Application Note

Small Molecule Pharmaceuticals and Generics

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USP Assay Method Transfer for Cephradine from a Traditional 5 µm Column to Agilent InfinityLab Poroshell 120 Columns

Authors

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Abstract

A United States Pharmacopeia (USP) assay method for cephradine, originally run on a conventional 4.6 × 250 mm, 5 µm column, was successfully transferred to 2.7 µm Agilent InfinityLab Poroshell 120 EC-C18 columns under the revised USP General Chapter <621> guidelines. The modernized methods met all USP system suitability requirements without revalidation. Compared to the original method, the InfinityLab Poroshell 120 EC-C18 columns delivered equivalent performance with up to 80 to 86% reduction in analysis time and 62 to 75% savings in solvent consumption, depending on the column format and flow rate. These results highlight the efficiency and sustainability benefits of InfinityLab Poroshell 120 columns for modernizing compendial methods.

Introduction

Because most USP monographs use HPLC methods for quality control, they are routinely used by pharmaceutical manufacturers. These methods mostly use old column technology such as conventional 5 or 10 µm totally porous particle (TPP) columns. Due to their low efficiency, longer columns are often required, leading to long analysis times and high solvent consumption. Therefore, it is necessary to modernize existing methods to take advantage of new column technologies, including smaller and superficially porous particle (SPP) technologies. Also, analysts must modernize their existing USP methods without making significant changes that would require revalidation. The new version of USP <621>, effective 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 cephradine[2] isocratic method requiring a 4.6 × 250 mm, 5 µm column was adjusted to 2.7 µm SPP Agilent InfinityLab Poroshell 120 EC-C18 columns. In addition, two different combinations of particle size and column length 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 Quaternary Pump (G7111B)	N/A
Agilent 1260 Infinity II Vial Sampler (G7129A)	– 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) – InfinityLab Quick Change inline filter assembly for UHPLC (p/n 5067-1603) with InfinityLab Quick Change filter disc, 4.6 mm id, 0.2 µm (p/n 5067-1612)
Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)	– 4-pos/10-port valve 600 bar (p/n 5067-4287) – Standard flow heater (G7116-60015) – Heater and column: InfinityLab Quick Connect assembly, 105 mm, 0.12 mm (p/n 5067-5957)
Agilent 1260 Infinity II Diode Array Detector 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	N/A

All reagents and solvents were HPLC grade. Methanol, sodium acetate, and acetic acid were purchased from ANPEL Laboratory Technologies (Shanghai, China). Water was purified using an ELGA LabWater PURELAB Chorus system (High Wycombe, UK). Cephradine and cephalexin standards were purchased from USP.

Sample preparation

The resolution solution was prepared as described in the USP assay method for cephradine. The solution used for the system suitability analyses contained 0.5 mg/mL of cephradine and 0.5 mg/mL of cephalexin in mobile phase.

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	Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm (p/n 959990-902)	– Agilent InfinityLab Poroshell 120 EC-C18 HPLC column, 4.6 × 100 mm, 2.7 µm (p/n 695975-902) – Agilent InfinityLab Poroshell 120 EC-C18 HPLC column, 3 × 100 mm, 2.7 µm (p/n 695575-302) – Agilent InfinityLab Poroshell 120 EC-C18 HPLC column, 4.6 × 75 mm, 2.7 µm (p/n 697975-902)
Mobile Phase	A mixture of water, methanol, 0.5 M sodium acetate, and 0.7 N acetic acid (782:200:15:3)	
Flow Rate	1 mL/min	The adjusted flow rates 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 2.5 Hz	DAD signal 254 nm, ref off 40 Hz

Results and discussion

The original method used an isocratic HPLC separation. Per the revised USP <621> guidelines, there are two options to modernize it. The first is to adjust the method using smaller particle TPP columns, based on the requirement described in USP <621>: "The particle size and/or length of the column may be modified, provided that the ratio of the column length (L) to the particle size (dp) remains constant or in the range between −25 to +50% of the prescribed L/dp ratio."[1] The second is to adjust the method using SPP columns. According to USP <621>: "For the application of particle-size adjustment from totally porous to superficially porous particles, other combinations of L and dp can be used, provided that the plate number (N) is within −25 to +50%, relative to the prescribed column."[1]

In this application note, the author demonstrated the method with a 4.6 × 250 mm, 5 µm Agilent ZORBAX Eclipse Plus C18 column. Then, the method was adjusted using InfinityLab Poroshell 120 EC-C18 columns. The allowable range of N values is between −20 and 50% (an additional requirement was stated in USP <621> for adjusted to < 3 µm columns) of that achieved using the ZORBAX Eclipse Plus C18 column. The combination of column length and particle size should be chosen from the range of N values provided in 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 the flow rate was adjusted for both the change in column diameter and particle size using the equation:

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

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

Based on USP <621> for isocratic flow rate adjustment, an additional flow rate change of ±50% is permitted. The actual flow rate was adjusted in this study to fit the pressure limit of the column and LC system.

The injection volume was adjusted based on the equation:

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

- 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 internal diameter specified in the USP monograph (mm)
- dc_2 = new column internal diameter (mm)

System suitability data including N, symmetry factor (As), resolution (R), and relative standard deviation (RSD) of peak areas for six replicates of standard solution were recorded on each column and summarized in Table 3. The original method, using a ZORBAX Eclipse Plus C18 column, and the modernized methods, using InfinityLab Poroshell 120 EC-C18 columns, meet all system suitability requirements for USP monograph cephradine analysis. Therefore, no additional method validation is required. The chromatograms obtained using the ZORBAX Eclipse Plus C18 and InfinityLab Poroshell 120 EC-C18 are shown in Figure 1.

Various flow rates were compared using the InfinityLab Poroshell 120 columns, and the chromatograms are shown in Figures and 3.

Table 3. Results achieved with the evaluated SPP columns.

Column Dimension	Flow Rate (mL/min)		Injection Volume (µL)	System Suitability Requirements						
				Plates Number (N)		Symmetry factor (As)		Resolution Between Peak 1 and 2 (R)	RSD (n = 6) (%) of Area	
				Peak 1	Peak 2	Peak1	Peak 2		Peak 1	Peak 2
Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm (p/n 959990-902)	Allowable range	Actual applied flow rate	Proportional to column volume	9,794–18,363 for particle size < 3 µm)	10,369–19,442 for particle size < 3 µm)	0.8 ≤ 1.8		≥ 2.0	≤ 2.0	≤ 2.0
	0.5–1.5	1.0	10	12,242	12,961	0.86	0.84	10.9	0.15	0.17
Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-902)	0.9–2.8	1.9	4	13,137	13,951	0.88	0.85	11.0	0.09	0.09
		1.4		15,005	15,775	0.86	0.83	11.7	0.06	0.07
		1.0		16,817	17,344	0.84	0.81	12.3	0.13	0.32
Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 µm (p/n 697975-902)	0.9–2.8	1.9	3	9,967	10,381	0.88	0.85	9.4	0.46	0.10
		1.4		11,070	11,423	0.86	0.83	9.8		0.32
		1.0		12,391	12,731	0.85	0.82	10.3		0.29
Agilent InfinityLab Poroshell 120 EC-C18, 3 × 100 mm, 2.7 µm (p/n 695575-302)	0.4–1.2	0.8	1.7	10,507	11,490	0.86	0.82	9.8		0.13
		0.6		11,223	12,398	0.85	0.80	10.2		0.17
		0.4		12,418	13,198	0.84	0.79	10.6		0.10

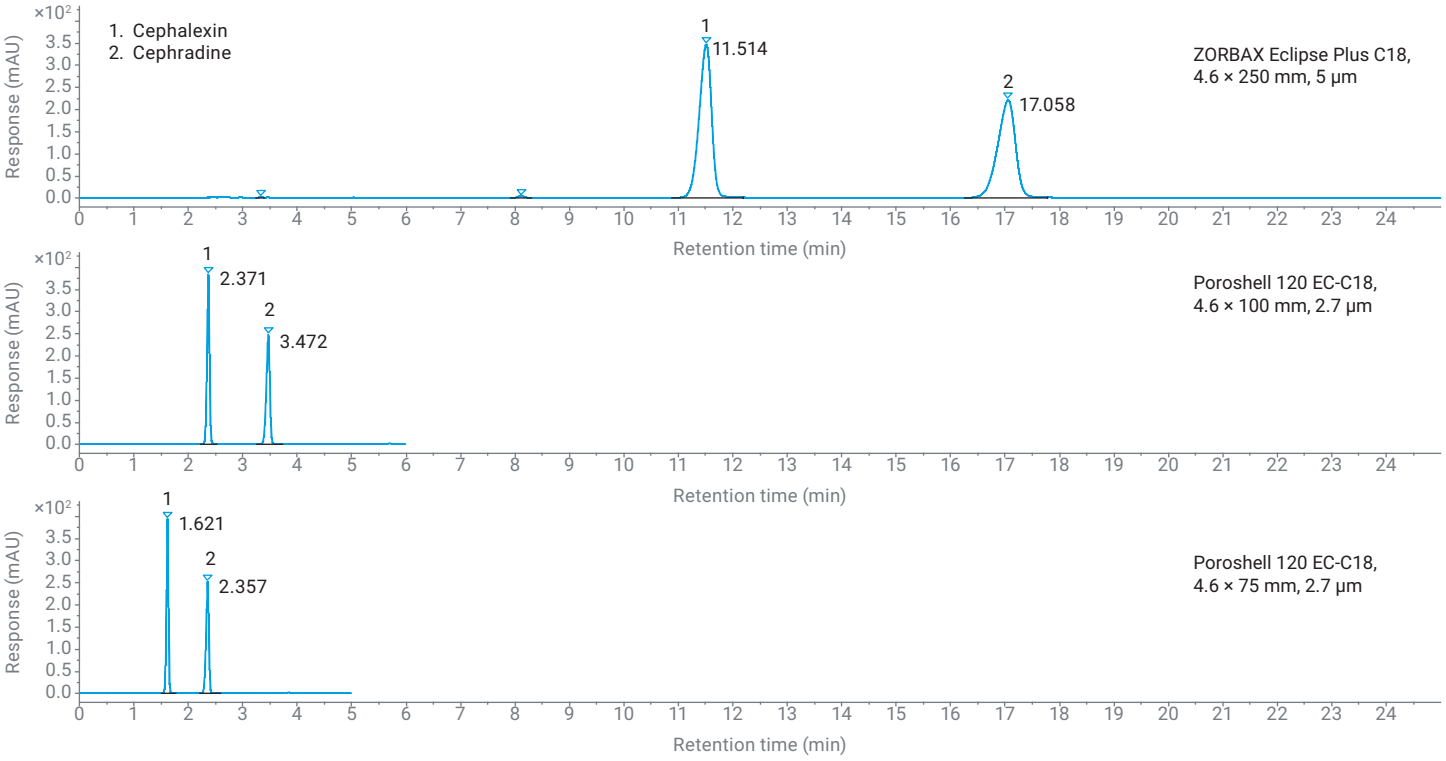

Figure 1. Chromatograms obtained using a conventional 4.6 × 250 mm, 5 µm Agilent ZORBAX Eclipse Plus C18 column compared to those obtained using Agilent InfinityLab Poroshell 120 EC-C18 columns.

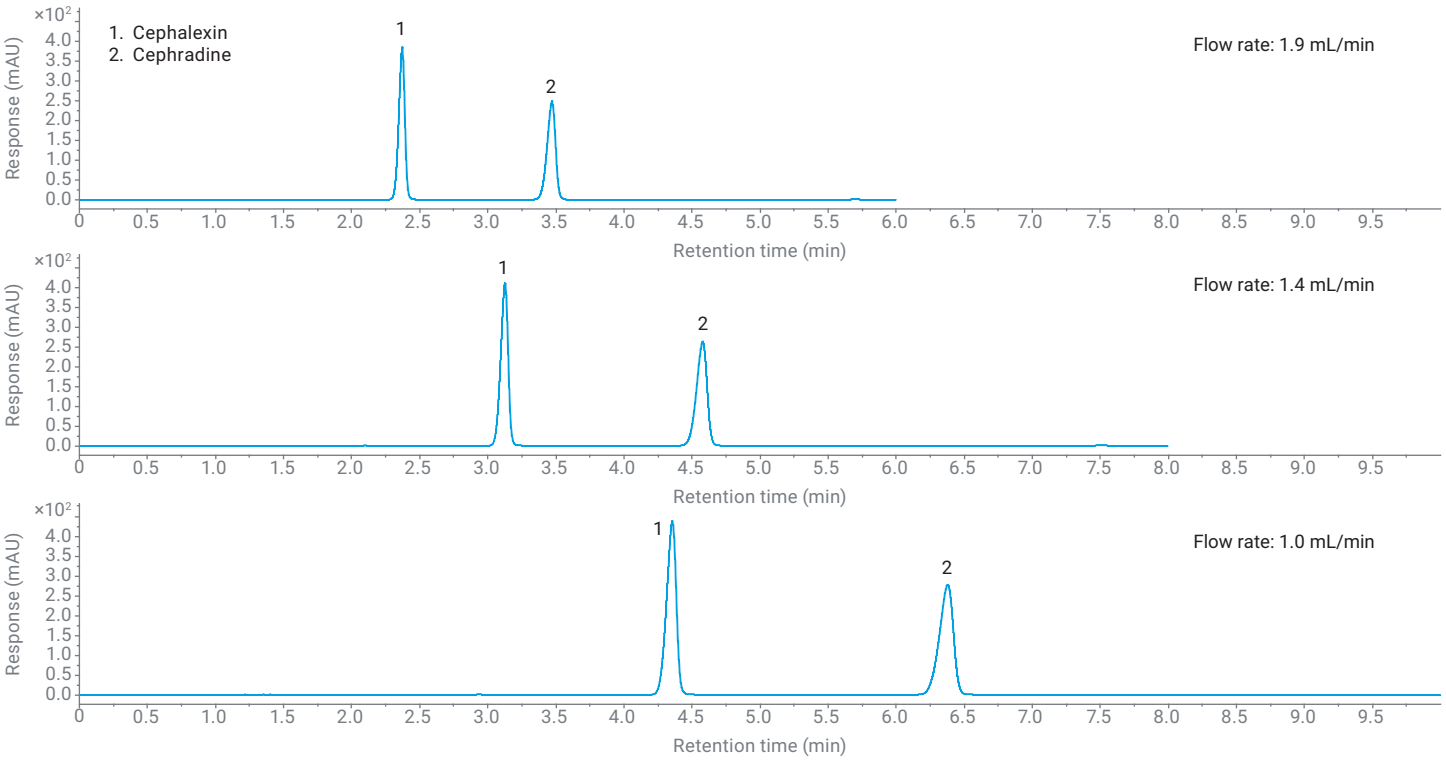

Figure 2. Chromatograms obtained at various flow rates using the 4.6 × 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 EC-C18 column.

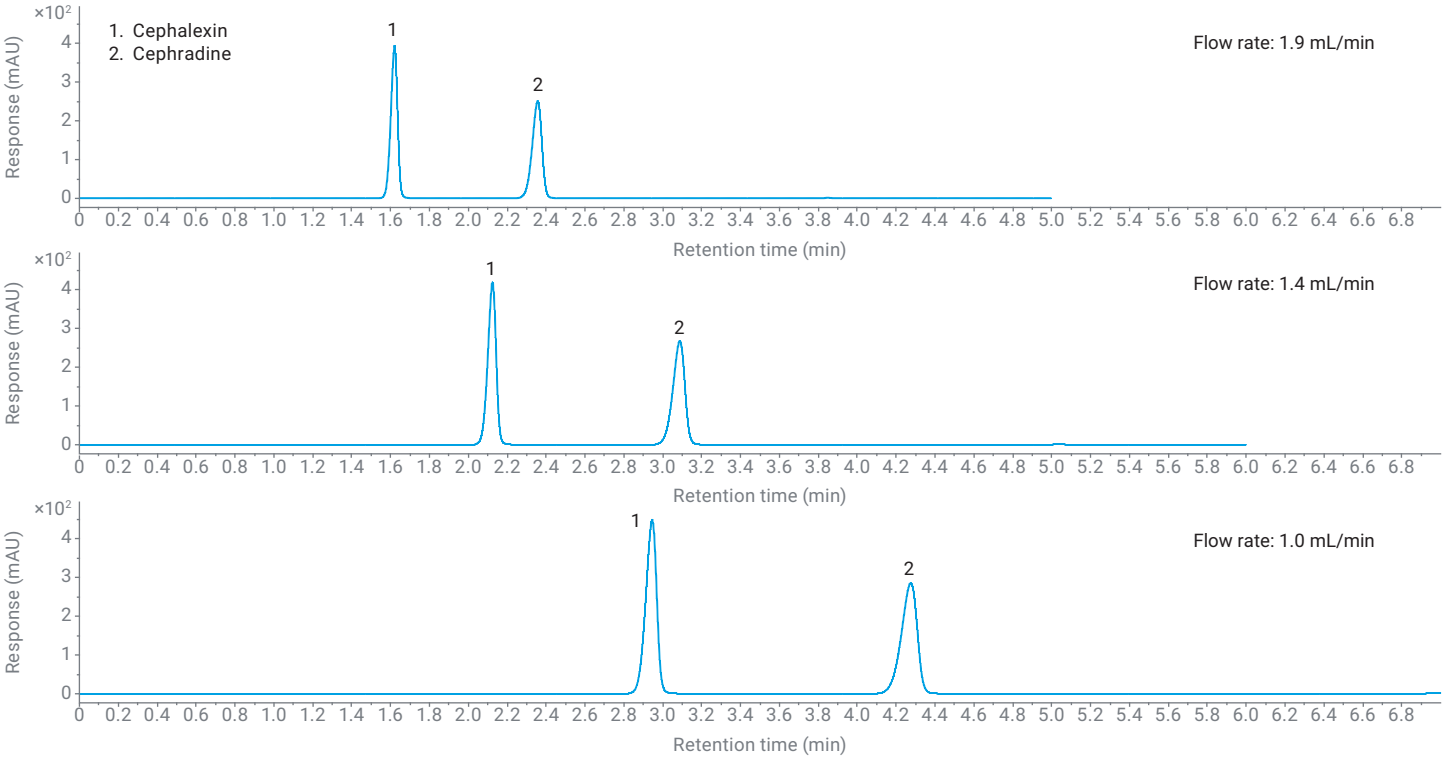

Figure 3. Chromatograms obtained at various flow rates using the 4.6 × 75 mm, 2.7 µm Agilent InfinityLab Poroshell 120 EC-C18 column.

Compared to the original method, both overall method run time and solvent consumption were reduced when using the smaller-particle InfinityLab Poroshell 120 columns evaluated here. A detailed comparison of the time and mobile phase savings is shown in Table 4 and Figure 4.

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

Column	Flow Rate (mL/min)	Time Saved (%)	Solvent Saved (%)
Agilent ZORBAX Eclipse Plus C18 Column, 4.6 × 250 mm, 5 µm	1	–	–
Agilent InfinityLab Poroshell 120 EC-C18 Column, 4.6 × 100 mm, 2.7 µm	1.0–1.9	62–80	62
Agilent InfinityLab Poroshell 120 EC-C18 Column, 4.6 × 75 mm, 2.7 µm	1.0–1.9	75–86	75

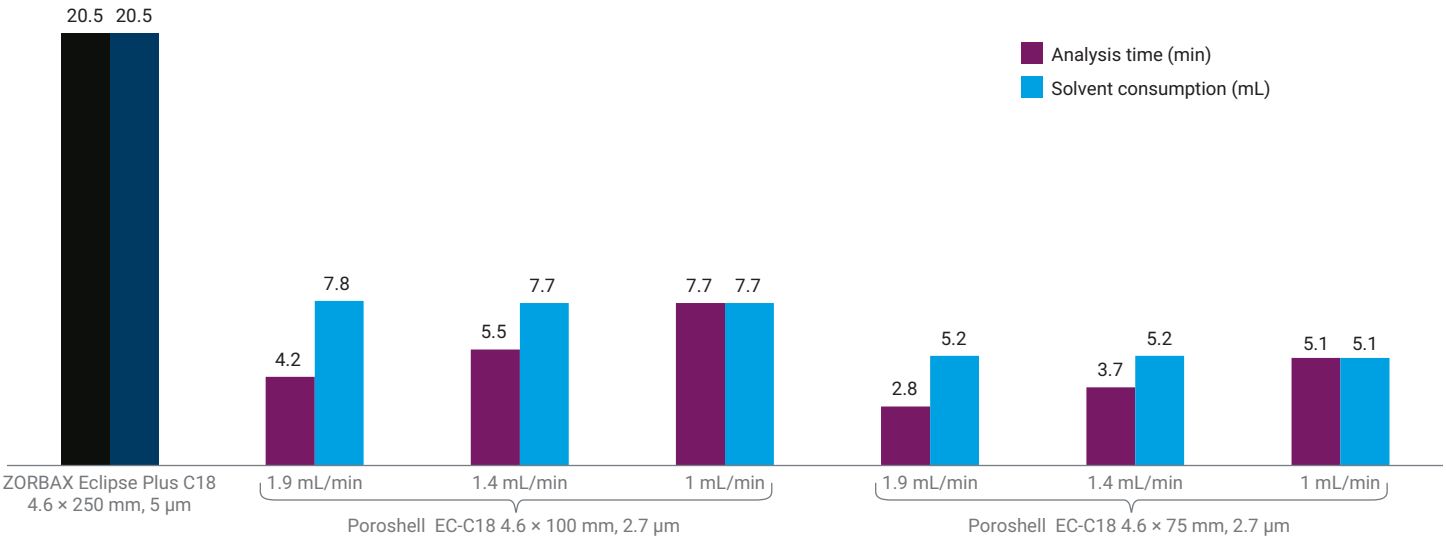

Figure 4. Chart displaying comparison of analysis time and solvent consumption.

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 like SPP columns can provide similar results while significantly reducing analysis times and mobile phase consumption.

This application note demonstrated the adaptation of an isocratic HPLC method for the USP cephradine assay, transitioning from older to more modern column technology. Specifically, a method that used a conventional 4.6 × 250 mm, 5 µm column was modernized to take advantage of 2.7 µm Agilent InfinityLab Poroshell 120 EC-C18 columns of various dimensions, without need for revalidation. The revised methods met system suitability requirements and provided reductions in both analysis time and solvent consumption.

References

1. USP Harmonized Standards Home Page. Supplement USP Stage 4 Harmonization, Official, December 1, 2022.
2. USP Monographs: Cephradine, United States Pharmacopeia, 2024.

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