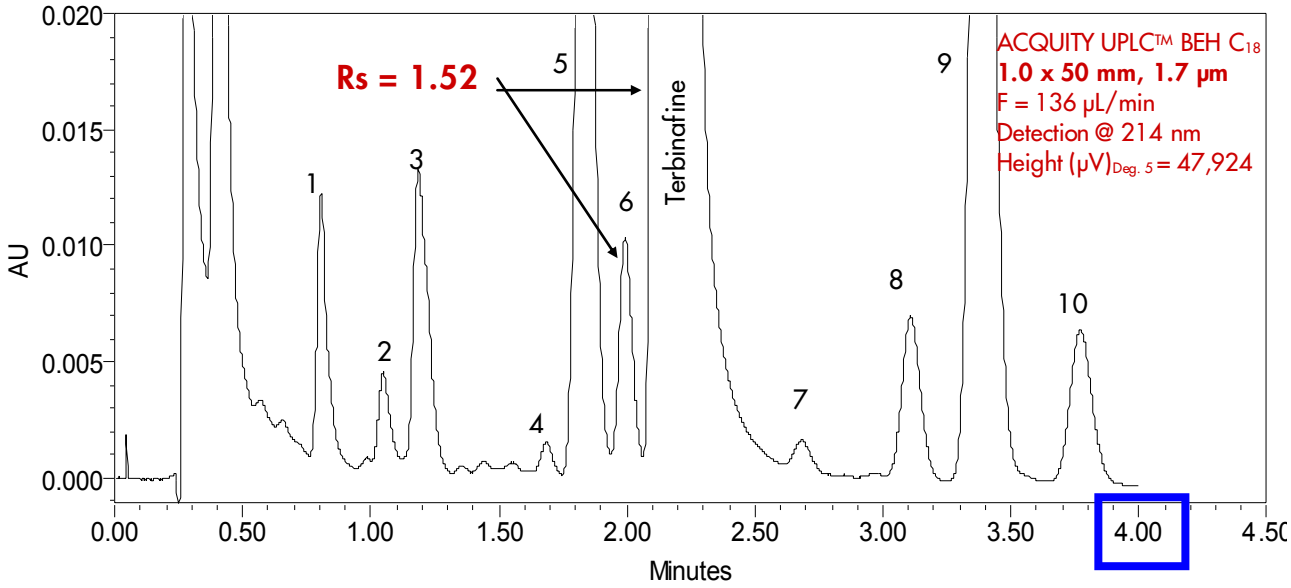
Choices available:

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

Waters

Improved Throughput and Sensitivity: 1.0 mm i.d. Column





Speed ↑ 6X

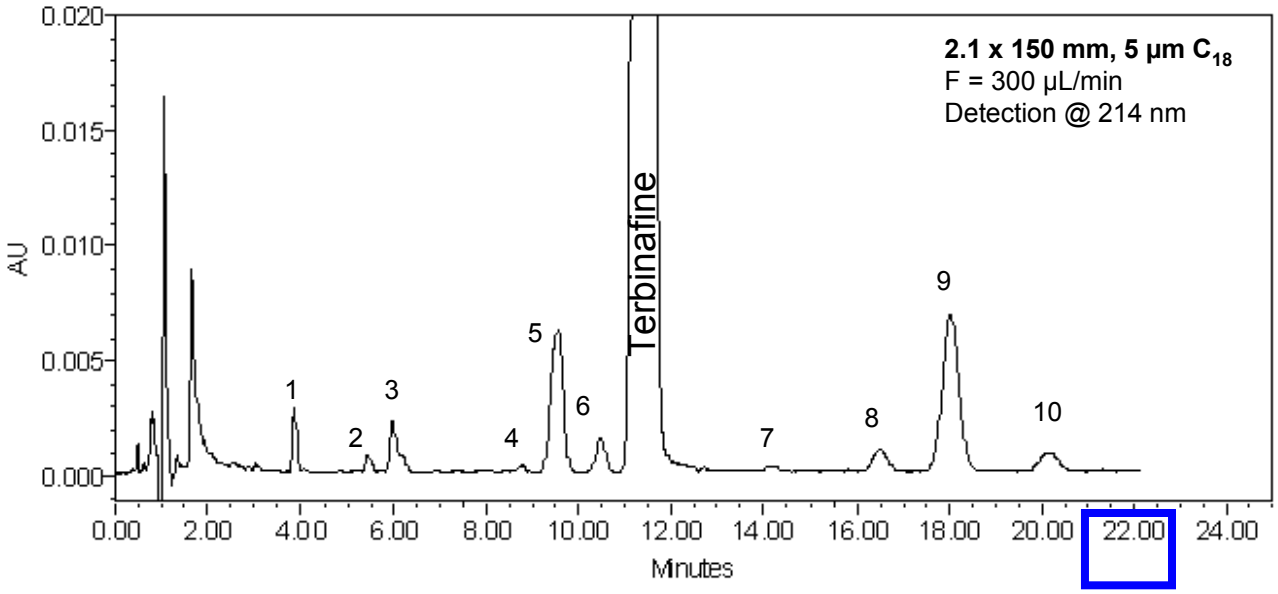
Sensitivity ↑ 8X

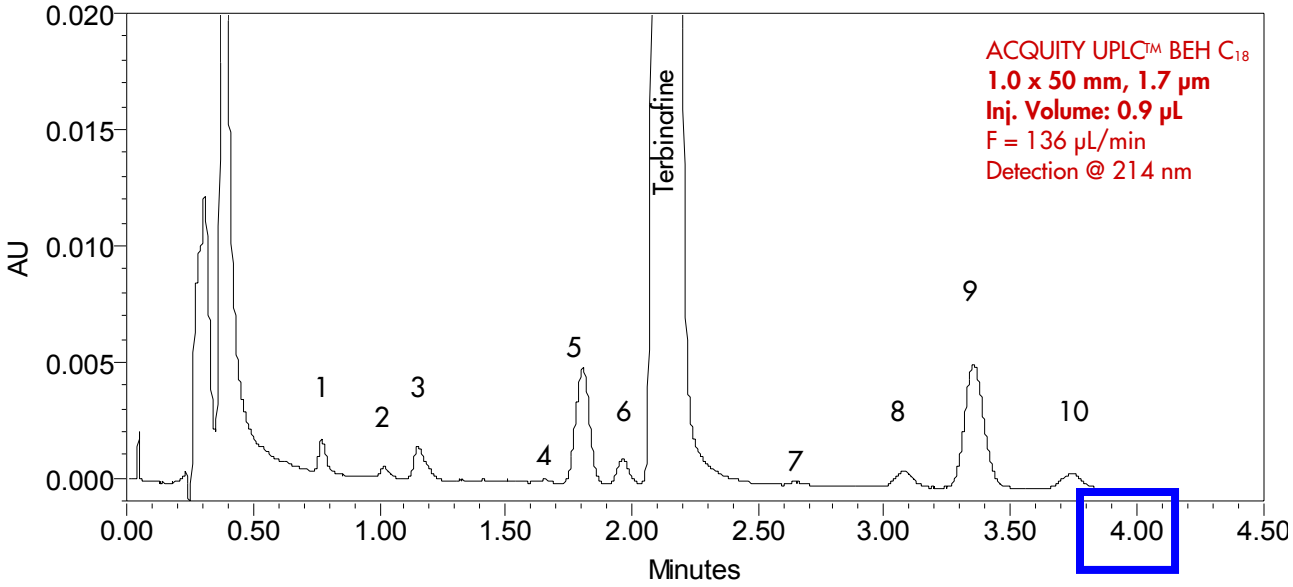
Rs = ↓ 1.55X

- **Same amount injected on column results in loss in Rs**
- **This is due to column overload**

Waters

Improved Throughput and Sensitivity:
1.0 mm i.d. Column Scaled Injection Volume





Scaled injection volume

4.0 µL → 0.9 µL

Waters

Can the Separation be Further Improved?

- Greater sensitivity
  - Inject same volume onto a 1.0 mm i.d. column packed with 1.7 µm particles

- Greater resolution
  - Use a 100 mm column packed with 1.7 µm particles (L/dp = 58,820)

Waters

UPLC™ Column Selection

Ratio of Column Length to Particle Size

L/dp		RATIO
$\frac{150\text{mm}}{5\mu\text{m}}$	=	30,000
$\frac{50\text{mm}}{1.7\mu\text{m}}$	=	29,500
$\frac{100\text{mm}}{1.7\mu\text{m}}$	=	58,820

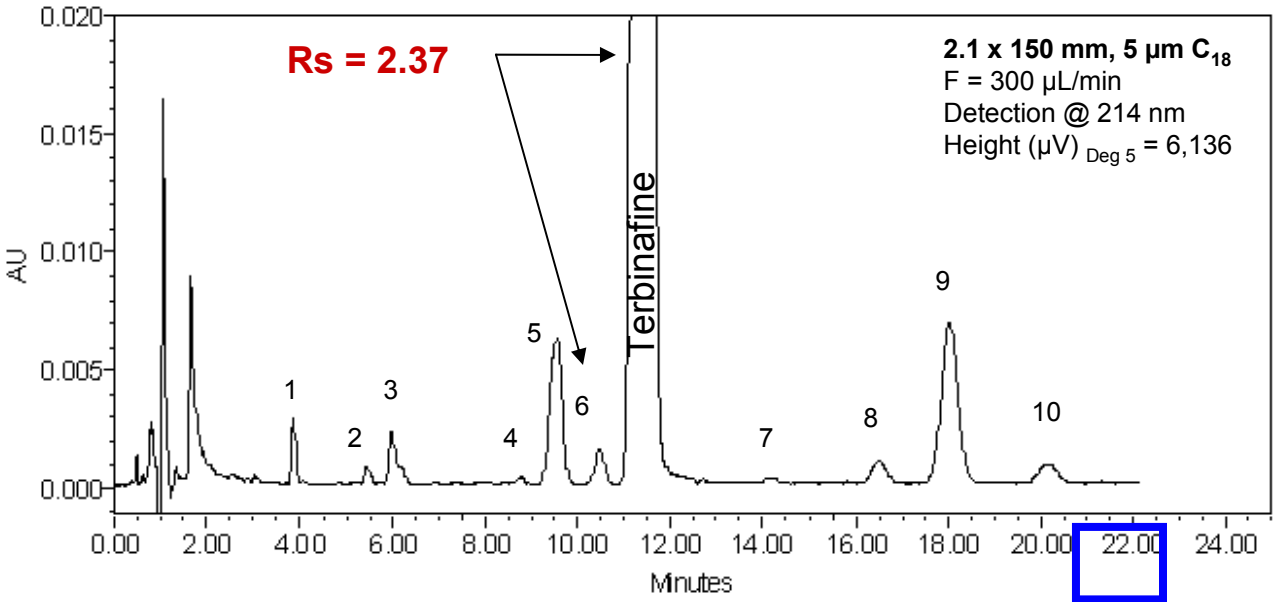
Implementing UPLC™

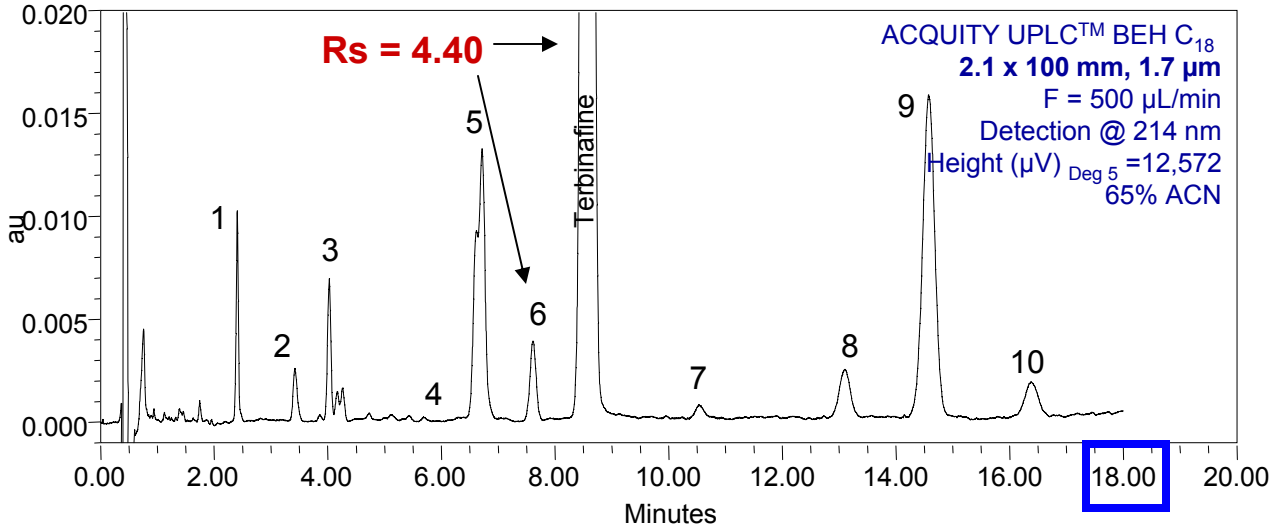
Choices available:

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

Waters

Improved Resolution and Sensitivity:
100 mm, 1.7 µm column





Speed = 1X

Sensitivity ↑ 2X

Rs = ↑ 1.8X

Waters

Conclusions

Benefits of applying UPLC™ to stability-indicating assays:

- Develop faster and more sensitive assays while maintaining existing resolution (2.1 mm i.d.)
  - **Speed ↑ 6X**
  - **Sensitivity ↑ 2.5X**
- Develop highly sensitive and faster assays (1.0 mm i.d.)
  - **Sensitivity ↑ 8X**
  - **Speed ↑ 6X**
- Develop sensitive, higher resolution assays within the same analysis time
  - **Rs = ↑ 1.8X**
  - **Sensitivity ↑ 2X**