Optimizing System Dispersion on the Agilent 1290 Infinity II LC

The Agilent Ultralow Dispersion Kit

Technical Overview

Authors

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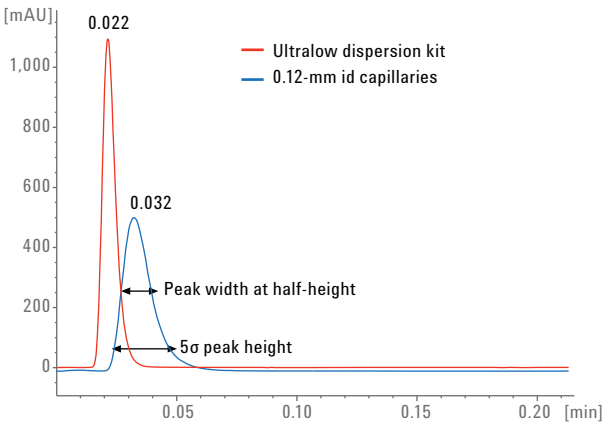
Abstract

The Agilent 1290 Infinity II LC is a universal system suitable for any HPLC or UHPLC application. For specific UHPLC analyses such as ultrafast or isocratic runs on small inside diameter columns, the performance can be further optimized by using the ultralow dispersion kit. With this kit, the system dispersion is minimized for increased peak capacity and resolution.

This Technical Overview compares the 1290 Infinity II LC equipped with the ultralow dispersion kit to an UHPLC system from another vendor, optimized for such UHPLC analyses. The 1290 Infinity II LC with ultralow dispersion kit provides less band-broadening (expressed in microliters) compared to the other vendor's system, resulting in higher plate count or peak capacity.



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Introduction

In general, system dispersion in liquid chromatography is defined as the volume between the effective injection point and the effective detection point of a system, and includes all connections between the autosampler and detector. Especially for ultrafast UHPLC analyses on small inside diameter columns, the system dispersion must be minimized to achieve the best possible number of theoretical plates (for isocratic runs) or peak capacity (for gradient runs). The Agilent 1290 Infinity II LC is a universal system for any HPLC or UHPLC application. To avoid loss of chromatographic efficiency during ultrafast or isocratic analyses on sub-2 µm particle columns, the system dispersion must be minimized for improved peak capacity or resolution. To achieve this, the 1290 Infinity II LC can be optimized using the 1290 Infinity II Ultra Low Dispersion Kit. With the installation of this kit, all standard capillaries with 0.12-mm id are exchanged with 0.075-mm id capillaries. Also, an Agilent Ultra-Low Dispersion Max-Light Cartridge Flow Cell with 10-mm path length and 0.6-µL V(σ) is installed instead of the standard Max-Light cartridge cell with 10-mm path length and 1-µL V(σ). Further, the needle seat in the autosampler and the heat exchanger in the column compartment are exchanged to reduce system dispersion to a minimum.

Agilent A-Line Quick Turn LC fittings[1] were used for all capillary connections. The fitting has a novel spring-loaded design that constantly pushes the tubing against the receiving port, delivering a reproducible connection with zero dead volume. The stem length of the spring-loaded fitting is self-adjustable and makes the fitting compatible with all types of HPLC and UHPLC columns. It seals up to 600 bar by finger-tightening, and up to 1,300 bar using wrenches.

The effects of the ultralow dispersion kit for the 1290 Infinity LC for different column inside diameters were described in a previous Technical Overview[2]. The effects of the 1290 Infinity II Ultra Low Dispersion Kit (both capillaries and flow cell) were evaluated regarding peak width, number of theoretical plates, and peak capacity. Figure 1 illustrates the recommendations for the use of the 1290 Infinity II Ultra Low Dispersion Kit, based on the experiments in this Technical Overview. The highest improvement regarding peak width, number of theoretical plates, and peak capacity are achieved for ultrafast and isocratic runs on small inside diameter columns. For standard analyses, the 1290 Infinity II Ultra Low Dispersion Kit can still be used, however, performance improvement is small. For analyses on 4.6-mm id columns, the ultralow dispersion kit is not recommended because the small inside diameters of the capillaries lead to high system backpressure.

This Technical Overview shows the performance, separation, and dispersion of the 1290 Infinity II LC with the ultralow dispersion kit, and compares the performance to the standard system setup using 0.12-mm id capillaries, and to an optimized system from another vendor[3]. System dispersion is measured, and performance results are shown for an isocratic and gradient separation with different compounds.

Experimental

Instrumentation

All experiments were carried out on an Agilent 1290 Infinity II LC system comprising the following modules:

- Agilent 1290 Infinity II High-speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (G7117B), equipped with a 10-mm path length Max-Light standard cartridge flow cell, V(σ) = 1 µL
- Agilent 1290 Infinity II Ultra Low Dispersion Kit (p/n 5067-5963)
- Agilent Ultra-Low Dispersion Max-Light Cartridge Flow Cell, 10 mm, V(σ) = 0.6 µL (p/n G4212-60038)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS systems, version C.01.07 [22]

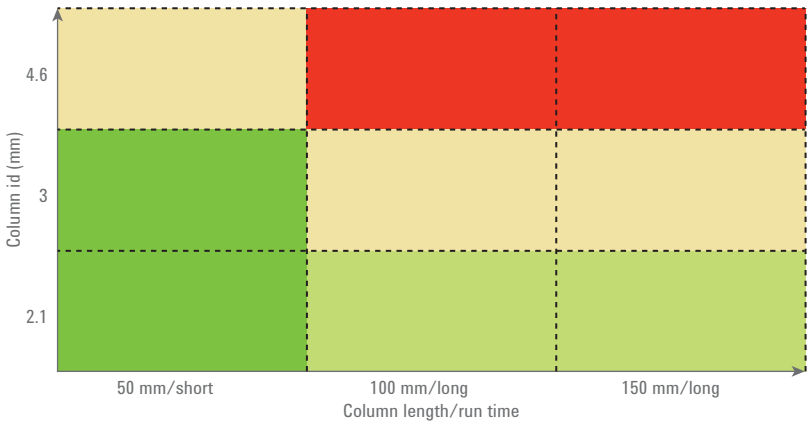

Figure 1. Overview improvements with ultralow dispersion kit for sub-2 µm particle columns (dark green – highly recommended, light green – highly recommend for isocratic runs, yellow – conditionally recommended, red – not recommended).

Samples

Caffeine and isocratic standards were from Agilent (p/n 8500-6762, p/n 01080-68704). The anesthetics were purchased from Sigma-Aldrich, St. Louis, USA

Chemicals

All solvents used were LC grade. Acetonitrile was purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak). Ammonium bicarbonate was purchased from Sigma-Aldrich, St. Louis, USA.

Results and Discussion

Determination of number of theoretical plates and peak width using an isocratic standard

The 1290 Infinity II Ultra Low Dispersion Kit leads to the highest performance improvements when used for ultrafast isocratic runs on 50-mm columns, especially for 2.1- and 3.0-mm id[2]. To determine the efficiency improvements, the plate counts were determined (according to Equation 1) for the system equipped with the 1290 Infinity II Ultra Low Dispersion Kit as well as for a standard system with 0.12-mm id capillaries using the isocratic sample in a short isocratic separation.

$$N = 5.54\left(\frac{RT}{W_{1/2}}\right)^2$$

Equation 1. Determination of plate count (N) from retention time (RT) and peak width at half-height ($W_{1/2}$).

Analysis conditions for caffeine

Parameter	Value
Sample	Caffeine 160 µL/mL
Column	Fused silica/PEEK capillary, 10 cm × 50 µm (p/n G1375-87325)
Column temperature	25 °C
Flow rate	0.5 mL/min
Mobile phase	50/50 (v/v) water/acetonitrile
Stop time	1 minute
Data rate	80 Hz
Wavelength	273 nm/4 nm
Injection volume	0.5 µL

Chromatographic method for isocratic standard

Parameter	Value
Sample	Agilent isocratic sample (p/n 01080-68704)
Column	2.1 × 50 mm, 1.7-µm ACQUITY UPLC BEH C18
Column temperature	45 °C
Flow rate	1.5 mL/min
Mobile phase	65 % acetonitrile in water
Stop time	1 minute
Data rate	160 Hz
Wavelength	254 nm/4 nm
Injection volume	0.1 µL

Chromatographic method for anesthetics

Parameter	Value
Sample	Benzocaine, procaine, prilocaine, lidocaine, tetracaine, and bupivacaine
Column	2.1 × 50 mm, 1.7-µm ACQUITY UPLC BEH C18
Column temperature	60 °C
Flow rate	1.25 mL/min
Mobile phase	A) Water + 10 mM ammonium bicarbonate B) Acetonitrile
Gradient	0 minutes, 25 %B 0.75 minutes, 75 %B 1 minute, 95 %B
Stop time	1.5 minutes
Post time	2 minutes
Data rate	160 Hz
Wavelength	220 nm/4 nm
Injection volume	0.5 µL

Figure 2 shows an overlay of the chromatograms of the isocratic standard measured with the system with the 1290 Infinity II Ultra Low Dispersion Kit installed and for a standard system equipped with 0.12-mm id capillaries.

Due to the reduced system dispersion with the 1290 Infinity II Ultra Low Dispersion Kit, the peaks are significantly narrower compared to the peaks from the analysis on the system with 0.12-mm id capillaries (Figure 2). Therefore, the early eluting compounds showed a substantial higher plate count with up to 141 %. However, for the later eluting peaks, the plate count is similar or even lower because the higher retention times get more influence than the reduced peak according to Equation 1.

Determination of system dispersion with caffeine

The system dispersion is determined by the injection of caffeine directly onto a 10 cm by 50-µm PEEKSil tubing. Figure 3 shows the effect of the 1290 Infinity II Ultra Low Dispersion Kit in comparison to the standard configuration using 0.12-mm id capillaries. At half peak-height, 6.5-µL system dispersion was determined for the LC system equipped with 0.12-mm id capillaries compared to 3 µL for the system equipped with the 1290 Infinity II Ultra Low Dispersion Kit. As a comparison, a system optimized for low system dispersion from another vendor showed a system dispersion of 3.55 µL[3].

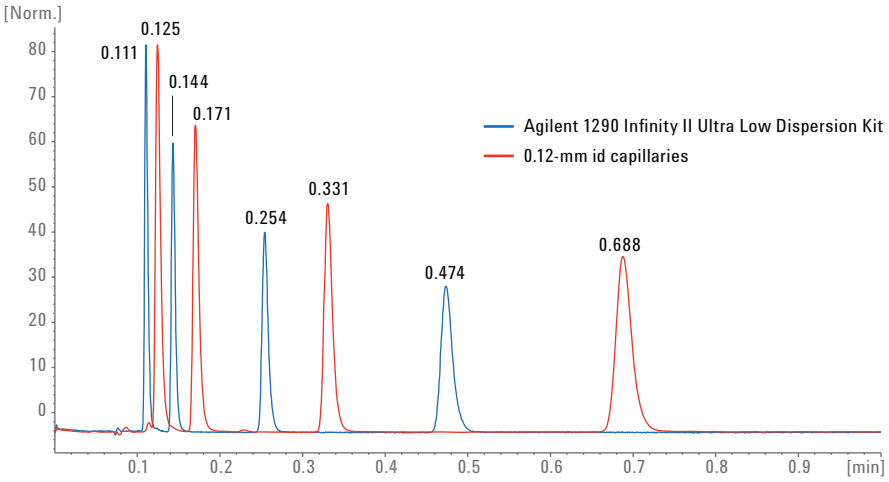

Figure 2. Isocratic separation of dimethylphthalate, diethylphthalate, biphenyl, and *o*-terphenyl using an Agilent 1290 Infinity II LC equipped with the Agilent 1290 Infinity II Ultra Low Dispersion Kit (blue), and a standard Agilent 1290 Infinity II LC equipped with 0.12-mm id capillaries (red).

	Agilent 1290 Infinity II LC with Agilent 1290 Infinity II Ultra Low Dispersion Kit		Agilent 1290 Infinity II LC with standard capillaries		
	Plate count	Peak width at half-height	Plate count	Peak width at half-height	Increase in plate count (%)
Dimethylphthalate	4,266	0.004	1,766	0.007	141
Diethylphthalate	4,959	0.005	3,306	0.007	50
Biphenyl	5,585	0.008	5,016	0.011	11
o-Terphenyl	5,532	0.015	5,946	0.021	–7

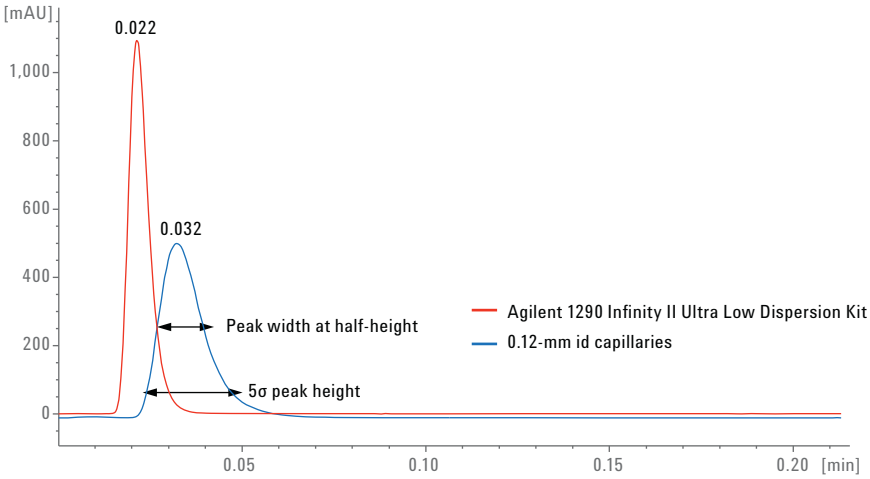

Figure 3. System dispersion measured at half-peak height and 5σ for caffeine. The measured system dispersion was compared to a standard Agilent 1290 Infinity II LC equipped with 0.12-mm id capillaries, and to a system from another vendor.

System dispersion	Agilent 1290 Infinity II LC with standard capillaries	Agilent 1290 Infinity II LC with Agilent 1290 Infinity II Ultra Low Dispersion Kit	System from other vendor
Half-peak height	6.5 µL	3 µL	3.55 µL
5σ peak height	16 µL	7 µL	7.5 µL

The calculations of the peak width at half height and 5σ were automatically done with the Agilent OpenLAB CDS Intelligent Reporter. To calculate the dispersion in microliters, Equation 2 was used.

$$\text{Half height peak width or } 5\sigma \text{ (in min)} \times \text{ flow rate } \left(500\ \frac{\mu L}{min}\right) = \text{band – spread in } \mu L$$

Equation 2. Determination of the system dispersion.

Determination of peak capacity for the gradient elution of anesthetics

In contrast to isocratic elution on short columns, less improvement regarding peak widths is achieved using the 1290 Infinity II Ultra Low Dispersion Kit for gradient elution[2]. For comparison of separation efficiency of the 1290 Infinity II LC and the other vendor's system, a gradient elution of six anesthetics was performed. The anesthetic mix was separated within a short gradient of 0.75 minutes (Figure 4). The separation efficiency was determined by peak capacity – describing the number of peaks that can be resolved within the elution of the first and last peak in the chromatogram. The peak capacity was calculated according to Equation 3. Compared to the dispersion volume optimized system from another vendor[3], the 1290 Infinity II LC equipped with the 1290 Infinity II Ultra Low Dispersion Kit provided higher peak capacity, for example, up to 72 % for the last peak.

$$n = \frac{t_g}{W_{1/2}}$$

Equation 3. Determination of peak capacity (n) from gradient run time (t_g) and peak width at half-height ($W_{1/2}$).

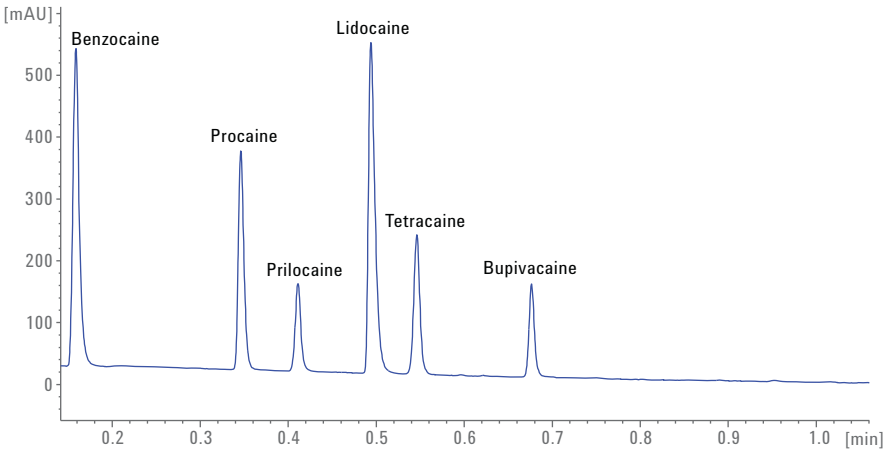

Figure 4. Gradient separation of six anesthetics using the Agilent 1290 Infinity II LC equipped with the Agilent 1290 Infinity II Ultra Low Dispersion Kit.

Peak capacity	Agilent 1290 Infinity II LC with Agilent 1290 Infinity II Ultra Low Dispersion Kit	System from other vendor	Improvement (%) in peak capacity
Benzocaine	107.1	89.5	20
Procaine	107.1	88.1	22
Prilocaine	107.1	79.9	34
Lidocaine	93.8	78.6	19
Tetracaine	107.1	81.5	31
Bupivacaine	125	72.8	72

Conclusion

The Agilent 1290 Infinity II Ultra Low Dispersion Kit of the 1290 Infinity II LC comprises capillaries with a low internal diameter of 0.075 mm, Agilent A-line Quick Turn fittings, needle seat, and heat exchanger. The comparison with an optimized system from another vendor revealed less system dispersion for the 1290 Infinity II LC equipped with the 1290 Infinity II Ultra Low Dispersion Kit (3.55 µL versus 3.0 µL). For isocratic runs, the ultralow dispersion kit showed significant performance improvements compared to a standard 1290 Infinity II LC because higher plate counts were achieved. For gradient runs, the 1290 Infinity II LC with the 1290 Infinity II Ultra Low Dispersion Kit provided higher peak capacity compared to the system from another vendor.

References

1. Agilent A-Line UHPLC Fittings, *Agilent Technologies Technical Overview*, publication number 5991-5525EN, **2015**.

2. Schneider, S., Agilent 1290 Infinity LC System – Applications requiring the Agilent Ultralow Dispersion Kit, *Agilent Technologies Application Note*, Publication Number 5991-0826EN, **2012**.

3. Scientific poster from other vendor presented at 38th Symposium of HPLC, **2011**.

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