Rapid Separation of Ibuprofen, Ibuprofen-Related Compound C, and Valerophenone Using Advanced UHPLC and Sub-2 µm Solid Core Column Technologies

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Application Note 21183

Key Words
Accucore Vanquish, Vanquish UHPLC, UHPLC, USP Monograph, rapid separation, high resolution, Ibuprofen, Ibuprofen-related compound C, valerophenone

Goal

To demonstrate the advantages of using the Thermo Scientific™ Accucore™ Vanquish™ C18+ 1.5 µm UHPLC column and the Thermo Scientific Vanquish UHPLC system for the separation of ibuprofen, ibuprofen-related compound C, and valerophenone. The advanced capabilities of the Vanquish UHPLC system allow the Accucore Vanquish UHPLC columns to be operated at higher flow rates that enable development of rapid analytical methods while maintaining performance.

Introduction

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) and is a commonly used painkiller. It is particularly useful for relieving the symptoms of inflammation. This application note demonstrates the use of an Accucore Vanquish C18+ UHPLC column to improve chromatographic performance in the impurity analysis of ibuprofen. The updated method provides a reduction in analysis time compared to a 4 µm column while maintaining the U.S. Pharmacopeial Convention (USP) requirements for resolution, asymmetry, and retention time reproducibility.

Accucore Vanquish C18+ UHPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. This next-generation column features 1.5 µm solid core particles that are not totally porous, but instead have a solid core and a porous outer layer. The optimized phase bonding creates a high-coverage, robust phase. This coverage results in a significant reduction in secondary interactions and delivers highly efficient peaks. The tightly controlled 1.5 µm diameter of Accucore Vanquish particles and refined manufacturing processes result in a column that delivers the increased chromatographic efficiency required for rapid assays.

The Accucore Vanquish UHPLC column and the Vanquish UHPLC systems were designed in combination to achieve the best possible chromatographic performance. The system is optimized to reduce extra column band dispersion and allow users to significantly improve the separation power in their analytical assays. The 1500 bar pressure capability of the Vanquish pump enables an extended range of flow rates to be employed, allowing for faster separations and higher throughput.

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Experimental

Consumables and Apparatus

- Accucore Vanquish C18+ 1.5 µm UHPLC column, 100 × 2.1 mm (P/N 27101-102130)
- Accucore XL C18 4 µm LC column, 150 × 4.6 mm (P/N 74104-154630)
- Thermo Scientific™ Virtuoso™ Vial Identification System (P/N 60180-VT100)
- Thermo Scientific™ Virtuoso™ 9 mm wide opening screw thread vial, 2 mL, clear glass vial with V-patch and red PTFE/white silicone septum (P/N 60108-VT402)
- Fisher Scientific™ HPLC grade water (P/N 10221712)
- Fisher Scientific HPLC grade acetonitrile (P/N 10616653)

Sample Preparation

Per the USP method, a solution of ibuprofen (0.35 mg/mL) and valerophenone (12 mg/mL) was made in mobile phase and spiked with 0.1% ibuprofen–related compound C (0.012 mg/mL).

Instrumentation

Analyses were performed using a Vanquish UHPLC system consisting of:

- System base (P/N VH-S01-A)
- Binary pump H (P/N VH-P10-A)
- Split sampler HT (P/N VH-A10-A)
- Column compartment H (P/N VH-C10-A)
- Diode array detector HL (P/N VH-D10-A)

UHPLC Conditions

Method 1: Accucore XL C18, 4 µm column

Mobile phase	Water + 1% chloroacetic acid (pH 3)/acetonitrile (40:60 v/v)
Flow rate	2 mL/min
Column temp.	30 °C
Injection volume	5 µL
Detection	UV at 254 nm (0.1 s rise time, 50 Hz, 8 nm slit width)
Viper connections	Autosampler to column: 0.1 × 250 mm (P/N 6040.2225)
	Column to detector: 0.075 × 350 mm (P/N 6041.5735)
UHPLC column	Accucore XL C18, 4 µm, 150 × 4.6 mm

Method 2: Accucore Vanquish C18+, 1.5 µm column

Mobile phase	Water + 1% chloroacetic acid (pH 3)/acetonitrile (40:60 v/v)
Flow rate	0.65 mL/min
Column temp.	30 °C
Injection volume	1 µL
Detection	UV at 254 nm (0.1 s rise time, 50 Hz, 8 nm slit width)
Viper connections	Autosampler to column: 0.1 × 250 mm (P/N 6040.2225)
	Column to detector: 0.075 × 350 mm (P/N 6041.5735)
UHPLC column	Accucore Vanquish C18+, 1.5 µm, 100 × 2.1 mm

Software

The Thermo Scientific™ Dionex™ Chromeleon™ 7.2 SR2 Chromatography Data System was used for data acquisition and analysis.

Results and Discussion

A scale down of the ibuprofen impurity USP analysis was achieved with an Accucore Vanquish C18+ 1.5 µm UHPLC column. The original USP method is based on a L1 150 × 4.0 mm, 5 µm column and is demonstrated with an Accucore XL C18 150 × 4.6 mm column to give a method time of 2.5 minutes and meet the USP criteria for resolution and peak shape. Switching to a 100 × 2.1 mm column packed with Accucore Vanquish C18+ 1.5 µm particles reduced the analysis time to 1.5 minutes, while maintaining the USP criteria for resolution and peak shape as shown in Figure 1. The Accucore Vanquish UHPLC column in combination with the Vanquish UHPLC system provided excellent retention time reproducibility as shown in Table 1.

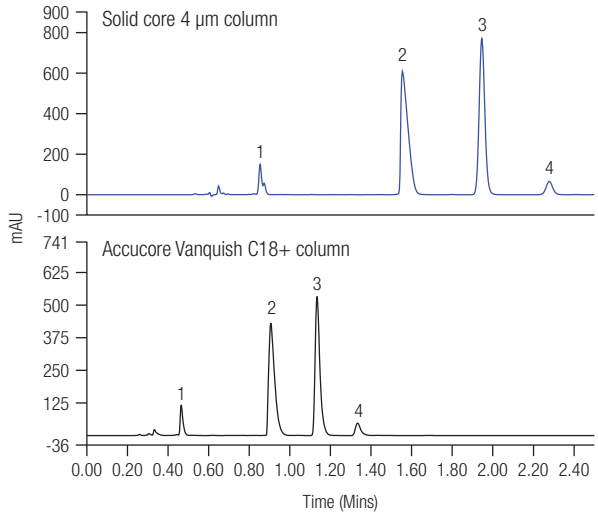

Figure 1. Chromatographic separation of ibuprofen (2), valerophenone (3), and ibuprofen-related compound C (4). Peaks labeled (1) are unknown impurities.

Table 1. Resolution, asymmetry, and injection reproducibility data for Accucore Vanquish 1.5 µm and Accucore XL C18 (n=6) columns.

	Resolution (USP)		Asymmetry (USP)		Retention Time Reproducibility (% RSD)	
	Accucore XL	Accucore Vanquish	Accucore XL	Accucore Vanquish	Accucore XL	Accucore Vanquish
Ibuprofen	6.56	4.76	2.67	1.86	0.04%	0.00%*
Valerophenone	5.99	4.62	1.13	1.53	0.03%	0.00%*
Ibuprofen-related compound C	n/a	n/a	1.08	1.49	0.00%	0.031%

* No retention time variation seen for replicate injections measured to three decimal places

Conclusion

The chromatographic data shown demonstrates the advantages of scaling a method from a 4 µm solid core column to the Accucore Vanquish 1.5 µm UHPLC column. The separation of ibuprofen and its impurity was achieved within the required criteria using the Accucore XL column in 2.5 minutes. By taking advantage of the performance of the Accucore Vanquish UHPLC column, in combination with the low internal volume and advanced capabilities of the Vanquish UHPLC, the method time was reduced to 1.5 minutes while maintaining the required performance criteria.

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