Agilent 1290 Infinity LC System – Applications requiring the Agilent Ultra-Low Dispersion Kit

Technical Overview

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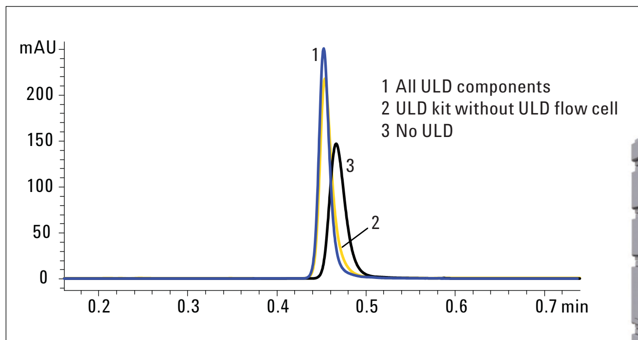

Abstract

Improvements with respect to peak width, peak capacity, and plate number were evaluated using the Agilent Ultra-Low Dispersion Capillary Kit and the Agilent Ultra-Low Dispersion Flow Cell for the Agilent 1290 Infinity LC System. Based on the experimental results presented in this Technical Overview, we recommend using the Ultra-Low Dispersion (ULD) Kit for short runs with 50-mm columns, especially for columns with 2.1-mm and 3-mm id. For longer runs with 2.1-mm and 3-mm id columns, the improvements using the ULD kit were negligible. However, for 100-mm and 150-mm × 4.6-mm columns, the increased pressure, for example, due to smaller capillary ids, exceeds the limit of 600 bar for sub-2 µm 4.6-mm id columns when used with gradient runs. Therefore, the ULD kit is not compatible with these columns or for long gradient or low-organic isocratic runs.

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Introduction

Extra-column volume leads to dispersion and therefore increased peak widths and lower peak heights. Depending on the columns used, the effect can be observed in different degrees. The extra-column volume is defined by the International Union of Pure and Applied Chemistry (IUPAC) as the volume between the effective injection point and the effective detection point, excluding the part of the column containing the stationary phase. It is composed of the volumes of the injector, connecting lines, and detector. Equation 1 displays the ratio of the extra-column dispersion (σ_{col}) and the column dispersion (σ_{ex}) to the total dispersion (σ_{tot}).

$$\sigma_{tot} = (\sigma_{col}^{2} + \sigma_{ex}^{2})^{0.5} \qquad \text{Equation 1}$$

Extra-column dispersion is especially critical for low id columns, because it accounts for a higher percentage of the total extra-column volume of the system. The Ultralow Dispersion (ULD) Capillary Kit in combination with the Agilent Max-Light Ultra-low Dispersion Cartridge Flow Cell reduces the extra-column volume of the LC system to a minimum using 75-µm capillaries and a ULD flow cell with V(σ) = 0.6 µL instead of V(σ) = 1 µL.

This Technical Overview shows the effect of low dispersion components on factors such as peak width and resulting calculations of peak capacities and plate numbers for different common column ids and lengths (4.6, 3, and 2.1 mm × 50, 100, and 150 mm) with the Agilent Eclipse Plus stationary phase for isocratic and gradient runs. The results of these experiments, provide recommendations as to which applications require the ULD components for better chromatographic results.

Experimental

Instruments

The Agilent 1290 Infinity LC System consisted of the following modules:

- Agilent 1290 Infinity Binary Pump (G4220A) with 35-µL Jet Weaver
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostat (G1330B)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector (G4212A), equipped with a standard 10-mm flow cell
- Agilent Ultra-Low Dispersion Capillary Kit (5067-5189)
- Agilent Max-Light Ultra-Low Dispersion Cartridge Flow Cell V(σ) = 0.6 µL, 10 mm (G4212-60038)

Columns

- Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 100 mm and 50 mm, 1.8 µm
- Agilent ZORBAX RRHD Eclipse Plus C18, 3 × 150 mm, 100 mm and 50 mm, 1.8 µm
- Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 100 mm and 50 mm, 1.8 µm

Software

Agilent OpenLAB CDS ChemStation Edition for LC & LC/MS Systems, Rev. C.01.03 [32]

Solvents and samples

Solvents: A = H_2O_{dd}
B = acetonitrile

For isocratic runs: 70% acetonitrile and 30% H_2O_{dd}

Samples: RRLC Checkout Sample (p/n 5188-6529) including acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, benzophenone, and acetanilide

All solvents used were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak).

Chromatographic conditions

	Time (min)	% B
Gradient	0	30
	x	95
50 mm	2 and 5	
100 mm	10	
150 mm	20	
Post time	10	
Isocratic with 70% B		
50 mm	2	
100 mm	3.30	
150 mm	5	
Flow rate		
4.6 mm	2.4 mL/min	
3 mm	1 mL/min	
2.1 mm	0.5 mL/min	
Injection volume		
4.6 mm	2 µL	
3 mm	0.9 µL	
2.1 mm	0.4 µL	
Thermostat	4 °C	
Column temperature	50 °C	
UV	245 nm/10 nm Ref.: 400 nm/100 nm	
Peak width	0.0063 min (0.13 s response time) (40 Hz)	

Results and discussion

The reduction of the extra-column volume by using the ULD components was evaluated with the RRLC standard sample consisting of a mix of nine phenones (see Figure 1).

The effect of the ULD capillary kit (including capillaries, ULD needle seat and ULD heat exchanger) is displayed in Figure 2 for the first peak, demonstrating the effective reduction of peak width, which results in sharper and more intense peaks. The use of the ULD flow cell increased this effect.

Peak width reduction, due to the use of the ULD components (both capillary set and flow cell), was significant especially for the first peaks in a fast 2-minute gradient using a 2.1 × 50-mm column as shown in Figure 3. The mean peak widths at 5σ were used for all graphics and calculations of peak capacity for gradient runs.

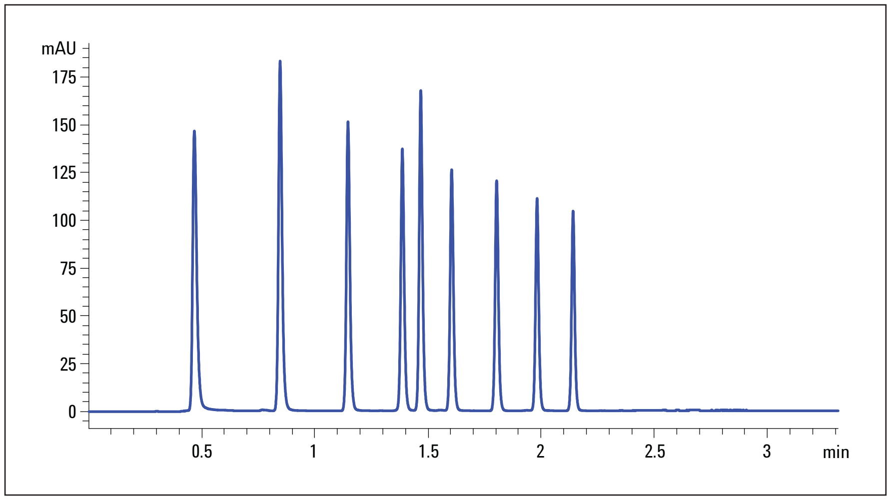

Figure 1
RRLC sample.

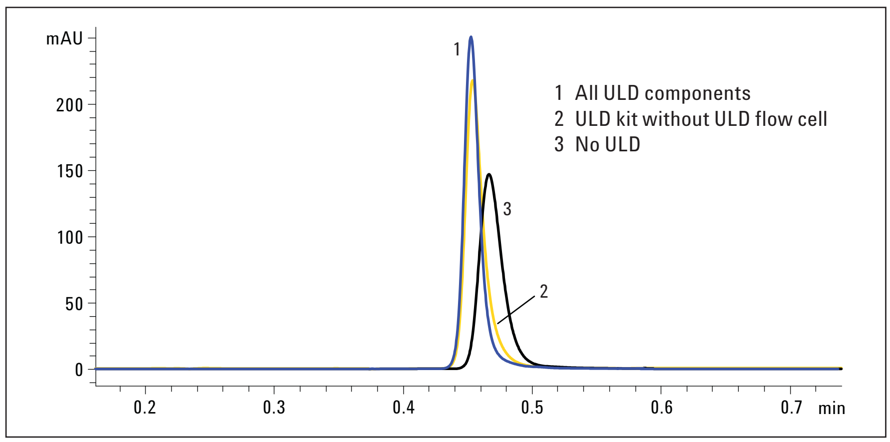

Figure 2
Overlay peak 1 showing the advantages of the ULD components for 2.1 × 50 mm, 1.8 µm with a 2-minute gradient.

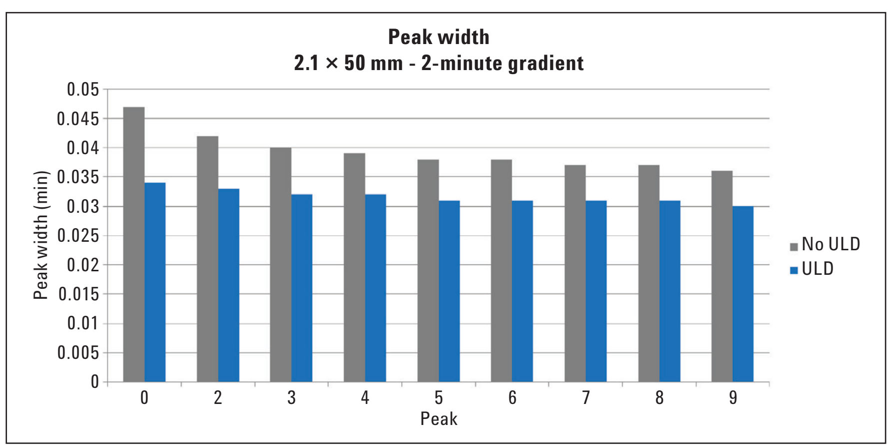

Figure 3
Comparison of peak widths for 2.1 × 50-mm column with a 2-minute gradient with and without ULD components.

Peak capacity n was calculated using Equation 2 (t_p = time from first to last peak, $w_{5\sigma}$ = mean peak width at 5σ).

$$n = \frac{t_p}{w_{5\sigma}} \qquad \text{Equation 2}$$

Figure 4 shows the increase in peak capacity with the use of the ULD components compared to the standard capillaries and flow cell. Note that an improvement of 25% in peak capacity can be achieved with the ULD components for short 2-minute gradients with 2.1-mm id columns and 50-mm length.

Using the ULD components, the peak width was reduced, leading to sharper and more intense peaks. However, depending on the application, the ULD flow cell with an internal volume of 0.6 µL might show increased noise values due to the reduced diameter. Figure 5 shows the signal-to-noise (S/N) ratios of all nine peaks of the short 2-minute gradient with 2.1 x 50 mm columns. The improvement of S/N, as seen for the first peak is not consistent for the residual eight peaks. The increase in peak height due to reduced peak widths with the ULD components does not compensate the increase in noise of the ULD flow cell for all peaks.

Figure 6 shows an overview of the calculated peak capacities of 2.1-mm columns of different lengths for gradient runs. The shorter the run (and the shorter the column), the bigger the ULD effect resulting in increased peak capacity especially for the 2-minute runs with the 2.1 × 50-mm column.

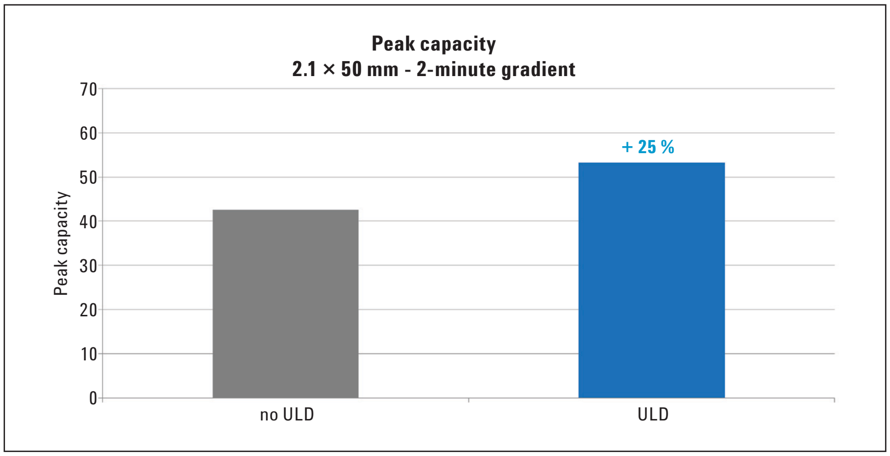

Figure 4
Peak capacity for 2.1 × 50-mm columns with 2-minute gradients.

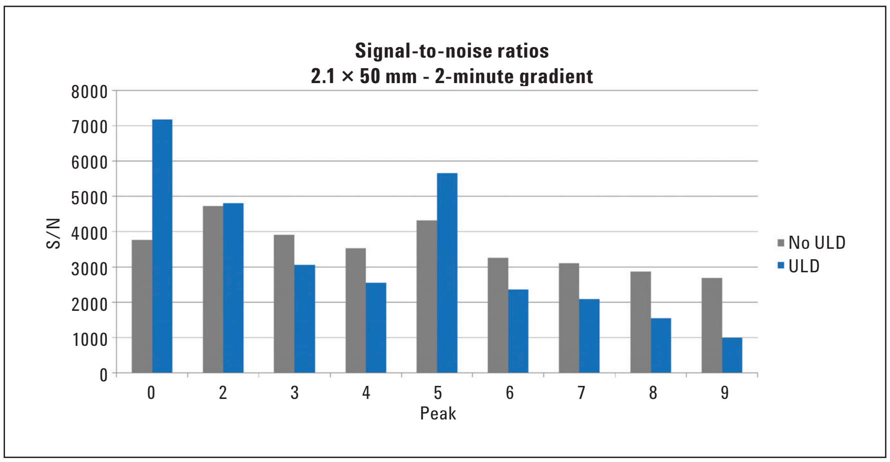

Figure 5
Signal-to-noise ratios of the RRLC sample using 2.1 × 50-mm column with a 2-minute gradient.

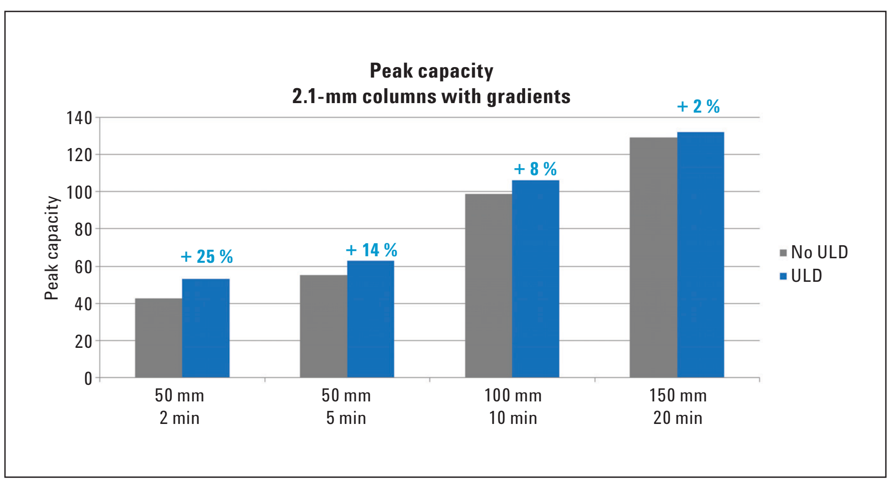

Figure 6
Peak capacity 2.1-mm columns.

This effect is evident with short gradients using 50-mm length columns with 3-mm id, but decreases strongly for longer gradients to 0 % improvement for 150-mm columns with 20-minute gradients (see Figure 7).

For short runs with 4.6-mm id columns, a small improvement is still shown (Figure 8). However, the gradients runs with 100-mm and 150-mm columns could not be conducted due to the 600-bar pressure limit of the column. Generally, 4.6-mm id columns with a particle size of sub-2 µm are limited to a pressure of 600 bar. With the implementation of the ULD components, the pressure increased more than double especially with the high flow rates used with the 4.6-mm id columns (see Figure 9).

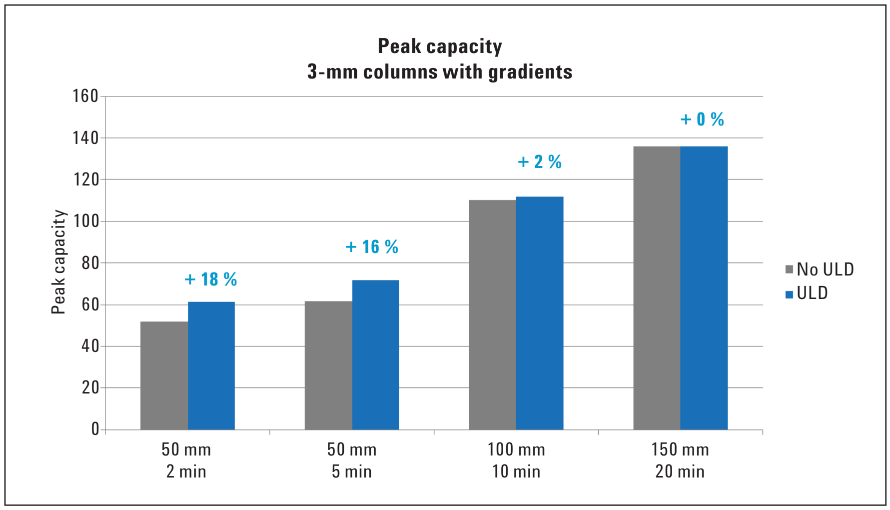

Figure 7
Peak capacity 3-mm columns.

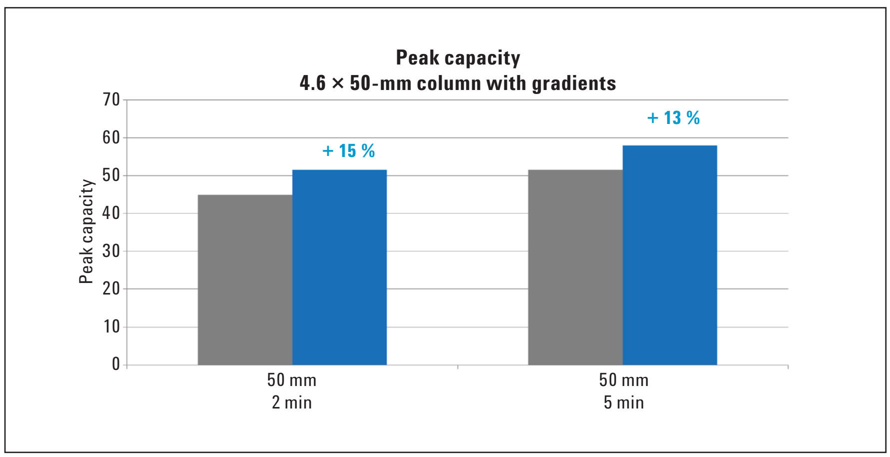

Figure 8
Peak capacity 4.6 × 50-mm columns.

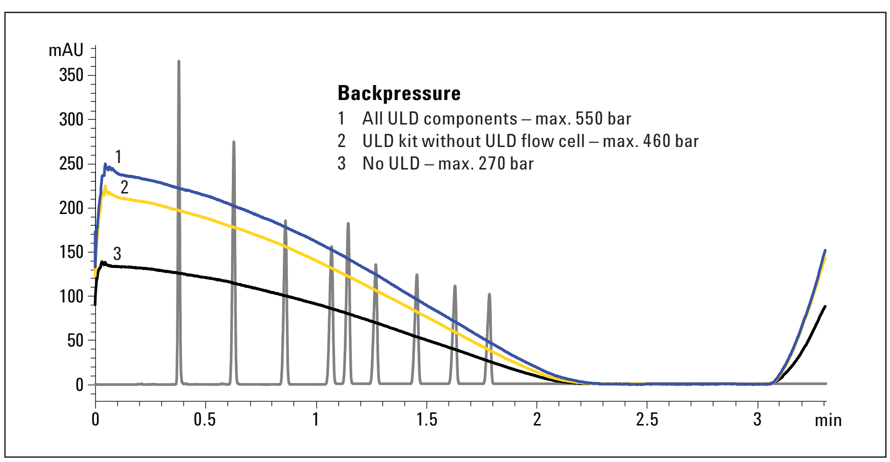

Figure 9
Increase in backpressure using the ULD components - 4.6 × 50-mm column with gradient.

For the evaluation of isocratic runs, the plate number N was calculated according to Equation 3 (RT = retention time, w_{50} = peak width at half height) and averaged over all nine peaks.

$$N = 5.54 * \left(\frac{RT}{w_{50}}\right)^2 \qquad \text{Equation 3}$$

For isocratic runs, the improvement of the calculated plate number was prominent for the 2.1-mm id columns, especially for the short runs, with 50-mm id columns (Figure 10). For short isocratic runs, use of 3-mm id columns produced an improvement of 30%. For 100-mm and 150-mm, 3-mm id columns, and for all 4.6-mm id columns, no significant ULD effect regarding peak width and plate number was observed.

Figure 11 shows an overview of 4.6-mm and 2.1-mm id columns regarding plate number in isocratic runs. Note that larger id columns typically lead to higher plate numbers compared to smaller id columns for any given extra-column volume, whether the volume reduced using the ULD kit or not. Therefore, for highest separation performance, larger id columns are preferred.

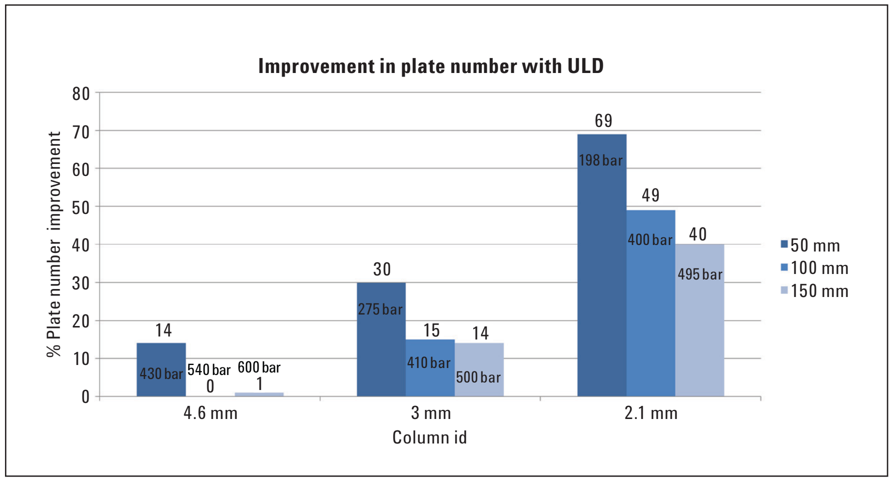

Figure 10
Improvement plate numbers isocratic runs with ULD.

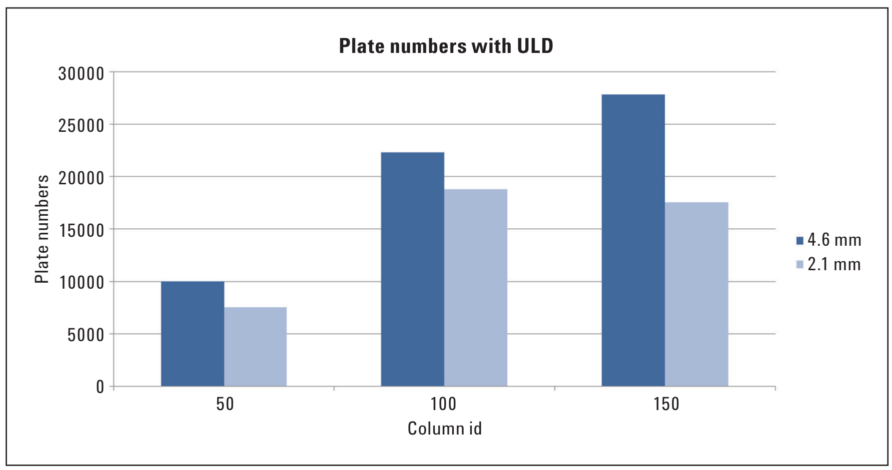

Figure 11
Plate numbers from isocratic runs with ULD.

Conclusion

The effects of the Ultra-Low Dispersion Kit (both capillaries and flow cell) were evaluated with respect to peak width, and as a result, plate numbers and peak capacity. Figure 12 illustrates the recommendations for the use of the ULD kit, based on the experiments in this Technical Overview. The green color represents "highly recommended", green-hatched "highly recommend for isocratic runs", yellow "conditionally recommended", red "not recommended".

The Ultra-Low Dispersion Kit (both capillaries and flow cell) shows the best effects, regarding plate numbers and peak capacities, when used for short runs with 50-mm columns, especially for 2.1- and 3-mm id. For short 2.1-mm id gradient runs, the improvement in peak capacity was about 25%, whereas the improvement in plate number for 2.1-mm id columns was calculated as 69%. For longer isocratic runs using 2.1-mm id columns, the improvement in plate number was 49% for 100-mm and 40% for 150-mm column length. The price for this improvement is a slight loss in sensitivity due to increased noise of the ULD flow cell. The improvements due to the ULD kit are negligible for 2.1- and 3-mm id columns regarding long gradient runs, but the ULD kit does not interfere with the analysis as the pressure limits of the used columns are 1,200 bar.

However, for 100-mm and 150-mm × 4.6-mm id columns, the pressure reaches or even exceeds the limit of 600 bar of sub-2 µm 4.6-mm id columns when used with gradient runs. Therefore, the ULD kit is not recommended for these columns and long runs (gradients or low-organic isocratic runs).

In summary, the ULD kit is highly recommended for short runs (gradient and isocratic) using 2.1-mm and 3-mm id columns (Figure 12, green boxes) and also for long isocratic runs with 2.1-mm id columns (Figure 12, green-hatched boxes).

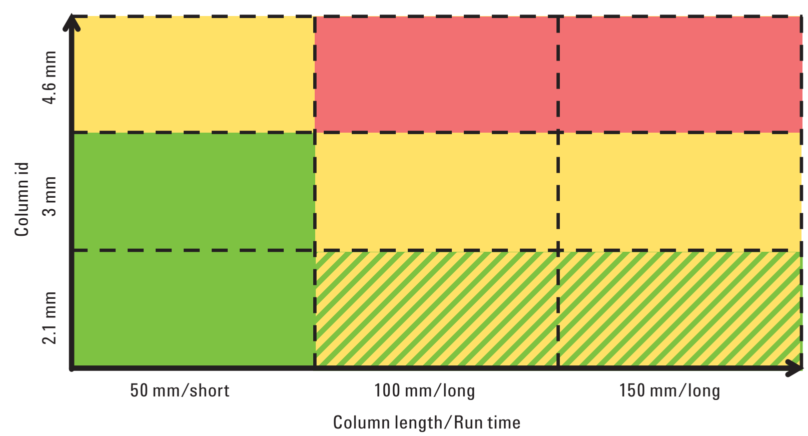

Figure 12
Overview improvements with ULD for sub-2 µm columns (green – highly recommended, green-hatched – highly recommend for isocratic runs, yellow – conditionally recommended, red – not recommended).

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