Maximizing peak capacity with UHPLC

Siji Joseph [(1)], Syed Lateef [(1)], Edgar Naegele [(2)], Christian Gotenfels [(2)]

[(1)] Agilent technologies , Bangalore, India
[(2)]Agilent Technologies, Waldbronn, Germany

HPLC 2011
P1-G-014-TU

Agilent Technologies

Introduction

The most commonly used measure for the performance of a chromatographic separation is plate counts. This value mainly depends on the particle size, column length and the packing quality of the column. However, plate counts are not an effective indicator of gradient separations. Peak capacity is a performance measure that describes the number of peaks that can be separated during a gradient run with a certain resolution and is a parameter used to evaluate the performance of a chromatographic separation. Higher peak capacity values are important for the separation of complex samples with an unknown number and variety of analytes.

In this study, the effect of changing gradient parameters, such as flow rate, gradient time, column length and slope of the gradient, on peak capacity are measured and discussed in the context of current literature. Agilent ZORBAX Eclipse Plus columns with an inner diameter of 2.1 mm, 1.8 µm particle size and various column lengths (50 mm, 100 mm and 150 mm) were used for this evaluation. An Agilent 1290 Infinity LC was used for the study because of its broad flow rate and pressure-range.

Experimental

The study was performed using a typical standard spike mix of small molecules. The individual components of the sample were 1) uracil, 2)phenol, 3) methyl paraben, 4) ethyl paraben, 5) propyl paraben, 6) N, N-diethyl-m-toluamide 7) butyl paraben, 8) heptyl paraben and 9) toluene. In this study, the peak width is uniform throughout the gradient chromatogram and as a mid-eluting peak, the peak width of N, N-diethyl-m-toluamide was very close to the observed average peak width value. Therefore, the peak width measured at 4σ of N, N-diethyl m- toluamide was selected for the peak capacity calculation.

Instrument configuration

Agilent 1290 Infinity LC controlled by ChemStation (Version B.04.02) and equipped with

- ➢Agilent 1290 Infinity Binary Pump with integrated vacuum degasser
- ➢Agilent 1290 Infinity Autosampler
- ➢Agilent 1290 Infinity Thermostatted Column Compartment
- ➢ Agilent 1290 Infinity Diode Array Detector with 10 mm flow cell.

Columns

Agilent ZORBAX Eclipse Plus C18, 50 mm 2.1 mm, 1.8 µm
Agilent ZORBAX Eclipse Plus C18, 100 mm 2.1 mm, 1.8 µm
Agilent ZORBAX Eclipse Plus C18, 150 mm 2.1 mm, 1.8 µm

LC Parameters

Parameter	Details	
Mobile phase A	0.1% Formic acid in water	
Mobile phase B	100% Acetonitrile	
Flow rate	Variable (0.2mL/min, 0.4mL/min, 0.6mL/min, 0.8 mL/min, 1.0 mL/min, 1.2 mL/min,1.4 mL/min, 1.6 mL/min, 1.8 mL/min or 2.0 mL/min.	
Needle wash	Flush port activated for 3 seconds using mobile phase B	
Detection	254/4nm; Reference off	
Post run time	3 minutes	
sampling acquisition rate	80 Hz	
Gradient	%B	Time (min)
	20 to 100	Variable (5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 min)

Figure 1: Agilent 1290 Infinity LC system

Experimental

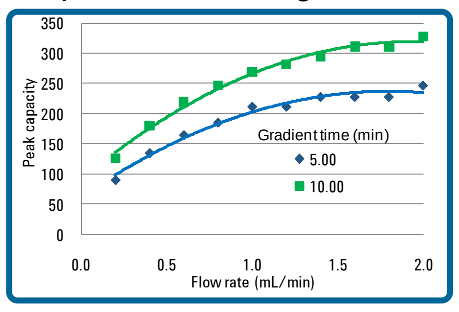
Procedure

Samples were injected and separated using 10 different gradients (5 min to 50 min). Each gradient was run with 10 different flow rates. An Agilent ZORBAX Eclipse Plus 50 mm × 2.1 mm, 1.8 µm column was used for a flow rate range of 0.2 to 2.0 mL/min (10 different trials), a 100 mm × 2.1 mm, 1.8 µm column was used up to1.4 mL/min (seven trials) and a 150 mm x 2.1 mm, 1.8 µm column was used up to 1.0 mL/min (five different trials). The use of higher flow rates was restricted by a high backpressure limit of 1200 bar when longer 1.8 µm columns were used. 4σ peak width for N,N-diethyl-m-toluamide was measured for each gradient and used for peak capacity calculation.

Results and Discussion

Effect of flow rate on peak capacity

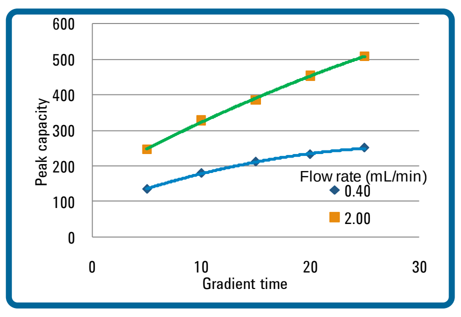
The analyte mix was first separated on Agilent ZORBAX Eclipse Plus 50 mm × 2.1 mm, 1.8 µm column using several flow rates. The peak capacity values are smaller for lower flow rates and increases dramatically with higher flow rates, because peak widths decrease with increasing flow rates.



Effect of gradient time on peak capacity

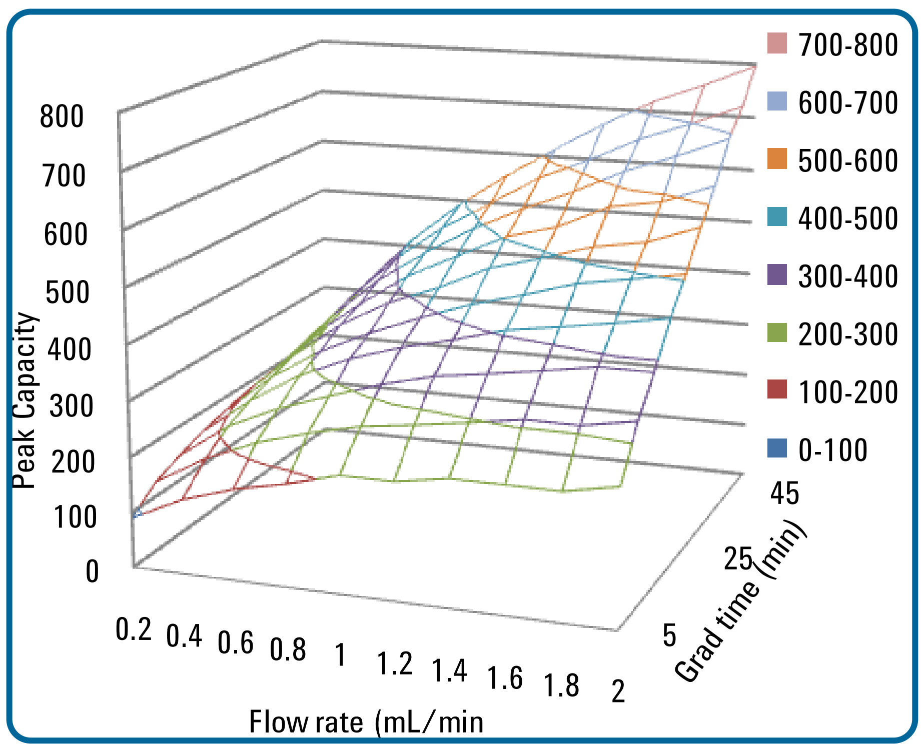
Peak capacity versus gradient time was determined for different flow rates on a ZORBAX Eclipse Plus 50 mm × 2.1 mm column with 1.8 µm particles.

The results illustrate that a 2.0 mL/min flow rate gave a peak capacity value of about 250 when using a 5-min gradient while the peak capacity value was 500 when the gradient time was increased to 25 min.



Increased peak capacity with an increase in the gradient time for a selected flow rate using sub-2-µm columns are consistent with existing literature.

Combined effect of gradient time and flow rate
On peak capacity



Results show that the highest peak capacity values are observed for the higher flow rates for each examined gradient time. Typical gradients for 50 mm 2.1 mm, 1.8 µm columns are about 5 or 10 minutes long and peak capacities of about 250 and 330 can be achieved, respectively.

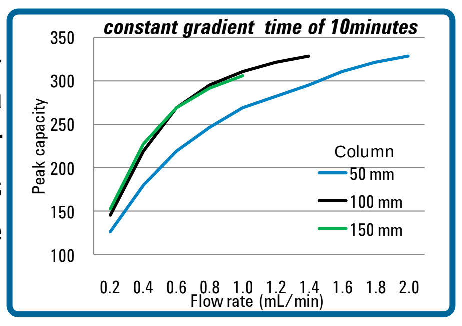
Peak capacity values are higher using a shorter gradient with higher flow rates than a longer gradient at lower flow rates for the given gradient volume. For example, the peak capacity value for a 50-min gradient at 0.2 mL/min flow rate was 211, whereas for a 5-min gradient with a 2.0 mL/min flow rate the value was 246 (~17% more). ***This shows that 45 minutes can be saved for a particular analysis with the same or even better peak capacity performance.*** At 2 mL/min flow rate ~1200 bar back pressure was observed. The Agilent 1290 Infinity LC can support column backpressures of up to 1200 bars.

Results and Discussion

Effect of column length on peak capacity

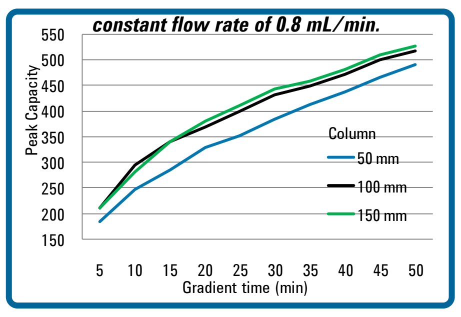
Experimental data were collected for N, N-diethyl-m-toluamide at various flow rates and gradient times on 50 mm, 100 mm and 150 mm 2.1 mm, 1.8 µm ZORBAX Eclipse Plus columns. Peak capacity increases with an increase in column length. However at low flow rates (for example, 0.2 mL/min), peak capacity using 150-mm column for a short gradient time (10 min) are marginally better than for a 50 mm column.

At a flow rate of 2.0 mL/min, the 50-mm column delivers a peak capacity of about 330 for a short 10-min gradient. This value is comparable to the value observed on100-mm column at 1.4 mL/min.



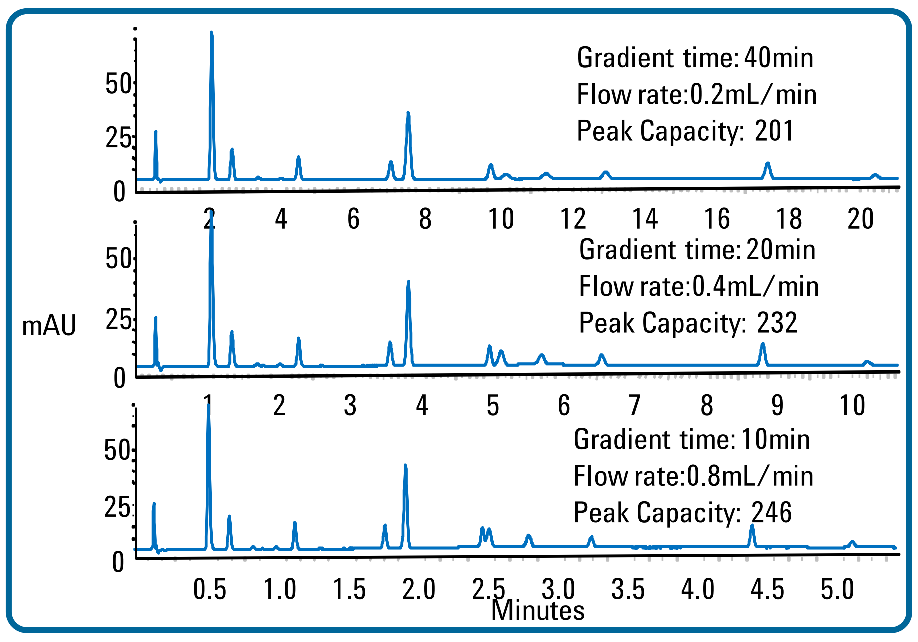
The longer column delivers higher peak capacities with longer gradient times. Peak capacity values achieved on a long column can be reproduced using a short column by adjusting the gradient time and flow rates.

Within the given pressure limit of 1200 bar, a 50-mm column delivers higher peak capacity values because it can permit higher flow rates when compared to longer columns of length 100 and 150 mm.



Peak capacity among gradients with same slope

For a given gradient slope, the peak capacity value is higher for those gradients with higher flow rates. The following figure shows that a gradient with the same slope and similar selectivity is achieved for a given separation within only a tenth of the time if the flow rate is increased by a factor of ten. The Agilent 1290 Infinity LC, with pressure range up to 1200 bar makes this possible.



Conclusions

- ➢Higher peak capacities for a gradient separation can be achieved by considering a) number of peaks and required peak capacity, b) column, c) gradient length, d) flow rate according to system and column, e) pressure limitations.
- ➢The results presented here show how to maximize the peak capacity and improve the chromatographic separation performance by varying the method parameters.
- ➢In general, peak capacity value increases with gradient time and flow rate.
- ➢The highest separation efficiency is achieved by increasing the flow rate with the Agilent 1290 Infinity LC, which can go up to 1200 bar while providing the highest possible flow rate for smaller particle sized columns.
- ➢The Agilent 1290 Infinity LC enables a shorter run time, while maintaining the gradient and providing the same selectivity.