Analysis of DNPH-derivatized aldehydes and ketones on 1-mm id columns with the Agilent 1290 Infinity LC System

Application Note

Environmental

Author

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Abstract

This Application Note demonstrates the ability of the Agilent 1290 Infinity LC System to use 1-mm id columns. The example shows the separation and detection of a sample containing 13 aldehyde and ketone derivatives. Using 1-mm id columns with sub-2-micron particles at UHPLC pressures offers fast separation at high resolution and reduced solvent consumption. In addition, 1-mm id columns allow smaller sample volumes and optimized flow rates for mass spectrometers.

Introduction

Aldehydes and ketones are frequently used compounds in the chemical industry. For example, those with medium-length carbon chains are used as intermediate products during the production of gum, synthetic resin, and plastic products. The most essential aldehyde is formaldehyde, which is used for the production of engineered wood and synthetic resins. Additionally, it is one of the most often used disinfectant and preservative agents. Another frequently used chemical is acetaldehyde. This is used as an organic solvent and as an important intermediate product in many industries. The majority of the analytical methods use the derivatization with 2,4-dinitrophenylhydrazine (DNPH), yielding the corresponding 2,4-dinitrophenylhydrazone. The dinitrophenylhydrazone derivatives can be analyzed by HPLC with UV detection at 360 nm.

In this Application Note, 13 aldehyde and ketone derivatives were analyzed on the Agilent 1290 Infinity LC System modified with the ultra low dispersion kit using 1-mm id columns.

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Experimental

Instruments

For method development, an Agilent 1290 Infinity LC System was used, consisting of:

- Agilent 1290 Infinity Binary Pump with integrated degasser (G4220A)
- Agilent 1290 Infinity High Performance Autosampler (G4226A) with thermostat (G1330A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector (G4212A) with 10-mm Agilent MaxLight flow cell (G4212-60008)
- Agilent 1290 Infinity Ultra Low Dispersion Kit (5067-5189)

Analyte mixture

The mixture of aldehyde-2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones is a certified reference material from Sigma-Aldrich (Catalog No. 47651-U) diluted in acetonitrile. In the mixture, each analyte has a concentration of 30 µg/mL of carbon.

The elution order for all analytes depicted in all Figure 1 is:

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-dinitrophenylhydrazone
8. 2-Butanone-2,4-dinitrophenylhydrazone
9. Butyraldehyde-2,4-dinitrophenylhydrazone
10. Benzaldehyde-2,4-dinitrophenylhydrazone
11. Valeraldehyde-2,4-dinitrophenylhydrazone
12. m-Tolualdehyde-2,4-dinitrophenylhydrazone
13. Hexaldehyde-2,4-dinitrophenylhydrazone

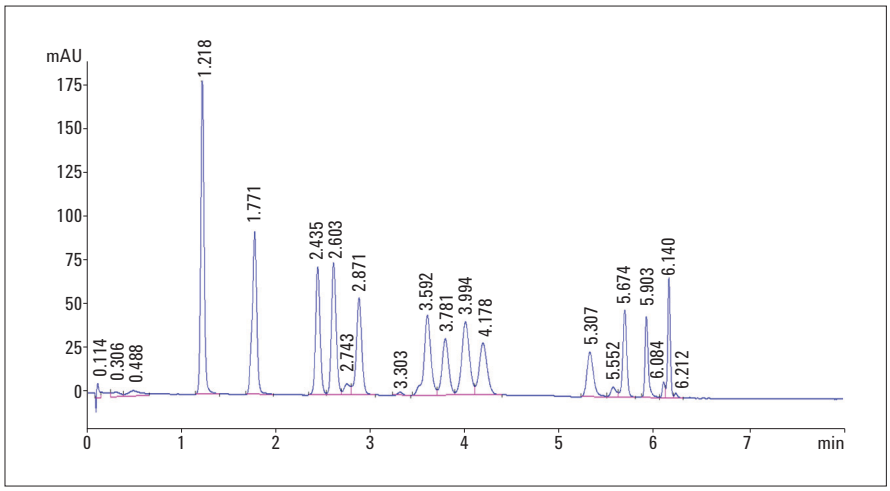

Figure 1
Analysis of 13 aldehydes and ketones on an Agilent 1290 Infinity LC System with 1.0-mm id column.

Method

Column:	Agilent ZORBAX Eclipse Plus RRHD SB C18, 1.0 × 50 mm, 1.8 µm (custom made)
Flow:	0.5 mL/min Solvent A: Water Solvent B: Acetone
Gradient:	0 min 20% B, 1 min 30% B, 3 min 38% B, 4.5 min 38% B, 6 min 65% B
Stop time:	8 min
Post time:	2 min
Injection:	0.2 µL sample, 5 s needle wash in 50% AcN
Sample cooling:	4 °C
Column temperature:	45 °C
Detection:	DAD 360 nm/10
Data rate:	40 Hz

Results and discussion

Because aldehydes and ketones are common intermediate products and also widely used as end products themselves, they are major pollutants in work environments but also occur in natural environment. To make them easily detectable by UV, they are derivatized with a reagent containing a UV chromophore. The separation of these derivatives can be done on an HPLC system. Identification is possible by retention time with a given method adjusted with standard compounds. To increase throughput as well as resolution, the method has to be transferred to an UHPLC system to separate a large number of the UV active derivatives of the most commonly used aldehydes and ketones. This can be achieved using the 1290 Infinity LC System and columns with 1.8-µm particles. To save solvent compared to standard measurement on 4.6-mm id columns, columns with lower inner diameter can be used. This example uses a column with an id of 1.0 mm and a length of 50 mm. To achieve the necessary separation performance, the columns is packed with 1.8-µm particle size material.

With this instrument set up, it was possible to develop a method which separates 13 aldehyde and ketone derivative standards in a very short time, under 7 minutes (Figure 1). Each compound was completely separated and was identified and quantified. The separation was done at an elevated flow rate to achieve the necessary speed and to minimize system delay time compared to flow rates typically applied with 1.0-mm columns. With the applied flow rate of 0.5 mL/min and a system delay volume of 110 µL there is only an approximate 15-second delay time, and hence minimum influence on the separation by isocratic pre-elution effects. The 1290 Infinity LC System is able to deliver the necessary pressure to operate 1.0-mm id columns even with solvents of higher viscosity such as methanol as organic eluent.

In typical standard HPLC systems, band broadening due to the large inner delay volume between injection port and column is a known problem. This is excluded by the low delay volume of the 1290 Infinity LC System. Due to the use of a 1.0-mm id column, this effect becomes even more important. To minimize the effect, the 1290 Infinity LC System was equipped with the Agilent 1290 Ultra Low Dispersions Kit which provides capillaries and an injection seat with minimized dead volume.

Conclusion

This Application Note shows that the Agilent 1290 Infinity LC System in combination with the Agilent 1290 Ultra Low Dispersions Kit is the perfect instrument to work with columns with 1.0-mm id. It provides the necessary backpressure to run even fast applications at high flow rates without isocratic pre-elution influences due to the minimum system delay volume. This is demonstrated by the fast separation of 13 compounds under 7 minutes.

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