Transferring a Method for Analysis of DNPH-Derivatized Aldehydes and Ketones from HPLC to UHPLC

Application Note

Environmental

Author

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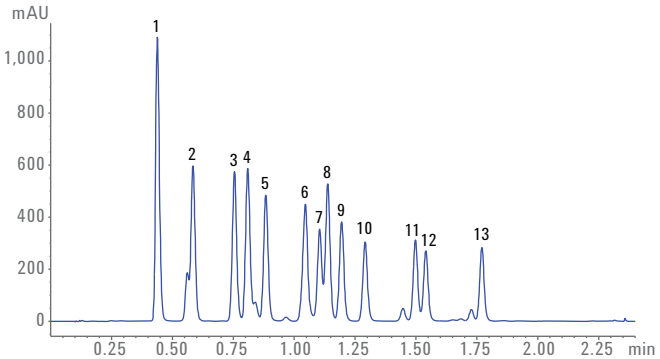
Abstract

This Application Note shows the transfer of a standard HPLC method to a UHPLC method for the analysis of 13 DNPH-derivatized aldehydes and ketones. By transferring the method from HPLC to UHPLC, the analysis time and solvent consumption was reduced by approximately 90 %. The results demonstrate the benefit of transferring an HPLC method to UHPLC conditions without compromising precision, linearity, or limits of detection and quantification.



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Introduction

Carbonyl compounds, such as aldehydes and ketones, are emitted into the atmosphere by anthropogenic and biogenic sources or, with an even higher impact, as air pollutants from various industrial processes and motor vehicles[1]. In addition, formaldehyde, one of the most abundant carbonyl compounds, is emitted from wooden products such as furniture or toys, and has a great impact on indoor pollution.

Long-term exposure to carbonyls is known to have diverse negative health effects such as irritation of eye or pulmonary function. In addition, formaldehyde was classified as a human carcinogen by the International Agency for Research on Cancer (IARC) in 2004[2].

Due to the adverse health effects, accurate analytical methods are important to determine the concentration of aldehydes and ketones in several environmental matrices. Today, the most common method for the analysis of aldehydes and ketones is based on a derivatization step using 2,4-dinitrophenylhydrazine (DNPH) followed by high performance liquid chromatography (HPLC) for separation and detection by UV-Vis or mass spectrometry[3].

This Application Note describes the transfer of an HPLC method to an UHPLC method for the analysis of DNPH-derivatized aldehydes and ketones using the Agilent 1290 Infinity II LC. This system is able to manage a pressure of up to 1,300 bar, which allows the use of sub-2 µm (STM) packing material. This facilitates the use of shorter columns with smaller inner diameters, which, in turn, leads to reduced analysis time and reduced solvent consumptions compared to traditional HPLC methods.

Experimental

Instrumentation

The Agilent 1290 Infinity II LC used for the experiments consisted of 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 Max-Light cartridge cell

Columns

- Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm (p/n 959993-902)
- Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959759-902)

Software

Agilent OpenLab CDS ChemStation Edition for LC and LC/MS systems, Version C.01.07 [27]

Chemicals

All solvents were LC grade. Acetone and acetonitrile were purchased from Merck, Darmstadt, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA).

The sample mixture consisted of 13 aldehyde-2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones purchased from Sigma-Aldrich, Steinheim, Germany (Catalog No. 47651-U). In the mixture, each analyte had a concentration of 30 µg/mL of carbon. For calibration, the mixture was further diluted with acetonitrile

Table 1. Elution order of aldehyde and ketone mixture.

Peak	Substance
1	Formaldehyde-2,4-dinitrophenylhydrazone
2	Acetaldehyde-2,4-dinitrophenylhydrazone
3	Acrolein-2,4-dinitrophenylhydrazone
4	Acetone-2,4-dinitrophenylhydrazone
5	Propionaldehyde-2,4-dinitrophenylhydrazone
6	Crotonaldehyde-2,4-dinitrophenylhydrazone
7	Methacrolein-2,4-dinitrophenylhydrazon
8	2-Butanone-2,4-dinitrophenylhydrazon
9	Butyraldehyde-2,4-dinitrophenylhydrazon
10	Benzaldehyde-2,4-dinitrophenylhydrazon
11	Valeraldehyde-2,4-dinitrophenylhydrazon
12	*m*-Tolualdehyde-2,4-dinitrophenylhydrazon
13	Hexaldehyde-2,4-dinitrophenylhydrazon

Method

Table 2. Chromatographic conditions for 4.6 × 150 mm, 5 µm HPLC column.

Parameter	Value
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm
Mobile phase	A) Water B) Acetone
Gradient	0 minutes – 45 %B 12 minutes – 53 %B 28 minutes – 67 %B 32 minutes – 67 %B 33 minutes – 95 %B
Stop time	34 minutes
Post time	20 minutes
Flow rate	1.2 mL/min
Injection volume	5 µL from stock solution, at 4 °C, draw speed 100 µL/min, 3 seconds needle wash
Column temperature	45 °C
Detection	360/10 nm, ref. wavelength off, 20 Hz

Table 3. Chromatographic conditions for 2.1 × 50 mm, 1.8 µm UHPLC column.

Parameter	Value
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm
Mobile phase	A) Water B) Acetone
Gradient	0 minutes – 45% B 4 minutes – 53 % B 9.5 minutes – 67 % B 11 minutes – 67 % B 11.05 minutes – 95 % B
Stop time	11.5 minutes
Post time	7 minutes
Flow rate	0.250 mL/min
Injection volume	1.25 µL from stock solution, at 4 °C, draw speed 100 µL/min, 3 seconds needle wash
Column temperature	45 °C
Detection	360/10 nm, ref. wavelength off, 20 Hz

Table 4. Chromatographic conditions for 2.1 × 50 mm, 1.8 µm UHPLC column, optimized for speed.

Parameter	Value
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm
Mobile phase	A) Water B) Acetone
Gradient	0 minutes – 45 % B 0.75 minutes – 53 % B 1.8 minutes – 67 % B 2.0 minutes – 67 % B 2.2 minutes – 95 % B
Stop time	2.4 minutes
Post time	1.5 minutes
Flow rate	1.25 mL/min
Injection volume	1.25 µL from stock solution, at 4 °C, draw speed 100 µL/min, 3 seconds needle wash
Column temperature	45 °C
Detection	360/10 nm, ref. wavelength off, 80 Hz

Results and Discussion

For the separation of DNPH-derivatized aldehydes and ketones under HPLC conditions, an Agilent ZORBAX Eclipse Plus C18 column with a particle size of 5 µm was used. The analysis of the 13 carbonyl compounds and some impurities took approximately 26 minutes. Six consecutive runs were used to evaluate the retention time and area precision, as well as resolution. Figure 1 shows the results of the six consecutive runs. All RSDs for retention time were below 0.04 %, and the area precision was always below 0.4 %.

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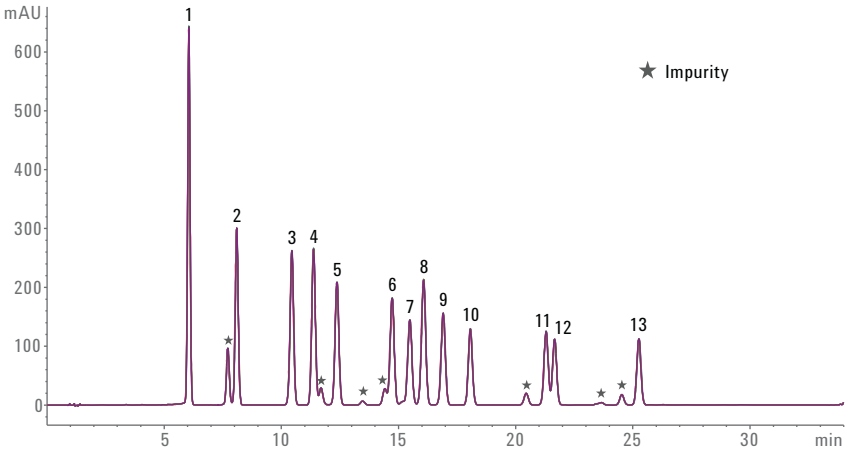

B

Compound	RT RSD (%)	Area RSD (%)	Resolution (mean)
1	0.04	0.24	–
2	0.03	0.21	1.61
3	0.03	0.13	8.96
4	0.03	0.28	3.22
5	0.03	0.37	3.31
6	0.03	0.26	7.5
7	0.03	0.11	2.25
8	0.03	0.33	1.71
9	0.03	0.25	2.44
10	0.03	0.30	3.46
11	0.02	0.24	2.45
12	0.02	0.15	1.06
13	0.02	0.20	2.14

Figure 1. Analysis of DNPH-derivatized aldehydes and ketones using an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm column. A) Overlay of six consecutive runs. B) Calculated values for retention time and area precision and resolution.

For the UHPLC method, an Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm column was used to enable shorter analysis times. All compounds were eluted after 9 minutes. The overall run time and solvent consumption was reduced by over 60 %. To investigate the precision regarding retention time and area for the UHPLC method, six consecutive runs were evaluated (Figure 2).

The RSDs for retention time and area were below 0.15 and 0.65 %, respectively, and were still good. The same was true for the resolution.

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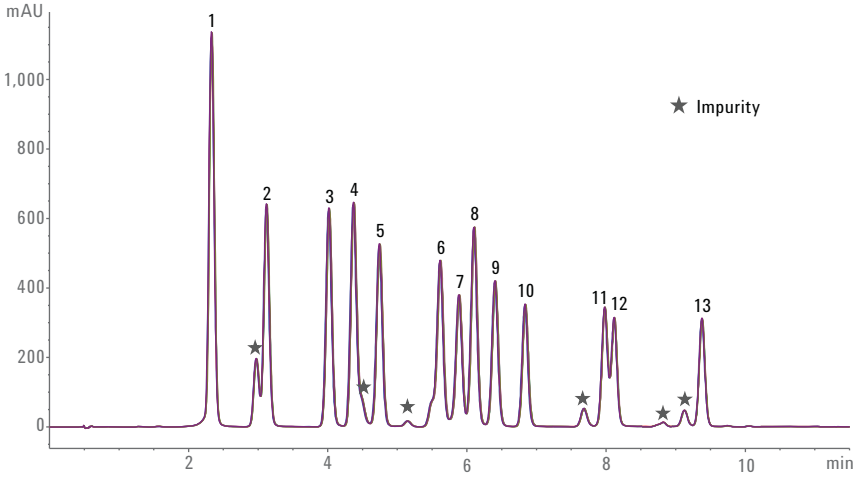

B

Compound	RT RSD (%)	Area RSD (%)	Resolution (mean)
1	0.06	0.465	–
2	0.15	0.512	1.09
3	0.15	0.520	6.33
4	0.14	0.534	2.36
5	0.13	0.518	2.43
6	0.12	0.570	3.09
7	0.11	0.578	1.69
8	0.10	0.551	1.34
9	0.10	0.586	1.92
10	0.09	0.519	2.77
11	0.06	0.609	1.83
12	0.06	0.498	0.79
13	0.04	0.604	1.63

Figure 2. Analysis of DNPH-derivatized aldehydes and ketones using an Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm column. A) Overlay of six consecutive runs. B) Calculated values for retention time and area precision and resolution.

To further reduce the analysis time and use the whole power range of the Agilent 1290 Infinity II LC, the flow rate was increased to 1.25 mL/min. The separation of the 13 carbonyl compounds was achieved in less than 2 minutes. The RSDs for retention time (below 0.15 %) and area (below 0.25 %) were found to be excellent, and the resolution values obtained were still acceptable (Figure 3).

To evaluate the limit of detection (LOD), limit of quantitation (LOQ), and linearity for all three methods, seven different concentration levels ranging from 30 µg/mL to 10 ng/mL were prepared. Table 5 shows high linearity values with correlation coefficients of 0.9998 and higher for all three methods.

The LOD and LOQ values were determined by a signal-to-noise ratio (S/N) of 3:1 and 10:1, respectively. For all aldehydes and ketones, the LOD and LOQ values were clearly improved under UHPLC conditions, in comparison to the HPLC conditions. Increasing the flow rate from 0.250 to 1.25 mL/min showed a further improvement in LOD and LOQ values for most of the compounds.

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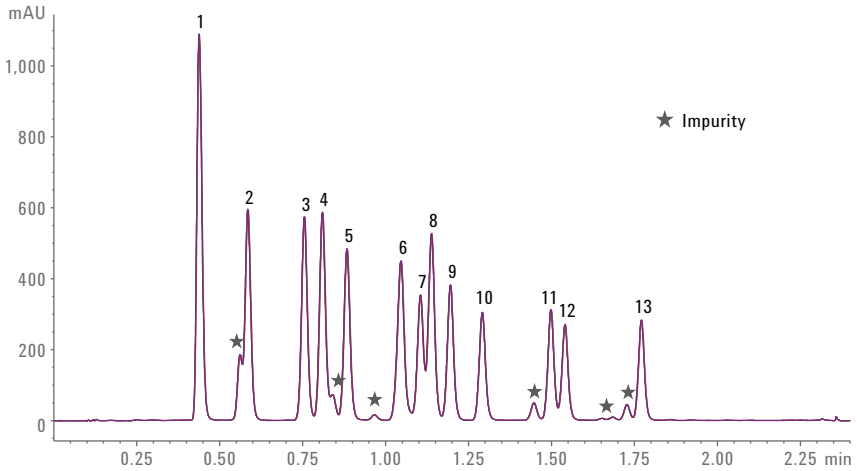

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11	0.06	0.609	1.83
12	0..06	0.498	0.79
13	0.04	0.604	1.63

Figure 3 Analysis of DNPH-derivatized aldehydes and ketones using an Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm column and a flow rate of 1.25 mL/min. A) Overlay of six consecutive runs. B) Calculated values for retention time and area precision and resolution.

Table 5. Overview of correlation coefficients for HPLC and the two UHPLC methods.

Substance	4.6 × 150 mm, 5 µm	2.1 × 50 mm, 1.8 µm	2.1 × 50 mm, 1.8 µm, 1.25 mL/min flow
Formaldehyde-2,4-dinitrophenylhydrazone	0.99989	0.99803	0.99994
Acetaldehyde-2,4-dinitrophenylhydrazone	0.99990	0.99999	0.99986
Acrolein-2,4-dinitrophenylhydrazone	0.99990	0.99998	0.99992
Acetone-2,4-dinitrophenylhydrazone	0.99990	0.99994	0.99994
Propionaldehyde-2,4-dinitrophenylhydrazone	0.99990	0.99998	0.99990
Crotonaldehyde-2,4-dinitrophenylhydrazone	0.99987	0.99994	0.99996
Methacrolein-2,4-dinitrophenylhydrazon	0.99986	0.99998	0.99991
2-Butanone-2,4-dinitrophenylhydrazon	0.99989	0.99989	0.99997
Butyraldehyde-2,4-dinitrophenylhydrazon	0.99990	0.99997	0.99992
Benzaldehyde-2,4-dinitrophenylhydrazon	0.99989	0.99994	0.99996
Valeraldehyde-2,4-dinitrophenylhydrazon	0.99990	0.99997	0.99996
m-Tolualdehyde-2,4-dinitrophenylhydrazon	0.99990	0.99993	0.99999
Hexaldehyde-2,4-dinitrophenylhydrazon	0.99990	0.99997	0.99991

Conclusion

This Application Note shows the analysis of DNPH-derivatized aldehydes and ketones under HPLC and UHPLC conditions using the Agilent 1290 Infinity II LC. The transfer of a standard HPLC method with a 4.6 × 150 mm, 5 µm column to a UHPLC method using a 2.1 × 50 mm, 1.8 µm column resulted in a time and solvent savings of approximately 60 %. In addition, the injection volume could be reduced by 75 %. In another step, the UHPLC method was optimized for speed. For the fast UHPLC method, a flow rate of 1.25 mL/min was used, which again resulted in a time and solvent savings of about 90 % in comparison to the original method. Under HPLC and UHPLC conditions, excellent precision and linearity were obtained. The determined LOD and LOQ values were lower for the UHPLC methods than for the HPLC method.

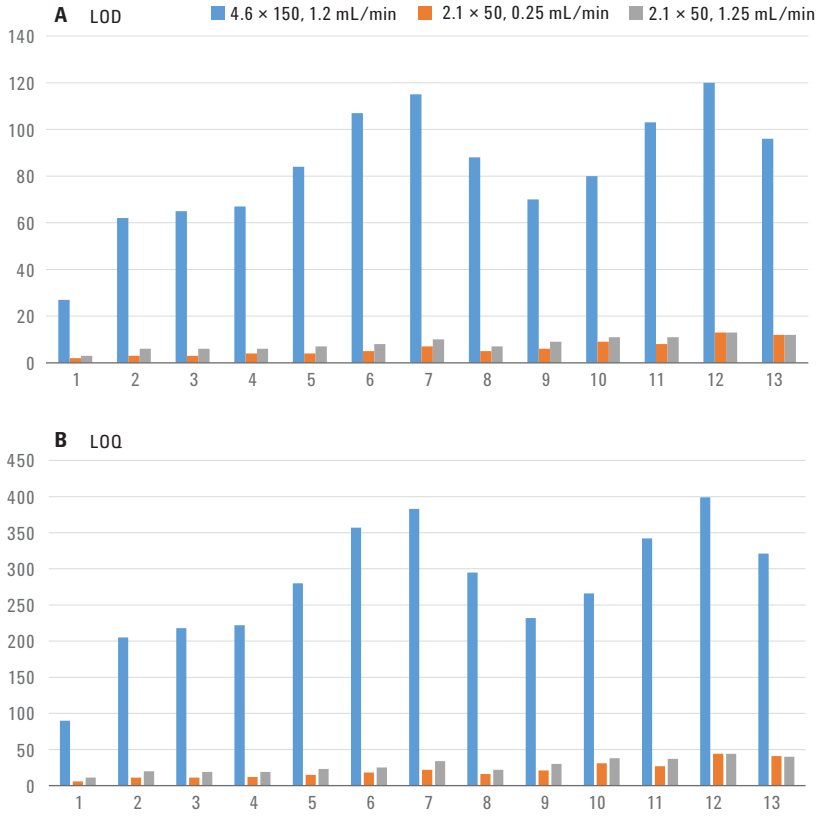

Figure 4. Bar chart for LOD and LOQ values for the HPLC and two UHPLC methods.

References

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