Application Note

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Working Within Allowable Changes to the U.S. Pharmacopoeia Naproxen Sodium Tablet Method

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Abstract

The USP method for naproxen is demonstrated using Agilent ZORBAX RR Eclipse Plus C8 and InfinityLab Poroshell 120 EC-C8 columns. When InfinityLab Poroshell 120 EC-C8 columns (4.6 × 75 mm and 4.6 × 50 mm, 2.7 µm) as well as ZORBAX RR Eclipse Plus C8 columns (4.6 × 100 and 4.6 × 75 mm, 3.5 µm) are used, analysis time is reduced to 22% of the original method time without need for revalidation using the InfinityLab Poroshell 120 EC-C8, 4.6 × 50 mm column.

Introduction

Pharmaceutical companies routinely adopt U.S. Pharmacopeia (USP) compendial methods for testing raw materials and finished products. Successful implementation of the USP methods and transferability between instruments are key steps to enhance throughput for routine analysis. Effective method transfer generates identical results for the same analysis independent of the laboratory, instrument, and the resources for a specific method. By ensuring successful lab-to-lab method transferability, companies can replicate methods at additional sites or with partners such as contract research or manufacturing organizations (CROs and CMOs). Transferring an HPLC-based USP method to UHPLC technology offers such organizations the additional opportunity to achieve productivity goals by reducing analysis time while ensuring reliable, high-quality chromatographic separations that are the basis for decisions about product quality. UHPLC technology offers QC and manufacturing facilities significant advantages in terms of increased throughput, improved quality, and reduced costs.

The costs associated with pharmaceutical testing can be reduced using adjustments to chromatography allowed under the general chapters in USP 621. These costs can be counted as chromatographic solvent and time. Of these two considerations, time is the most important.

The method for the analysis of naproxen impurities and naproxen tablets was updated as additional standards became available from the USP. In addition, the assay method and the impurities method can be run on the same column. In this application note, the method published in the USP is adjusted within allowable limits to increase sample throughput using superficially porous particle columns.

The costs associated with pharmaceutical testing are considerable and many prudent lab managers are seeking ways to reduce costs by reducing solvent use and improving productivity, while still using the LC instruments in their lab. Compendial methods from the USP are widely used in drug product and raw material testing. While efforts have been made to modernize these methods, they can be improved by taking advantage of newer technologies.

Naproxen is classified as a nonsteroidal anti-inflammatory drug (NSAID), and is available as generic tablets. It was patented in 1967, and while it remains a prescription-only drug in much of the world, the Food and Drug Administration (FDA) approved it as an over-the-counter (OTC) drug in 1994 in the United States. The USP tablet assay method previously used a 5 µm C18 or L1 column but has recently been revised to use a 5 µm C8 or L7 column with the same mobile phase.

The structure of naproxen sodium is shown in Figure 1. Its IUPAC name is (*S*)-6-methoxy-α-methyl-2-naphthaleneacetic acid sodium salt.

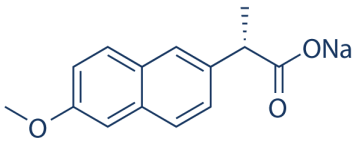

Figure 1. Structure of naproxen sodium.

InfinityLab Poroshell 120 columns are an LC column choice that can provide improved performance on a typical LC instrument. These columns have a 2.7 µm superficially porous particle that can provide faster analysis and high resolution in shorter columns for testing more samples in less time on existing instruments. The columns are available in many phases including L1 (C18), L7 (C8), L11 (Phenyl), L10 (Cyano), as well as many others. The work in this application note used the L7 phase (InfinityLab Poroshell 120 EC-C8).

Experimental

An Agilent 1260 Infinity II LC was configured using 0.17 mm tubing throughout for this work. Table 1 shows the details.

Fresh, glacial acetic acid used was ACS/USP grade, purchased from VWR. Acetonitrile was purchased from Honeywell (Burdick and Jackson

Table 1. Instrument configuration.

Agilent 1260 Infinity II LC	
Agilent 1260 Infinity II binary pump (G7117B)	
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 1260 Infinity II multicolumn thermostat (MCT; G7116A)	• Standard flow heater G7116-60015 • Heater and column: Agilent InfinityLab Quick Connect assembly, 105 mm, 0.12 mm (p/n 5067-5961)
Agilent 1260 Infinity II diode array detector FS (G7117A)	• 10 mm 1 µL flow cell (G4212-60008) • 80 Hz
Agilent OpenLab CDS, version C.01.07	

HPLC-certified grade). Water was produced on site using a Millipore Milli-Q system (0.2 µm filtered, 18 MΩ). USP naproxen sodium RS was purchased from United States Pharmacopeia. Mobile phase was prepared as per the USP method by mixing acetonitrile, water, and glacial acetic acid (500 mL:490 mL:10 mL).

Samples were prepared by creating a stock solution with not less than 20 tablets at 1.0 mg/mL. Since 20 tablets at approximately 220 mg/tablet would yield 4,400 mg, only a portion of the finely powdered tablets would be used in preparation of a stock solution. This stock solution could be prepared by transferring approximately 100 mg of finely ground tablet material into a 100 mL volumetric flask, to which 15 mL of water was added. The volumetric flask was then sonicated for 5 minutes, at which time 50 mL of prepared mobile phase (acetonitrile, water, and glacial acetic acid 500 mL:490 mL:10 mL) was added. The flask was then further sonicated for 30 minutes with intermittent shaking. The flask was allowed to cool, then brought to the appropriate volume with additional prepared mobile phase. An aliquot was then centrifuged for 5 minutes. 1 mL was then transferred to a 10 mL volumetric flask and diluted with mobile phase to produce a solution of 0.1 mg/mL.[1]

Columns used in this work

- Agilent ZORBAX Eclipse XDB-C8, 4.6 × 150, 5 µm (p/n 993967-906)
- Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 75 mm, 2.7 µm (p/n 697975-906)
- Agilent InfinityLab Poroshell 120 EC-C8, 4.6 × 50 mm, 2.7 µm (p/n 699975-906)
- Agilent ZORBAX RR Eclipse Plus C8, 4.6 × 100 mm, 3.5 µm (p/n 959961-906)
- Agilent ZORBAX RR Eclipse Plus C8, 4.6 × 75 mm, 3.5 µm (p/n 959933-906)

Results and discussion

Table 3 describes the allowable adjustments within the USP method without the need of method validation. One example of an allowed change is the L/dp rule. The ratio of column length to particle size is kept constant within a range of −25 to +50%. By keeping the efficiency of the column nearly constant, a new method is not created. The intent is not to create a more efficient method, just a faster method. No changes can be made to the detection without revalidation. No changes are made to the mobile phase. Finally, while injection volume may be adjusted as far as is consistent with precision and detection limits, and the injection volumes are scaled geometrically.[2] Precision is an important criterion in assay methods.

Following the USP method with the original column required, an analysis time of approximately 9 minutes can be met given a retention time of

Table 2. Initial LC method conditions.

Parameter	Value
Column	L7 (Agilent ZORBAX RR Eclipse Plus C8, 4.6 × 150 mm, 5 µm)
Mobile Phase	Premix (acetonitrile:water:glacial acetic acid 450:540:10)
Flow Rate	1.2 mL/min
Run Time	Not Less than twice retention of naproxen
Temperature (Column)	25 °C
Injection Volume	20 µL (geometrically scaled for smaller columns)
Sample Concentration	0.1 mg/mL of USP naproxen sodium RS in mobile phase sample nominally equivalent to 0.1 mg/mL naproxen sodium
Detector	UV: 254 nm
System Suitability Requirements	Tailing factor: not more than 2.0% Relative standard deviation: not more than 2.0%

Table 3. Summary of allowable adjustments per USP General Chapter <621>.

Parameters for System Suitability	USP37-NF32S1	
	Isocratic	Gradient
Particle Size	L/dp: −25 to +50% or N: −25 to +50%	No changes allowed
Column Length		
Column Inner Diameter	Flexible, w/ constant linear velocity	No changes allowed
Flow Rate	Based on dp: $F_2 = F_1 \times [(dc_2^2 \times dp_1)/(dc_1^2 \times dp^2)]$ Additional adjustments: ±50%, provided N decreases ≤20%	No changes allowed
Injection Volume	May be adjusted, as far as is consistent with precision and detection limits	May be adjusted, as far as is consistent with precision and detection limits
Column Temperature	±10 °C	±10 °C
Mobile Phase pH	±0.2 units	±0.2 units
Salt Concentration	within ±10% if the permitted pH variation is met	within ±10% if the permitted pH variation is met
Ratio of Components in Mobile Phase	Minor component (≤50%): ±30% relative, but cannot exceed ±10% absolute; may only adjust one minor component in ternary mixtures	No changes allowed * * Not specified in <621>, assume no changes are allowed
Wavelength of UV-Visible Detector	No changes allowed	No changes allowed

4.55 minutes with a system suitability requirement of not less than twice the retention. The L/dp ratio is 30,000. In addition, a tailing factor of not more than 2.0 is easily met with a tailing factor of 1.05. This chromatogram appears as Figure 2.

Applying the L/dp rule with a 75 mm column with a 2.7 µm particle, we find a ratio of 27,778, which is within the range of –25 to +50%. Using this rubric, we can find an allowable change that cuts analysis time in half, also saving 50% of mobile phase. Injection volume is cut geometrically proportionally, to 10 µL, half of the original 20 µL injection volume. Peak height is comparable to the original 5 µm, 150 mm method, and the tailing factor is 1.03. This chromatogram appears as Figure 3A. The USP recognizes that smaller particles have a higher optimal linear velocity, and so the flow rate of newly adjusted methods may be increased proportionately by the particle size ratio up to 1.5 times the original compendial method. These changes are governed by Equation 1.

Based on dp:

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

Additional adjustments: ±50%, provided N decreases ≤20%

Equation 1.

This equation can also be used to adjust to smaller diameter columns. In some cases, the change in particle size would indicate a bigger change in the flow rate, however, this increase is limited to +50% of the original flow rate. This chromatogram appears in Figure 3B. F_1 and F_2 refer to the initial and final flow rate, dc refers to the internal diameter of the column, and dp is the column particle diameter.

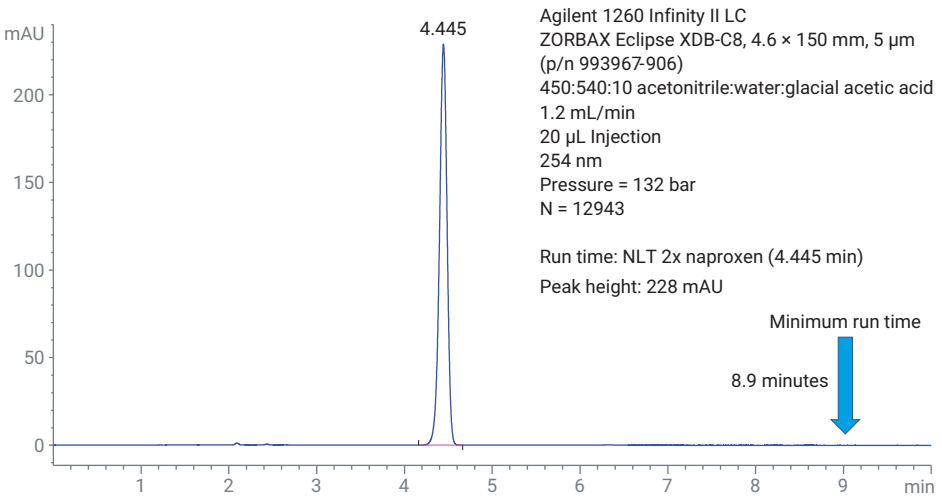

Figure 2. Chromatogram of naproxen sodium using USP-equivalent column.

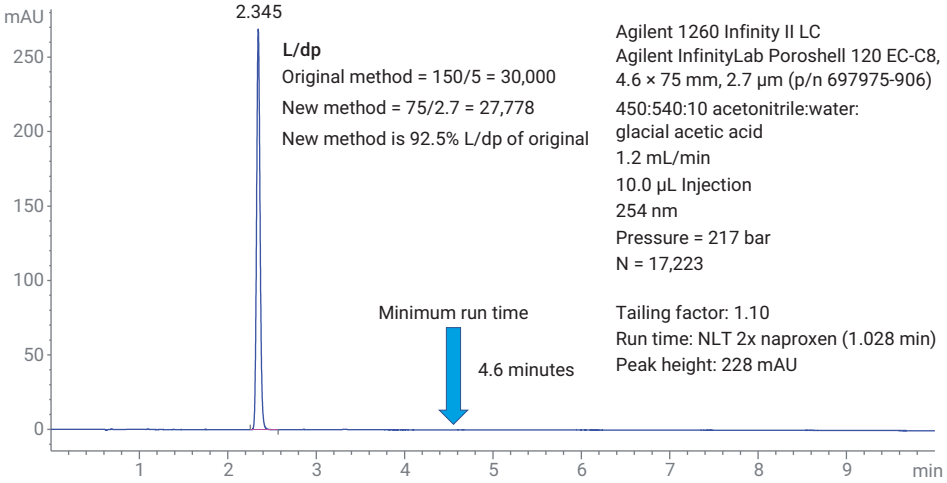

Figure 3A. Chromatogram of naproxen sodium using an Agilent InfinityLab Poroshell 120 EC-C8 column (4.6 × 75 mm, 2.7 µm) at 1.2 mL/min.

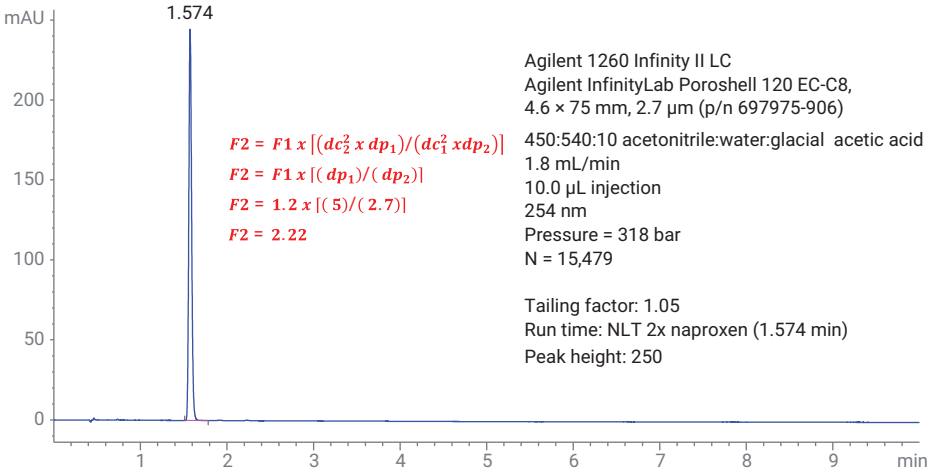

Figure 3B. Chromatogram of naproxen sodium using an Agilent InfinityLab Poroshell 120 EC-C8 column (4.6 × 75 mm, 2.7 µm) at 1.8 mL/min.

Table 4 summarizes the improvements in method throughput when adjusting the method from a 5 µm, 150 mm column to a 2.7 µm, 75 mm column. As shown, we are increasing throughput by 48% at 1.2 mL/min to 64% at 1.8 mL/min. A solvent saving of 50% is constant when changing to the shorter 75 mm column.

An alternative to using the L/dp ratio method is to keep the efficiency of the method with a shorter column. This rule is generally applicable to superficially porous columns. In the case of a 50 mm column with a 2.7 µm particle, the efficiency of the column is measured with the analyte of interest (naproxen). This is compared to the naproxen efficiency of the original column with a 5 µm particle. If the efficiency of the new shorter column is within the range of –25 to +50% of the efficiency of the original column, the adjustment is acceptable and only method verification needs to be carried out.

Figure 4A shows the chromatogram of naproxen sodium run at 1.2 mL/min using a 4.6 × 50 mm column. Since the column volume is a third of the original specified column, the injection volume is reduced similarly. In this example, an efficiency of 11,697 is achieved, which is 90.4% of the original 5 µm, 150 mm method. This is an acceptable solution. Further, the pressure at 160 bar is only slightly above the 132 bar of the original method. Analysis time is approximately 3 minutes. This adjustment will save approximately 66% of the original solvent and 66% of the analysis time. The tailing factor is 1.03. As shown in the previous example, the flow rate may be increased by up to 1.5 times the original linear velocity. Figure 4B shows the chromatogram of naproxen sodium run at 1.8 mL/minute using a 4.6 × 50 mm column. In this case, the new run time is 2.1 minutes. Our solvent saving is the same, but the time saving is 76% of the original method, at 244 bar, well within the capabilities of the instrumentation.

Table 4. USP allowable adjustments in naproxen sodium assay to Agilent InfinityLab Poroshell 120 EC-C8 column (4.6 × 75 mm, 2.7 µm).

Column	Column Length (L, mm)	Particle Size (dp, µm)	L/dp Ratio	Allowable L/dp Range (-25% to +50 %)	N Naproxen Standard	% Time Saving	Pressure (Bar)
Fully porous C8 L7	150	5	30,000	22,500–45,000	12943		132
Superficially porous C8	75	2.7	27,778	Meets specification	17223	48%	217 (1.2 mL/min)
Superficially porous C8	75	2.7	27,778	Meets specification	15479	64.7%	318 (1.8 mL/min)

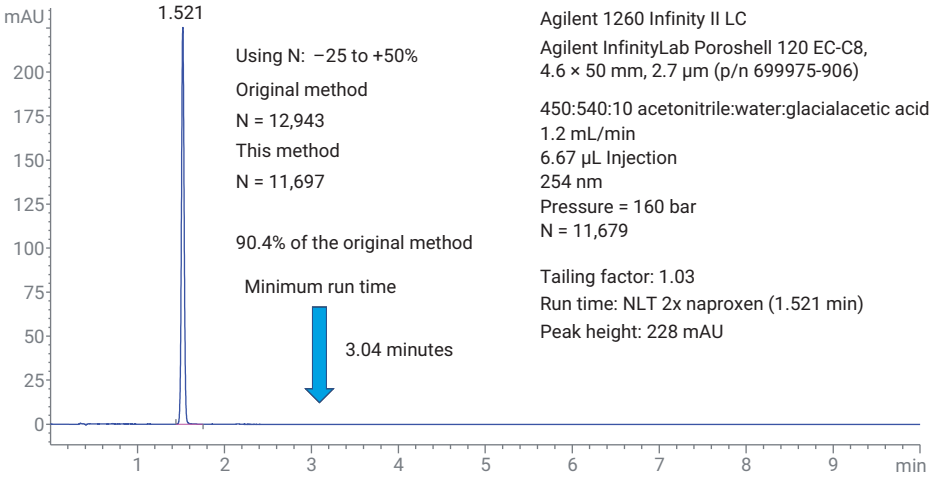

Figure 4A. Chromatogram of naproxen sodium using an Agilent InfinityLab Poroshell 120 EC-C8 column (4.6 × 50 mm, 2.7 µm) at 1.2 mL/min.

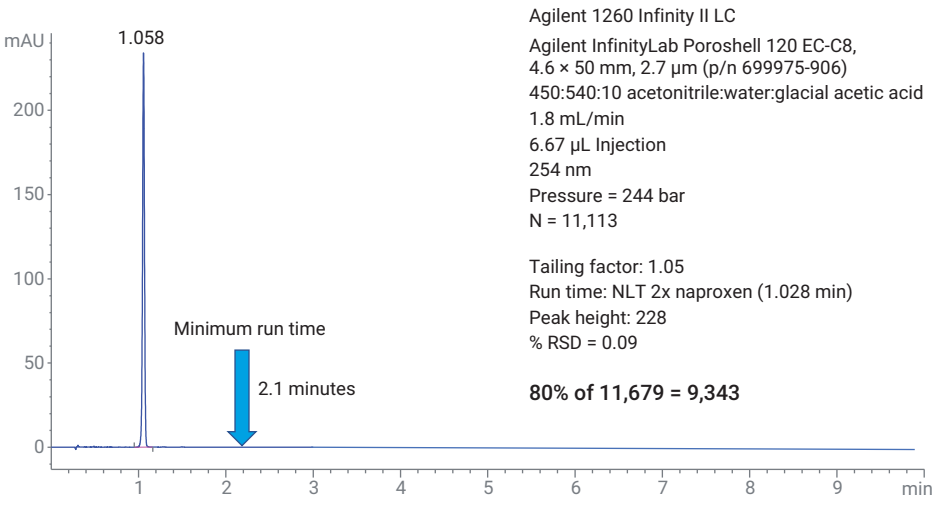

Figure 4B. Chromatogram of naproxen sodium using an Agilent InfinityLab Poroshell 120 EC-C8 column (4.6 × 50 mm, 2.7 µm) at 1.8 mL/min.

Table 5 summarizes the improvements in method throughput when adjusting the method from a 5 µm, 150 mm column to a 2.7 µm, 50 mm column. As shown, we are increasing throughput by 66% at 1.2 mL/min to 76 % at 1.8 mL/min. A solvent saving of 66% is constant when changing to the shorter 50 mm column.

A comparison to 3.5 µm totally porous columns was also carried out. 4.6 × 100 and 4.6 × 75 mm columns were evaluated at 1.2 and 1.8 mL/min. In Figure 5A, the chromatogram of naproxen sodium run at 1.2 mL/min using a 4.6 × 100 mm ZORBAX RR Eclipse Plus C8 column is shown. This column is within the allowed adjustments following the L/dp rule. Using these conditions, it is possible to save 33% of the original solvent and analysis time. It is also possible to increase the linear velocity up to 1.8 mL/minute. This chromatogram is shown in Figure 5B. A 4.6 × 75 mm, 3.5 µm column was also evaluated. For this case, the L/dp rule was not met, however, by applying the allowable N = −25 to +50% of the efficiency, the column falls into the allowable range at 1.2 mL/min. This chromatogram is shown in Figure 5C. Analysis time and solvent consumption are reduced by 50%. System pressure is the same as the original method at 130 bar. However, when the flow rate was increased to 1.8 mL/min (200 bar), the efficiency of the column dropped below the −25% range, and so this solution is not an acceptable adjustment. This chromatogram is shown in Figure 5D. These comparisons are summarized in Table 6.

Table 5. USP allowable adjustments of naproxen sodium assay to Agilent InfinityLab Poroshell 120 EC-C8 column (4.6 × 50 mm, 2.7 µm).

Column	Column Length (L, mm)	Particle Size (dp, µm)	L/dp Ratio	Allowable L/dp Range (-25% to +50%)	N Naproxen Standard	Allowable N Range (-25% to +50%)	% Time Saving	Pressure (Bar)
Fully porous C8	150	5	30,000	22,500 to 45,000	12,943			132 (1.2 mL/min)
Superficially porous C8	50	2.7	18,518	Does not meet specification	11,679	Meets Specification	66%	160 (1.2 mL/min)
Superficially porous C8	50	2.7	18,518	Does not meet specification	11,113	Meets Specification	76%	244 (1.8 mL/min)

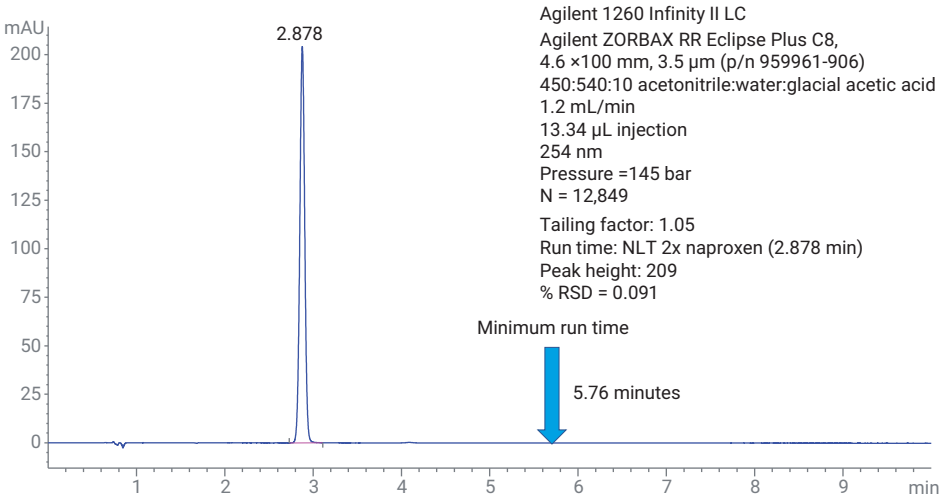

Figure 5A. Chromatogram of naproxen sodium using an Agilent ZORBAX RR Eclipse Plus C8 column (4.6 × 100 mm, 3.5 µm) at 1.2 mL/min.

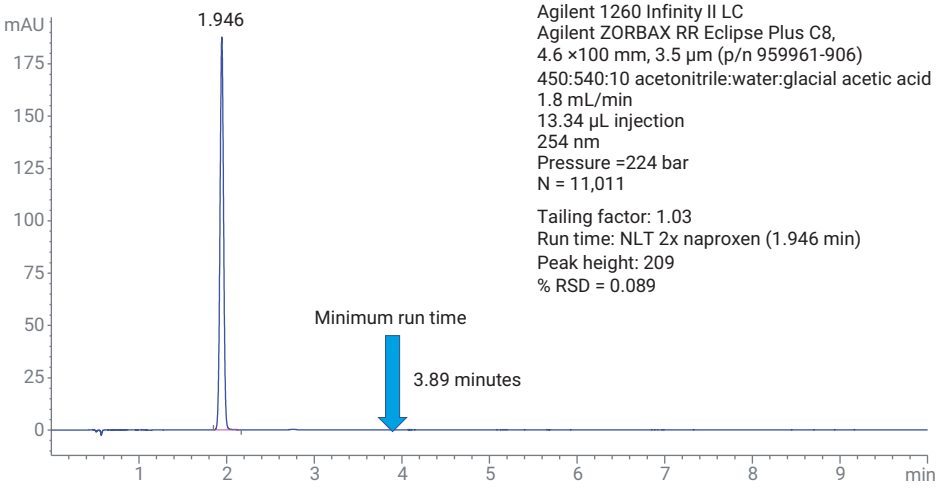

Figure 5B. Chromatogram of naproxen sodium usingan Agilent ZORBAX RR Eclipse Plus C8 column (4.6 × 100 mm, 3.5 µm) at 1.8 mL/min.

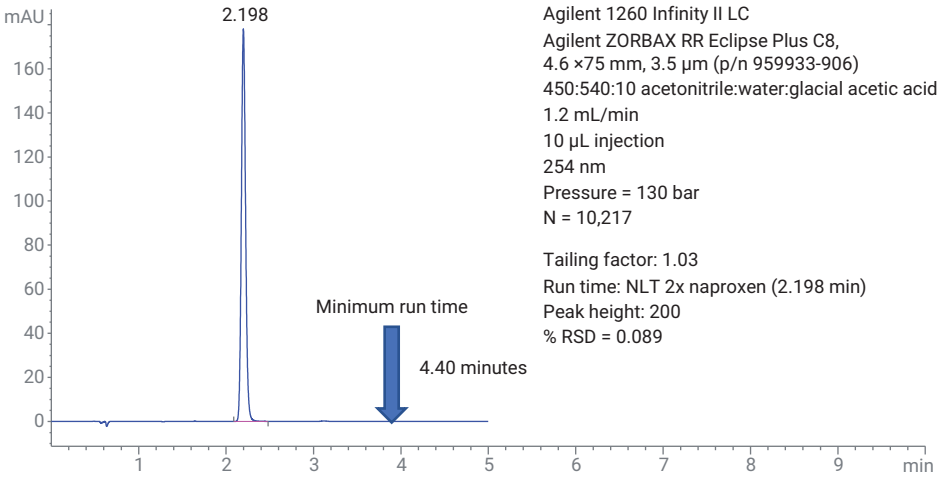

Figure 5C. Chromatogram of naproxen sodium using an Agilent ZORBAX RR Eclipse Plus C8 column (4.6 × 75 mm, 3.5 µm) at 1.2 mL/min.

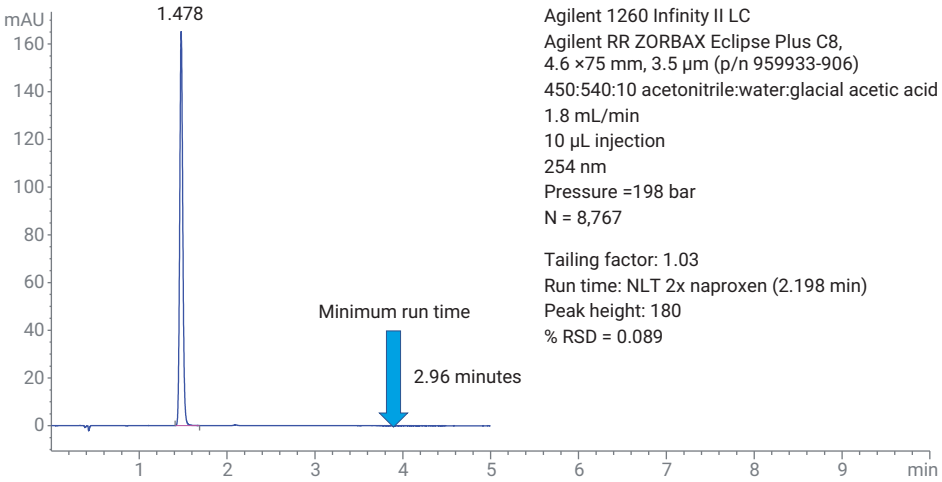

Figure 5D. Chromatogram of naproxen sodium usingan Agilent ZORBAX RR Eclipse Plus C8 column (4.6 × 75 mm, 3.5 µm) at 1.8 mL/min.

Table 6. USP allowable adjustments of naproxen sodium assay to Agilent ZORBAX EC-C8 column (4.6 × 100 and 75 mm, 3.5 µm).

Column	Column Length (L, mm)	Particle Size (dp, µm)	L/dp Ratio	Allowable L/dp Range (–25 to +50%)	N Naproxen Standard	Allowable N Range (–25 to +50%)
Fully porous C8	150	5	30,000	22,500-45,000	12,943	9,707-19,414
Fully porous C8	100	3.5	28,571	Meets specification	12,849	Meets Specification
Fully porous C8	100	3.5	28,571	Meets specification	11,011	Meets Specification
Fully porous C8	75	3.5	21,428	Does not meet specification	10,217	Meets Specification
Fully porous C8	75	3.5	21,428	Does not meet specification	8,767	Does not meet Specification

System suitability requirements are the acceptance criteria for adjustments. In the case of the naproxen sodium assay, we were able to reduce the analysis time from 10 minutes on the original method to 2 minutes on a method on an InfinityLab Poroshell 120 EC-C8, 4.6 × 50 mm, 2.7 µm column. In addition to the efficiency change, we also earn a 66% decrease in solvent consumption. This is typical of methods adapted to 50 mm InfinityLab Poroshell 120 2.7 µm columns. System suitability requirements using the naproxen sodium tablet method are not more than (NMT) 2.0%. This is easily met with a 0.064% area RSD and a 0.050% RSD. In addition, USP tailing factor of NMT 2.0 is also met, with a tailing factor of 1.05. This is summarized in Table 7.

Table 7.Results of the system suitability test and analysis time summary.

	System Suitability Requirements	Agilent InfinityLab Poroshell 120 EC-C8 (4.6 × 50 mm), 1.2 mL/min	Agilent InfinityLab Poroshell 120 EC-C8 (4.6 × 50 mm), 1.8 mL/min
USP Tailing Factor	NMT 2.0	1.03	1.03
RSD	NMT 2.0%	Area = 0.046%	Area = 0.064%
		Retention time = 0.036%	Retention time = 0.050%
Run Time ($2 \times t_r$)	Standard solution	3.042 minutes	2.056 minutes

Conclusion

Laboratories performing compendial analyses with fully porous 5 µm columns can benefit from the increased speed and solvent savings that superficially porous 2.7 µm InfinityLab Poroshell 120 columns and fully porous 3.5 µm columns can provide without needing to replace instrumentation. Faster analysis times, leading to higher throughput, can lead to a more productive laboratory. By applying allowed adjustments to these shorter columns, no additional validation is required. In this case, superficially porous columns can achieve faster results than 3.5 µm columns, resulting in a more productive laboratory while easily meeting system suitability requirements.

References

1. USP Naproxen Sodium Tablet Method, *United States Pharmacopeia 42 (4) Proposed IRA*, Rockville, MD **2017**.
2. USP General Chapter 621, USP 37-NF32, First supplement.

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