Achieving Shortest Run Time and Narrowest Peak Shape Using the Agilent 1290 Infinity II LC with Ultralow Dispersion Kit

Technical Overview

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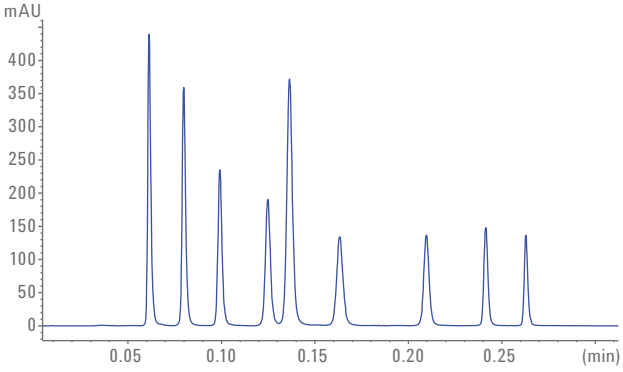
Abstract

The Agilent 1290 Infinity II LC equipped with an Agilent Ultralow Dispersion Kit offers an optimal prerequisite for establishing highly efficient high-throughput methods. Ultrashort chromatographic methods were developed for the separation of nine phenones within only 16 seconds. The reduction of extracolumn volume with the ultralow dispersion kit led to minimal peak widths for sharpest peaks. Excellent relative standard deviations of retention time and area were found at maximal pressures over 1,000 bar at 90 °C. With the described setup, the productivity and efficiency were greatly enhanced for ultrafast UHPLC analysis with short sub-2 µm columns.



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Introduction

The increased need for superfast and high-resolving analysis in different application fields, for example, in food or drug monitoring, led to the development of ultrahigh performance liquid chromatography (UHPLC) systems with lowest dispersion and high-pressure ranges. The combination, with short sub-2 µm (STM) columns, enables new possibilities regarding the development of ultrafast UHPLC methods, with run times far below 1 minute.

The Agilent 1290 Infinity II LC equipped with Ultralow Dispersion Kit provides a low dispersion setup with a reduction of extracolumn volume by using capillaries with a low inside diameter (id) of 0.075 mm, including all connections from the injection point to the detector. All capillaries with the 0.12 mm id of a normal UHPLC system are replaced by 0.075 mm id capillaries. In addition, a needle seat with a 0.075 mm id capillary, a 1-µL internal volume heat exchanger, and an ultralow dispersion Max-Light cartridge flow cell, 10 mm, V(σ) = 0.6 µL, were installed to reduce the dispersion of the whole system to a minimum. The lower the dispersion, the higher the performance of an UHPLC system regarding resolution, narrow peak width, and, therefore, peak capacity or plate count, leading to a significant increase in productivity. The effects of the Ultralow Dispersion Kit with different id columns were already demonstrated in previous Application Notes[1,2]. The quick and convenient use of the Agilent A-line Quick Turn UHPLC fittings[3] facilitated and accelerated the instrument setup, resulting in reduced lab time. 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.

This Technical Overview shows ultrashort, high-throughput runs for the separation of nine phenones using gradient and isocratic analysis methods. The performance, as well as the peak shape and widths, are evaluated.

Experimental

Instrumentation

All experiments were carried out on an Agilent 1290 Infinity II LC 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)
- Agilent Ultralow Dispersion Kit (p/n 5067-5963)
- Agilent Ultralow Dispersion Max-Light Cartridge Flow Cell, V(σ) = 0.6 µL, 10 mm (G4212-60038)

Column

Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8 µm (p/n 857700-902)

Software

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

Chromatographic conditions

Table 1. Chromatographic conditions for ultrashort isocratic analysis.

Parameter	Value
Mobile phase	Water/acetonitrile, 45/55
Flow rate	2.5 mL/min
Stop time	1 minute
Needle wash mode	Standard wash
Injection Volume	1 µL
Column temperature	90 °C
Detection	254/4 nm, reference 360/100 nm > 0.0016 minutes (0.031 seconds response time) (160 Hz)

Table 2. Chromatographic conditions for ultrashort gradient analysis.

Parameter	Value
Mobile phase	A) Water B) Acetonitrile
Flow rate	2.5 mL/min
Gradient	0 minutes, 55 %B 0.1 minutes, 95 %B 0.2 minutes, 95 %B
Stop time	0.5 minutes
Post time	0.5 minutes
Needle wash mode	Standard wash
Injection volume	1 µL
Column temperature	90 °C
Detection	254/4 nm, reference 360/100 nm > 0.0016 minutes (0.031 seconds response time) (160 Hz)

Samples

RRLC Checkout sample (p/n 5188-6529) containing (in order of elution):

1. Acetanilide
2. Acetophenone
3. Propiophenone
4. Butyrophenone
5. Benzophenone
6. Valerophenone
7. Hexanophenone
8. Heptanophenone
9. Octanophenone

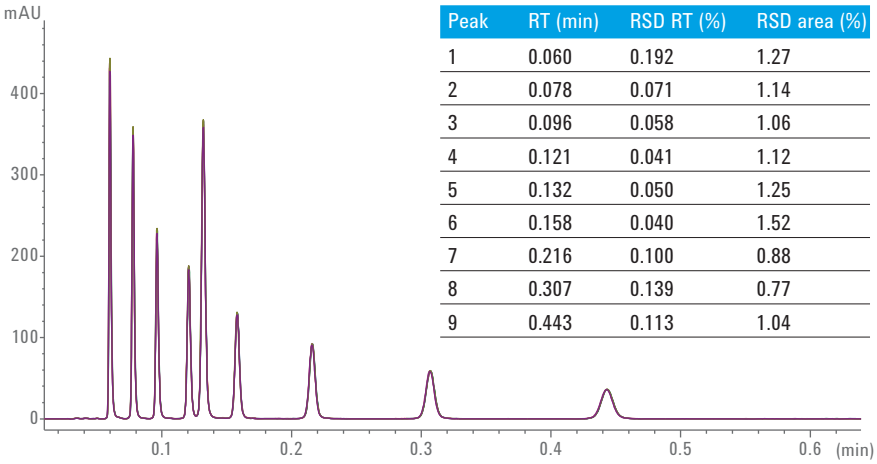
Chemicals

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

Results and Discussion

Determination of plate counts for ultrafast isocratic analysis

Ultrafast methods were developed for the analysis of a standard containing nine phenones. Figure 1 displays the ultrafast isocratic separation, showing an overlay of six subsequent runs. An isocratic separation was possible below 0.5 minutes with baseline-separated peaks. Excellent precision was found for retention time and area with relative standard deviations below 0.2 and 1.53 %, respectively.



Peak	RT (min)	RSD RT (%)	RSD area (%)
1	0.060	0.192	1.27
2	0.078	0.071	1.14
3	0.096	0.058	1.06
4	0.121	0.041	1.12
5	0.132	0.050	1.25
6	0.158	0.040	1.52
7	0.216	0.100	0.88
8	0.307	0.139	0.77
9	0.443	0.113	1.04

Figure 1. Overlay of six subsequent runs of the ultrafast isocratic separation of the standard containing nine phenones.

To determine the efficiency of the analysis, the plate count of the analysis was calculated using Equation 1.

$$N = 5.54\left(\frac{RT}{W_{5\sigma}}\right)^2$$

Equation 1. Determination of plate count (N), where RT is retention time and $W_{5\sigma}$ is peak width at 5σ.

Table 3 shows the peak widths at half height and at 5σ, as well as the calculated plate count. The effect of the Ultralow Dispersion Kit on plate count was already demonstrated in previous Application Notes[1,2]. Due to the reduced system dispersion with the Ultralow Dispersion Kit, the peaks are significantly narrower compared to the peaks from the analysis on the system with 0.12 mm id capillaries. Hence, early eluting compounds in an isocratic run show substantially higher plate counts.

Table 3. Peak widths, peak width at half height ($W_{1/2}$), peak width at 5σ ($W_{5\sigma}$), and calculated plate count.

Peak no.	$W_{1/2}$ (min)	$W_{5\sigma}$ (min)	Plate count, N
1	0.002	0.004	1,247
2	0.002	0.005	1,348
3	0.002	0.006	1,418
4	0.003	0.007	1,655
5	0.003	0.008	1,508
6	0.004	0.009	1,707
7	0.005	0.012	1,795
8	0.007	0.017	1,807
9	0.010	0.024	1,888

Determination of peak capacity for ultrafast gradient analysis

In addition to the isocratic analysis, an ultrafast gradient separation was also developed for the separation of nine phenones. The separation is displayed in Figure 2 as an overlay of six runs. Using a gradient, an ultrashort separation was possible for the nine phenones standard within only 16 seconds. Again, excellent precision was found for retention time and area with relative standard deviations below 0.03 and 0.96 %, respectively.

To determine the efficiency of the analysis, the peak capacity was calculated using Equation 2.

$$n = \frac{t_g}{W_{5\sigma}}$$

Equation 2. Determination of peak capacity (n), where t_g is gradient run time and $W_{5\sigma}$ is peak width at 5σ

Table 4 shows the peak widths at half height and at 5σ, as well as the calculated peak capacity. The calculated peak capacity results in up to 100 peaks per 30 seconds total run time, resulting from the ultrasharp peak shape of $W_{5\sigma}$ = 0.005 minutes, corresponding to 300 milliseconds.

Peak	RT (min)	RSD RT (%)	RSD area (%)
1	0.061	0.018	0.95
2	0.08	0.025	0.87
3	0.099	0.015	0.84
4	0.125	0.021	0.81
5	0.137	0.020	0.73
6	0.163	0.026	0.84
7	0.21	0.021	0.75
8	0.241	0.019	0.59
9	0.263	0.013	0.44

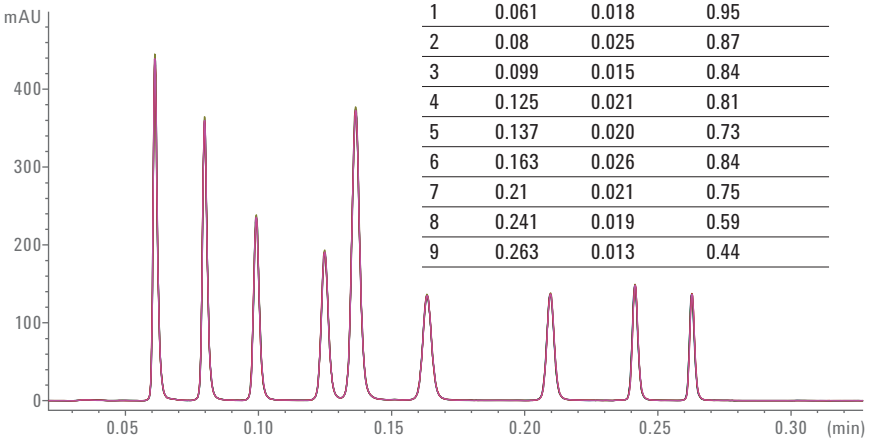


Figure 2. Overlay of six subsequent runs of the ultrafast gradient separation of the standard containing nine phenones.

Table 4. Peak widths, peak width at half height ($W_{1/2}$), peak width at 5σ ($W_{5\sigma}$), and calculated peak capacity.

Peak no.	$W_{1/2}$ (min)	$W_{5\sigma}$ (min)	Peak capacity, n
1	0.002	0.005	100
2	0.002	0.005	100
3	0.002	0.006	83
4	0.003	0.007	71
5	0.003	0.008	63
6	0.004	0.009	56
7	0.003	0.007	71
8	0.002	0.006	83
9	0.002	0.005	100

Conclusion

Ultrashort isocratic and gradient methods were developed for the analysis of nine phenones. The isocratic conditions enabled baseline separation of all nine peaks within 27 seconds due to sharp peak shapes. Minimal peak widths delivered substantially higher plate counts compared to a setup without the Agilent Ultralow Dispersion Kit. Using gradient conditions, the separation was possible in only 16 seconds, enabling peak capacities of 100 peaks in 30 seconds. The performance of both ultrashort analysis methods revealed excellent relative standard deviations of retention time and peak area, running at pressures over 1,000 bar and column temperatures above 90 °C. The combination of the Agilent 1290 Infinity II LC with the Ultralow Dispersion Kit offers optimal conditions to greatly enhance productivity and efficiency for ultrafast UHPLC methods.

References

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