

# Analysis of Cephalosporins on a Polar Endcapped C18 3 $\mu$ m Particle Column

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## Key Words

Synconis, aQ, cephalosporins, polar endcapping, antibiotics

## Abstract

The analysis of species containing polar groups using reversed phase HPLC can be challenging. The polar groups present on the analytes can result in secondary interactions with silanols on the silica surface. Furthermore, the overall polarity of the molecule sometimes calls for the use of mobile phases with very high aqueous content to ensure the analyte solubility, retention and elution, at the expense of stationary phase stability. In this application note we demonstrate the analysis of a mix of four analytes from the cephalosporin antibiotics family on a Thermo Scientific™ Synconis™ aQ 3  $\mu$ m particle column. The analysis yields excellent peak shape and resolution in short run times, as well as outstanding reproducibility.

## Introduction

One of the key goals for the chromatographer is to achieve a consistent, reproducible separation. The selection of a highly reproducible HPLC column is essential if this goal is to be attained. The Synconis column range has been engineered to provide exceptional reproducibility due to its highly pure, high surface area silica, dense bonding and double endcapping, all controlled and characterized through the use of rigorous testing. The aQ phase endcapping group has been further optimized to provide a controlled interaction mechanism that retains and resolves polar analytes, with added media stability in 100% aqueous mobile phase.

Cephalosporins are a class of antibiotics constituting a class of  $\beta$ -Lactam antibiotics first discovered and isolated from the fungus *Cephalosporium Acremonium*. The core structure of these antibiotics (Figure 1) features a number of hydrogen bond donors and acceptor groups, as well as an acid function. Together these groups contribute towards the polarity of this class of compounds.

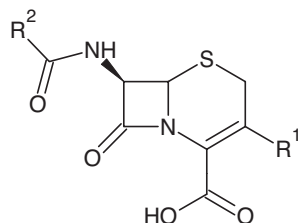
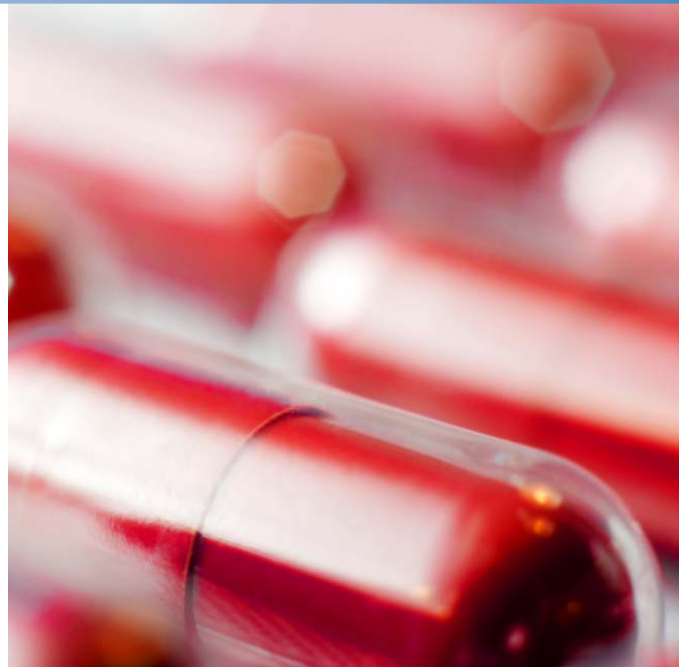


Figure 1: Core structure of cephalosporins



A group of four cephalosporins, listed in Figure 2, was chosen to be separated using a Synconis aQ column with a 3  $\mu$ m particle. Two of the components (cephalexin and cefradine) differ only in the presence of a phenyl group in cephalexin compared to a 1,4-cyclohexadiene in cefradine.

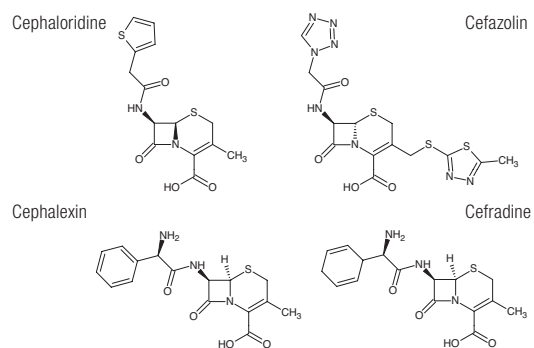


Figure 2: Cephalosporins mix used for this analysis

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## Experimental Details

<b>Consumables</b>		<b>Part Number</b>
Cephalexin, cephradine, cefazolin sodium salt and cephaloridine were all of a suitably high purity (97% or above)		
Fisher Scientific analytical reagent grade ammonium acetate		A/3440/53
Fisher Scientific OPTIMA grade acetic acid		A465-250
Fisher Scientific HPLC grade acetonitrile		A/0627/17
Thermo Scientific Smart2Pure® purified water (18.2 MΩ)		
<b>Sample Handling Equipment</b>		<b>Part Number</b>
Liquid handling hardware:	FinnPipette Kit 1	4700870
Thermo Scientific Premium:	8 mm standard opening screw thread vial convenience kit	60180-600 pack of 100
<b>Separation Conditions</b>		<b>Part Number</b>
Instrumentation:	Thermo Scientific Dionex Ultimate 3000 HPLC system	
Column:	Synchronis aQ, 100 x 3.0 mm, 3 μm	97303-103030
Mobile phase A:	25 mM ammonium acetate, adjusted to pH 5 with acetic acid	
Mobile phase B:	Acetonitrile	
Gradient:	Time (min)	%B
	0.0	10
	3.0	40
	4.0	40
	4.1	10
	6.0	10
Flow rate:	0.5 mL/min	
Run time:	6.0 min	
Column temperature:	35 °C	
Injection details:	1 μL of cephalosporins mix	
Injection wash solvent:	Water:acetonitrile 9:1 (v/v)	
UV detector wavelength:	254 nm	
<b>Solutions</b>		
Individual solutions of cephalexin, cephradine, cefazolin and cephaloridine were prepared by diluting 20 mg of compound ( $\pm$ 1 mg) in 20 mL 2:8 (v/v) of acetonitrile : water (acidified with 0.1% acetic acid). Each standard solution was sonicated for 10 minutes. A mixture of all four cephalosporins was prepared by diluting 100 μL of each standard in 600 μL of water (acidified with 0.1% acetic acid).		

## Results

Under the conditions described above, separation of the cephalosporins mixture above was achieved with a total run time of six minutes, with an elution window of 1.8 minutes for all four analytes. The separation chromatogram is shown in Figure 3. Good retention and separation of these polar compounds is achieved, demonstrating the controlled interaction mechanism and selectivity provided by the Synchronis aQ polar endcapping group.

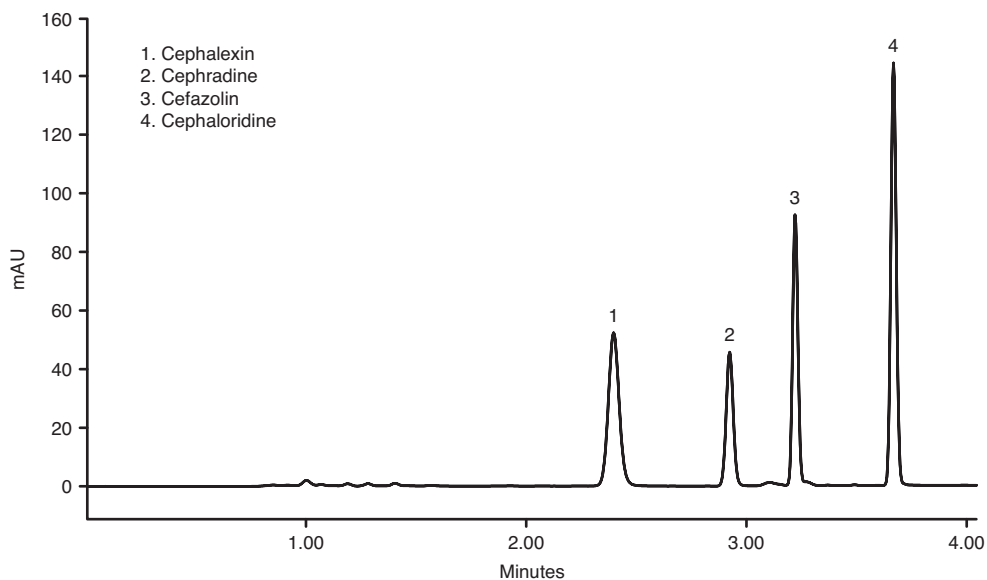


Figure 3: Separation chromatogram of a mixture of cephalosporins on a Synchronis aQ 3  $\mu\text{m}$  particle column

In Figure 4 the chromatograms obtained from six replicate injections are overlaid and % RSD values for the retention time of each analyte listed in the table inset. Excellent reproducibility was found, with % RSD values not exceeding 0.19%.

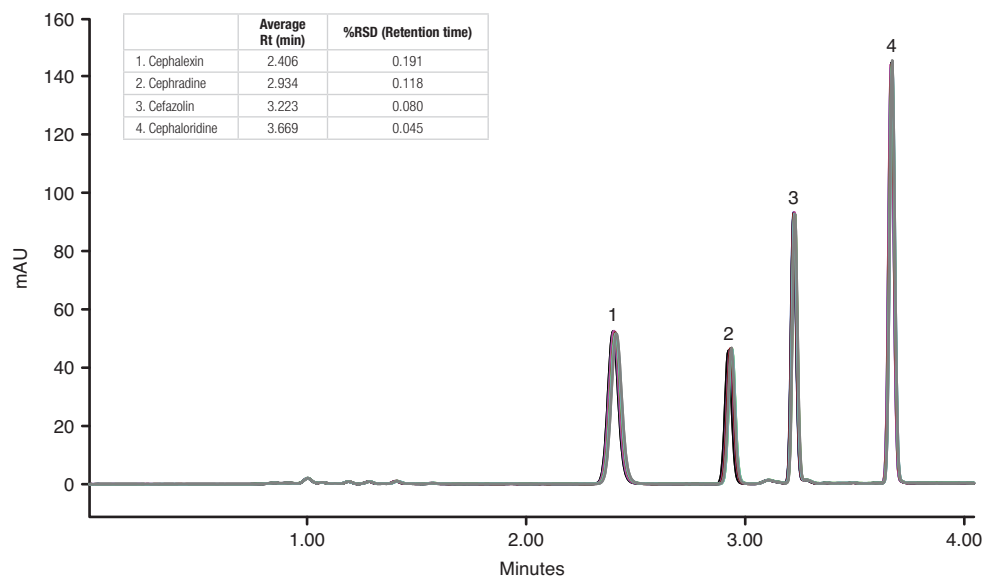


Figure 4: Overlay of six replicate injections of a mixture of four cephalosporins on Synchronis aQ 3  $\mu\text{m}$  particle column

Outstanding low asymmetry values were found for each analyte (Table 1), demonstrating the superior interaction mechanism and selectivity provided by Synchronis aQ polar endcapping group and lack of unwanted secondary interactions with the silica substrate.

	Average Asymmetry
<b>Cephalexin</b>	1.083
<b>Cephradine</b>	1.057
<b>Cefazolin</b>	1.075
<b>Cephaloridine</b>	1.035

Table 1: Average peak asymmetry value measured for each cephalosporin analyte

## Conclusion

- Synchronis aQ 3  $\mu\text{m}$  provides excellent resolution power within short run times
- The highly pure, high surface area silica combined with polar endcapping provides good retention and superior peak shape for polar analytes
- Exceptional reproducibility of results is achieved.

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