Analysis of Naproxen Using Poroshell 120 EC-C18: Headache Free Method Adjustment

Application Note

Pharmaceuticals

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Abstract

The USP method for Naproxen is demonstrated using Eclipse Plus C18 and Poroshell 120 EC-C18. When a 4.6 x 50 mm Poroshell 120 EC-C18 column is used, the modified method reduces analys time to 22% of the original method without revalidation.

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Introduction

The costs associated with pharmaceutical testing are considerable and many lab managers are seeking ways to reduce costs by reducing solvent usage and improving productivity while still using the LC instruments in their lab.

Compendia methods from the USP (United States Pharmacopeia) are widely used in pharmaceutical drug product and raw materials testing. However, not all methods in the USP use modern technologies and may be more time consuming than desired. These methods can be updated by making adjustments following the recommendations in USP chapter <621>. The ranges for adjustments that were used in this method are: column length, column material, particle size, and injection volume. While other parameters can be adjusted according to the USP, none of those were needed to improve the throughput of this method. Modifications outside these ranges are considered changes and require revalidation of the method.

Naproxen is classified as a non-steroidal anti-inflammatory drug or NSAID and is available as generic tablets. The USP contains a method for the analysis of Naproxen tablets, which uses an L1 (C18), 5 µm column. The structure of Naproxen is shown in Figure 1.

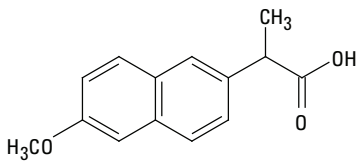

Figure 1. Structure of Naproxen.

Agilent 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 LC instruments. The columns are available in a C18 bonded phase, a typical L1 material. In Figure 1, a 4.6 mm × 150 mm, 5 µm L1 or C18 column is used as the starting point for the method. The conditions are unchanged and both an Agilent Poroshell 120 EC-C18 4.6 mm × 100 mm, 2.7 µm and an Agilent Poroshell 4.6 mm × 50 mm, 2.7 µm column are included for comparison.

Experimental

- Agilent 1200 Series Binary Pump SL, Mobile phase Channel A: Acetonitrile: Water: Glacial Acetic Acid (500:490:10); Flow rate was 1.2 mL/min, in some work the flow rate is increased up to 2.2 mL/min (G1312B)
- Agilent 1200 Series Automatic Liquid Sampler SL (ALS), injection volume was 20 µL for the 150 mm column, 13.34 µL for the 100 mm column, 6.67 µL for the 50 mm column (G1376C)
- Agilent 1200 Series Thermostatted Column Compartment SL(TCC), Temperature was 25 °C (G1316B)
- Agilent 1200 Series Diode Array Detector SL (DAD), wavelength used was 254, 4 nm, with a G1315-60024 micro flow cell (5-mm path, 6 µL volume) (G1316C)

Agilent ZORBAX Columns:

- Agilent Eclipse Plus C18, 4.6 mm × 150 mm, 5 µm p/n 959993-902
- Agilent Eclipse Plus C18, 4.6 mm × 100 mm, 3.5 µm p/n 959961-902
- Agilent Poroshell 120 EC-C18, 4.6 mm × 100 mm, 2.7 µm p/n 695975-902
- Agilent Poroshell 120 EC-C18 4.6 mm × 50 mm, 2.7 µm p/n 699975-902

Acetonitrile used was Burdick and Jackson ACS/HPLC Certified solvent, purchased from Honeywell. Glacial Acetic Acid used was ACS/USP Grade purchased from VWR. Water used was produced on site using a Millipore Milli-Q system,18 MΩ filtered to 0.2 µm. USP Naproxen was purchased from United State Pharmacopeia. Butyrophenone was purchased from Sigma-Aldrich. Sample and mobile phase preparation are from the USP method. [1]

Mobile Phase Preparation

The mobile phase is prepared by mixing acetonitrile, water, and glacial acetic acid (500 mL: 490 mL: 10 mL). [1]

Sample Preparation

Samples and internal standards are prepared in a mixture of acetonitrile and water (90:10). The internal standard is prepared by diluting 5 mL of butyrophenone with acetonitrile to make 100 mL. 1 mL of the resulting solution is diluted with acetonitrile to make 100 mL. Each mL of this solution contains about 0.5 µL of butyrophenone.

The USP Resolution Standard or USP Naproxen RS is prepared by dissolving an accurately weighed quantity of in Solvent mixture to obtain a solution having a known concentration of about 2.5 mg per mL. 1.0 mL of the resulting solution and 2.0 mL of Internal standard solution is transferred to a 100-mL volumetric flask, diluted with Mobile phase to volume, and mixed. This solution contains about 25 µg of USP Naproxen RS per mL. [1]

The chromatographic and performance requirements of the method are listed in the USP method. These are summarized below. [1]

- 4.6 mm × 150 mm column, L1 column (C18)
- N of the analyte not less than 4000 plates
- Resolution between the analyte and internal standard peaks is not less than 11.5

As can be seen in Figure 2, the efficiency and other chromatographic performance requirements of the USP method are easily met. During the course of a day using the USP method as written, an analysis can be performed every 9 minutes. This leads to a throughput of six to seven analyses per hour, or approximately 160 injections that can be made per day at 9 minutes per injection. Over the course of a week, 1120 injections can be performed using a 150 mm, 5-µm column. In many applications this throughput is sufficient. An increased throughput can be achieved by adjusting the method. The USP updated chapter <621> presents recommendations on how much a method can be modified such that the changes are considered an adjustment. [2]

- Column length ± 70%
- Column internal diameter ± 25%
- Column material particle size: Reduction of up to 50%, no increase
- Flow rate ± 50%
- Injection volume Changes are allowed as long as system suitability testing (SST) criteria are met
- Column temperature ± 10%
- pH of mobile phase ± 0.2
- UV wavelength: no change outside manufacturer specifications
- Concentration of salts in buffer ± 10%

Modifications outside these ranges are considered changes and require re-validation. If the analyst chooses to use a shorter column, such as a 4.6 mm × 100 mm, 3.5-µm column as shown in Figure 2, the same analysis could be accomplished in 67% of the time (approximately 6 minutes per sample). The method could also be easily applied to an Agilent Poroshell EC-C18 4.6 mm × 100 mm, 2.7 µm or an Agilent Poroshell EC-C18 4.6 mm × 50 mm, 2.7 µm. The 50 mm column is still within the allowed adjustment window and easily allows reduction of analysis time to 33% of the initial method time.

In order to achieve the best performance with Poroshell 120 or other small volume columns, it is necessary to optimize detector speed and minimize extra column volume. Typically, a data collection rate of 40 Hz is used. [3] To avoid column overloading, the injection volume is scaled geometrically as the column volume is reduced. This means a 150 mm column with a 20 µL injection is scaled to a 100 mm column with an injection volume equal to 20 × (100/150) or 13.67 µL and a 50 mm column is scaled to use an injection volume of 20 × (50/150) or 6.67 µL.

The performance requirements of the method are exceeded when changing from the 5 µm L1 columns to either of the superficially porous 2.7 µm C18 columns. The analysis on the 100 mm column is 2 × faster than the original method, and on the 50 mm long column the method is 4.5 × faster than the original method. Either column choice improves productivity.

One of the allowed adjustments is a change in flow rate by ± 50%. This would allow an increase of up to 1.8 mL/min under current rules. A suggested change is currently being discussed that would allow the linear velocity of the column and particle to remain constant, allowing a flow rate increase of almost 100% (up to 2.4 mL/min) without revalidation of the method. [4] Implementing these changes would increase through put even more.

Figure 3 shows the effect of increasing flow rate on an Agilent Poroshell 120 EC-C18 4.6 mm × 100 mm column. In this case, the flow is increased from 1.2 mL/min to 2 mL/min, while exceeding the efficiency and resolution requirements and remaining under 400 bar pressure. At 2.2 mL/min, the efficiency and resolution requirements are still met but we are now slightly above the 400 bar threshold.

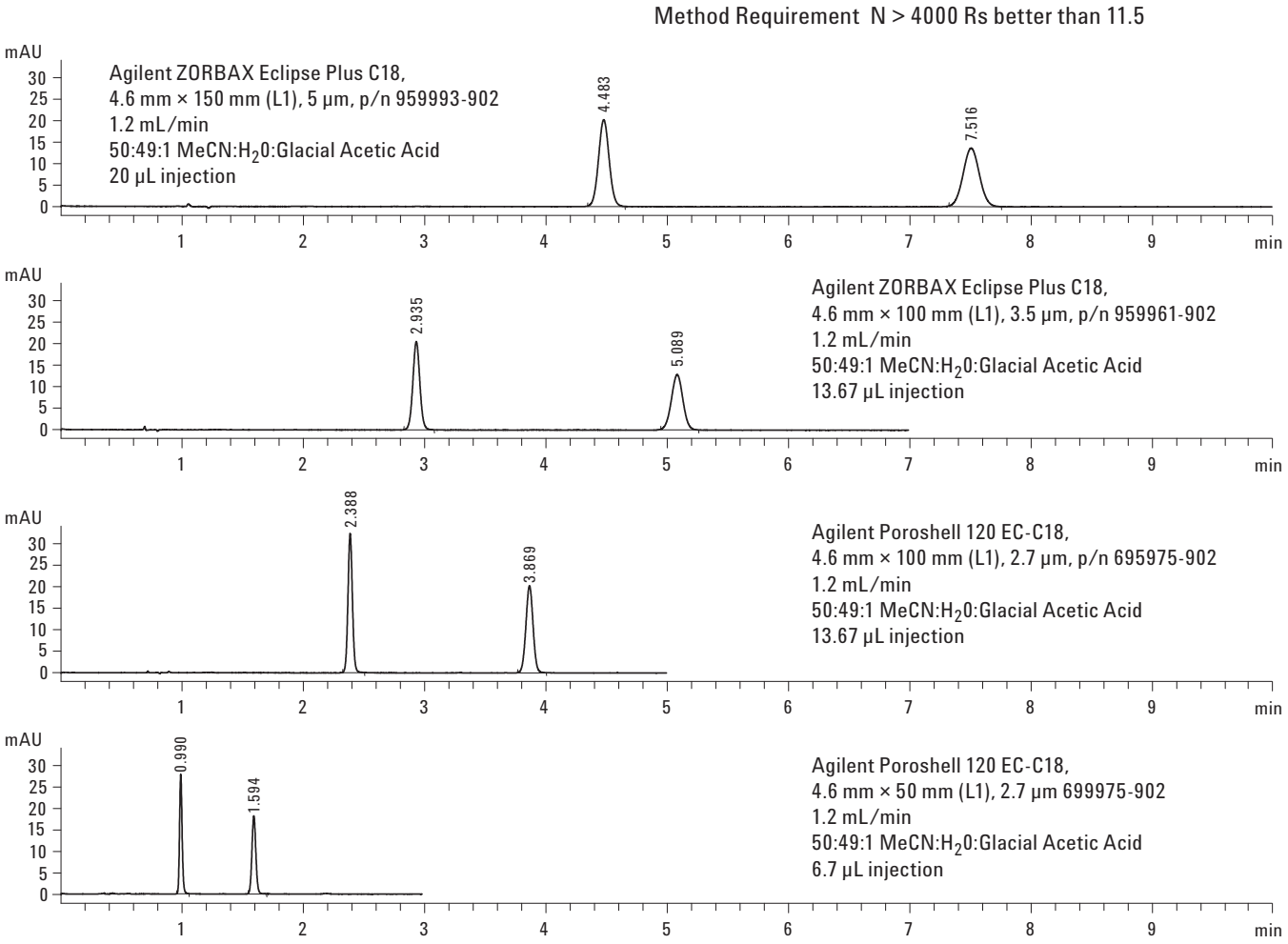

Figure 2. USP Naproxen Method Demonstrated on Varied Totally Porous and Superficially Porous Columns.

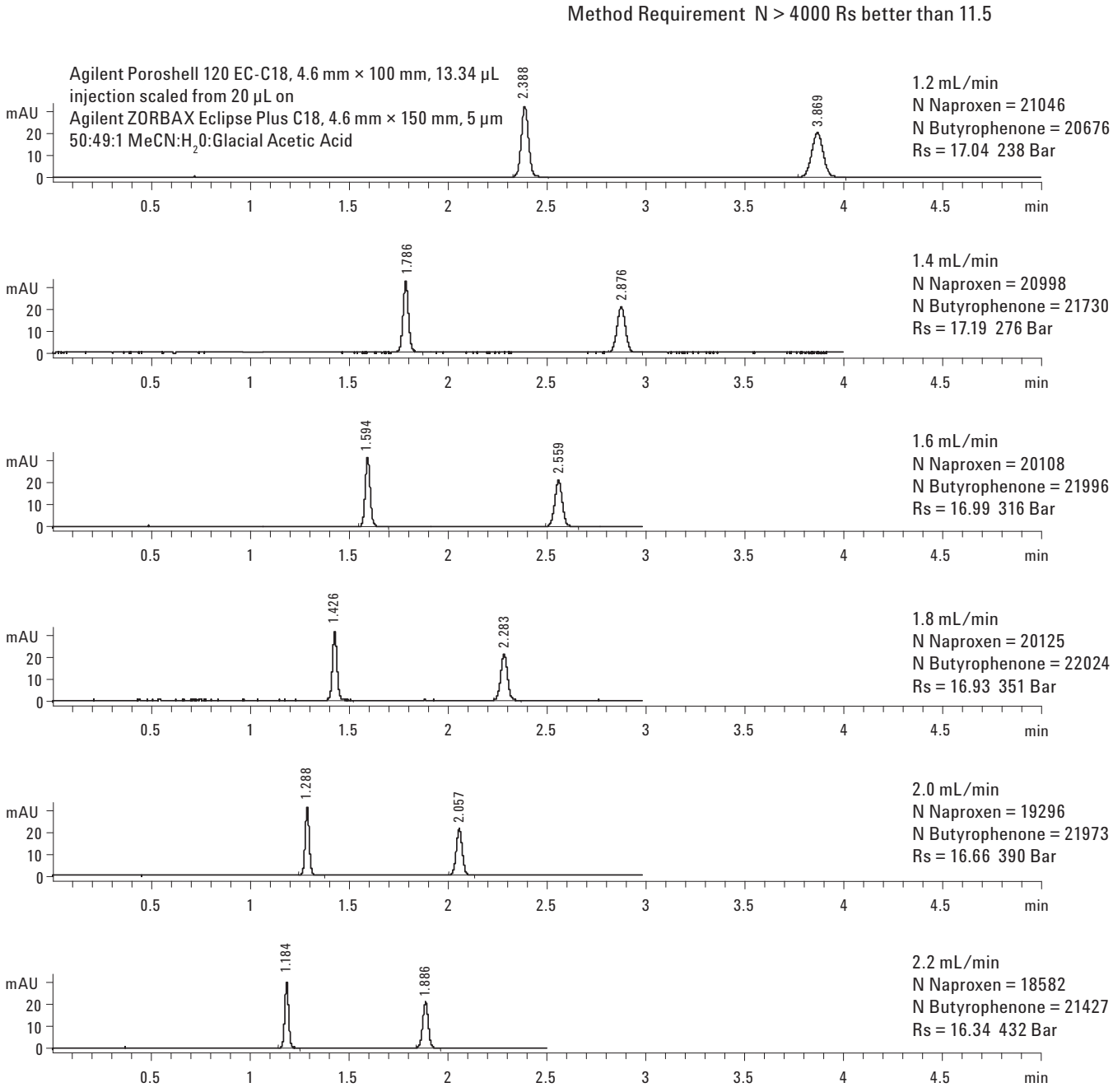

Figure 3. USP Naproxen Method Demonstrated on an Agilent Poroshell 120 EC-C18, 4.6 mm × 100 mm at varied flow rates.

Figure 4 shows the effect of increasing flow rate on an Agilent Poroshell 120 EC-C18, 4.6 mm × 50 mm column. In this case, the flow is increased from 1.2 mL/min to 2.4 mL/min, while exceeding the efficiency and resolution requirements and remaining under 300 bar pressure.

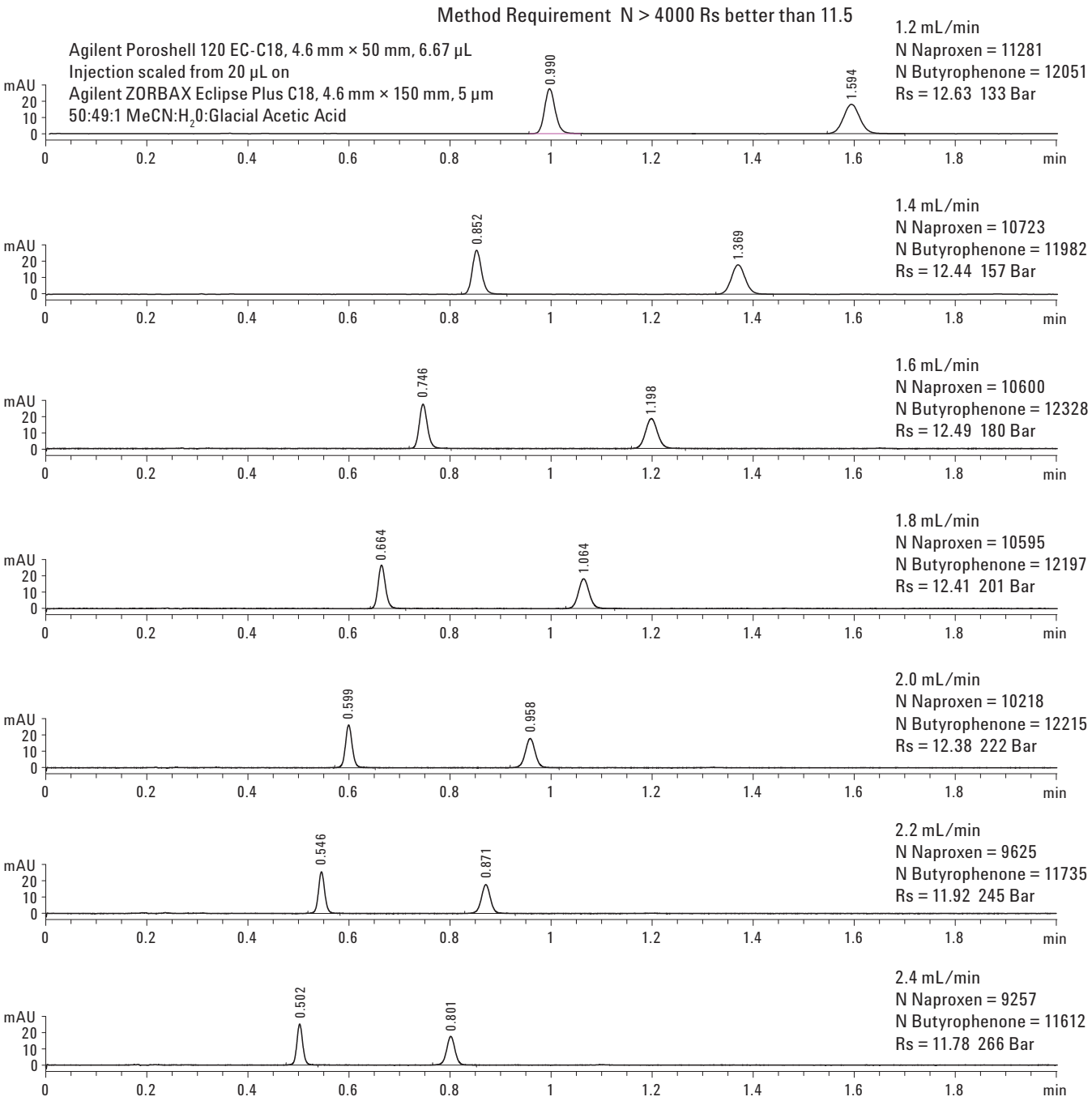

Figure 4. USP Naproxen Method Demonstrated on an Agilent Poroshell 120 EC-C18, 4.6 mm × 50 mm at varied flow rates.

Conclusions

Laboratories performing compendia analysis with fully-porous LC columns can benefit from the increased speed, resolution, and sensitivity that superficially porous, Agilent Poroshell 120 columns provide without having to replace existing instrumentation. Faster analysis times resulting in higher throughput and greater productivity can be achieved with Agilent Poroshell 120 columns. Method adjustments to these compendia methods with shorter length columns and the smaller 2.7 µm particle size provide these improved results.

References

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3. William J. Long, Anne E. Mack, John W. Henderson, Jr. "Optimization of HPLC Instrument for Use with Poroshell 120 Columns," Poster Number 2080-9 Pittsburg Conference 2010 Orlando, FL, USA.
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