Solid Core 4 µm Particles – High Peak Capacity for Complex Samples

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Key Words
Accucore XL, solid core, superficially porous, peak capacity

Abstract
Using green tea extract as a complex sample, separation of multiple compounds was performed to demonstrate peak capacity on the Thermo Scientific™ Accucore™ XL HPLC column. The benefits of shortening the Accucore XL HPLC column length to maximize the number of peaks per minute through peak capacity to analysis time ratio was shown.

Introduction

The ultimate goal in chromatography is to fully resolve all the compounds within a sample in the shortest possible time with the instrument at hand. Therefore it is important to maximize the efficiency of a separation. The separation can be measured through peak capacity or the peak capacity to analysis time ratio. Maximizing the efficiency can be achieved by improving separation properties of a packed column through the use of superficially porous or solid core particles. Also, as demonstrated in this technical note, peak capacity can be maximized through the use of longer columns or column chains while staying within the pressure restrictions of an instrument.

Based on Core Enhanced Technology™ using 4 µm solid core particles, Accucore XL HPLC columns allow users of conventional HPLC methods to enjoy performance far beyond that of columns packed with 5 µm or even 3 µm fully porous particles. Very high separation efficiencies using standard HPLC instruments and conditions provide increased peak resolution and lower limits of detection. An ultra-stable packed bed results in exceptionally robust columns that demonstrate excellent retention and response reproducibility. In addition, higher flow rates can be achieved without significantly affecting the separation efficiency, which means that faster separations can be performed without compromising performance.

Assessment of Peak Capacity for Gradient Separations

Peak capacity is a broad measure of the separation performance of a column. For gradient separations, peak capacity is calculated using equation 1 [1].

Equation 1

$$n_c = 1 + \left(\frac{t_g}{w}\right)$$

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When total peak capacity (n_c) is calculated based on a gradient elution, t_g is total gradient time and $\overline{w}$ is the average peak width. In this case peak width is measured at the baseline of the peak.

As shown in Equation 1, peak capacity is influenced by peak width, which is directly related to efficiency. Solid core particles maximize efficiency and therefore, under the conditions used for this application, peak capacity by reducing the degree of eddy and longitudinal diffusion through the column [2]. This means that Accucore XL HPLC columns exhibit greater peak capacities compared to columns packed with fully porous particles of a similar size.

The separation of highly complex samples is a major challenge in chromatography and the properties of the Accucore XL HPLC columns provide a means of providing highly efficient separations in a short analysis time. The separation properties, including assessment of efficiency through peak capacity of an Accucore XL HPLC column were demonstrated through the analysis of a complex mixture of a green tea extract. In addition, the peak capacity to analysis time ratio was calculated to show the benefits of shortening the column length on an Accucore XL HPLC column. This provides improvements in analysis times with some sacrifice in resolution and peak capacity and presents the user with a choice between maximizing peak capacity or reducing their analysis times for their separation.

Experimental Conditions

Sample Preparation

Green tea extract was removed from a 315 mg capsule and dissolved in 5 mL methanol / water (50:50 v/v). The sample was vortexed and centrifuged at 14,000 rpm for 10 minutes to remove insoluble particulates and also maximize the number of analytes in the sample for analysis. The supernatant was transferred and diluted 1:5 in mobile phase A for injection onto the HPLC.

Columns	Part Number
Accucore XL C18 4 µm, 150 × 2.1 mm	74104-152130
Accucore XL C18 4 µm, 300 × 2.1 mm	
Accucore XL C18 4 µm, 450 × 2.1 mm	

Separation Conditions	
Instrumentation:	Thermo Scientific™ Dionex™ UltiMate™ 3000 HPLC system
Column temperature:	30 °C
Injection volume:	2 µL (partial loop)
Flow rate:	0.3 mL/min
UV detection:	254 nm (data rate 20 Hz)
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Gradient conditions:	Dependent on column length (Table 1a-c)

Results and Discussion

Using a gradient composed of 0.1% formic acid in methanol and water in conjunction with the Accucore XL HPLC column, separation of a complex green tea extract was performed. Column lengths of 450 mm, 300 mm, and 150 mm were examined to investigate the effect of column length on peak capacity, as well as peak capacity to analysis time ratio. The gradient timetables were adjusted to keep the % B change per unit column length constant. Therefore, a similar separation of the compounds on the different column lengths based on the critical pair was achieved. The gradient timetables for three column lengths are shown in Tables 1a-c.

(a) Accucore XL 4 µm, 150 × 2.1 mm

Gradient Timetable	
Time	% B
0.0	5
0.5	5
13.0	75
15.0	75
15.1	5
20.0	5

(b) Accucore XL 4 µm, 300 × 2.1 mm

Gradient Timetable	
Time	% B
0.0	5
1.0	5
26.0	75
30.0	75
30.2	5
40.0	5

(c) Accucore XL 4 µm, 450 × 2.1 mm

Gradient Timetable	
Time	% B
0.0	5
1.5	5
39.0	75
45.0	75
45.3	5
60.0	5

Tables 1a-c: Gradient timetables for Accucore XL HPLC column lengths of 150 mm (a), 300 mm (b), and 450 mm (c)

The lower backpressures generated on the Accucore XL HPLC column allowed column lengths of up to 450 mm to be used to maximize peak capacity with backpressures ≤600 bar. This means, if necessary, for complex samples highly efficient separations can be performed within the pressure limits of conventional HPLC instrumentation.

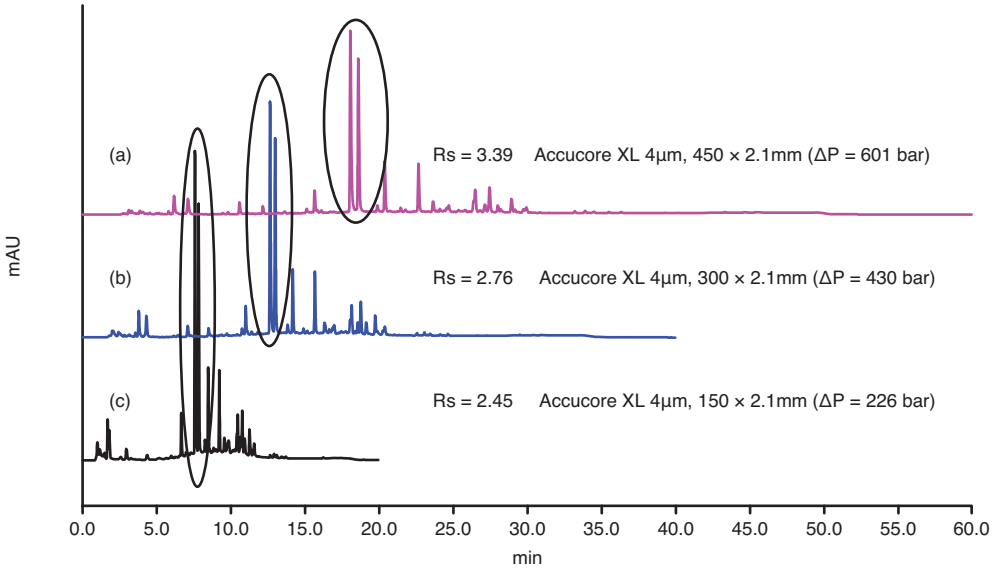

Figure 1: Overlaid chromatograms for the separation of green tea extract on Accucore XL HPLC column lengths 150 mm (a), 300 mm (b), and 450 mm (c). The critical pair is circled on each chromatogram and the resolution for each critical pair is indicated. There is low resolution for the early eluters on the 150 mm column (c), as the focus of the gradient is based on the critical pair.

The Accucore XL HPLC column is shown to offer the analyst a choice of either maximizing peak capacity or sample throughput. Resolution and peak capacity can be maximized using the longer 450 mm column, as seen in Figure 1 and a summary of the peak capacities achieved on each column is seen in Table 2. However, by shortening the column length of the Accucore XL HPLC column, the number of detectable peaks per minute (peak capacity to analysis time ratio) increased by up to 50%, as seen in Figure 2, with only a 28% loss in resolution of the critical pair. This means that sample throughput can be increased through a reduced analysis time without significantly affecting the quality of the data produced based on the critical pair.

Column Length (mm)	Run Time (mins)	Peak Capacity
150	20	167
300	40	280
450	60	336

Table 2: Summary of the peak capacities achieved on the different Accucore XL HPLC column lengths

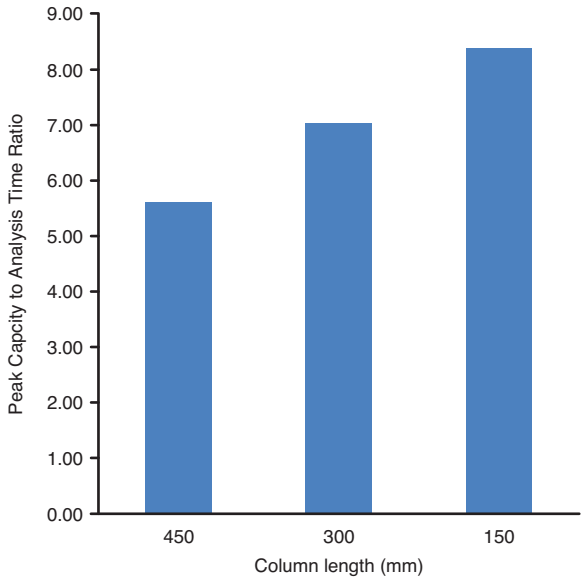

Figure 2: The number of peaks per minute increases by up to 50% as column length decreases by 67%. Therefore, shorter analysis times with good resolution can be achieved using the shorter column.

Statistical examination of the critical pair on the different column lengths, seen in Tables 3a-c, show that the data is matched with excellent precision. Run-to-run reproducibility is of particular importance when analyzing complex mixtures, as it enables the examination of batch-to-batch differences between complex samples. In addition, the selectivity between the critical pair does not change with decreasing column length, which indicates that the separation remains reproducible for the analysis of this complex sample on the different Accucore XL HPLC column lengths.

(a) 450 mm

	Peak 1 RT (min)	Peak 2 RT (min)	Resolution	Selectivity
Rep 1	18.06	18.59	3.35	1.03
Rep 2	18.05	18.58	3.40	1.03
Rep 3	18.07	18.61	3.42	1.03
Average	18.06	18.59	3.39	1.03
%RSD	0.05	0.06	1.06	0.02

(b) 300 mm

	Peak 1 RT (min)	Peak 2 RT (min)	Resolution	Selectivity
Rep 1	12.62	12.97	2.83	1.03
Rep 2	12.65	13.00	2.72	1.03
Rep 3	12.64	12.99	2.73	1.03
Average	12.64	12.99	2.76	1.03
%RSD	0.16	0.09	2.20	0.06

(c) 150 mm

	Peak 1 RT (min)	Peak 2 RT (min)	Resolution	Selectivity
Rep 1	7.58	7.82	2.45	1.03
Rep 2	7.58	7.83	2.45	1.03
Rep 3	7.58	7.82	2.45	1.03
Average	7.58	7.83	2.45	1.03
%RSD	0.03	0.04	0.00	0.01

Tables 3a-c: Statistical examination of the critical pair detected in the complex mixture for Accucore XL HPLC column lengths of 450 mm (a), 300 mm (b) and 150 mm (c)

Conclusion

The high efficiency of the Accucore XL HPLC column has been demonstrated. It has been shown longer Accucore XL HPLC columns provide the greatest separation and peak capacity. However, separation of a complex mixture can be maintained when decreasing the column length to improve analysis times. The shortest Accucore XL HPLC column was shown to provide the greatest number of peaks per minute without compromising the separation.

Therefore, the high resolution offered by the Accucore XL HPLC column can be used to improve complex separations through an increase in peak capacity to analysis time ratio, which makes it an ideal candidate for improving the overall performance of a separation.

References

[1] Giddings J. C. Maximum Number of Components Resolvable by Gel Filtration and Other Elution Chromatographic Methods. *Anal.* Chem. **1967**, *39*, 1027.

[2] Gritti F. The Mass Transfer Mechanism of Columns Packed With sub-3 µm Shell Particles and its Reproducibility for Low- and High-Molecular Weight Compounds. *Chromatography Today* **2012**, *5*(2), 4-11.

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