Adapt the USP Naproxen Tablet Method for Agilent Poroshell 120 4 µm Columns

Application Note

Small Molecule Pharmaceuticals

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Introduction

Naproxen is classified as a nonsteroidal anti-inflammatory drug or NSAID, and is available as generic tablets. It is also known by the trade names Aleve, Anaprox, Apronax, Naprelan, or Naprosyn. Naproxen is commonly used for the reduction of pain, fever, inflammation, and stiffness caused by conditions including migraine, arthritis, kidney stones, gout, and tendinitis. Naproxen is available as an over-the-counter drug (OTC) in the United States, but is available only by prescription in much of the world.

The United States Pharmacopeia (USP) has 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. The costs associated with pharmaceutical testing are considerable, and many lab managers look for ways to reduce costs by reducing solvent usage and improving productivity, while still using the LC instruments in their lab. Compendial methods from the USP are widely used in pharmaceutical drug product and raw material testing. However, not all methods in the USP use modern technologies and can be more time consuming than desired. These methods can be updated by making adjustments following the recommendations in USP chapter <621>. The range of adjustments used for the naproxen tablet method includes column length, column material, particle size, and injection volume. While other parameters can be adjusted according to the USP, none were needed to improve the throughput of the method in this study. Modifications outside these ranges are considered changes and require revalidation of the method. In August 2014, changes were made in the USP dealing with allowable modifications to methods. This application note discusses how these allowable modifications can be implemented with an Agilent Poroshell 120 EC-C18, 4 µm column.

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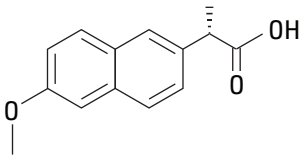

Figure 1. Naproxen structure.

Experimental

Instrumentation

- Agilent 1260 Infinity Binary Pump SL (G1312B)
- Agilent 1260 Infinity Diode Array Detector SL (G4212)
- Agilent 1260 Infinity Automatic Liquid Sampler SL (G1376C)
- Agilent 1260 Infinity Thermostatted Column Compartment SL (G1316B)

Conditions

Columns:	Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm (p/n 959993 902), Agilent Poroshell 120 EC-C18, 4.6 × 150 mm, 4 µm (p/n 695975 902), Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 4 µm (p/n 699975 902)
Mobile phase:	Acetonitrile:water:glacial acetic acid (500:490:10) [1]
Flow rate:	1.2 mL/min, or up to 2.2 mL/min
Injection volume:	20 µL for the 150 mm columns, 13.34 µL for the 100 mm column
Temperature:	25 °C
Detection:	254, 4 nm, with a micro flow cell (p/n G4220 60024)

Acetonitrile used was Burdick and Jackson ACS/HPLC certified, purchased from Honeywell. Glacial acetic acid was ACS/USP grade purchased from VWR. Water was produced on site using a Millipore Milli Q system,18 MΩ filtered to 0.2 µm. USP Naproxen was purchased from USP. Butyrophenone was purchased from Sigma-Aldrich, Corp. Sample and mobile phase preparation was from the USP method [1].

Sample preparation

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

The USP Resolution Standard or USP Naproxen RS was prepared by dissolving an accurately weighed quantity in the solvent mixture to obtain a solution having a known concentration of about 2.5 mg/mL. One milliliter of the resulting solution and 2.0 mL of internal standard solution was transferred to a 100 mL volumetric flask, diluted with mobile phase to volume, and mixed. This solution contained about 25 µg USP Naproxen RS per mL [1]. The chromatographic and performance requirements of the method are listed in the USP method. These are summarized as:

- 4.6 mm × 150 mm column, L1 column (C18) 5 µm particle
- N of the analyte not less than 4,000 plates
- Resolution between the analyte and internal standard peaks is not less than 11.5

Results and Discussion

As can be seen in Figure 2, the efficiency and other chromatographic performance requirements of the USP method were easily met by the Agilent columns, with a nearly 80% efficiency improvement using a Poroshell 120, 4.6 × 150 mm, 4 µm column as a drop-in replacement. The pressure measured on the 4.6 × 150 mm, 5 µm Eclipse Plus C18 column was 95 bar and the pressure on the 4.6 × 150 mm, 4 µm Poroshell 120 EC-C18 column was 165 bar. The pressure from a slightly shorter, 4.6 × 100 mm, 4 µm column was found to be 98 bar. As the backpressure was still under 200 bar, these columns are ideal replacements on 400 bar instruments. 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 per day at 9 minutes per injection. Over the course of a week, 1,120 injections can be performed using a 150 mm, 5 µm column. In many applications, this throughput is sufficient. However, 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 [2,3].

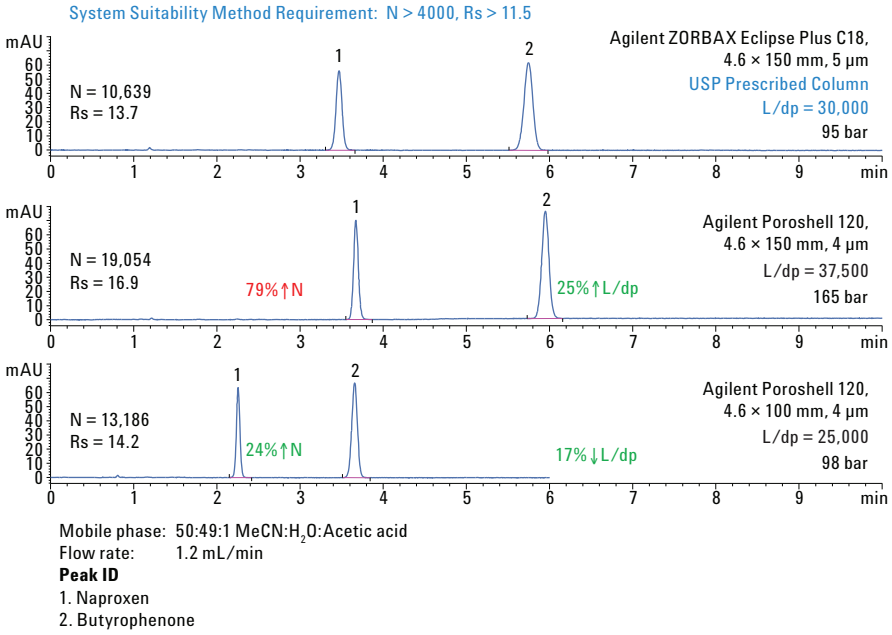

Figure 2. USP analysis of naproxen using Agilent Poroshell 120, 4 µm and Agilent ZORBAX Eclipse Plus, 5 µm columns. The 4 µm particles deliver a 79% increase in efficiency at roughly the same run time in the 150 mm column, or a 24% increase in efficiency with a 33% shorter run time in the 100 mm column.

On 1 August 2014, changes were made to how allowable changes are implemented (Table 1). Many changes were made to make method adjustments more clear. One important change was to define the particle size of a compendial method as the largest possible size within the range of the column definition, unless the particle size was stated in the method. For example, the L1 column is defined as being a C18 column with a particle size between 10 and 1.7 µm. However, in the naproxen tablet method, the particle size is listed as 5 µm. This can be an important consideration as the L/dp ratio can be used to determine if a method change is allowed. For example, a 150 mm column with a 5 µm particle size has an L/dp ratio of 30,000. A 4 µm, 150 mm column has a L/dp ratio of 37,500, a 25% increase, which is allowable under the USP37 NF32S1 guidelines.

Table 1. USP compendial summary.

	USP36-NF31 before July 31, 2014	**USP37-NF32S1 after August 1, 2014**	
Chromatographic parameter	**Isocratic/Gradient**	**Isocratic**	**Gradient**
Particle diameter	–50%	Constant L/dp or N:–Changes up to 25% to +50%	No changes allowed
Column length	± 70%		
Flow rate	± 50%	Based on dp ± 50%	No changes allowed
Column diameter	Flexible	Flexible	No changes allowed
Injection volume	Flexible	Flexible	Flexible
Column temperature	± 10°	± 10°	± 10°
Mobile phase pH	± 0.2 units	± 0.2 units	± 0.2 units
Salt concentration	Within ± 10% if the permitted pH variation (see above) is met	Within ± 10% if the permitted pH variation (see above) is met	Within ± 10% if the permitted pH variation (see above) is met

In addition, a 4 µm, 100 mm column has a L/dp ratio of 25,000, a 17% decrease that is also allowable. However, a 4 µm, 50 mm column has an L/dp ratio of 12,500, which is a 58% change and not allowed without revalidation. Figure 2 shows a comparison of the 5 µm reference column, and the two allowable changes. The Poroshell 120 4 µm particles in Figure 2 show a 79% increase in efficiency at roughly the same run time for the 150 mm column, or a 24% increase in efficiency with a 33% shorter run time in the 100 mm column. The injection volume was changed proportionately to account for changes in column volume.

Conclusions

This work showed that the Agilent Poroshell 120 EC-C18, 4 µm column can be easily used for the compendial analysis of naproxen tablets. Use of the 100 mm column in this test decreases analysis time by 33% with a corresponding saving in solvent cost. Implementation of testing improvements can help decrease drug manufacturing costs. Furthermore, the Poroshell 120 4 µm column offers a bridge to the Poroshell 120 Fast LC column family, offering significant laboratory improvements.

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

References

1. *USP Naproxen Tablet Method, United States Pharmacopeia 31 NF 26*; United States Pharmacopeia, Rockville, MD, **2008**.
2. *USP Method Validation Guidance, United States Pharmacopeia 30 Supplement 2 <621>*; United States Pharmacopeia, Rockville, MD, **2013**.
3. *USP Method Validation Guidance, United States Pharmacopeia 30 Supplement 2 <621>*; United States Pharmacopeia, Rockville, MD, **2014**.

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