Application Note 21001

Rapid Separation of Eighteen Herbicides Using Advanced UHPLC and Sub-2 µm Solid Core Column Technologies

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Key Words

Accucore Vanquish, Vanquish UHPLC, pesticides, herbicides, rapid separation, high resolution

Abstract

This application shows the advantages of using the Thermo Scientific™ Accucore™ Vanquish™ C18 1.5 µm UHPLC column and the Vanquish UHPLC system for the separation of 18 herbicides. The advanced capabilities of the Vanquish UHPLC system allow the Accucore Vanquish columns to be operated at higher flow rates that enable development of rapid, high-performance analytical methods. Comparisons are made with other commercially available sub-2 µm solid core columns, demonstrating improved peak capacity and resolution.

Introduction

Environmental monitoring of herbicides used in the production of food crops is becoming more widespread due to their increased use and inevitable leaching from soil into water sources. The Accucore Vanquish UHPLC column and Vanquish UHPLC system allow for fast separation of complex samples at low levels, with improved chromatographic resolution.

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, which 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 Vanquish UHPLC systems were designed in combination to achieve the best possible chromatographic performance. The optimized fluidic flow path of the Vanquish UHPLC

system and the low dispersion Thermo Scientific™ Dionex™ Viper™ fingertight fitting capillaries allow the user to take full advantage of the separation power of the Accucore Vanquish UHPLC column. 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 Details

Consumables	Part Number
Accucore Vanquish C18 1.5 µm UHPLC column, 100 × 2.1 mm	17101-102130
Fisher Scientific™ HPLC grade water	10221712
Fisher Scientific HPLC grade acetonitrile	10616653
Thermo Scientific™ Virtuoso™ 9 mm wide opening, 2 mL screw thread vial and cap kit (recommended)	60180-VT400

Sample Preparation
Pesticide mix 18 obtained from Neochema (Bodenheim/Manz, Germany) containing the following pesticides at 10 µg/mL in acetonitrile: (1) desethylatrazine, (2) metoxuron, (3) hexazinone, (4) simazine, (5) cyanazine, (6) methabenzthiazuron, (7) chlorotoluron, (8) atrazine, (9) monolinuron, (10) diuron, (11) isoproturon, (12) metobromuron, (13) metazachlor, (14) sebuthylazin, (15) propazine, (16) terbuthylazine, (17) linuron, (18) metolachlor
Sample was diluted 1:1 with water to produce a working solution with a 5 µg/mL concentration

Instrumentation	Part Number
Vanquish UHPLC system consisting of:	
Binary pump H	VH-P10-A
Split sampler HT	VH-A10-A
Column compartment H	VH-C10-A
Diode array detector HL	VH-D10-A
Thermo Scientific™ Virtuoso™ Vial Identification System	60180-VT-100

Separation Conditions	
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Column temperature:	43 °C
Injection details:	0.5 µL
Detection:	UV at 230 nm (0.1 s rise time, 50 Hz, 8 nm slit width)
UHPLC Column 1:	Accucore Vanquish C18 1.5 µm, 100 × 2.1 mm
Flow rate:	0.65 mL/min

Time (min)	% A	% B
0.00	80	20
4.00	60	40
7.00	20	80
7.50	80	20
12.00	80	20

Table 1: Gradient conditions for the Accucore Vanquish 1.5 µm and competitor columns (i) and (ii)

UHPLC Column 2:	Solid core C18 2.6 µm, 100 x 2.1 mm
Flow Rate:	0.38 mL/min

Time (min)	% A	% B
0.00	80	20
6.93	60	40
12.13	20	80
13.00	80	20
20.80	80	20

Table 2: Gradient conditions for the 2.6 µm solid core column

Data Processing	
Software:	Thermo Scientific™ Dionex™ Chromeleon™ 7.2 Chromatography Data System (CDS)

Results

A comparative study of the Accucore Vanquish 1.5 µm and sub-2 µm competitor columns was carried out using the same herbicide mixture and chromatographic method with a flow rate of 0.65 mL/min and a two-stage binary gradient from 20% to 80% acetonitrile over 7 minutes. Baseline separation of 16 out of 18 compounds was achieved on all columns in less than 6 minutes (Figure 1).

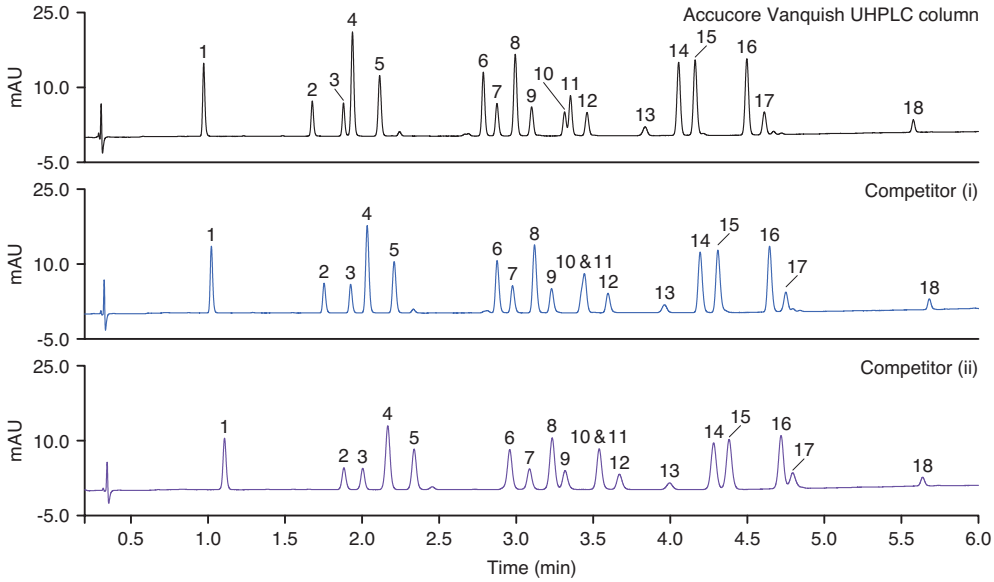

Figure 1: Chromatographic separation of eighteen herbicides on Accucore Vanquish UHPLC column (black), competitor (i) (blue) and competitor (ii) (purple) columns

The Accucore Vanquish UHPLC column demonstrated superior performance with average peak widths 21% and 60% lower (Figure 2) than competitor (i) and (ii), respectively. As a consequence peak capacity was 17% higher than competitor (i) and 37% higher than competitor (ii).

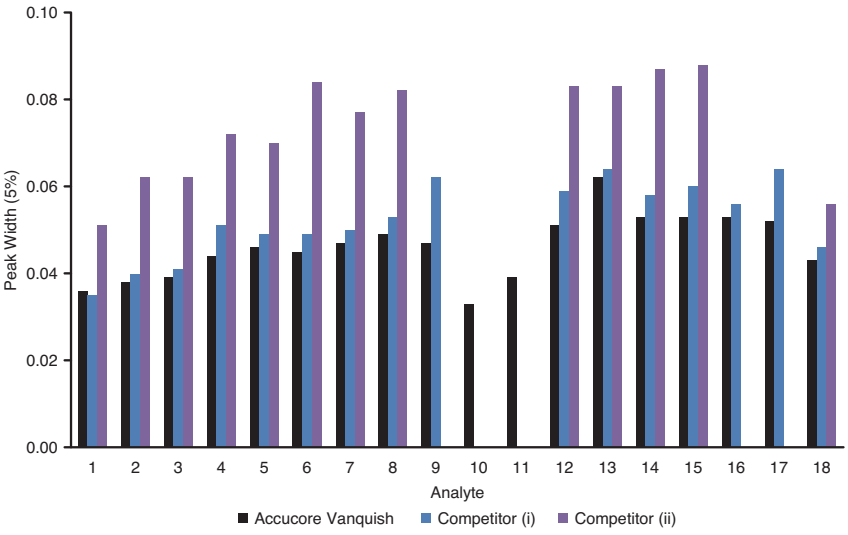

Figure 2: Comparison of peak widths for the Accucore Vanquish UHPLC column (black), competitor (i) (blue) and competitor (ii) (purple) columns. Peaks 10 and 11 coelute on competitor columns (i) and (ii).

Overall, average resolution on the Accucore Vanquish UHPLC column was 6% and 31% greater than that achieved by competitor (i) and (ii), respectively (Figure 3). Peak heights were 11% and 23% higher than competitor (i) and (ii), respectively (Figure 4). In addition, the Accucore Vanquish column was less retentive with average retention times being 3% and 5% lower than those on competitor (i) and (ii) (Figure 5).

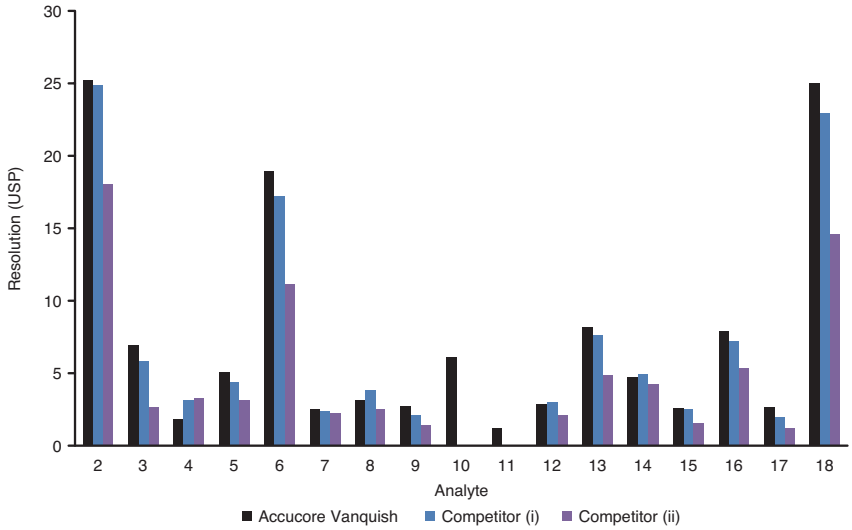

Figure 3: Comparison of resolution between the Accucore Vanquish UHPLC column (black), competitor (i) (blue) and competitor (ii) (purple) columns. Peaks 10 and 11 coelute on competitor columns (i) and (ii).

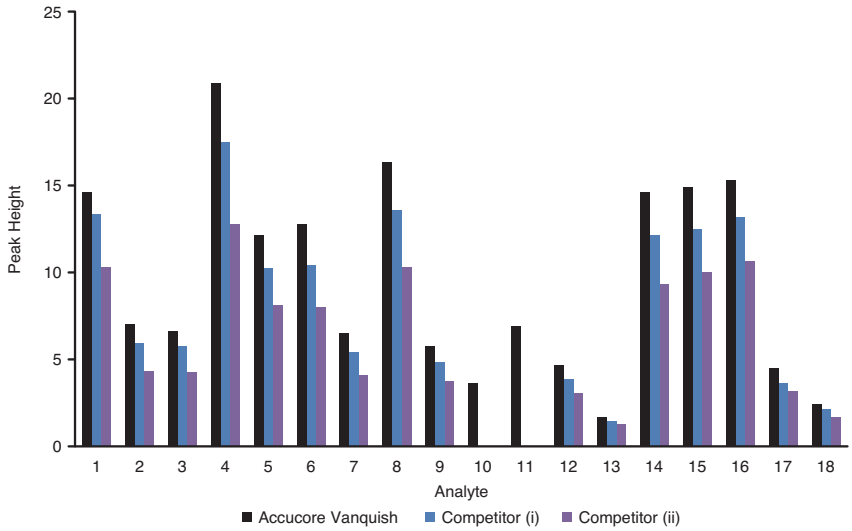

Figure 4: Comparison of peak height on the Accucore Vanquish UHPLC column (black), competitor (i) (blue) and competitor (ii) (purple) columns. Peaks 10 and 11 coelute on competitor columns (i) and (ii).

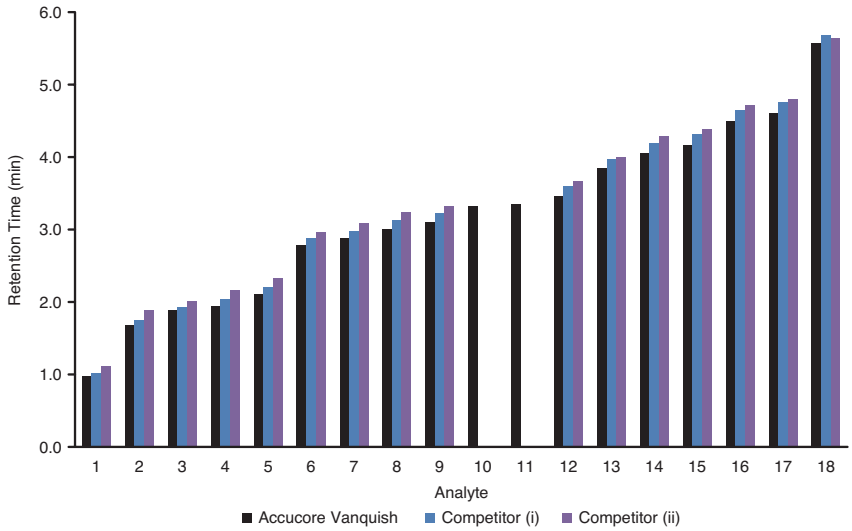

Figure 5: Comparison of retention times on the Accucore Vanquish UHPLC column (black), competitor (i) (blue) and competitor (ii) (purple) columns. Peaks 10 and 11 coelute on competitor columns (i) and (ii).

The chromatographic data shown in Figure 6 and subsequent figures demonstrate the advantages of scaling a method from a 2.6 µm solid core column to the Accucore Vanquish 1.5 µm UHPLC column. The separation of 18 herbicides was achieved 40% faster on the Accucore Vanquish 1.5 µm UHPLC column used in conjunction with the Vanquish UHPLC system.

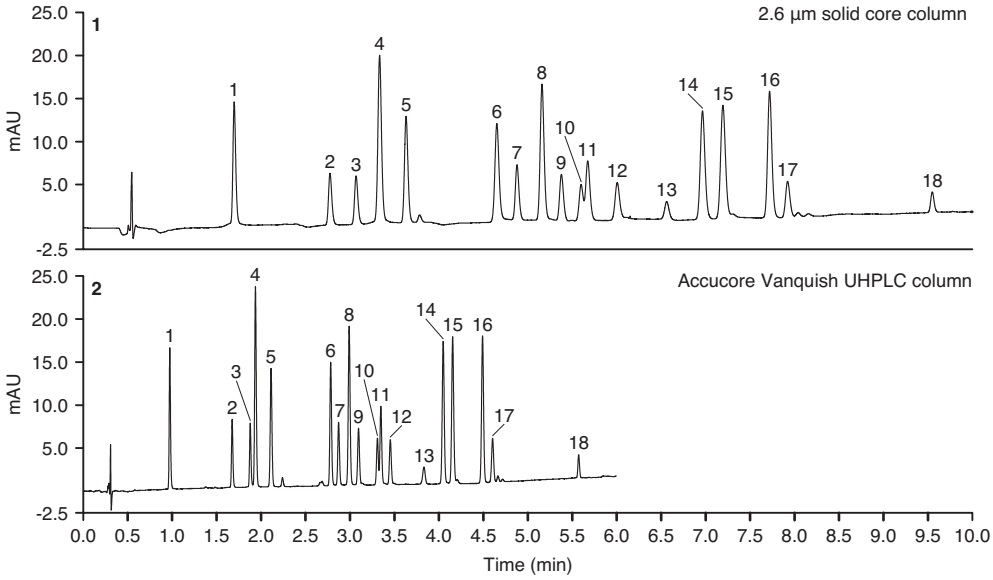

Figure 6: Chromatographic separation of 18 herbicides on a 2.6 µm solid core column (top) and the Accucore Vanquish UHPLC column (bottom)

In addition, peak widths were reduced by 56%, peak heights were improved by 17% (Figure 7), peak capacity was improved by 31%, and, as a result, average resolution was improved by 24% (Figure 8).

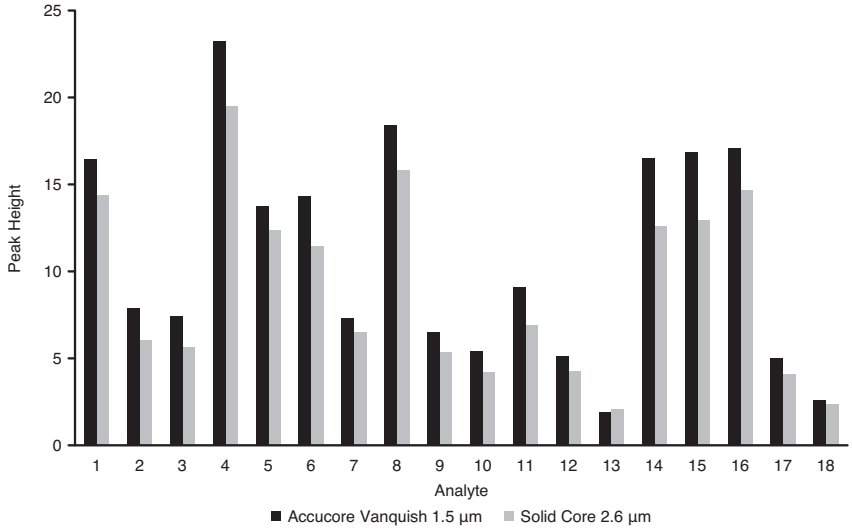

Figure 7: Comparison of peak height between the Accucore Vanquish UHPLC column and a 2.6 µm solid core column

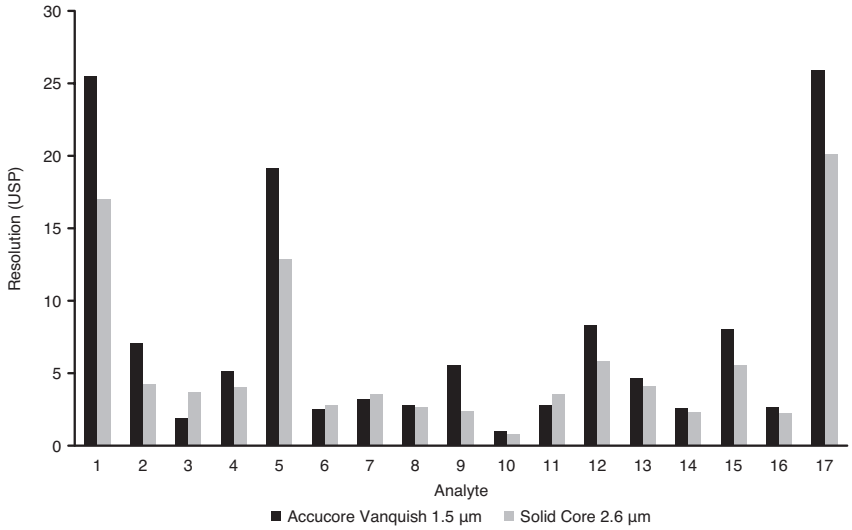

Figure 8: Comparison of resolution between the Accucore Vanquish UHPLC column and a 2.6 µm solid core column

Conclusion

This application demonstrates the advantages of using the Accucore Vanquish C18 1.5 µm UHPLC column and Vanquish UHPLC system for the analysis of herbicides. The solution delivers separation of 18 herbicides in less than 6 minutes.

When compared to competitor sub-2 µm solid core columns it delivers:

- Greater peak capacity
- Increased resolution
- Increased sensitivity

When compared to a solid core 2.6 µm particle packed column it delivers:

- Reduced analysis time
- Increased resolution
- Improved sensitivity
- Greater peak capacity

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