Analysis on Cephalosporins on a Polar Endcapped C18 3 µm Particle Column

Valeria Barattini, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words
Syncronis, 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™ Syncronis™ aQ 3 µm 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 Syncronis 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 β-Lactam antibiotics first discovered and isolated from the fungus *Cephalosporum Acremonium*. The core structure of these antiobitics (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.

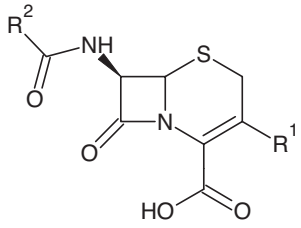
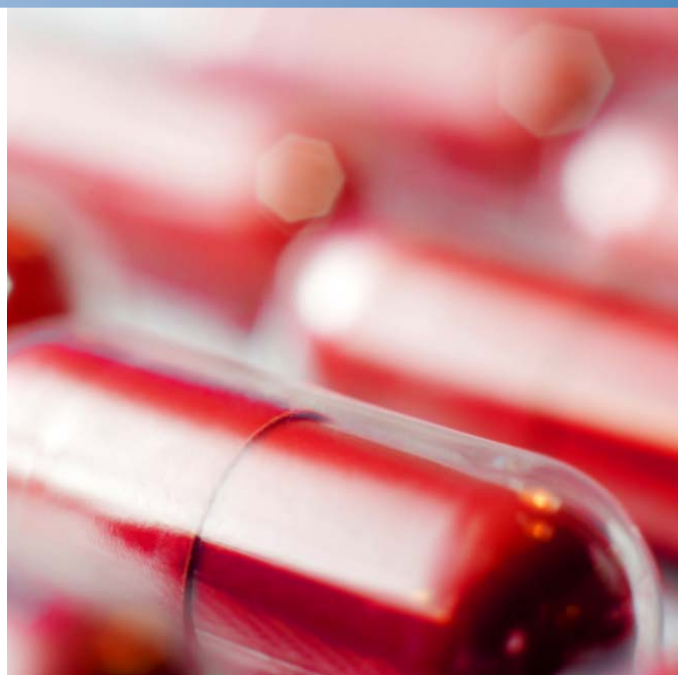


Figure 1: Core structure of cephalosporins



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

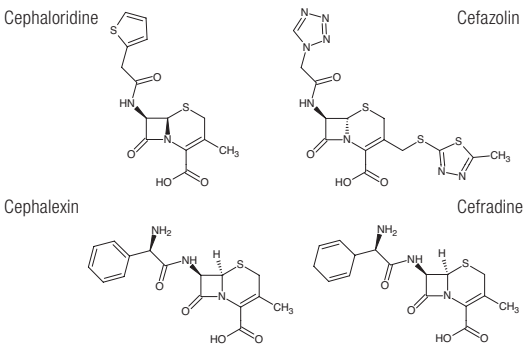


Figure 2: Cephalosporins mix used for this analysis

Thermo SCIENTIFIC

Experimental Details

Consumables	Part Number
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)	

Sample Handling Equipment		Part Number
Liquid handling hardware:	FinnPippette Kit 1	4700870
Vials and closures:	8 mm standard opening screw thread vial Convenience kit	60180-600 pack of 100

Separation Conditions			Part Number
Instrumentation:	Thermo Scientific Dionex Ultimate 3000 HPLC system		
Column:	Syncronis 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:acetontrile 9:1 (v/v)		
UV detector wavelength:	254 nm		

Solutions	
	Individual solutions of cephalexin, cephradine, cefazolin and cephaloridine were prepared by diluting 20 mg of compound (± 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 Syncronis aQ polar endcapping group.

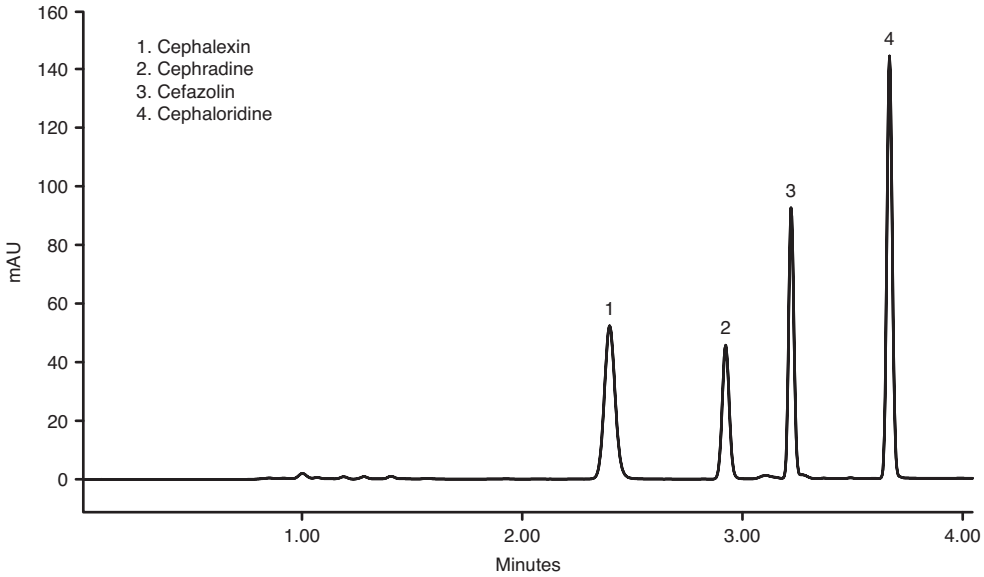


Figure 3: Separation chromatogram of a mixture of cephalosporins on a Syncronis aQ 3 µm particle column

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%.

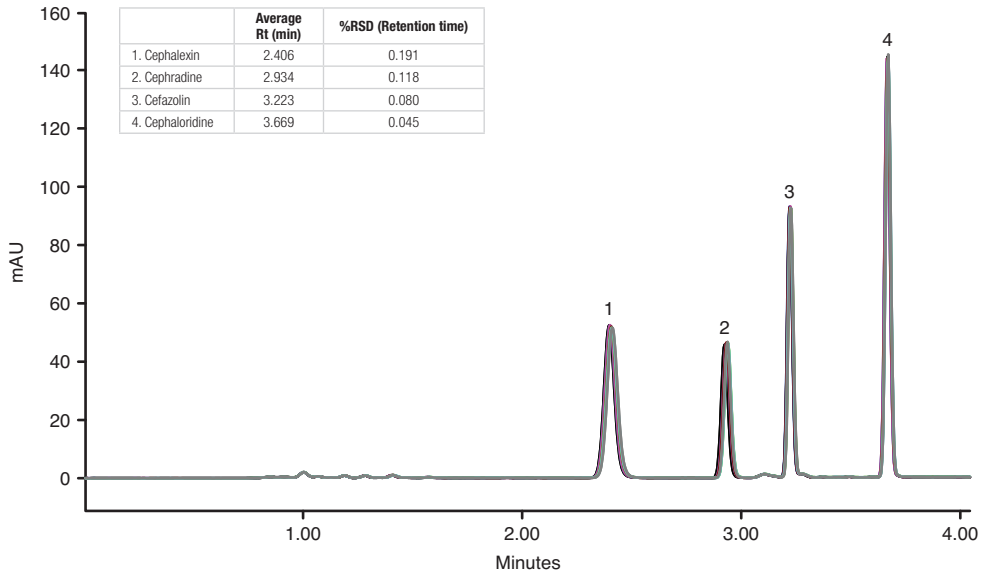


Figure 4: Overlay of six replicate injections of a mixture of four cephalosporins on Syncronis aQ 3 µm particle column

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

	Average Asymmetry
Cephalexin	1.083
Cephradine	1.057
Cefazolin	1.075
Cephaloridine	1.035

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

Conclusion

- Syncronis aQ 3 µm 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.

thermoscientific.com/chromatography

USA and Canada +1 800 332 3331
France +33 (0)1 60 92 48 34
Germany +49 (0) 2423 9431 20 or 21
United Kingdom +44 (0)1928 534110
Japan +81 3 5826 1615

China +86 21 68654588 +86 10 84193588
+86 20 83145199 800 810 5118
India +91 22 6742 9494 +91 27 1766 2352
Australia 1 300 735 292 (free call domestic)
New Zealand 0800 933 966 (free call domestic)
All Other Enquiries +44 (0) 1928 534 050

Technical Support
North America +1 800 332 3331
Outside North America +44 (0) 1928 534 440

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific

AN20672_E 11/12S