Fast Liquid Chromatography and Liquid Chromatography/Mass Spectrometry Analysis of Antibiotics Using Rapid Resolution HT HPLC Columns with Sub Two-Micron (1.8 µm) Particles

Application

Authors

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Abstract

ZORBAX Rapid Resolution High Throughput HPLC columns using 1.8 µm particles provide outstanding efficiency in short column lengths for high sample throughput of liquid chromatography and liquid chromatography/mass spectrometry methods. These columns can be used for either isocratic or gradient analyses with high quality, reliable results.

Introduction

Fast, high-throughput HPLC is performed by using short (15–50-mm) columns that dramatically reduce analysis times. When these columns are packed with small particles, high resolution is maintained. High resolution and fast analysis times are ideal for checking pharmaceutical samples for precursor, related, or degradation products. These analyses can be performed by isocratic or gradient HPLC depending on the range of polarity of the compounds present. One example of this approach is the analysis of clindamycin phosphate, an antibiotic, and its precursor product, lincomycin. This analysis can be performed by isocratic or gradient HPLC, but gradient elution on Rapid Resolution High Throughput (HT) columns offers the highest sample throughput because of the shorter overall analysis time.

To provide accurate identification of the products, a corresponding liquid chromatography/mass spectrometry (LC/MS) method was developed using a narrow-bore, 2.1-mm id Rapid Resolution HT column packed with 1.8-µm particles. LC/MS provides quick, accurate identification of the antibiotic and its precursor. The 1.8 µm particles provide extremely high efficiency resulting in high resolution. This allows the use of very short (15–50 mm) columns ideal for LC/MS. This added resolution provides more accurate analyte identification by reducing co-elution and related MS signal suppression. This high resolution is especially critical in resolving critical analytes from early eluting matrix or excipient components.

Experimental Conditions

LC Methods

Clindamycin and lincomycin standards were dissolved in water (1 mg/mL). They were diluted 1:10 with 10% acetonitrile: 90% 20 mM NaH_2PO_4, pH 2.8. The isocratic separations were done on a ZORBAX Rapid Resolution SB-C18 4.6 × 50 mm, 3.5-µm column and a ZORBAX Rapid Resolution HT SB-C18 4.6 × 30 mm, 1.8-µm column. The mobile phase for the isocratic separation was 85% 20 mM NaH_2PO_4 in water: 15% acetonitrile at a flow rate of 1 mL/min. The gradient analysis was done using

the ZORBAX Rapid Resolution HT SB-C18, 4.6 × 30 mm, 1.8-µm column. The mobile phase consisted of A: 20 mM NaH_2PO_4 and B: acetonitrile. Gradient conditions were 10%–40% B in 2 minutes at a flow rate of 1.5 mL/min. Injection volume was 1 µL. Ultraviolet (UV) diode array (DAD) detection was at 210 nm. The separation was performed on an Agilent 1100 HPLC with a G1389A microautosampler and G1315B DAD equipped with a 5-µL micro flow cell. The instrument was plumbed with 0.12-mm id tubing.

LC/MS Method

Clindamycin and lincomycin standards were dissolved in water (1 mg/mL). They were diluted 1:10 with 10% acetonitrile: 90% 0.2% formic acid, pH 2.8. A ZORBAX Rapid Resolution HT SB-C18 2.1 × 30 mm, 1.8-µm column was used. The mobile phase consisted of A: 0.2% formic acid in water and B: acetonitrile + 0.2% formic acid. Gradient conditions were 15%–50% B in 1 minute, hold for 1.5 minutes at a flow rate of 0.5 mL/min. A very short 1.5 minute post time was used to provide very high-throughput. Injection volume was 1 µL. The separation was performed on an Agilent 1100 Series liquid chromatography/mass selective detector (LC/MSD) SL equipped with a well plate autosampler using the Automatic Delay Volume Reduction (ADVR) On feature. When this feature is turned on, the system bypasses extra autosampler volume without any additional programming of the ChemStation software.

The quadrupole MS was used with an atmospheric pressure chemical ionization (APCI) interface. Positive ion APCI mode was used to detect clindamycin and lincomycin.

Results

The isocratic analysis of lincomycin and clindamycin was completed in less than 8 minutes on a 4.6 × 50 mm long Rapid Resolution SB-C18 (3.5 µm) column (Figure 1). This is a reasonable analysis time but is not high-throughput for a simple analysis. The 4.6 × 30-mm Rapid Resolution HT SB-C18 (1.8-µm) column (as shown in Figure 1) can decrease analysis time. On this column the analysis took less than 6 minutes. The efficiency of the Rapid Resolution HT (1.8-µm) column exceeded that of the Rapid Resolution (3.5-µm) column, providing the same resolution in less time due to the shorter column length. This is a typical result when transferring an analysis to a Rapid Resolution HT column.

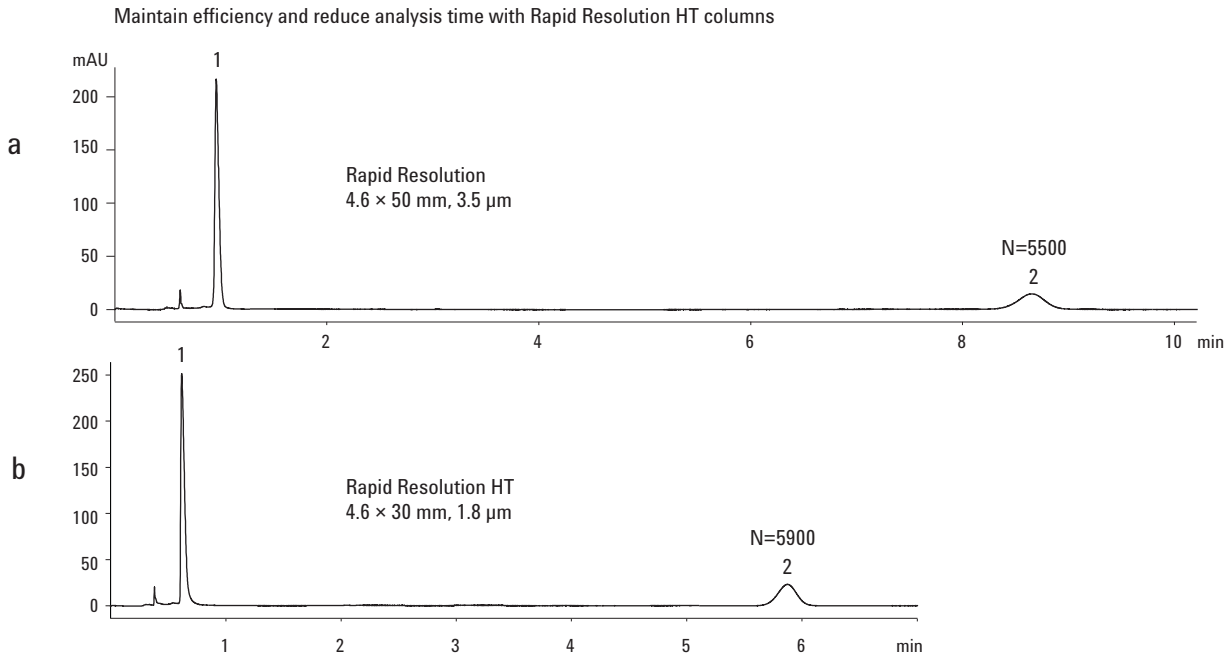

Figure 1. Chromatograms of lincomycin and clindamycin on Rapid Resolution (a) and Rapid Resolution HT (b) columns. Lincomycin (1) and clindamycin (2) are resolved on the Rapid Resolution SB-C18 4.6 × 50 mm, 3.5-µm column and the Rapid Resolution HT SB-C18 4.6 × 30 mm, 1.8 µm with the same efficiency. Mobile phase: 85% 20 mM NaH_2PO_4 in water pH, 2.8: 15% ACN, Flow rate: 1.0 mL/min, Temperature: Ambient, Detection: UV 210 nm.

But, the lincomycin precursor product is substantially more polar than the final antibiotic product, clindamycin. This polarity difference means that a rapid gradient analysis can effectively reduce total analysis time. When this same analysis was done using a gradient on the 4.6 × 30 mm, 1.8-µm Rapid Resolution HT SB-C18 column the compounds were separated in under 1.5 minutes (Figure 2) and the column was re-equilibrated in 1.5 minutes for a total cycle time of 3.0 minutes.

For confirmation of the products present, an LC/MS method was developed. To maintain a high linear velocity and perform the separation within the flow rate range compatible with the MS, a narrow-bore 2.1-mm id Rapid Resolution HT column was the best choice. The flow rate was 0.5 mL/min, a high flow rate for a 2.1-mm id column, but it is used very effectively with the short Rapid Resolution HT columns to reduce analysis time for LC and LC/MS. This flow rate is compatible with most LC/MS ionization techniques.

Formic acid was chosen as the volatile LC/MS mobile phase additive at 0.2% to maintain the pH of 2.8. APCI was selected based on the structures of the molecules and provided good sensitivity for these analytes (Figure 3). The spectra of the two components (Figure 4) show predominantly the expected $[M+H]^+$ ions for both lincomycin and clindamycin phosphate.

The HPLC was equipped with a well plate autosampler for rapid sample handling and the ADVR option was selected. The ADVR option minimizes gradient delay volume and makes it possible to keep gradient analysis times very short. The analysis time of the gradient separation modified for LC/MS was under 2 minutes. Therefore, high-throughput LC/MS is possible with the Rapid Resolution HT columns.

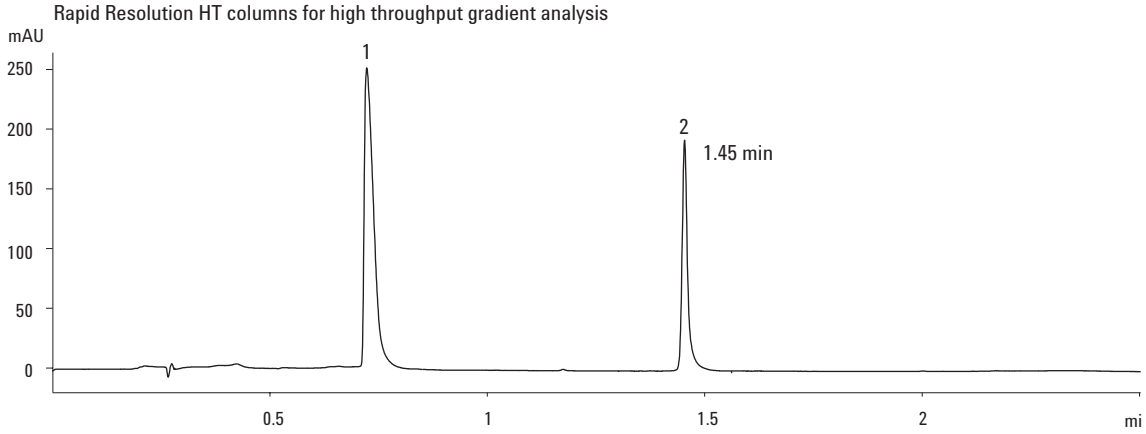

Figure 2. Gradient separation of lincomycin (1) and clindamycin (2) on the Rapid Resolution HT SB-C18 4.6 × 30 mm, 1.8-µm column in 2 minutes with re-equilibration in 1.5 minutes. Mobile phase: Gradient: 10% – 40% B in 2 minutes, A: 20 mM NaH_2PO_4 in water, pH 2.8 B: ACN, Flow Rate: 1.5 mL/min, Temperature: Ambient, Detection: UV 210 nm.

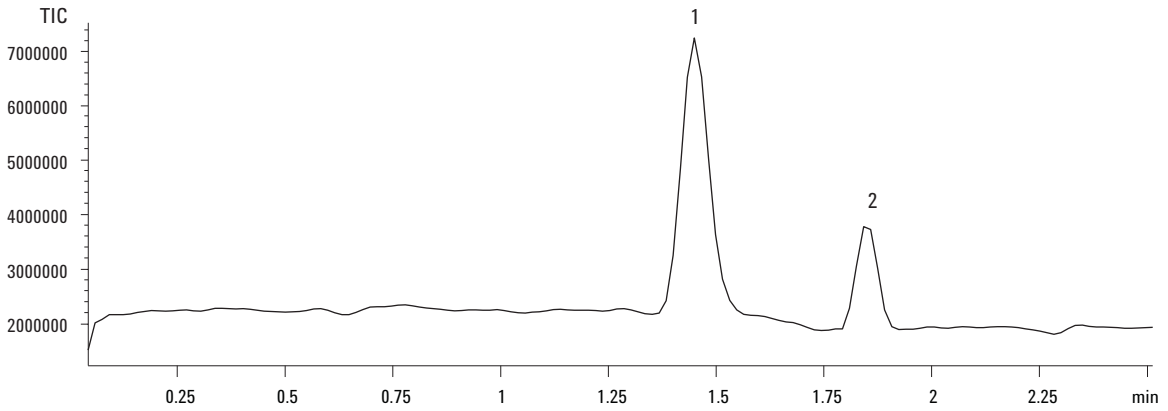

Figure 3. TIC (Total Ion Chromatogram) of lincomycin (1) and clindamycin (2) on the Rapid Resolution HT SB-C18, 2.1 × 30 mm, 1.8-µm column, Mobile phase: Gradient: 15% – 50% B in 1 minute, hold for 1.5 minutes, A: 0.2% formic acid in water pH 2.8,B: ACN + 0.2% formic acid, Post time: 1.5 minutes, Flow Rate: 0.5 mL/min, Injection volume: 1 µL, Temperature: Ambient, HPLC: Agilent 1100 with WPS and ADVR on Detection: APCI, Positive ion, MS Conditions: Peak width: 0.10 minutes Scan: 150–600 Da, step 0.1 Fragmentor: 70, Gas temp: 350 °C, Vaporizer: 350 °C, Drying gas: 12 L/min, Nebulizer pressure 50 psi, V_{cap} +3000V, Corona: 4.0 mA.

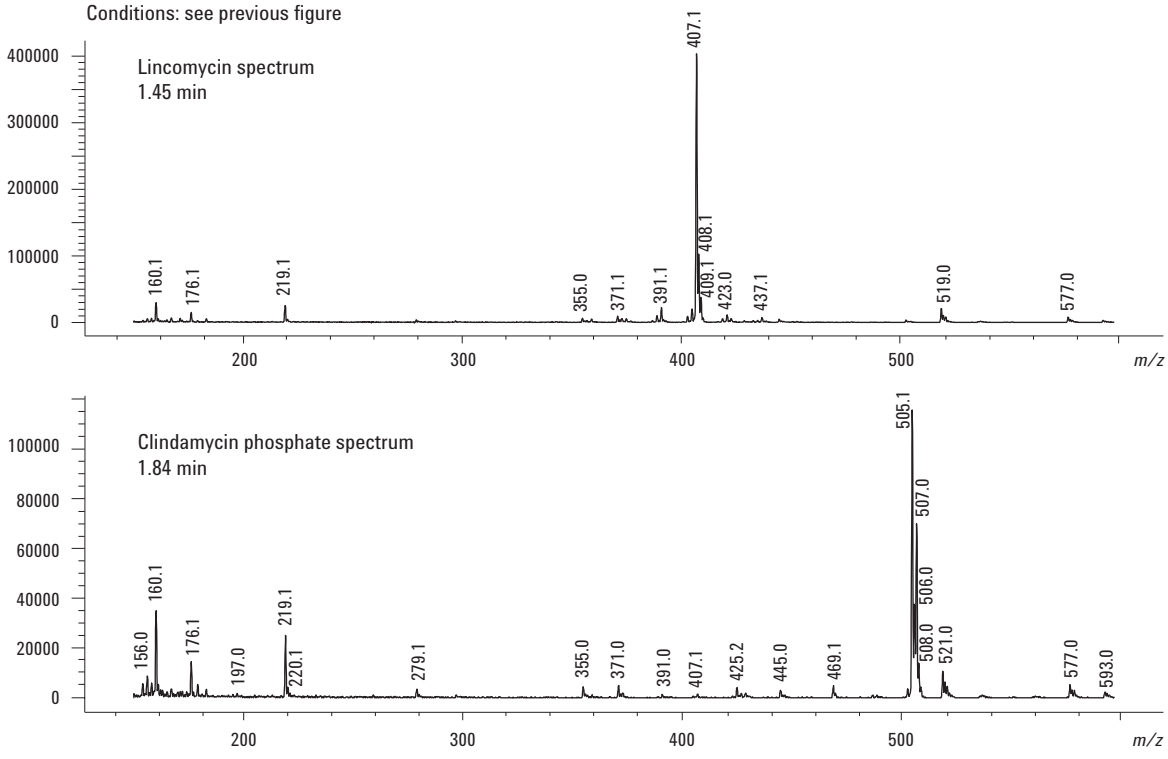

Figure 4. APCI-MS Spectra of lincomycin and clindamycin phosphate.

Conclusions

The new Rapid Resolution HT column with 1.8-µm particles provides high efficiency in short column lengths for high sample throughput using isocratic or gradient analysis. The gradient separation of lincomycin and clindamycin can be done in 40% less time than the isocratic analysis and results in higher sample throughput, making new Rapid Resolution HT columns ideal for fast analyses.

Narrow-bore 2.1-mm id Rapid Resolution HT columns are ideal for high-throughput LC/MS of this antibiotic and its precursor. At high linear velocities high sample throughput can be achieved without compromising resolution or sample identification in LC/MS separations with these short, 1.8-µm particle size columns.

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