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Increased Throughput and Sensitivity Obtained with 1.0 mm i.d. Columns Packed with 1.7 µm Particles for High Resolution, Ultra Fast Stability-Indicating Method Development

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Overview

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.

Experimental Approach

Terbinafine was forcefully degraded by acid then analyzed by LC/UV/MS for spectral identification of the degradant peaks and to determine spectral purity of terbinafine. The selectivity of the separation was optimized so all degradants were resolved from the parent compound.

The resolution and sensitivity of a traditional HPLC and UPLC™ were compared using 2.1 mm i.d. columns packed with 5 µm and 1.7 µm bridged ethyl-siloxane hybrid (BEH) particles. A constant column length inversely proportional to the particle diameter was maintained to demonstrate improved sensitivity and reduced analysis times for 1.7 µm particles while maintaining resolution of critical pairs for stability-indicating assays.

Additionally, a 1 mm i.d. column packed with 1.7 µm BEH particles was used to demonstrate improved limits of detection compared to traditional 2.1 mm i.d. columns packed with 5 µm particles.

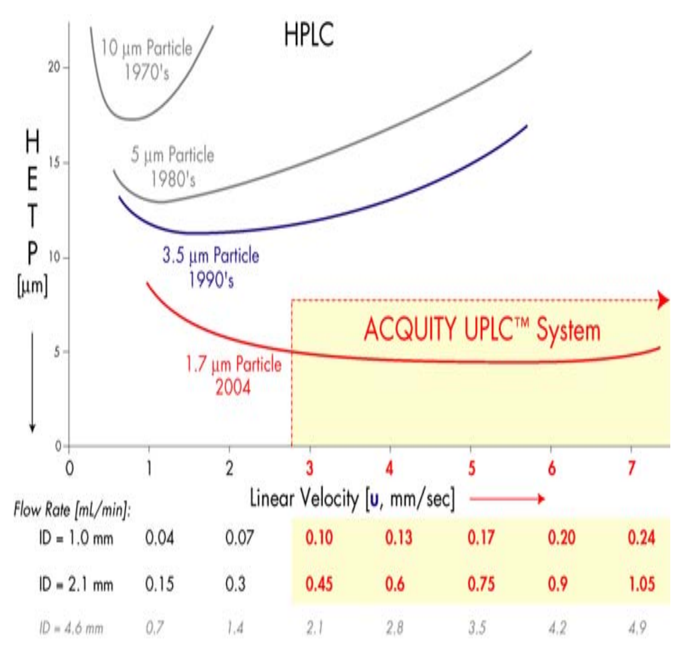
Chromatographic Theory

OPTIMAL SEPARATION EFFICIENCY: van Deemter Equation

$$H = \underbrace{a(dp)}_{\text{A term}} + \underbrace{\frac{b}{u}}_{\text{B term}} + \underbrace{c(dp)^2 u}_{\text{C term}}$$

u = Linear Velocity (Flow Rate)
dp = Particle Size
C term Is impacted by Both

A term Eddy Diffusion/ Interparticle channels. It is particle size dependant
B term Molecular Diffusion (Axially). It is inversely proportional to velocity
C term Mass Transfer Kinetics. It is directly proportional to velocity, and the particle size squared.



To achieve optimal separation efficiency, 1.7 µm particles are operated at higher linear velocities than larger 3.5 µm and 5 µm particulate columns. Additionally, the van Deemter curves are flatter for the 1.7 µm particle allowing for a large linear velocity range in which resolution can be maintained while speed of analysis is improved.

CONSTANT RATIO OF COLUMN LENGTH INVERSELY PROPORTIONAL TO PARTICLE SIZE

Productivity and speed at constant L/dp
Efficiency, N, is directly proportional to column length, L, and inversely proportional to particle size, dp

$$N \propto \frac{L}{dp}$$

For same N and, therefore, same Rs,

N = 1X, Rs = 1X
dp ↓ 3X, L ↓ 3X, , F ↑ 3X, T ↓ 9X

Sensitivity at constant L/dp
Assuming same efficiency, peak height is inversely proportional to column length, L

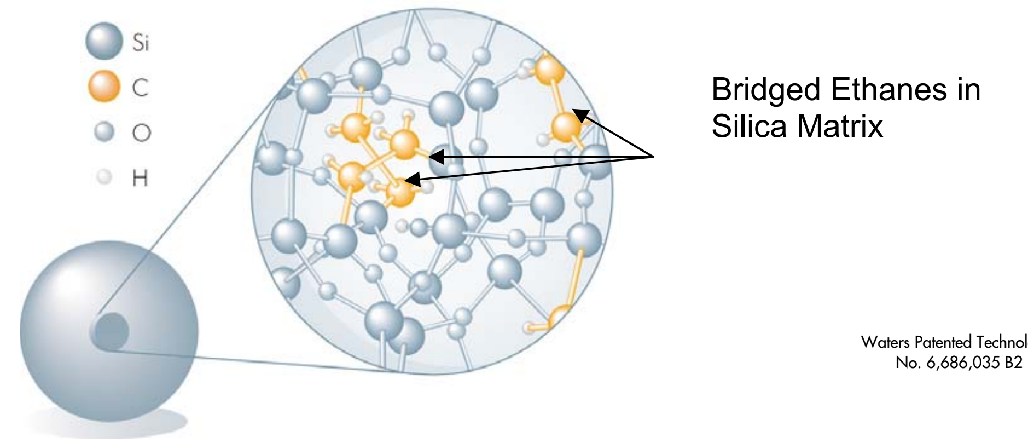
$$Height = \frac{1}{L}$$

For same efficiency, column length, L is decreased proportionally to particle size, dp (constant L/dp)

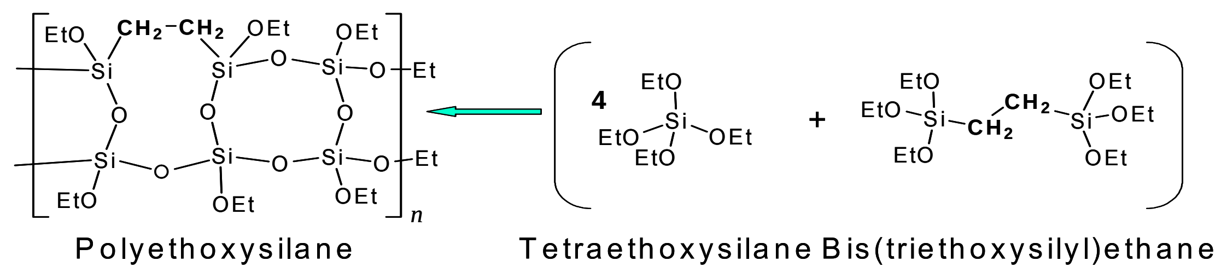
N = 1X, Rs = 1X
dp ↓ 3X, L ↓ 3X, , F ↑ 3X, T ↓ 9X
Sensitivity ↑ 3X

A New Generation of Hybrid Packings

Optimal linear velocities of sub-2 µm particles require operations at higher pressures. Inorganic-organic hybrid materials have demonstrated increased mechanical stability compared to traditional silica based materials*[1]. A 1.7 µm bridged ethyl-siloxane hybrid (BEH) particle was designed to meet the demands of operation at pressures as high as 15,000 PSI, as well as mobile phase pH in the range of 1—12.



Waters Patented Technology
No. 6,686,035 B2



Polyethoxysilane (BPEOS)
Tetraethoxysilane (TEOS)
Bis(triethoxysilyl)ethane (BTEE)

System Considerations

In order to benefit from the chromatographic theory discussed previously, specialized instrumentation was designed to meet the requirements of sub-2 µm packings. These requirements include low system volumes (150 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 methods.

Experimental Conditions

Sample Preparation:

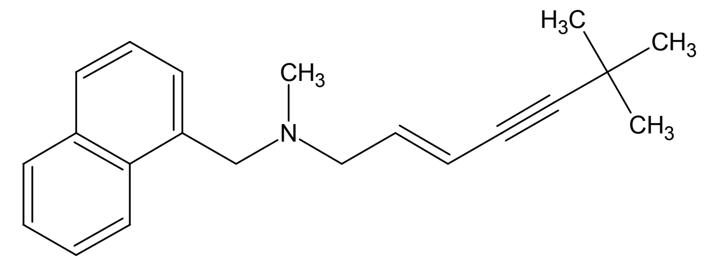
- Terbinafine HCl was forcefully degraded with 8.0 N hydrochloric acid
- A 10 mg/mL solution was stirred in a 60 °C water bath for 60 minutes
- A 1 mL aliquot was then neutralized with sodium hydroxide.
- This 5 mL solution was then diluted with 10 mL of water and 15 mL of acetonitrile for analysis on the UPLC™/UV/MS system.

Instrument:
ACQUITY UPLC™ with TUV optical detector and Waters ZQ™ mass spectrometer

Chromatographic Conditions:
Column: ACQUITY UPLC™ BEH C_{18} 2.1 x 50 mm, 1.7 µm,
ACQUITY UPLC™ BEH C_{18} 1.0 x 50 mm, 1.7 µm,
BEH C_{18} 2.1 x 150 mm, 5.0 µm (prototype)
Mobile Phase A: 20 mM ammonium bicarbonate pH 10.0
Mobile Phase B: acetonitrile
Flow Rate: 0.6 mL/min (2.1 mm i.d. 1.7 µm)
0.136 mL/min (1.0 mm i.d. 1.7 µm)
0.2 mL/min (2.1 mm i.d. 5 µm)
Isocratic: 73% B
Injection Volume: 4.0 µL (0.9 µL 1.0 mm i.d. 1.7 µm scaled)
Sample Diluent: 50ACN with 10 mM NH_4HCO_3 pH 10
Temperature: 30 °C

TUV Optical Detector Settings:
Wavelength: 214 nm
Sampling Rate: 40 Hz
Time Constant:: 0.1 seconds

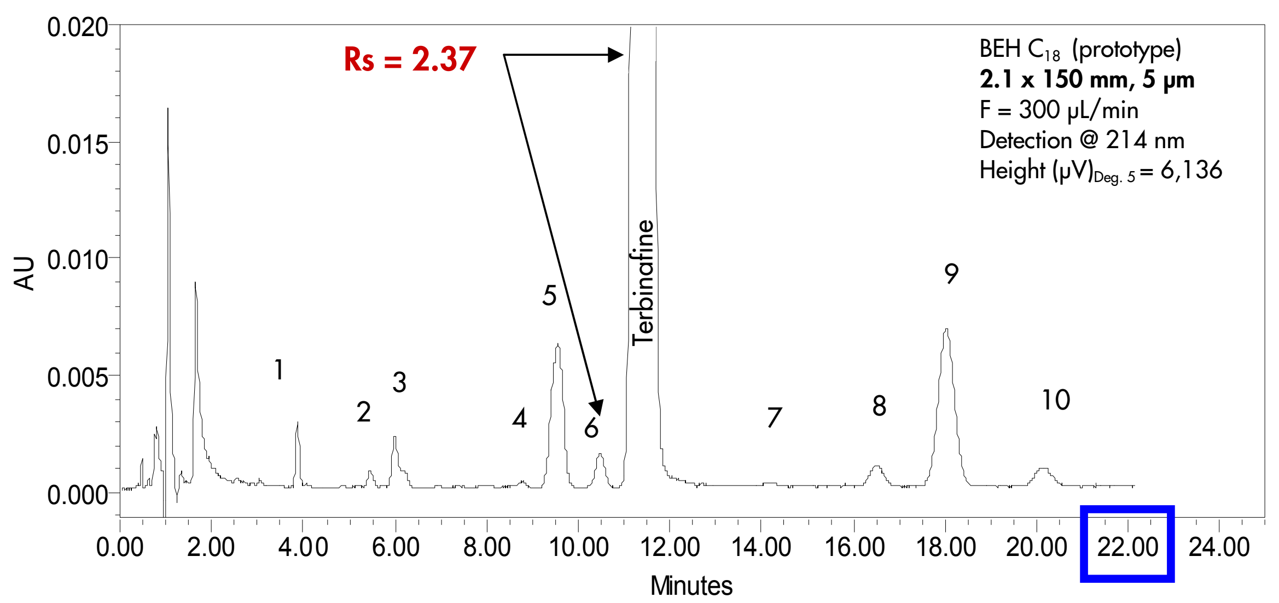
Mass Spectrometer Settings (ESI+):
Capillary (kV): 1.0
Cone (V): 25
Extractor: 3 V
RF Lens: 0.5 V
Source Temperature (°C): 150
Desolvation Temperature (°C): 350
(450 @ 0.6 mL/min)
Cone Gas Flow (L/Hr): 50
Desolvation Gas Flow (L/Hr): 550
(700 @ 0.6 mL/min)
SCAN m/z: 50 to 600
ScanTime: 0.2 seconds
Interscan Delay: 0.1 seconds



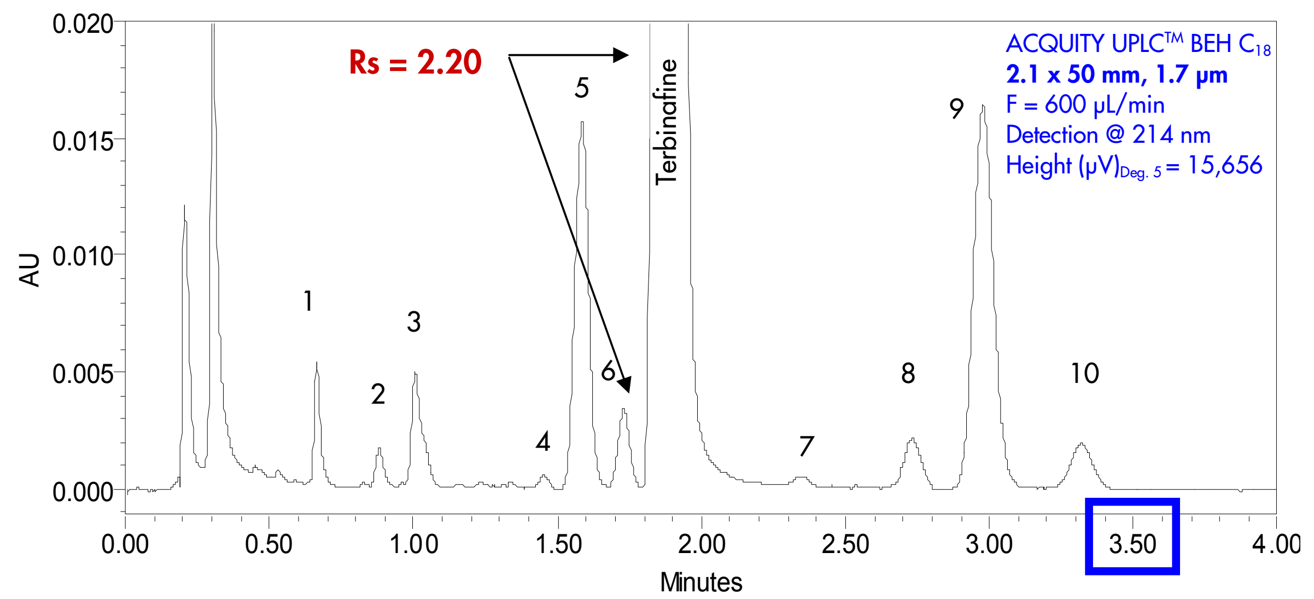
Terbinafine
mw = 291.43

Improved Throughput and Sensitivity

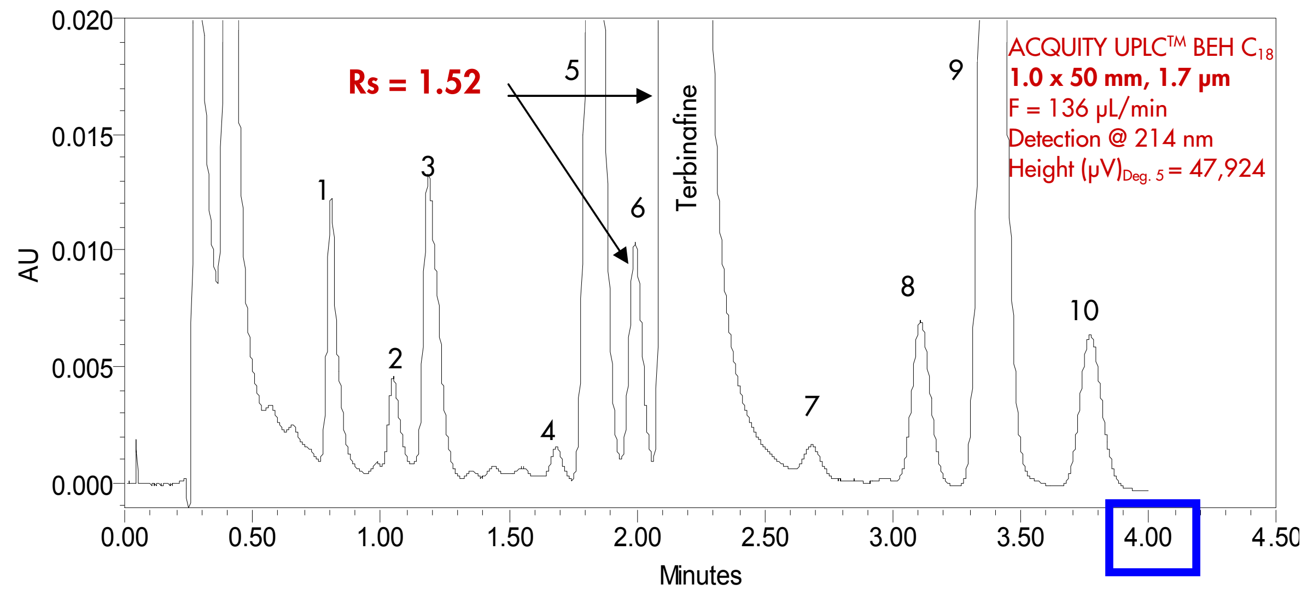
Improvements in throughput and sensitivity are demonstrated for the UPLC™ 1.7 µm particulate columns when analyzing the degraded drug product by scaling the column length inversely proportional to the particle diameter. Additionally, each particle size was run at its optimal linear velocity.



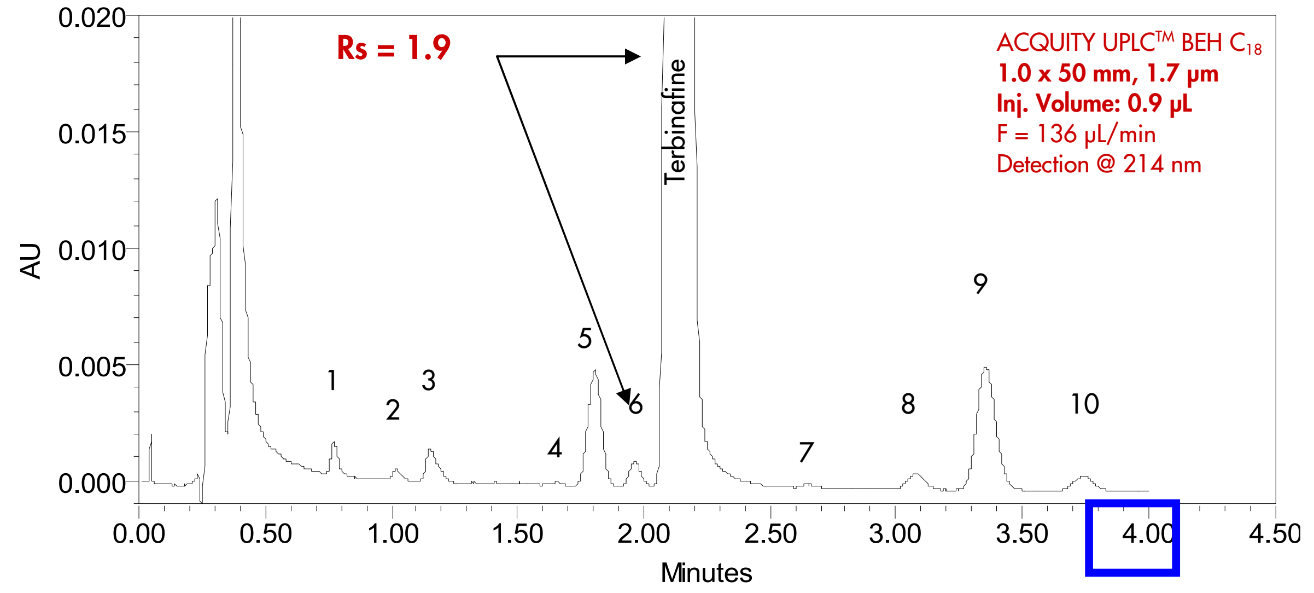
The chromatogram above depicts a typical stability-indicating assay using a 15 cm, 5 µm stationary phase. A resolution of 2.37 is achieved for the critical pair (degradant 6 and terbinafine). Additionally, a lengthy run time of 22 minutes is needed to resolve all components.



For the **1.7 µm 2.1 mm i.d. column**, results indicate that **sample throughput improved 6X** and **sensitivity improved 2.5X** while maintaining the original resolution between the critical pair. These improvements are in agreement with theory (F ↑2X, L ↓ 3X).



An **8X improvement in sensitivity** is observed on the **1.7 µm 1.0 mm i.d. column** when injecting the same sample volume on column. A reduction in resolution is observed due to volume overload. This can be addressed by scaling the injection volume appropriately to 0.9 µL.

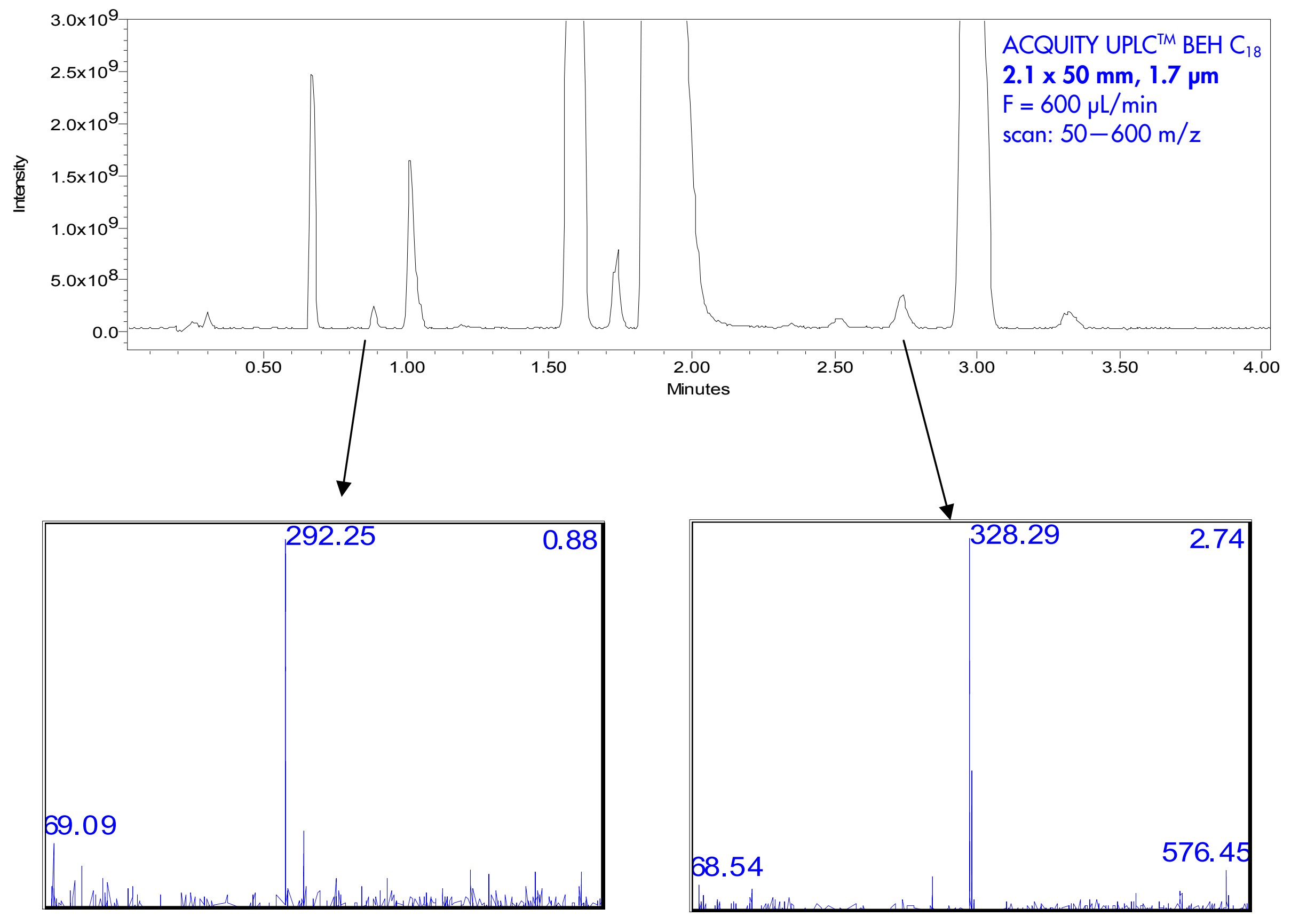


By scaling the injection volume appropriately from 4.0 µL to 0.9 µL, resolution of the critical pair is improved. Additionally, the sample loop was reduced from 10.0 µL to 2.0 µL to minimize peak dispersion.

For the UPLC™ separations, peak widths were as low as 3 seconds making a fast optical detector essential for accurate quantitation of peak resolution.

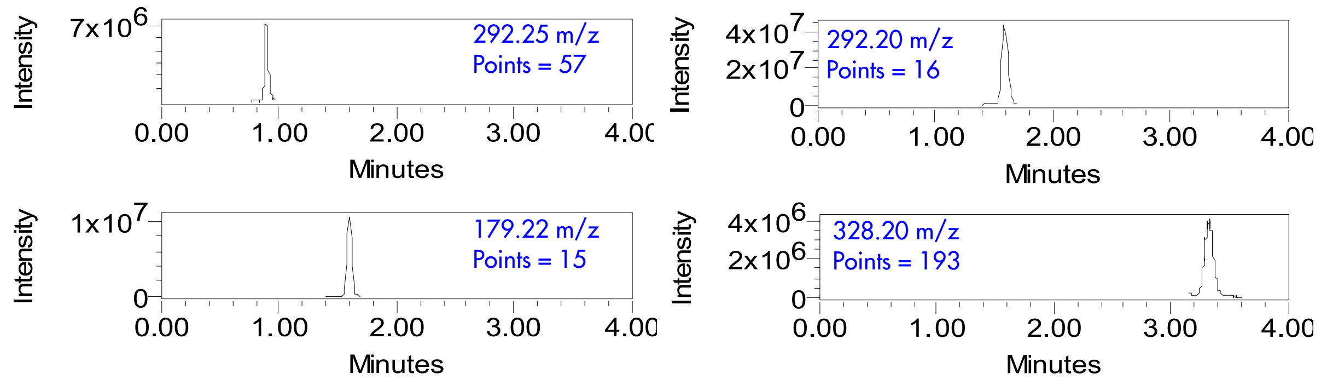
Degradant Identification and Quantitation Utilizing UPLC™/MS

A single quadrupole MS was used in scan mode from 50—600 m/z to identify the degradant peaks. Fast scans of 5000 Da/sec are necessary for identification of trace level components.



To demonstrate the possibility of quantitation for stability-indicating assays, SIR channels were determined from the MS scan. Due to the high concentrations of the parent compound injected on column, an injection volume of 0.5 µL was used to minimize signal overload of the MS detector.

It is necessary when performing fast analysis to consider the data collection parameters when setting up an MS method. At least 15 points across a peak are needed for accurate quantitation.



Conclusions

- Faster and more sensitive stability-indicating assays can be developed using 1.7 µm particulate columns with UPLC™ instrumentation.
- Fast, low dispersion optical detectors and mass spectrometers are essential for quantitation and identification of small volume, degradant peaks

1. Wyndham, K.D., O' Gara, J.E., Walter, T.H., Glose, K.H., Lawrence, N.L., Alden, B.A., Izzo, G.S., Hudalla, C.J. and Iraneta, P.C. Anal, Chem. 2003, 75, 6781-6788