

# Application Examples with Rapid Resolution HT HPLC Columns

**Technical Overview** 

Rapid Resolution HT HPLC columns are a new family of LC and LC/MS columns packed with 1.8 µm particles and designed for very fast high-resolution separations. As the particle size of the column packing decreases to 1.8 µm, column efficiency increases dramatically. This allows for more complex separations to be completed on shorter columns or permits a separation to be transferred from a longer column to a shorter column with equivalent results. Therefore, these cartridge columns are very short (15-50 mm) for the fastest possible separations and are available in the two most popular ZORBAX C18 bonded phases - Eclipse XDB-C18 and StableBond C18 – for compatibility with mobile phases from pH 1-9. A wide variety of rapid LC and LC/MS methods can be developed with these columns, and examples of a few applications are shown below. These examples are just excerpts from the complete application notes.

For copies of the application notes shown here go to www.agilent.com/chem/rrht and download the complete application note.

## Fast LC and LC/MS Analysis of Antibiotics Using Rapid Resolution HT Columns with Sub Two-Micron (1.8 µm) Particles

See Agilent Technologies publication 5989-0025EN

To provide accurate identification of the antibiotics, an LC/MS method was developed using a narrow-bore,  $2.1 \times 30$  mm Rapid Resolution HT column with 1.8 µm particles. LC/MS provides quick, accurate identification of the antibiotic and its precursor. With the very high efficiency possible with these small particles, high resolution can be achieved with these very short columns, thus making them an ideal choice for LC/MS applications.

Formic acid was chosen as the volatile LC/MS mobile phase additive at 0.2% to maintain a pH of 2.8. Atmospheric pressure chemical ionization (APCI) was selected based on the structures of the molecules, and it provided good sensitivity for these analytes (Figure 1).



Figure 1. APCI Analysis of clindamycin and lincomycin.



#### Increasing Sample Throughput Using Sub-Two Micron Particles in Short HPLC Columns

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These Rapid Resolution HT columns are used to demonstrate fast analysis times compared with traditional sized columns (250 mm long, 5  $\mu$ m particles) without compromising component resolution. The added benefit when using Rapid Resolution HT columns is the reduction in mobile phase usage that can significantly reduce the cost of analysis.

The remaining chromatograms in Figure 2 further illustrate the results of decreasing column length and particle size. Chromatogram B shows the same sample on a shorter Eclipse XDB-C18 column packed with smaller 3.5 µm particles. The actual results show a decrease in retention of 60% and baseline separation of all components. The Rapid Resolution HT columns show up to an 80% reduction in analysis time and baseline separation of all components.

1		4.6 × 250 mm, 5 μm			
Α	2 3 1	4 _5	6		
<sup>1</sup> 2 В	3 4 <sup>5</sup>	6 7	<b>Rapid Reso</b> 4.6 × 150 m	l <b>ution</b> ım, 3.5 µm	
cl <sup>2</sup> 3 4 <sup>5</sup>	6 7 <b>Rapid</b> I ∧ 4.6 × 7	<b>Resolution</b> 5 mm, 3.5 µm			
12 34 <sup>5</sup> 6 7 <b>Rapid Resolution</b> D 4.6 × 50 mm, 3.5 μm					
1 2 3 4 5 6 7 <b>Rapid Resolution HT</b> E 4.6 × 50 mm, 1.8 μm					
0 2	4	6	8	10	12min
Columns: Mobile phase: Flow rate: Temperature: Detection:	ZORBAX Eclips 73% MeOH, 27 1 mL/min Ambient UV 254 nm	e XDB-C18 % 20 mM Pho	sphate buffe	r, pH 7.0	

Figure 2. Reduce analysis time by 80% using rapid resolution HT columns.

## The Influence of Sub Two-Micron Particles on HPLC Performance

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The optimum flow rate for Rapid Resolution HT columns is higher than for larger particle size columns. Figure 3 shows an example of a Van Deemter curve on a  $4.6 \times 30$  mm, 1.8 µm column with methanol: water mobile phase.

The Rapid Resolution HT columns are designed to give twice the efficiency of a 3.5  $\mu$ m column with the same column dimensions. Figure 4 shows a comparison of a 4.6  $\times$  50 mm Rapid Resolution HT column with a comparable column made with 3.5  $\mu$ m particles.

#### www.agilent.com/chem



Figure 3. Example of a Van Deemter curve.



#### Figure 4. Efficiencies for RRHT and 3.5 µm SB C18 columns.

#### **For More Information**

For more information on our products and services, visit our Web site at www.agilent.com/chem/rrht.

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