Application Note

Pharmaceutical

Modernizing the USP Ceftizoxime Sodium HPLC Method Following the Revised USP <621> Guidelines

Realizing the benefits of smaller particle size columns without revalidation

Author

Rongjie Fu
Agilent Technologies
(Shanghai) Co. Ltd.

Abstract

The original United States Pharmacopeia (USP) Ceftizoxime Sodium HPLC assay was modernized to take advantage of smaller particle columns, including totally porous particle (TPP) and superficially porous particle (SPP) columns, following the newly revised USP <621> guidelines. The updated methods replaced conventional 10 µm TTP columns with 5 and 3.5 µm Agilent ZORBAX Eclipse Plus C18 columns and Agilent InfinityLab Poroshell 120 EC-C18 columns without revalidation. All system suitability requirements were met and significant reductions in both analysis time and solvent consumption were achieved.

Introduction

Because most USP monographs use HPLC methods for quality control, they are routinely used by pharmaceutical manufacturers. These methods mostly employ old column technology such as conventional 5 or 10 µm TPP columns. Due to their low efficiency, longer columns are often required, leading to long analysis times and high solvent consumption. Therefore, there are needs to modernize the existing methods to take advantage of new column technologies, including smaller and superficially porous particle technologies. Also, analysts need to modernize their existing USP methods without making significant changes that would require revalidation. The new version of USP <621> guidelines that became effective in December 2022 allows laboratories to transfer their isocratic and gradient methods from conventional TPP columns to both TPP columns and SPP columns.[1]

In this application note, per the current USP <621> guidelines, a USP ceftizoxime sodium isocratic method[2], which used 4.0 × 300 mm, 5 to 10 µm columns, was adjusted to allow the use of smaller particle size 5 and 3.5 µm ZORBAX Eclipse Plus C18 columns and 4 and 2.7 µm InfinityLab Poroshell 120 EC-C18 columns. In addition, various column particle sizes and dimensions were evaluated.

Experimental

Instruments and materials

An Agilent 1260 Infinity II LC system with 0.12 mm tubing throughout was used to evaluate the columns. The instrument configuration is listed in Table 1.

Table 1. Instrument configuration.

Agilent 1260 Infinity II LC system	
Agilent 1260 Infinity II Binary Pump (G7112B)	4-pos/10-port valve, 600 bar (p/n 5067-4287)
Agilent 1260 Infinity II Multisampler (G7167A)	Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (p/n 5182-0716) Cap, screw, blue, PTFE/red silicone septa, 100/pk (p/n 5182-0717)
Agilent Infinity II Multicolumn Thermostat (MCT)	Standard flow heater (G7116-60015) Heater and column: InfinityLab Quick Connect assembly, 0.12 × 105 mm (p/n 5067-5957)
Agilent 1260 Diode Array Detector (DAD) WR (G7115A)	Standard flow cell 10 mm, 13 µL (p/n G1315-60022) Long-life deuterium lamp (p/n 2140-0820)
Agilent OpenLab CDS, Version 2.8	

All reagents and solvents were HPLC grade. Acetonitrile, methanol, citric acid monohydrate, dibasic sodium phosphate, monobasic potassium phosphate, salicylic acid, and ceftizoxime sodium were purchased from Anpel Laboratory Technologies (Shanghai, China). Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK).

Sample preparation

The internal standard solution and standards were prepared as described in the USP method.[2] The standard solution used for the system suitability analyses contained 0.02 mg/mL ceftizoxime and 0.3 mg/mL of salicylic acid in pH 7.0 buffer.

LC conditions

The LC conditions used for the original and updated methods are provided in Table 2.

Table 2. LC conditions.

	Original USP Method	Adjusted Method
Column	L1, 4 × 300 mm, 5 to 10 µm	Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm (p/n: 959993-902) Agilent ZORBAX Eclipse Plus C18, 3.0 × 150 mm, 3.5 µm (p/n: 959963-302) Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 3.5 µm (p/n: 959793-902) Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 4 µm (p/n: 695970-902) Agilent InfinityLab Poroshell 120 EC-C18, 3 × 75 mm, 2.7 µm (p/n: 697975-302) Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 50 mm, 4 µm (p/n: 699970-902) Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm (p/n: 699775-902)
Mobile Phase	A mixture of pH 3.6 buffer (1.42 g of citric acid monohydrate and 1.73 g of dibasic sodium phosphate were dissolved in water to obtain 1,000 mL of solution) and acetonitrile (9:1)	
Flow Rate	2 mL/min	The adjusted volumes are shown in Table 3
Temperature	30 °C	
Injection Volume	10 µL	The adjusted volumes are shown in Table 3
Detection	DAD signal 254 nm, ref off 20 Hz	DAD signal 254 nm, ref off 80 Hz

Results and discussion

The original method used an isocratic HPLC separation, and per the revised USP <621> guidelines, there are two options to modernize it. The first is to adjust the method to use smaller particle TPP columns following the steps described in Case Study 1 of the Agilent white paper "Understanding the Latest Revisions to USP <621>."[3] To update the method, different length and particle size columns can be used if the ratio of column length to particle diameter (L/dp) remains constant or in the range between −25 to +50% of the prescribed ratio.

In this application note, the author chose to evaluate the ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm; ZORBAX Eclipse Plus C18, 3.0 × 150 mm, 3.5 µm; and ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 3.5 µm columns. The L/dp ratios of these three columns are in the allowable range (Table 3). The flow rate was adjusted because smaller-particle columns require higher linear velocities to obtain the same performance. The particle size was changed, and flow rate was adjusted for both the change in column diameter and particle size using Equation 1.

Equation 1.

$$F_2 = F_1 \times [(dp_1 \times dc_2^2) / (dp_2 \times dc_1^2)]$$

- F_1 = flow rate specified in the USP monograph (mL/min)
- F_2 = adjusted flow rate (mL/min)
- dc_1 = internal diameter (id) of the column specified in the USP monograph (mm)
- dc_2 = id of the column used (mm)
- dp_1 = particle size specified in the USP monograph (µm)
- dp_2 = particle size of the column used (µm)

After adjusting for the change in column dimensions, an additional flow rate change of ±50% is permitted. The actual flow rate used in this study was the lowest value within the range due to column and instrument pressure limits. The greatest benefits of method modernization are obtained when using sub-2 µm particle size columns. However, the flow rate calculated using Equation 1 is high and exceeds the column and instrument pressure limits. For example, when the method is adjusted from a 10 to 1.8 µm particle size for a 4.6 mm id column, the calculated flow rate range is 7.3 to 22 mL/min, which is not possible using a conventional LC system. The acceptable flow rate range is 3.1 to 9.4 mL/min for 3.0 mm id columns and 1.5 to 4.6 mL/min for 2.1 mm id columns. Even when applying the lowest permitted flow rate, the pressure exceeds LC system limits. For this reason, the author recommends using a 3.5 µm particle size with smaller 3.0 or 2.1 id columns to shorten analysis times. In addition, considering the pressure limit of the column and instrument and the desire to reduce solvent consumption, the minimum flow rate of the permissible range should be used. In sum, by using 3.5 µm instead of sub-2 µm particle columns, the method can run on conventional UHPLC systems which are not rated for the extreme pressures generated by sub-2 µm particle columns.

The injection volume was adjusted based on Equation 2.

Equation 2.

$$V_2 = V_1 \times [(L_2 \times dc_2^2) / (L_1 \times dc_1^2)]$$

- V_1 = injection volume specified in the USP monograph (µL)
- V_2 = adjusted injection volume (µL)
- L_1 = column length specified in the USP monograph (cm)
- L_2 = new column length (cm)
- dc_1 = column id specified in the USP monograph (mm)
- dc_2 = new column id (mm)

Five replicates of standard solution were analyzed on each column using the flow rates, L/dps, and injection volumes listed in Table 3. Per the USP monograph, the relative standard deviations (RSDs) cannot exceed 2.0%. The RSDs obtained for all three columns were less than 2.0% (Table 3). The changes made to the method here were acceptable and did not require revalidation. Additionally, the system suitability criteria were met (Table 3). The chromatograms obtained using the three different dimension ZORBAX Eclipse Plus C18 columns are shown in Figure 1.

Table 3. Results achieved using the different TPP columns evaluated.

Column Dimension	Flow Rate (mL/min)		L/dp	Injection Volume (µL)	System Suitability Requirements						
					Plate Number (N)		Tailing Factor (Tf)		Resolution Between Peak 1 and 2 (R)	%RSD (n = 5) of Area	
					Peak 1	Peak 2	Peak 1	Peak 2		Peak 1	Peak 2
L1, 4 × 300 mm, 5 to 10 µm	2.0		30,000*	10	–	–	–	–	–	–	–
	Allowable range	Actual applied flow rate	22,500 to 45,000 (–25% to +50%)	Proportional to column dimension	≥ 2,000	≥ 2,000	≤ 2.0	≤ 2.0	≥ 4.0	≤ 2.0	≤ 2.0
Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm	2.6 to 7.9	2.6	30,000	7	4,463	7,830	1.01	1.09	14.3	0.07	0.12
Agilent ZORBAX Eclipse Plus C18, 3.0 × 150 mm, 3.5 µm	1.6 to 4.8	1.6	42,900	3	7,822	12,256	1.06	1.08	16.3	0.46	0.21
Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 3.5 µm	0.8 to 2.4	0.8	28,600	1	3,551	6,545	1.12	1.23	11.5	0.52	0.86

* Assumes the original column used for the analysis is 10 µm.

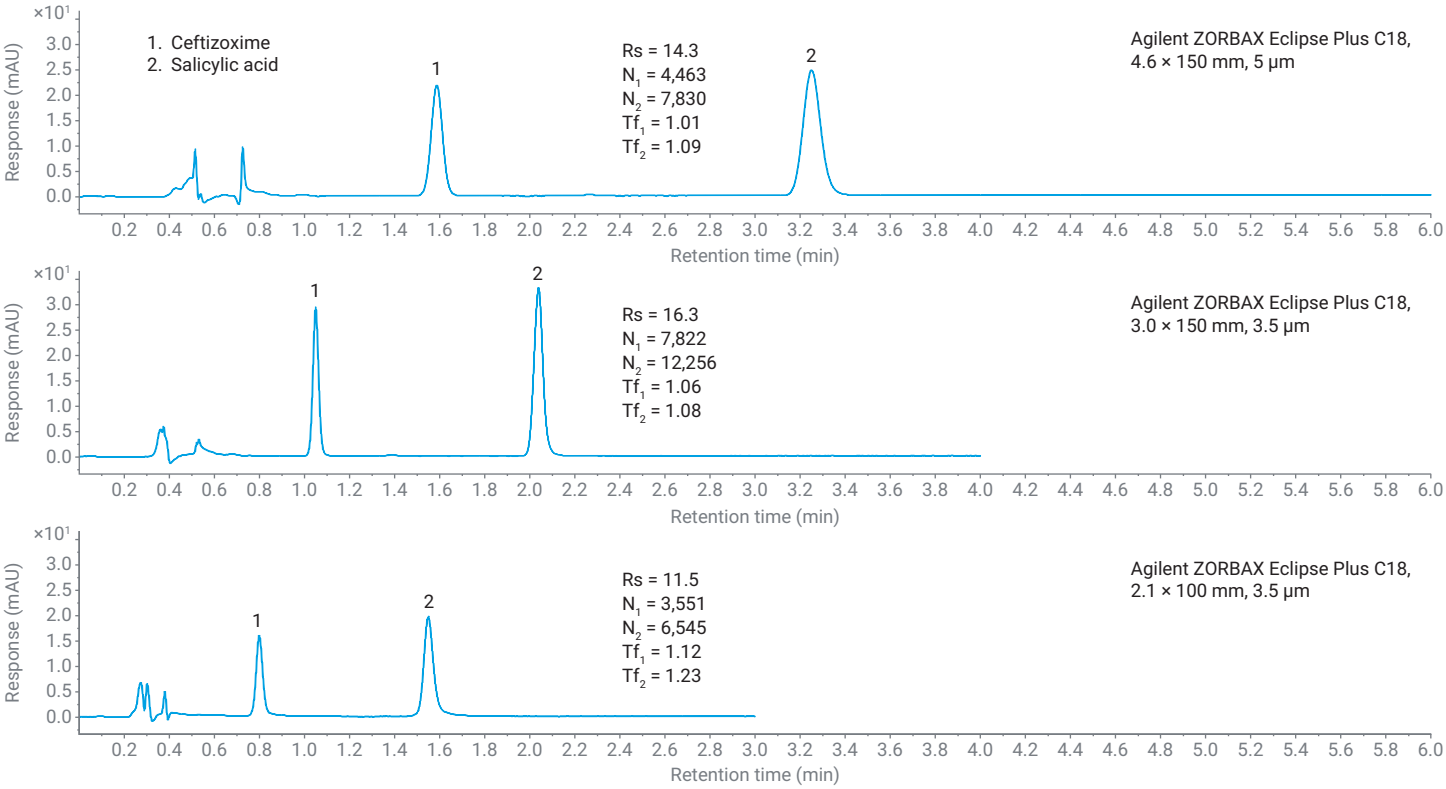

Figure 1. Chromatograms obtained from analysis of the ceftizoxime sodium system suitability solution for the various length and particle size TPP columns.

The second modernization option for an isocratic method is to update it for smaller particle SPP columns following the steps described in Case Study 2 of the Agilent white paper "Understanding the Latest Revisions to USP <621>."[3] The steps used to adjust the method to SPP columns are the same as those used for TPP columns except that different combinations of L and dp (column length and particle size) are applied. The plate number (N) of the SPP column must be within −25 to 50% of that of the original column.

Because they have the same L/dp ratio (30,000), the author assumed that the N achieved using the ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm is the same as that of the original column. The allowable range of N values is within −25 to 50% (−20 to 50% for < 3 µm column) of that achieved using the ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm column. The combination of column length and particle size should be chosen from the range of N values provided in Table 4. Similar to the method adjustment for TPP columns, sub-2 µm particle SPP columns were not selected because the column and instrument pressure limits were exceeded in the allowable flow rate range. Here, 4 and 2.7 µm SPP columns of appropriate lengths were selected for the method modernization study (Table 4). The N values achieved using the InfinityLab Poroshell 120 EC-C18, 4.6 × 50 mm, 4 µm column were out of the required range, so it is not recommended for method modernization. The other column lengths and particle sizes evaluated met the allowable range of N values as well as the system suitability requirements (Table 4). The chromatograms obtained from the analysis of the system suitability solution for the various length and particle size SPP columns are shown in Figure 2.

A longer column with smaller particles, such as the InfinityLab Poroshell 120, 3 × 75 mm column, overpressurized the analytical system when operated at the minimum allowable flow rate range. In this case, the column temperature was adjusted to 40 °C to ensure that the pressure was less than the maximum pressure limit of column and instrument. Per USP <621> guidelines, this temperature adjustment is allowable for isocratic methods.

Table 4. Results achieved using the different SPP columns evaluated.

Column Dimension	Flow Rate (mL/min)		Injection Volume (µL)	System Suitability Requirements						
				Plate Number (N)		Tailing Factor (Tf)		Resolution Between Peak 1 and 2 (R)	%RSD (n = 5) of Area	
				Peak 1	Peak 2	Peak 1	Peak 2		Peak 1	Peak 2
L1, 4 × 300 mm, 5 to 10 µm	2.0		10	4,463*	7,830*	–	–	–	–	–
	Allowable range	Actual applied flow rate	Proportional to column volume	≥ 2,000 (3,347 to 6,695 for particle size ≥ 3 µm 3,570 to 6,695 for particle size < 3 µm)	≥ 2,000 (5,872 to 11,745 for particle size ≥ 3 µm 6,264 to 11,745 for particle size < 3 µm)	≤ 2.0	≤ 2.0	≥ 4.0	≤ 2.0	≤ 2.0
Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 4 µm	3.3 to 9.9	3.3	4	5,310	9,306	1.15	1.34	14.3	0.75	0.47
Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 50 mm, 4 µm	3.3 to 9.9	3.3	2	3,597	5,038 (not meets)	1.07	1.08	11.1	0.69	0.82
Agilent InfinityLab Poroshell 120 EC-C18, 3 × 75 mm, 2.7 µm	2.1 to 6.3	2.1**	1.4	4,950	8,455	1.19	1.50	11.0	0.46	0.39
Agilent InfinityLab Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm	2.1 to 6.3	2.1	1.0	3,608	6,166	1.04	1.03	10.9	0.69	1.50

* Assumes that the plate number of the original column is also achieved using the ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm column, which has the same L/dp ratio as the 300 mm, 10 µm column.

** This was run under 40 °C to make sure the pressure was below 600 bar.

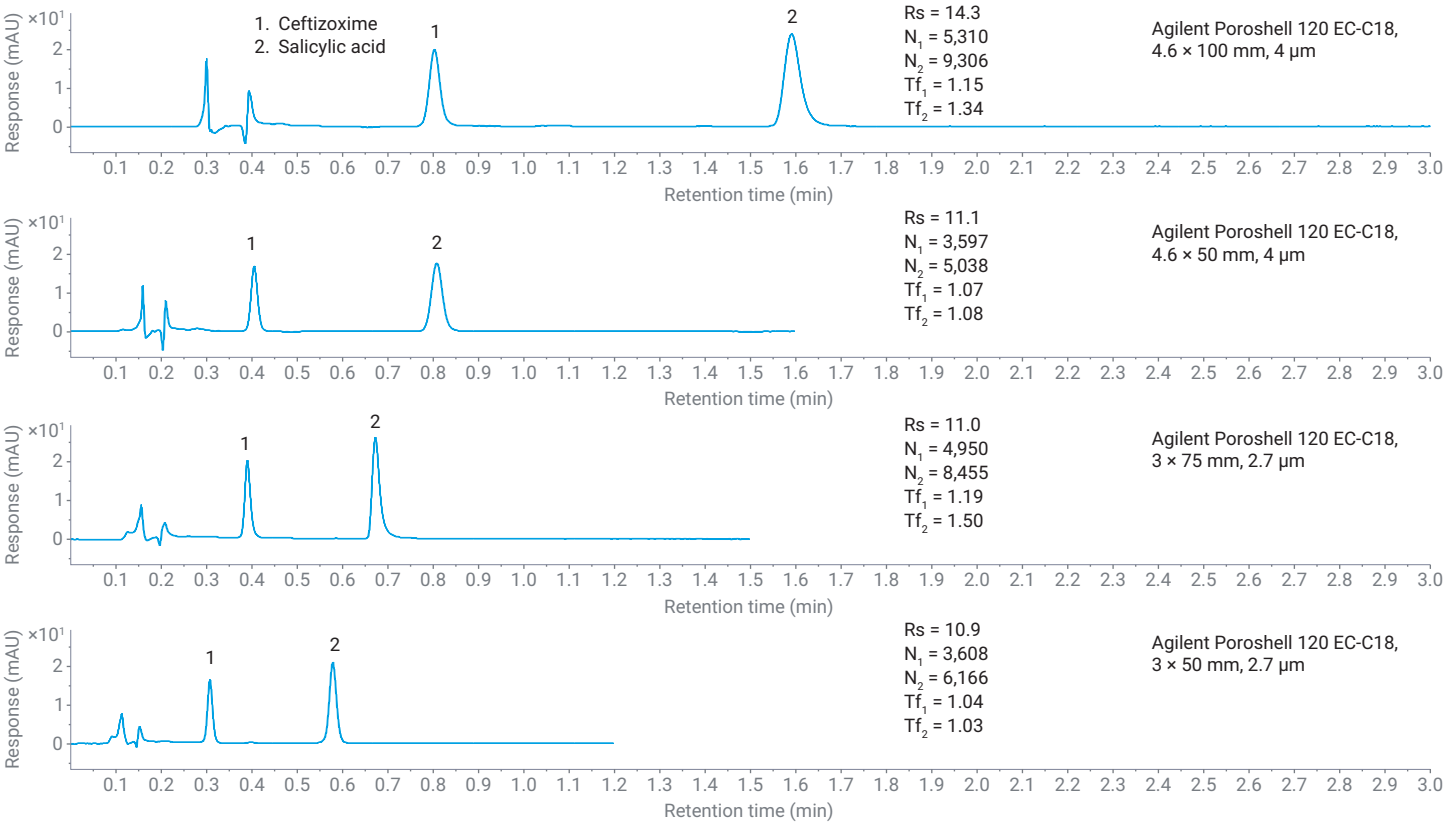

Figure 2. Chromatograms obtained from analysis of the ceftizoxime sodium system suitability solution for the various length and particle size SPP columns.

During method adjustment from the original column to the smaller particle size or smaller internal diameter column, the LC system may require additional modifications to minimize extracolumn band broadening due to factors such as instrument connections, detector cell volume and sampling rate, and injection volume. For this application note, an optimized Infinity 1260 system containing 0.12 mm id tubing throughout was used. A smaller flow cell can be used for 3.0 and 2.1 mm id columns. To achieve even better performance, a UHPLC system with low extracolumn volume such as the Agilent 1290 Infinity II System can be used with extremely small id columns, including 2.1 × 50 mm, 2.7 µm columns.

Compared to the original methods, overall method run time and solvent consumption were reduced when using the smaller particle columns evaluated here. When using narrower id columns, such as 3.0 mm id columns, additional solvent is saved due to a reduced flow rate. In this case an InfinityLab Poroshell 120 EC-C18, 3.0 × 100 mm, 4 µm column can be used to replace the 4.6 mm id column to save an additional 24% solvent. A detailed comparison of the time and mobile phase savings is shown in Table 5.

Assay modernization can save substantial analysis time and solvent. Both the ZORBAX Eclipse Plus C18 and InfinityLab Poroshell 120 EC-C18 columns are good platforms for method transfer, as these families of columns include a wide range of particles sizes and column dimensions suitable for HPLC and UHPLC analyses. The scalability of particle sizes allows for modernization of older USP monograph methods quickly, easily, and with minimal rework.

Table 5. Comparison of the analysis time and mobile phase consumption for the original and modernized methods.

Method	Column Dimension	Flow Rate (mL/min)	Analysis Time/Injection (min)	Mobile Phase Consumption/Injection (mL)	Solvent Saved (%)	Analysis Time Saved (%)
Original Method	L1, 4 × 300 mm, 10 µm	2.0	13*	26	–	–
Modernized Methods	Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm	2.6	6.5	16.9	35	50
	Agilent ZORBAX Eclipse Plus C18, 3.0 × 150 mm, 3.5 µm	1.6	4.2	6.7	74	68
	Agilent ZORBAX Eclipse Plus C18, 2.1 × 100 mm, 3.5 µm	0.8	3.1	2.5	90	76
	Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 4 µm	3.3	3.2	10.6	59	75
	Agilent InfinityLab Poroshell 120 EC-C18, 3 × 75 mm, 2.7 µm	2.1	1.3**	2.7	90	90
	Agilent InfinityLab Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm	2.1	1.1	2.3	91	92

* Assumes the retention factor of the original column is the same as that of the ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm column.
** The method was run under 40 °C due to column and instrument pressure limits.

Conclusion

USP monograph methods that use older column technology can be modernized to newer column technology by following the guidelines provided in USP general chapter <621>. Use of newer column technology, including smaller particle size and SPP columns, can provide similar results while reducing analysis times and mobile phase consumption.

This application note demonstrated the adaptation of an isocratic HPLC method for the USP Ceftizoxime Sodium assay from older to more modern column technology. Specifically, a method that used a conventional 4.6 × 300 mm, 10 µm column was modernized to take advantage of Agilent ZORBAX Eclipse Plus and Agilent InfinityLab Poroshell 120 columns of various particle sizes and dimensions, without the need for revalidation. The revised methods met system suitability requirements and provided reductions in both analysis time and solvent consumption.

References

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3. Fu, R.; Grover, M.; Freeman, R.; and Long, W. Understanding the Latest Revisions to USP <621>. *Agilent Technologies white paper*, publication number 5994-6618EN, **2023**.

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