

Case study for Solvent Saving with USP method of Ceftizoxime Sodium using Agilent ZORBAX Eclipse Plus C18 Columns

Application Note

Pharmaceuticals

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Abstract

Ceftizoxime sodium is a parenteral third-generation cephalosporin, which is prescribed for use worldwide. This application shows several different columns that could be selected for the USP method of ceftizoxime sodium analysis in order to reduce solvent use. The USP method uses a 4.0 mm × 300 mm, 5-10 μm L1 column. This method could be quickly converted for use on an Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 250 mm, 5 μm column without any modifications. The method can also be switched to the Agilent ZORBAX Solvent Saver Eclipse Plus C18, 3.0 mm × 150 mm, 5 μm and Agilent ZORBAX Rapid Resolution, 4.6 mm × 100 mm, 3.5 μm columns with simple modifications and to the Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 μm column with method validation, in order to save the most time and solvent.



Agilent Technologies

Introduction

Ceftizoxime sodium, a parenteral third-generation cephalosporin, is highly resistant to a broad spectrum of beta-lactamases and is active against a wide range of both aerobic and anaerobic gram-positive and gram-negative organisms. [1] It has few side effects and is reported to be safe and effective in aged patients and in patients with hematologic disorders. [1] It is a widely prescribed drug throughout the world. The structures of the ceftizoxime sodium analyzed in this application are depicted in Figure 1.

The USP chromatographic method for content measurement of ceftizoxime sodium recommends using an L1 column with 4.0 mm × 300 mm, 5-10 μm particle size and 2 mL/min flow rate. However, under the revised general chapter <621>, the USP allows adjustments in the chromatographic parameters to be made but only when, "adjustments have improved the quality of the chromatogram in meeting system suitability requirements." [2] In this application note, we have taken advantage of the allowable adjustments from the USP to produce a method with substantial solvent savings.

Table 1 shows the original method for ceftizoxime sodium in the USP and the permitted adjustable range according to the USP. The ZORBAX Eclipse Plus C18 columns of 4.6 mm × 250 mm, 5 μm and 3.0 mm × 150 mm, 5 μm and 4.6 mm × 100 mm, 3.5 μm are all within USP limits. While the 4.6 mm × 50 mm, 1.8 μm column is out of the USP limits for an adjustment with this method, it can also be used with additional method validation.

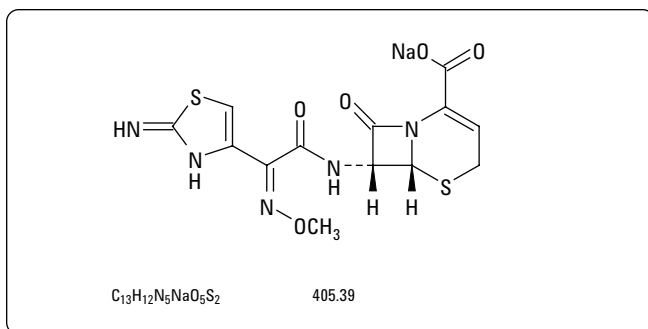


Figure 1. Structures of Ceftizoxime Sodium.

Table 1. The Permitted Adjustments for the USP Ceftizoxime Sodium Method

Permitted adjustments	Original method	Permitted limits
Column length (± 70%)	300 mm	90 mm–510 mm
Column id (± 25%)	4.0 mm	3.0 mm–5.0 mm
Particle size (-50%)	5-10 μm	2.5 μm–5 μm
Flow rate (± 50%)	2.0 mL/min	1-3 mL/min
Injection volume (reduced until it is consistent)	10 μL	Variable
Column temperature (± 10 °C)	Not specified	Variable
Mobile Phase Composition	10% organic	7–13% organic
pH of Mobile Phase (± 0.2)	3.6	3.4–3.8

Experimental

Citric acid monohydrate, dibasic sodium phosphate, monobasic potassium phosphate and acetonitrile were purchased from Merck. Water was made using a Milipore system, and USP ceftizoxime sodium was purchased from the United States Pharmacopeia and the active pharmaceutical ingredient (API) of ceftizoxime sodium was provided by Qi-Lu Pharmaceutical Co. LTD. CHINA.

High performance liquid chromatography (HPLC) analysis was performed with the Agilent 1200 Series Rapid Resolution LC (RRLC) system:

- G1312B binary pump SL, Mobile phase Channel A: pH 3.6 buffer in water (1.42 g of citric acid monohydrate and 1.73 g of dibasic sodium phosphate in 1000 mL water), Channel B: Acetonitrile, (90/10); Flow rate was 2 mL/min (conditions varied for robustness testing)
- G1376D automatic liquid sampler SL (ALS), injection volume was 10 μL for the 4.6 mm × 250 mm column, 4 μL for the 4.6 mm × 100-mm column, 2.6 μL for the 3.0 mm × 150 mm column, 2 μL for the 4.6 mm × 50-mm column
- G1316B Thermostatted Column Compartment SL (TCC), Temperature was 25 °C
- G1316C Diode Array Detector SL (DAD), wavelength used was 254, 4 nm, with a G1315-60022 standard flow cell (10-mm path, 13 μL volume)

ZORBAX Columns:

- Eclipse Plus C18, 4.6 mm × 250 mm, 5 µm p/n 959990-902
- Solvent Saver Eclipse Plus C18, 3.0 mm × 150 mm, 5 µm p/n 959993-302
- Rapid Resolution Eclipse Plus C18, 4.6 mm × 100 mm, 3.5 µm p/n 959961-902
- Rapid Resolution HT Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 µm p/n 959941-902

Standard preparation, assay preparation and mobile phase were prepared following the USP ceftizoxime sodium analysis method. [3] The method requires that the column efficiency determined from the analyte peak is not less than 2000 theoretical plates; the tailing factor for the analyte peak is not more than 2; the resolution (R), between the analyte and internal standard peaks is not less than 4; and the relative standard deviation for replicate injections is not more than 2%. [3]

As seen from the chromatogram in Figure 2 and performance data in Table 1, the chromatographic performance requirements of the USP method are easily met. No method adjustment should be made to the USP method of ceftizoxime sodium analysis when using the Eclipse Plus C18, 4.6 mm × 250 mm, 5 µm column. This is a traditional method with a total analysis time of 12 minutes.

To reduce the solvent use, the column diameter, length, or particle size can be changed within USP adjustment criteria without method revalidation. Agilent provides various dimensions and configurations of columns with the same packing to meet customers' needs. Here the ZORBAX Solvent Saver Eclipse Plus C18 3.0 mm × 150, 5 µm and the ZORBAX Rapid Resolution Eclipse Plus C18 4.6 mm × 100, 3.5 µm columns are both allowable choices within USP adjustment criteria. So the method can be easily modified for them. The injection volume should be adjusted according to the column volume and the flow rate should be adjusted to the internal diameter. Figures 3 and 4 show chromatograms on both columns. As can be seen, both the analysis time and solvent are reduced.

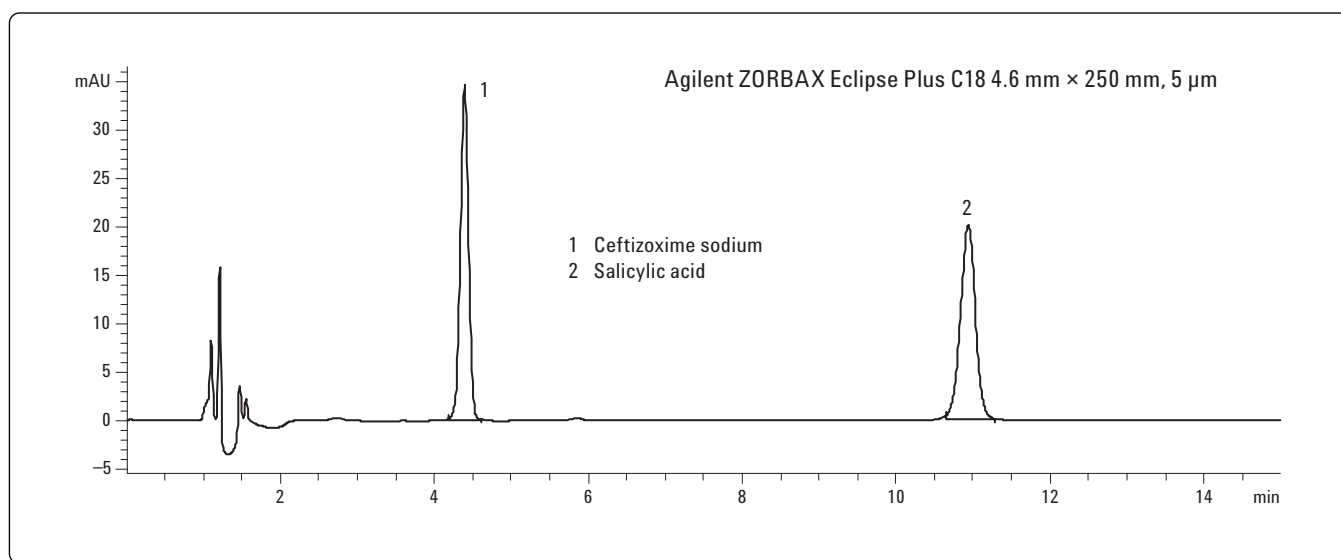


Figure 2. Chromatogram on Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 250 mm, 5 µm column.

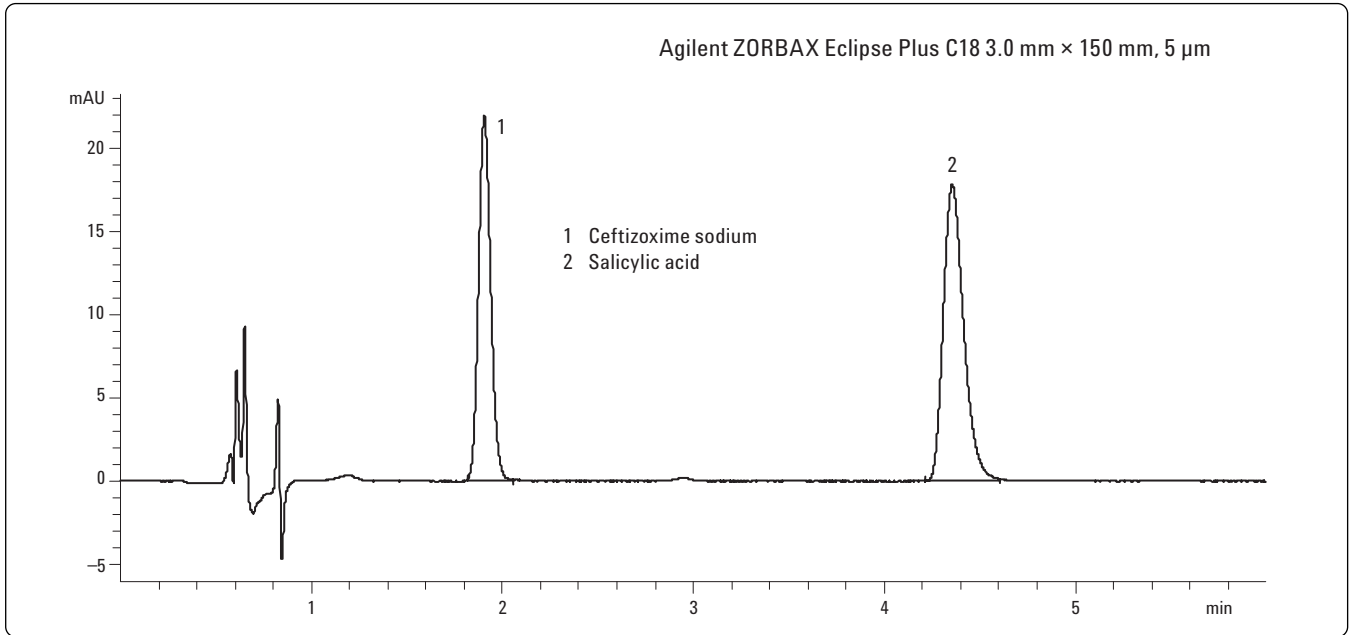


Figure 3. Chromatogram on Agilent ZORBAX Eclipse Plus C18, 3.0 mm × 150 mm, 5 μm column.

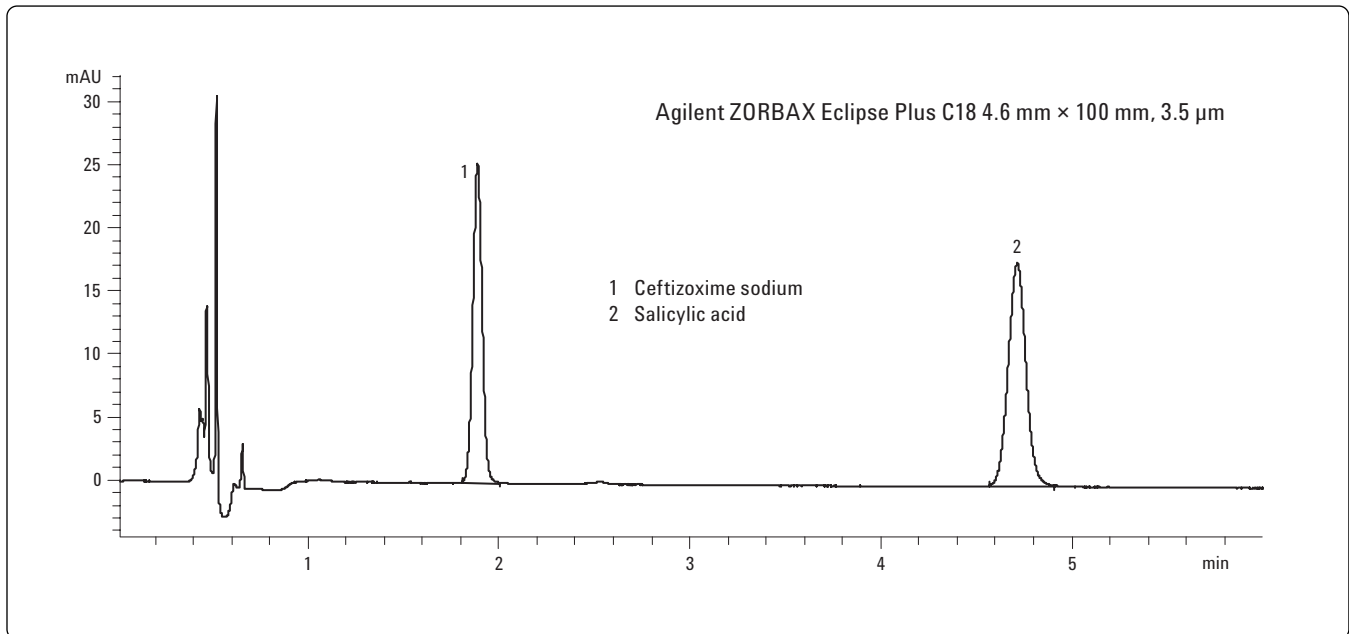


Figure 4. Chromatogram on Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 100 mm, 3.5 μm column.

Also the analysis could be performed using an Agilent ZORBAX Rapid Resolution HT (RRHT) Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 μm column as shown in Figure 5.

Data in Table 2 demonstrate that the USP requirement can be easily met with columns of different configurations. Table 2 also shows the dramatic savings in solvent and analysis time that are possible by moving to columns with smaller diameters or short columns with smaller particles.

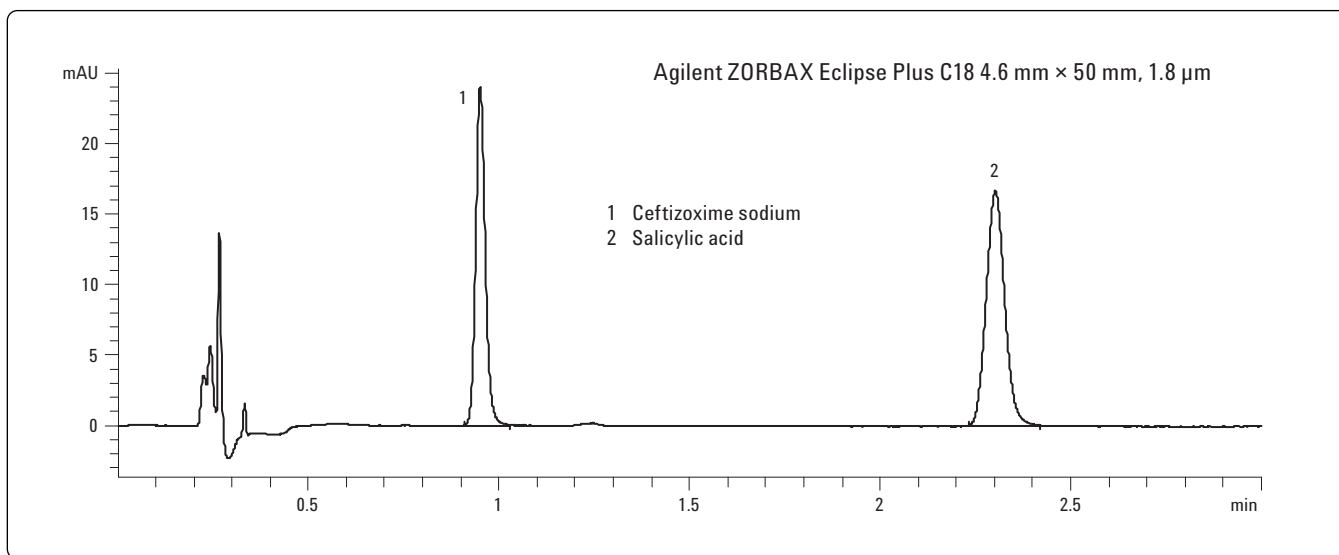


Figure 5. Chromatogram on Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 μm column.

Table 2. Data for System Suitability on Different Column Configurations

Column configuration	Peak	Theoretical plates	USP tailing factor	Resolution	Decrease in analysis time (%)	Decrease in solvent use (%)
Agilent ZORBAX Eclipse Plus C18 4.6 mm x 250 mm, 5 μm	1 2	8524 15865	0.96 0.95	24.3	–	–
Agilent ZORBAX Eclipse Plus C18 3.0 mm x 150 mm, 5 μm	1 2	4554 8332	1.12 1.30	16.1	62	81
Agilent ZORBAX Eclipse Plus C18 4.6 mm x 100 mm, 3.5 μm	1 2	7338 12725	1.02 1.03	22.2	60	60
Agilent ZORBAX Eclipse Plus C18 4.6 mm x 50 mm, 1.8 μm	1 2	7167 11334	1.13 1.09	20.3	80	80
USP method	1 and 2	N ≥ 2000	Tf < 2.0	≥ 4	–	–

While the performance requirements of the USP method are easily met, it is necessary to revalidate the method as the column choice is outside the allowable limits of 621 method adjustment. The length has been reduced by over 80%, and the particle size has been reduced by 62%.

Validation items that are required include linearity and robustness testing for temperature, pH and organic content.

Figure 6-1 shows the plot for linearity where the correlation coefficient (r) for the linearity is 0.99991. The range is set from 50% to 150% of the prepared sample which covers a range of 80% to 120% required for recovery test.

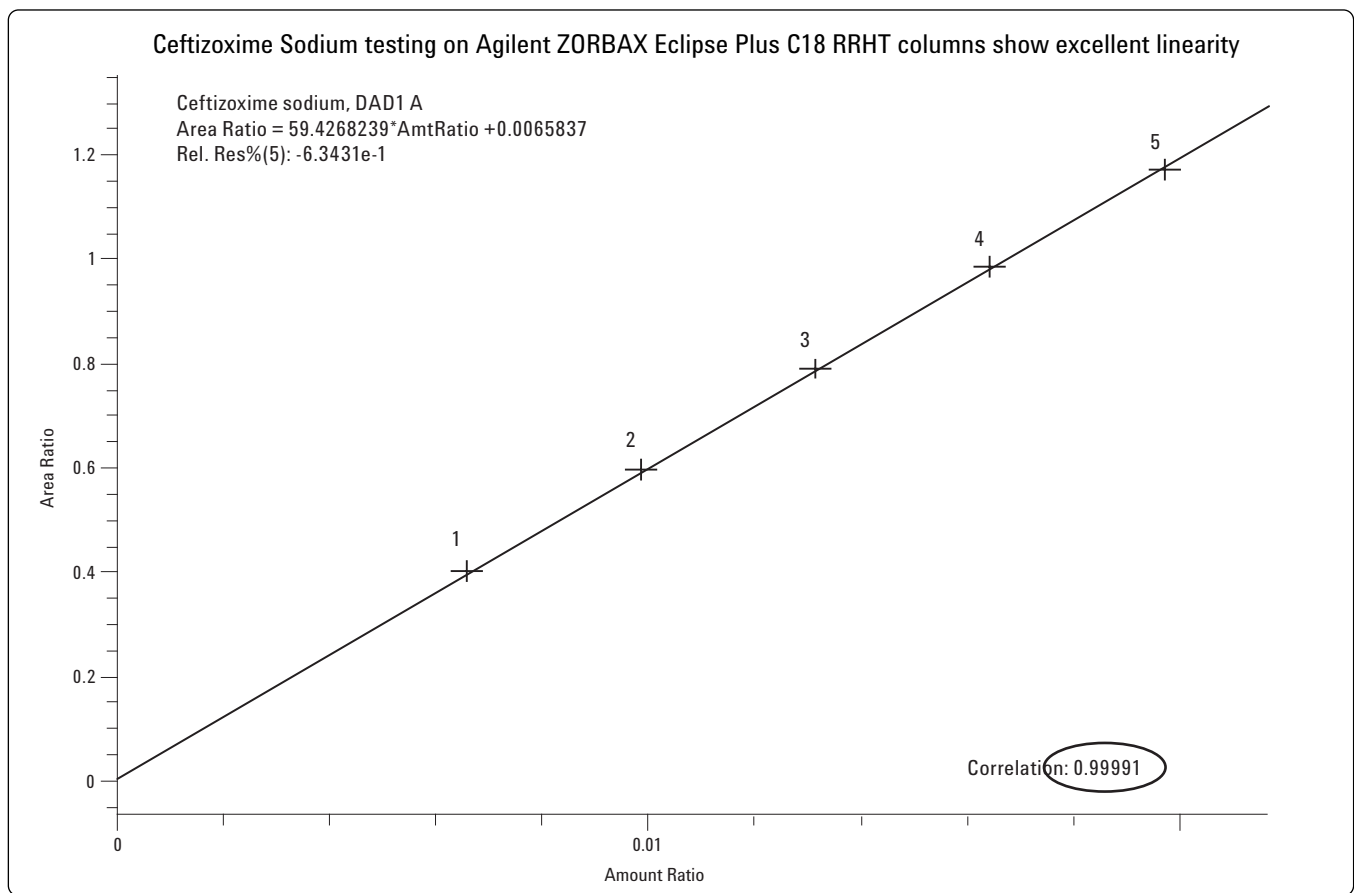


Figure 6-1. Linearity plot for ceftizoxime sodium analysis.

Figures 6-2, 6-3, 6-4, 6-5 and 6-6 are the chromatograms generated during robustness testing. The theoretical plates, USP tailing factor and the resolution between the two peaks are shown in Table 3. Over the test range, all the data are within the USP performance requirements for this method.

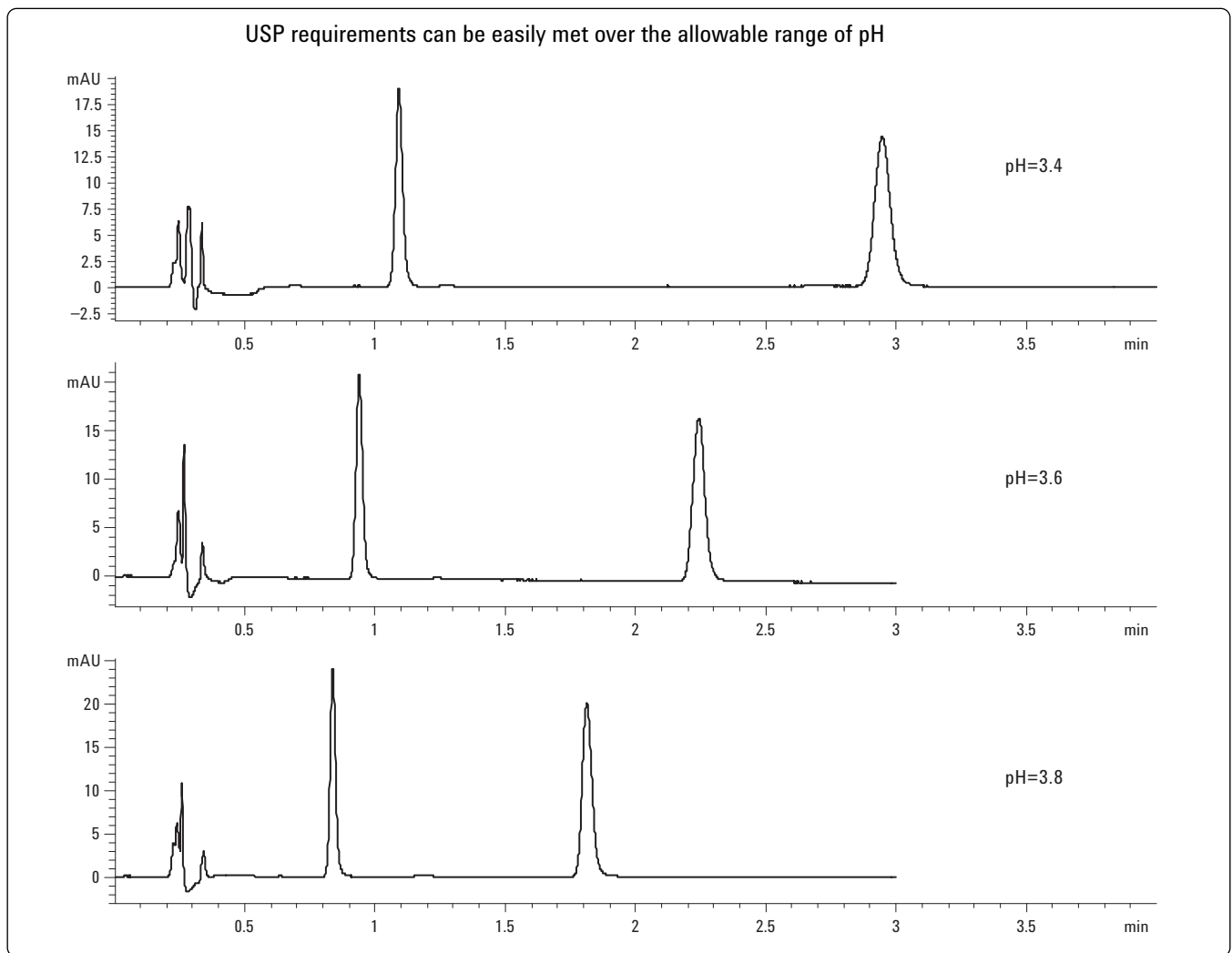


Figure 6-2. Overlay chromatograms for adjustment of pH.

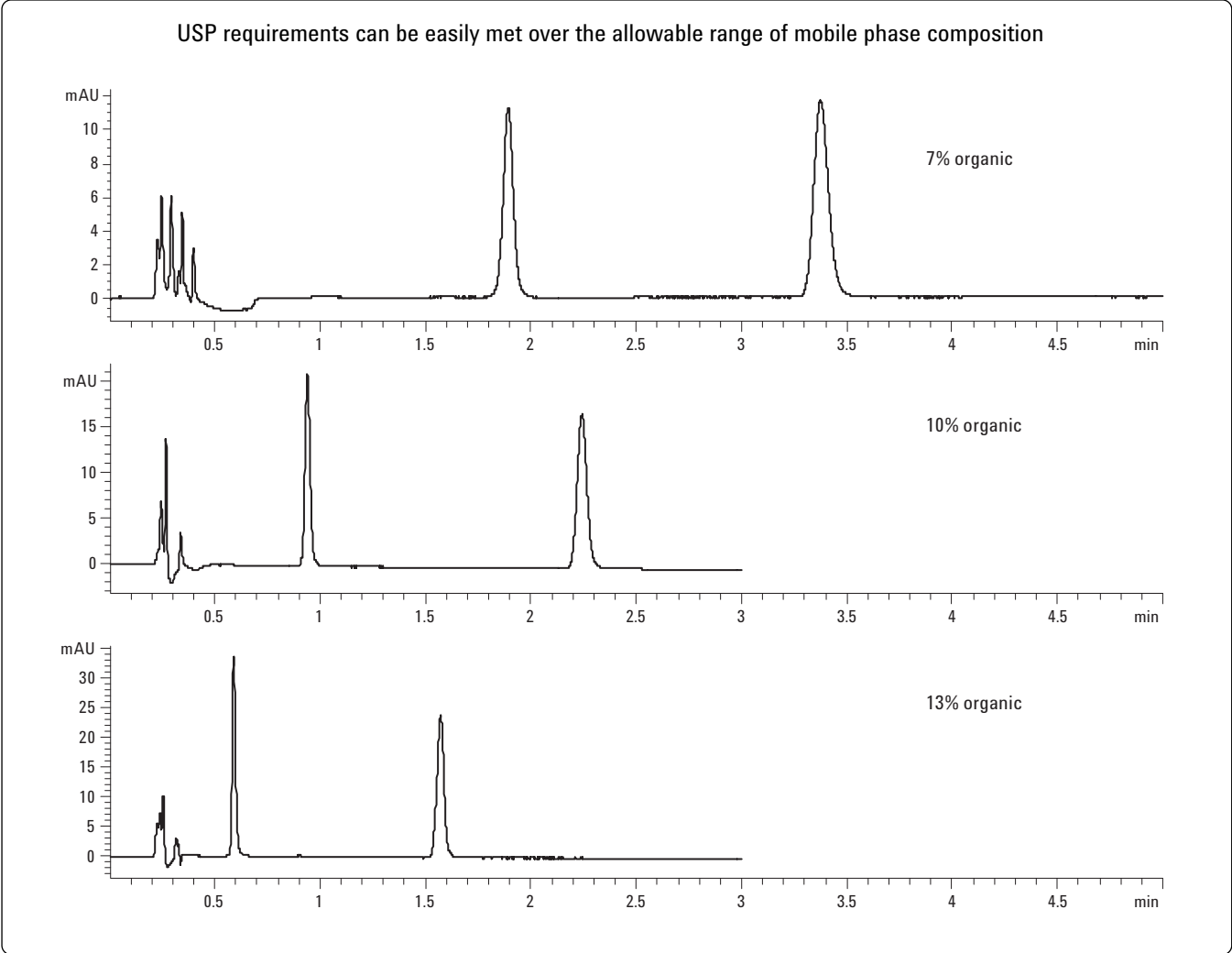


Figure 6-3. Comparison of analysis with change in organic.

USP requirements can be easily met over the allowable range of temperature

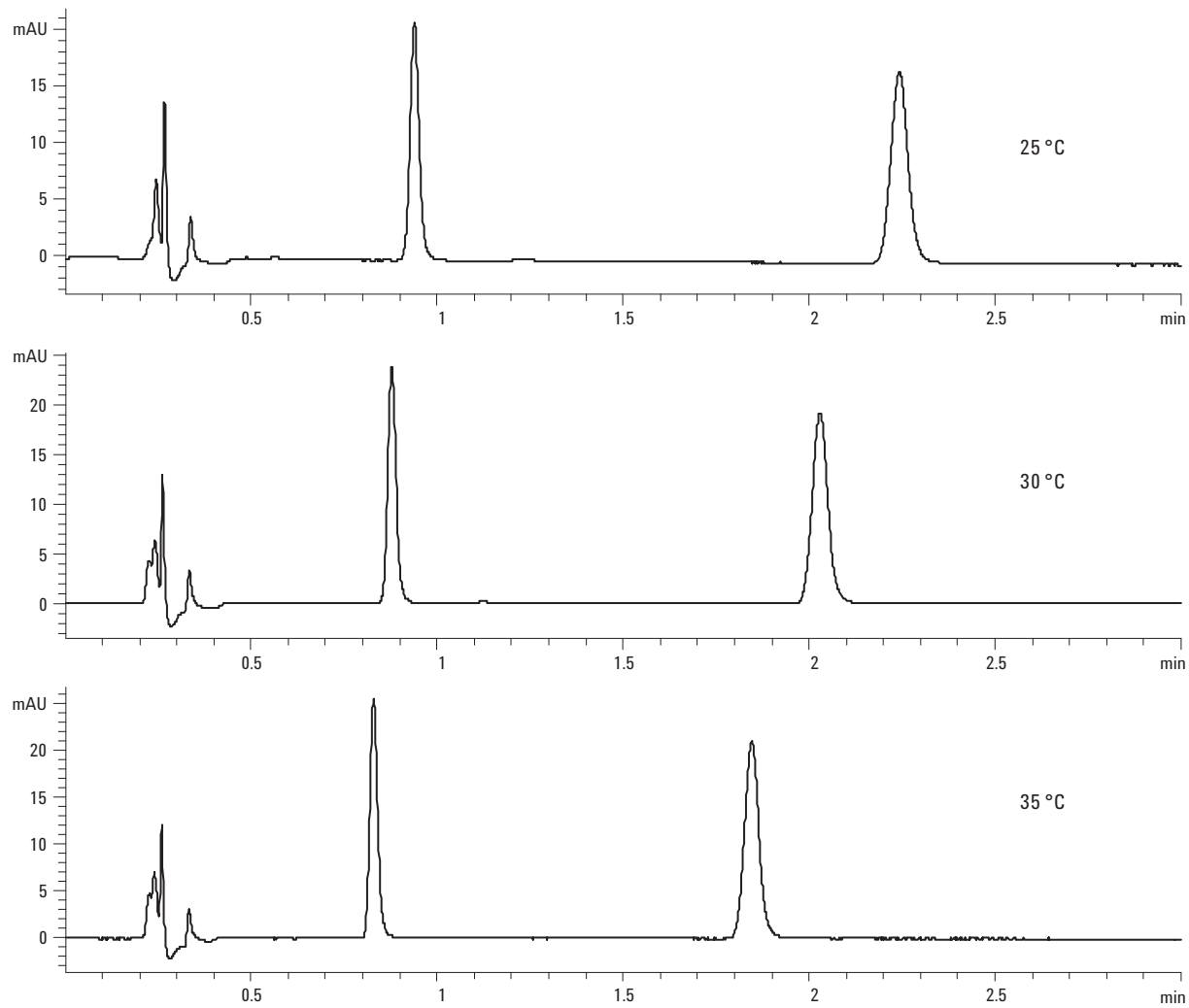


Figure 6-4. Comparison of the separation with change in temperature.

USP requirements can be easily met over the allowable range of flow rates

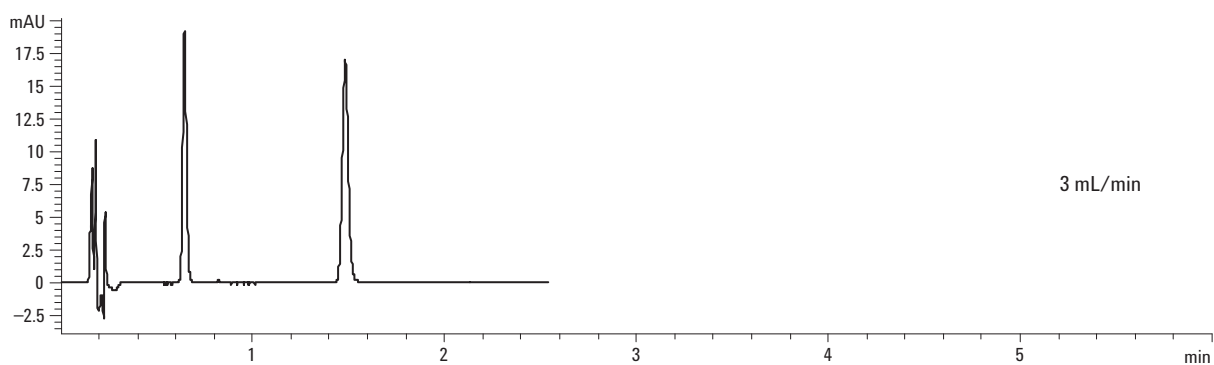
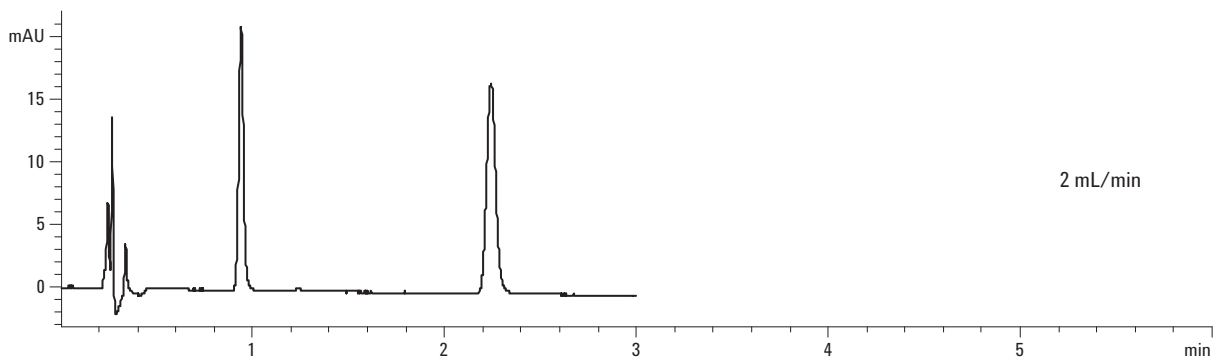
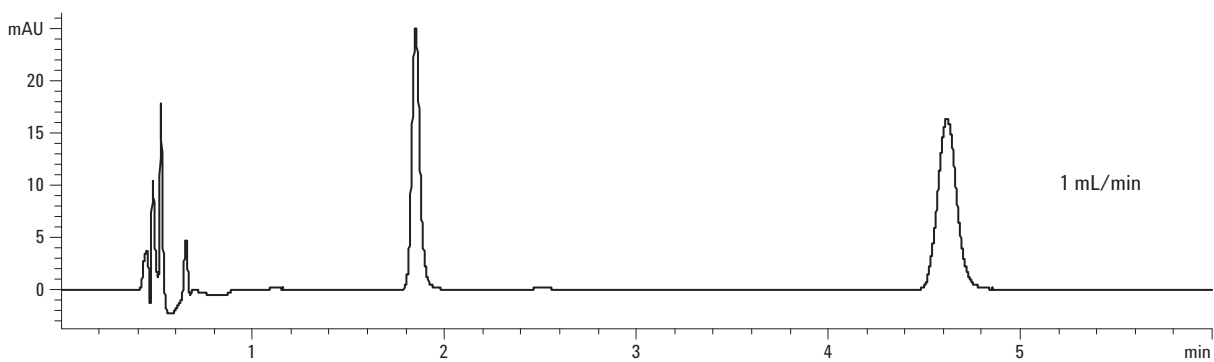


Figure 6-5. Comparison of separation at different flow rates.

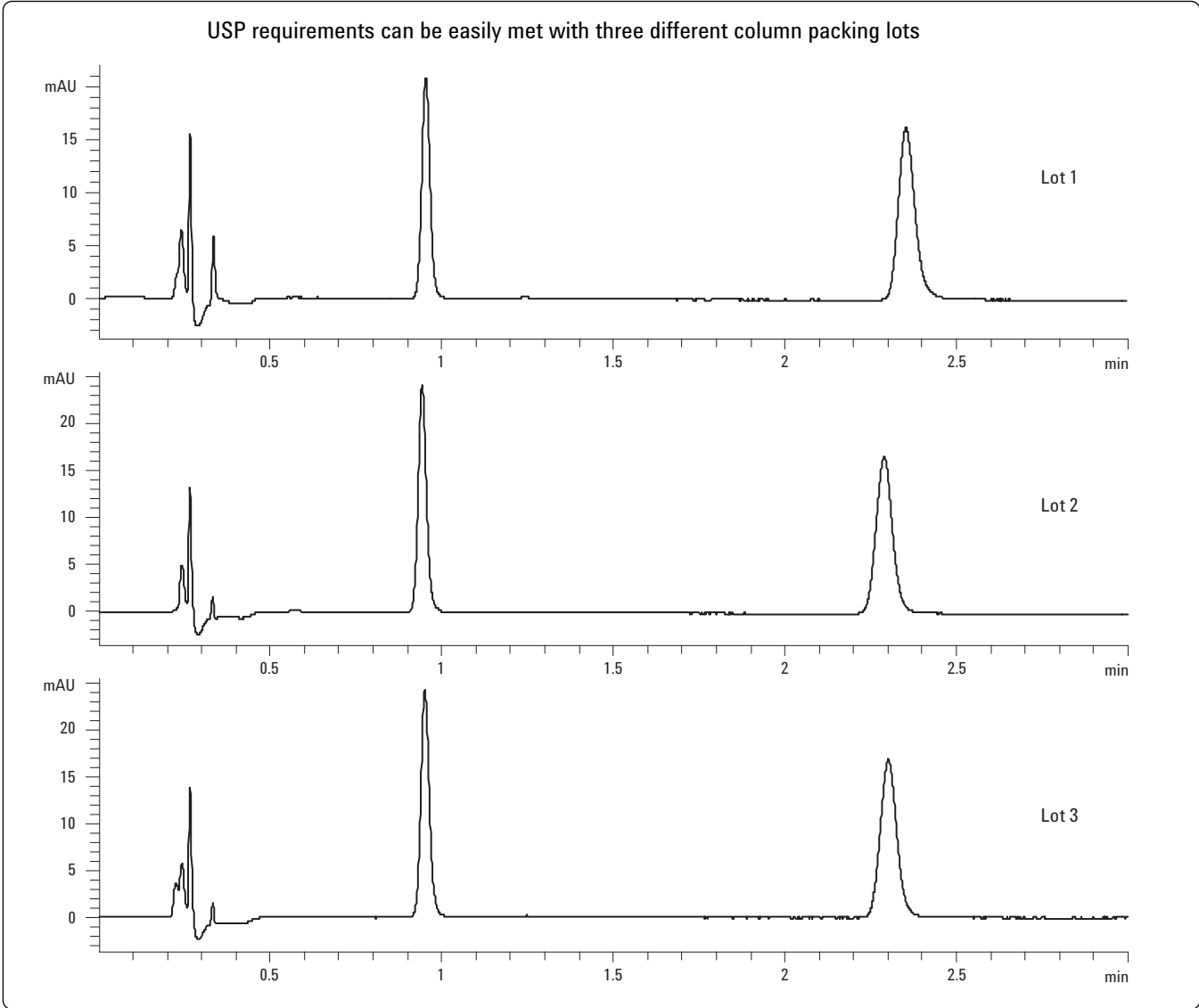


Figure 6-6. Method robustness testing with different column packing lots.

Table 3. Data of System Suitability for Robustness Test on Agilent ZORBAX Rapid Resolution HT Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 μm Column

Parameters for robustness	Variation of parameters	Peak	Theoretical plates	USP tailing factor	Resolution
pH	3.4	1	7475	1.11	22.8
		2	11099	1.10	
	3.6	1	7176	1.12	20.3
		2	11334	1.09	
	3.8	1	7488	1.14	18.4
		2	11622	1.15	
	7%	1	7555	1.02	13.8
%Organic	10%	2	11222	1.18	20.3
		1	7167	1.12	
		2	11334	1.09	
	13%	1	6993	1.21	21.8
		2	10659	1.01	
	Temperature (°C)	25	1	7167	1.12
2			11334	1.09	
30		1	7846	1.17	19.8
		2	11265	1.10	
	35	1	7980	1.19	18.9
		2	11094	1.07	
	Flow rate (mL/min)	1	1	9712	1.17
2			10550	1.09	
2		1	7167	1.12	20.3
		2	11334	1.09	
	3	1	6097	1.10	18.4
		2	10428	1.10	
	Column lot	1	1	7724	1.06
2			11678	1.16	
2		1	7167	1.12	20.3
		2	11334	1.09	
	3	1	7856	1.18	20.9
		2	11408	1.10	
USP regulation		1 and 2	N ≥ 2000	Tf < 2.0	≥ 4

Conclusion

Labs have been looking for more economic ways to accomplish many HPLC analyses. The price of HPLC solvents may remain higher following the recent shortage of acetonitrile. The approaches to reduce solvent use include reducing the column internal id, the column length or particle size. All the changes should follow USP criteria, or method validation may be required.

The Agilent ZORBAX Solvent Saver columns with 3.0 mm id are reasonable for USP methods that started with 4.0 mm id columns, such as the method considered here for the analysis of ceftizoxime sodium. The solvent use is reduced by more than 40 percent and the columns are compatible with almost any LC system.

Short columns with smaller particle size provide fast analysis with solvent savings while giving the same performance. USP methods with 5 μm particle columns can be easily transferred to Agilent ZORBAX Rapid Resolution columns with 3.5 μm particles without method revalidation. Using ZORBAX Rapid Resolution HT columns with 1.8 μm particles will require method revalidation, and shorter length columns like 30 mm and 50 mm can be run on standard HPLC systems while yielding substantial solvent savings.

Reference

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2. U.S. Pharmacopeia 31-NF 26, second supplement, 2008
3. U.S. Pharmacopeia 31-NF 26, Official Monographs: Cefprozil Sodium

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Printed in the USA
August 13, 2009
5990-4494EN



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