

# Separation and Purification of Highly Polar Pharmaceutical Compounds on a RPLC Column at Prep Scale:

## Are You Thinking about Atlantis™ dC<sub>18</sub>?

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Atlantis™ dC<sub>18</sub> columns are a fully LC/MS compatible line of reversed-phased (RP) columns designed for retaining and separating both polar and non-polar compounds. This new silica-based packing material has excellent stability in acidic mobile phases and is fully compatible with 100% aqueous mobile phases. With Atlantis™ dC<sub>18</sub> columns, direct scale-up from analytical to preparative dimensions is simple. To demonstrate the utility of Atlantis™ dC<sub>18</sub> columns, we separated and purified three mixtures of polar compounds. Our results indicate that Atlantis™ dC<sub>18</sub> preparative columns provide excellent efficiency, high mass loading, and ease of scale-up.

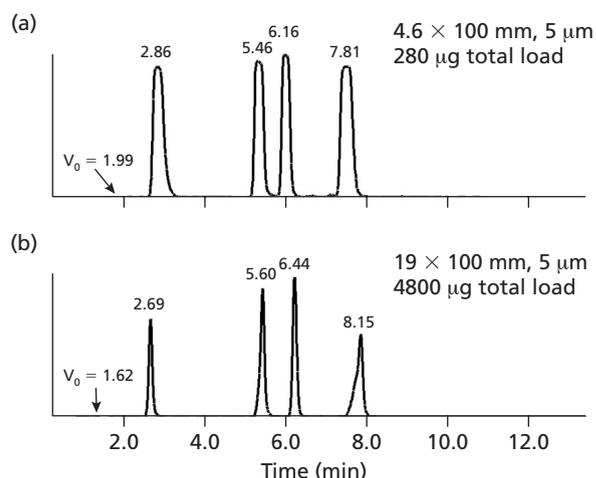
### Introduction

Chromatographers have often struggled with retaining polar compounds on traditional RP columns (e.g., C<sub>18</sub> stationary phase). These columns are too hydrophobic to retain the polar analytes. Additionally, these traditional columns often dewet under the 100% aqueous conditions necessary for retaining these polar analytes. The Atlantis™ dC<sub>18</sub> material was designed with the best combination of pore size, ligand density and ligand type to retain polar analytes and to operate in 100% aqueous conditions without dewetting. We have developed separations of polar mixtures on analytical dimension Atlantis™ dC<sub>18</sub> columns. We then scaled-up the separations to preparative dimensions to demonstrate the utility of these columns for compound purification.

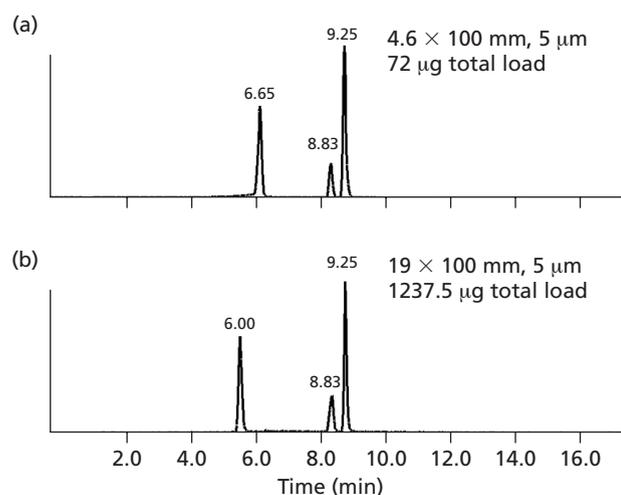
### Experimental Conditions

Analytical separations were performed on an Atlantis™ dC<sub>18</sub> 4.6 × 100 mm, 5 μm column. Preparative separations were performed on an Atlantis™ dC<sub>18</sub> 19 × 100 mm, 5 μm column. All experiments were conducted at ambient temperatures. The sample in Figure 1 is a mixture of 1 mg/mL each of sulphanilamide, sulphathiazole, sulphamethazine and sulphamethoxazole prepared in deionized (DI) water. The sample in Figure 2 is a mixture of pyrodoxal, folic acid and caffeine at 1, 0.25 and 1 mg/mL, respectively, in DI water with 0.2% ammonia to increase the solubility of folic acid. The sample in Figure 3 is a mixture of cinoxacin, oxolinic acid and nalidixic acid at 5, 0.5 and 1 mg/mL, respectively, dissolved in DMSO. The mobile phase consisted of A: water with 0.1% formic acid, and B: acetonitrile/water (90/10) with 0.1% TFA was used in place of formic acid

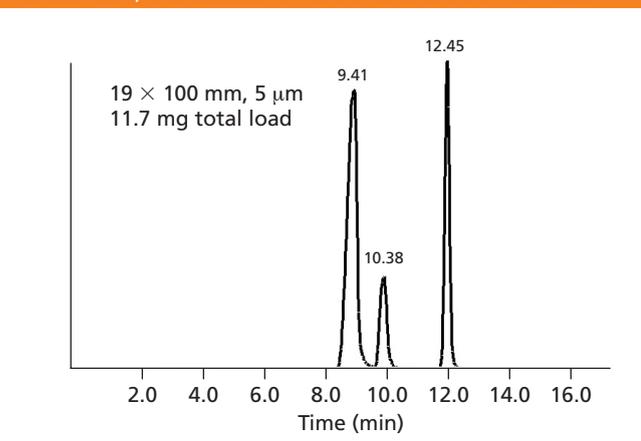
**Figure 1:** Separations of sulphonamides on analytical and preparative Atlantis™ dC<sub>18</sub> columns. A) Atlantis™ dC<sub>18</sub> 4.6 × 100 mm, 5 μm analytical column. Gradient conditions: 1 min hold at 85% A, 85% to 70% A in 1 min, and 70% to 30% A in 10 min for a total gradient time of 12 min. Injection volume: 70 μL. B) Atlantis™ dC<sub>18</sub> 19 × 100 mm, 5 μm preparative column. Gradient conditions: 3.02 min hold at 85% A, 85% to 70% A in 1 min, and 70% to 30% A in 10 min for a total gradient time of 14.02 min. Injection volume: 1200 μL. Analytes in order of elution: sulphanilamide, sulphathiazole, sulphamethazine and sulphamethoxazole. Detection: UV at 280 nm.



**Figure 2:** Water-soluble vitamin separations on Atlantis™ dC<sub>18</sub> analytical and preparative columns. A) Atlantis™ dC<sub>18</sub> 4.6 × 100 mm, 5 μm analytical column. Gradient conditions: 1 min hold at 100% A, 100% to 80% A in 5 min, and 80% to 60% A in 10 min for a total gradient time of 16 min. Injection volume: 32 μL. B) Atlantis™ dC<sub>18</sub> 19 × 100 mm, 5 μm preparative column. Gradient conditions: 2.53 min hold at 100% A, 100% to 80% A in 5 min, and 80% to 60% A in 10 min for a total gradient time of 17.53 min. Injection volume: 550 μL. Analytes in order of elution: pyrodoxal, folic acid and caffeine. Detection: UV at 280 nm.



**Figure 3:** Separation of nalidixic acid antibiotics on an Atlantis™ dC<sub>18</sub> 19 × 100 mm, 5 μm preparative column. Gradient conditions: 4.02 min hold at 80% A, 80% to 40% A in 10 min for a total gradient time of 14.02 min. Injection volume: 1800 μL. Analytes in order of elution: cinoxacin, oxolinic acid, and nalidixic acid. Detection: UV at 300 nm.



for the separation in Figure 2. Gradient methodology for each separation example is listed in each figure caption. The flow-rate was 1.0 mL/min for the 4.6 mm i.d. column and 17.06 mL/min for the 19 mm I.D. column. All experiments were run on the Waters® AutoPurification™ System, which consists of a Waters® 2525 Binary Gradient Module, a Waters® 2767 Sample Manager, a Waters® 2996 Photodiode Array Detector and a Waters® ZQ™ Mass Spectrometer.

## Results

The retention and separation of the sulphonamides on the analytical column is shown in Figure 1A. The total load is 280 μg and the flattened profiles reflect the saturation of the PDA detector. The mass load was proportionally scaled-up and run on the preparative column as shown in Figure 1B. Note the direct scale up, excellent peak shapes and total mass load of 4800 μg.

The retention and separation of pyrodoxal, folic acid and caffeine on the analytical column is shown in Figure 2A. The total load in this experiment is 72 μg. The mass load was proportionally scaled-up and run on the preparative column as shown in Figure 2B. Again, note the direct scale up, excellent peak shapes and total mass load of 1237.5 μg.

The final mixture was run directly on the preparative column and is shown in Figure 3. For this set of analytes, we achieved a total load of 11.7 mg.

## Conclusions

Atlantis™ dC<sub>18</sub> RPLC columns are useful tools for retaining and separating polar compounds under highly aqueous mobile phase conditions. Since Atlantis™ dC<sub>18</sub> columns are fully LC-MS compatible, they can be used in a mass-directed chromatographic purification system. Atlantis™ dC<sub>18</sub> preparative columns provide excellent efficiency, high mass loading, and ease of scale-up.

**Atlantis™**  
Columns

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