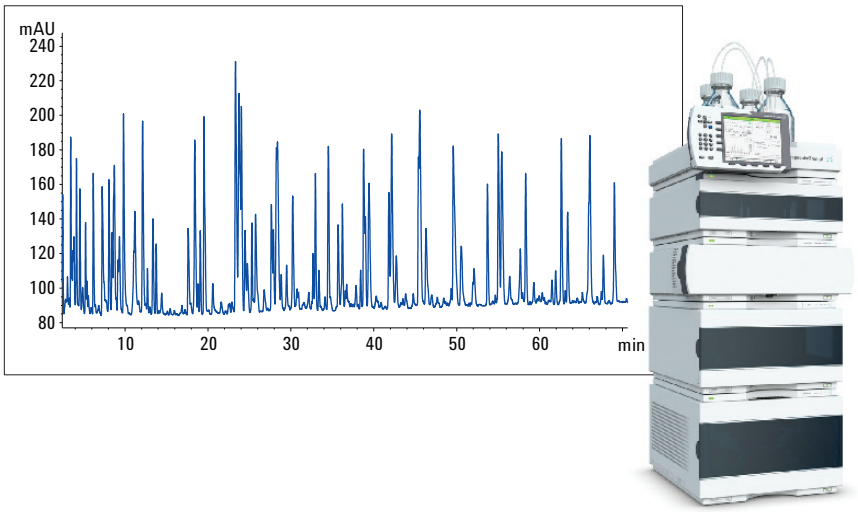
Optimizing performance of the Agilent 1290 Infinity LC System using 1-mm id columns

Enhancing UHPLC separation for peptide analysis

Technical Overview



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Abstract

The performance of the Agilent 1290 Infinity LC System was optimized for the analysis of peptides using 1-mm id columns. The 1290 Infinity LC System was equipped with an Agilent Ultra-low Dispersion Capillary Kit and an Agilent Max-Light Ultra-low Dispersion Cartridge Flow Cell. The experiments achieved the same or better performance compared to 2.1-mm id columns. The deployment of 1-mm id columns showed high sensitivity and resolution and reduced mobile phase consumption. In addition, the combination of 1-mm id columns with ESI-MS readout revealed high linearity and sensitivity of the analysis of HSA peptide standards.

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Introduction

Traditionally, (ultra) high performance liquid chromatography is performed with 4.6 to 2.1-mm id columns. This Technical Overview demonstrates the use of 1-mm id columns in combination with the Agilent 1290 Infinity LC System. The column id has an influence on flow rate and the sample amount that can be loaded onto the column. This leads to the following advantages of small id columns. By running lower flow rates, the mobile phase consumption is reduced, resulting in lower costs for solvents. Also, small volumes of mobile phase are easier to vaporize with electrospray mass spectrometry (ESI-MS) detection. Sensitivity is improved due to signal enrichment by the sample elution of higher concentrated chromatographic bands. In addition, less sample amount is needed due to the smaller bed volume of the column. This can be an important factor in areas, where only limited amount of sample is available, for example, in proteomics.

When transferring a method from higher to lower id columns, it is necessary to scale down flow rate and injection volume to maintain consistent gradient profiles. Formulas 1 and 2 display the correlation of flow rate (F), sample load (SL), length (L) and internal diameter (id):

$$F_2 = F_1 \times \left(\frac{id_2^2}{id_1^2} \right) \qquad (1)$$

$$SL_2 = SL_1 \times \left(\frac{id_2^2}{id_1^2} \times \frac{L_2}{L_1} \right) \qquad (2)$$

To take full advantage of low id columns, the LC system extra-column volume has to be minimized, including connecting capillaries, needle seats, heat exchangers, and detector flow cells. The Agilent Ultra-low Dispersion 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. The smaller the column id, the more critical is the LC system volume, as it accounts for a higher percentage of the system's extra-column volume, compared to a larger id column.

This Technical Overview compares the use of 2.1- and 1-mm id columns in combination with the Agilent 1290 Infinity LC System for the analysis of peptides with and without the reduction of extra-column volume using the Ultra-low Dispersion Capillary Kit in and the Max-Light Ultra-low Dispersion Cartridge Flow Cell.

Experimental

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

- Agilent 1290 Infinity Binary Pump (G4220A) with 100-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 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 (G4212-60038)
- Agilent 6490 Triple Quadrupole LC/MS System (G6490A)

Columns

- C18, 2.1 × 100 mm, < 2 µm
- C18, 1 × 100 mm, < 2 µm
- Agilent Poroshell 120 EC-C18, 1 × 150 mm, 2.7 µm (custom)

Software

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

Agilent MassHunter Workstation software, Version B.04.00, Build 4.0.479.0

Solvents and samples

Solvents

A = H_2O_{dd} + 0.1% trifluoroacetic acid (TFA) or for LC/MS formic acid (FA)

B = acetonitrile + 0.1 % TFA or for LC/MS formic acid (FA)

Samples

- HSA (human serum albumin) Peptides Standard Mix (G2455-85001)
- BSA (bovine serum albumin) Digest, alkylated

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

Gradient	Minutes	% B
HSA Peptide mix	0	5
	15	50
	16	95
	20	95
HSA Peptide mix MS read-out	0	5
	5.30	30
	7	50
	7.30	95
BSA digest	0	5
	70	30
	75	50
	76	95
	80	95
	81	5
Post time	10 minutes	
Flow rate		
2.1 mm	0.44 mL	
1 mm	0.1 mL	
1 mm MS readout	0.2 mL	
Injection volume		
2.1 mm	3 µL	
1 mm	0.7 µL	
1 mm MS readout	1 µL	
Thermostat	4 °C	
Column temperature	40 °C	
UV	214 nm/4 nm Ref.: 360 nm/100 nm	
Peak width	0.025 minutes (0.5 second response time) (10 Hz)	

Results and discussion

Separation of HSA peptide standard mix was performed using 2.1- and 1-mm id columns in combination with the Agilent 1290 Infinity LC System. Figure 1 shows the chromatograms of the HSA peptide standard mix separated with 2.1- (green) and 1-mm id column (blue).

To reduce extra-column volume of the system, the Agilent Ultra-low Dispersion Capillary Kit and the Agilent Max-Light Ultra-low Dispersion Cartridge Flow Cell were implemented in the system. A comparison of the chromatogram from peptide two to six is shown for the use with the ultra-low dispersion (ULD) components (blue) and without (red) with 1-mm id column (Figure 2).

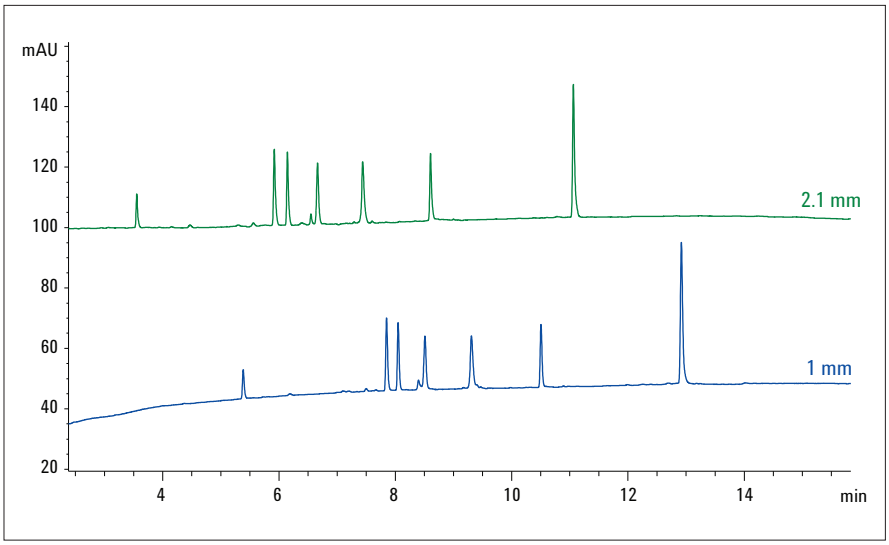

Figure 1
HSA peptide mix - Comparison of 2.1 and 1-mm id columns with the Agilent Ultra-low Dispersion Kit and the Agilent Ultra-low Dispersion Cartridge Flow Cell.

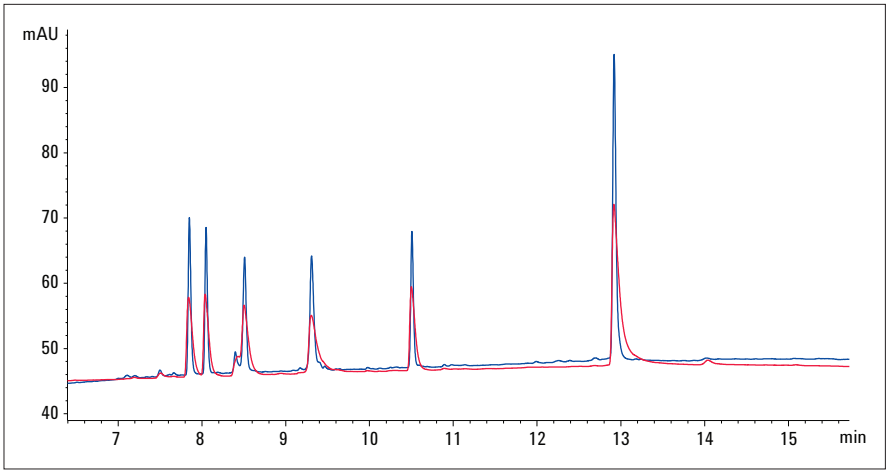

Figure 2
HSA peptide mix (peptide two to six) using 1-mm id column with (blue) and without ULD components (red).

The ULD components clearly led to huge improvements regarding peak width and resolution, resulting in more than doubled peak capacity for the 1-mm column, see Figure 3.

Using the ULD components, the peak width was reduced by 30% for the 2.1-mm columns and by 60% for the 1-mm columns. Peak capacity with ULD was found to be similar for 2.1- and 1-mm columns. Detailed results for peak width and peak capacities regarding the comparison of and 1-mm id column with and without ULD components are displayed in Table 1. Peak capacity n was calculated according to formula 3 (t_p = time from first to last peak, $w_{5\sigma}$ = peak width at 5σ).

$$n = \frac{t_p}{w_{5\sigma}} \qquad (3)$$

Tables 2, 3, and 4 show retention time precision, resolution, and signal-to-noise ratios for the seven HSA peptides. Retention time precision was comparable for 2.1- and 1-mm columns (Table 2).

As expected, the resolution for later eluting peaks became slightly worse for 1-mm columns compared to 2.1-mm id (Table 3). However, due to the bigger isocratic volume a better separation of early eluting peaks is possible.

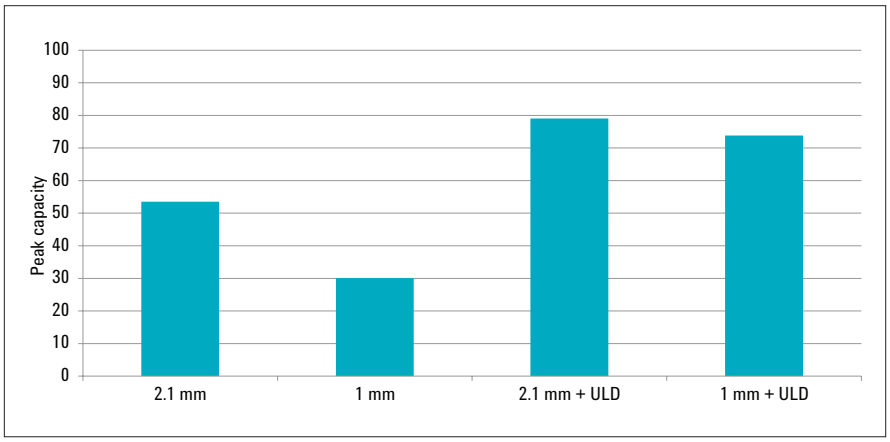

Figure 3
Peak capacity with and without ULD components.

Column	Averaged peak width at 5σ	t_p Peak 1 - Peak 7	Peak capacity n
2.1 without ULD	0.14	7.483	54
2.1 with ULD	0.09	7.497	79
1 without ULD	0.25	7.547	30
1 with ULD	0.10	7.533	74

Table 1
Peak width and peak capacities for 2.1 and 1-mm id column with and without ULD components.

Column	Peptide 1	Peptide 2	Peptide 3	Peptide 4	Peptide 5	Peptide 6	Peptide 7
2.1 without ULD	0.091	0.061	0.08	0.101	0.104	0.083	0.026
2.1 with ULD	0.082	0.093	0.087	0.067	0.068	0.066	0.069
1 without ULD	0.1	0.015	0.016	0.033	0.039	0.021	0.039
1 with ULD	0.115	0.103	0.107	0.091	0.047	0.023	0.037

Table 2
Retention time precision.

Column	Peptide 1	Peptide 2	Peptide 3	Peptide 4	Peptide 5	Peptide 6	Peptide 7
2.1 without ULD	-	24	2	5	7	11	26
2.1 with ULD	-	43	4	9	11	18	44
1 without ULD	-	20	2	4	5	9	18
1 with ULD	-	44	3	7	10	17	37

Table 3
Resolution.

Sensitivity increased from 2.1 to 1-mm columns without ULD components (Table 4). However, with ULD components it remained the same. For 1-mm columns, sensitivity decreased up to two times with the use of the 0.6-µL flow cell, due to increased noise of the cell.

Also, a tryptic BSA digest was analyzed using 2.1 and 1-mm id columns. Table 5 displays the peak widths and peak capacities for a 55-minute gradient analyzing tryptically digested BSA. With a custom-made Agilent Poroshell 120 EC-C18, 1 × 150 mm, 2.7 µm, it was possible to achieve even higher peak capacities, see Figure 4.

TFA is a common modifier used in peptide analysis as ion-pairing component of the mobile phases. It has a high absorbance and absorption fluctuations can show up as baseline noise, also referred to as mixing noise. To improve the noise values of the chromatogram due to the interference of TFA, it is possible to reduce the TFA concentration to 0.05% in water and 0.045% in acetonitrile. In addition, the use of a 380-µL Jet Weaver high performance mixer (G4220-60012) is optionally available for such demanding applications requiring 0.1% TFA. The 380-µL Agilent Jet Weaver strongly improves mixing and reduces, therefore, baseline noise resulting in higher detection sensitivity.

Column	Peptide 1	Peptide 2	Peptide 3	Peptide 4	Peptide 5	Peptide 6	Peptide 7
2.1 without ULD	44	100	96	92	98	97	289
2.1 with ULD	50	111	107	89	90	98	206
1 without ULD	96	222	233	197	156	236	443
1 with ULD	46	114	107	84	84	102	230

Table 4
Signal-to-Noise USP.

Column	Averaged peak width at 50% peak height	t_p	Peak capacity n
1-mm id sub-2-micron	0.16	55	339
1-mm id Poroshell	0.14	55	385

Table 5
Peak width and peak capacities for 1-mm columns with BSA digest.

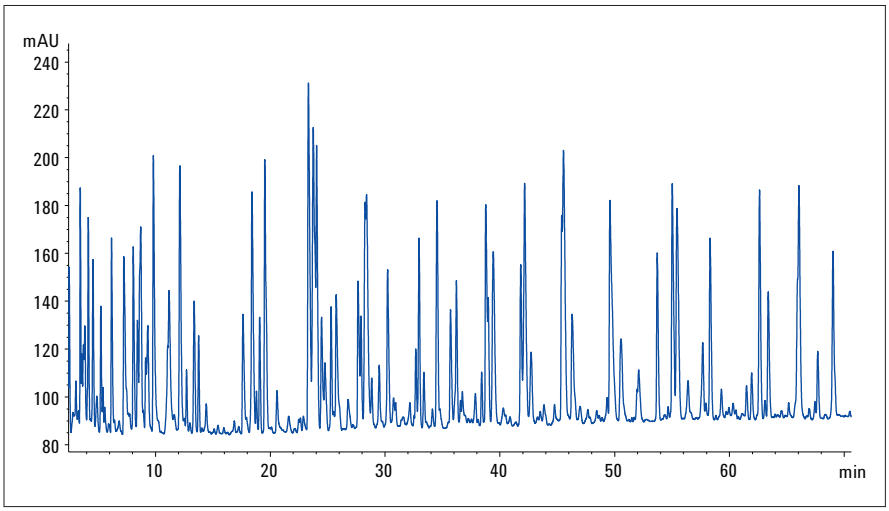

Figure 4
Separation of a tryptic BSA digest with an Agilent Poroshell 120 EC-C18, 1 × 150 mm, 2.7 µm column.

Small volumes of mobile phase are easier to vaporize with ESI-MS, which makes the use of low ids like 1-mm id columns very reasonable together with ESI-MS read-out. To prove the applicability of 1-mm id columns together with the ULD components for ESI-MS read-out, the HSA Peptide Standard Mix was analyzed with a Triple Quadrupole MS using Multiple Reaction Monitoring (MRM). Figure 5 displays the transitions for all seven HSA peptide standards.

In addition, a dilutions series of the HSA peptide standards was analyzed. Figure 6 shows the correlation curve of the MRM transitions from 100 fmol to 12.5 amol for one of the seven HSA peptide standards. With a determination coefficient of 0.995 and a limit of detection of 25 to 50 amol, the analysis of the dilution series revealed high linearity and sensitivity with the use of 1-mm id columns combined with Triple Quadrupole ESI-MS readout.

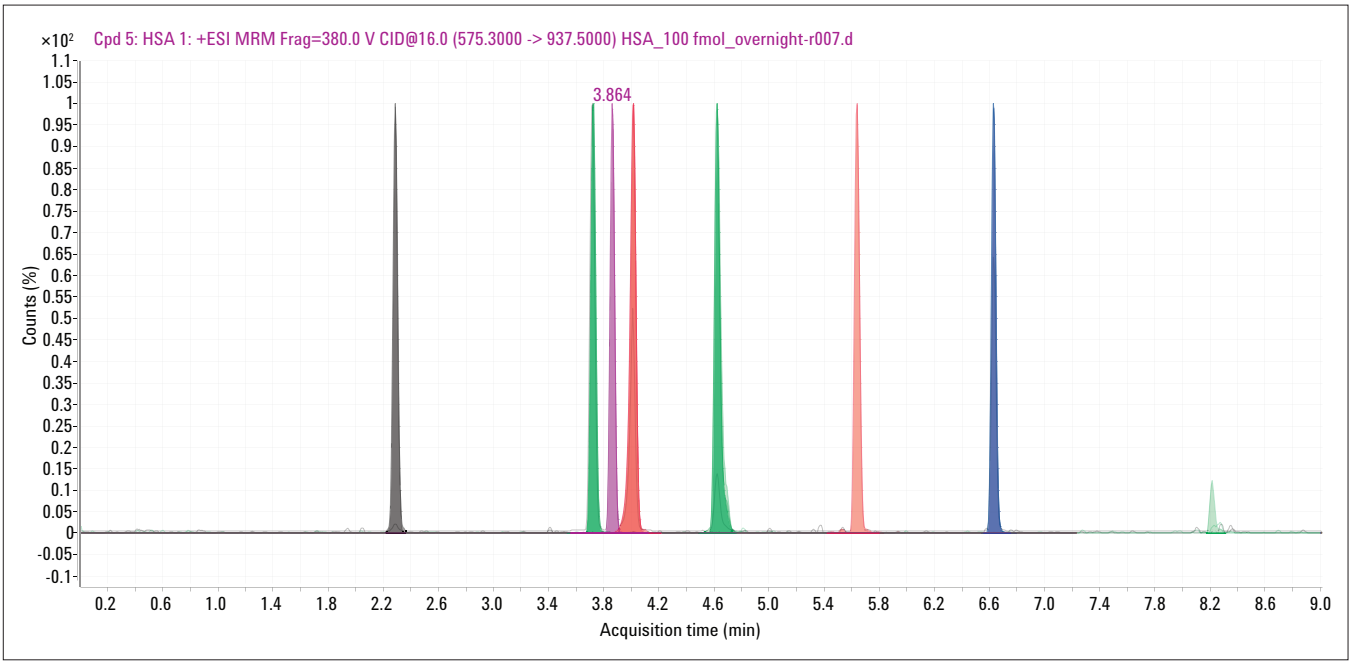

Figure 5
MRM transitions for all seven HSA peptide standards.

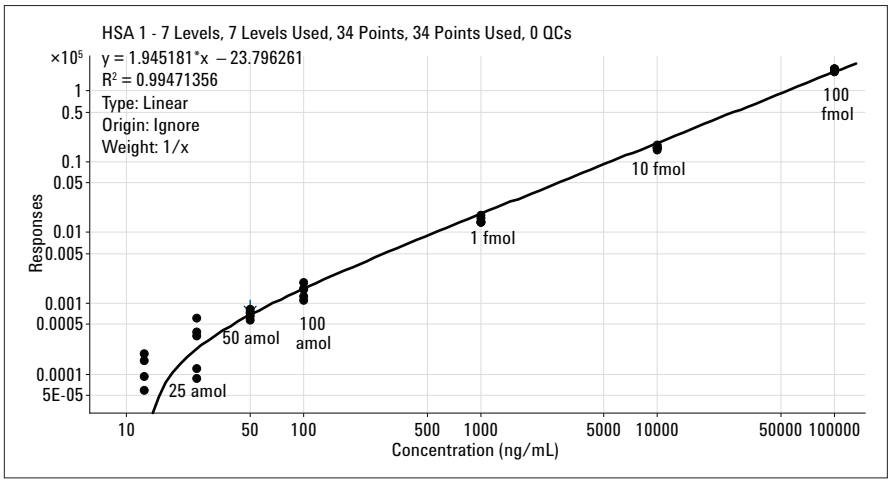

Figure 6
Linearity of a dilution series of HSA peptide standard.

Conclusion

The use of 1-mm id columns in combination with the Agilent Ultra-low Dispersion Cartridge Flow Cell and the Agilent Max-Light Ultra-low Dispersion Cartridge Flow Cell optimize the performance of the Agilent 1290 Infinity LC System for the separation of peptides. The experiments demonstrated the same or better performance as achieved with 2.1-mm id columns. Major benefits of using 1-mm id columns are the reduction of solvent costs (75% less solvent needed compared to 2.1-mm id columns), the reduced sample volume due to lower flow rates, and smaller column volume. In addition, the combination of 1-mm id columns with ESI-MS read-out revealed high linearity and sensitivity of the analysis of HSA peptide standards.

The Agilent 1290 Infinity LC System together with 1-mm id columns is an ideal solution for the analysis of peptides, resulting in high sensitivity and resolution together with reduced mobile phase consumption.

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