High-throughput method development for aldehydes and ketones using an Agilent 1290 Infinity LC system and an Agilent ZORBAX StableBond HD column

Application Note

Environmental

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Abstract

This Application Note describes the development of a fast method for the determination of 13 aldehyde and ketone derivates with the Agilent 1290 Infinity LC system. The method, which used acetone as organic co-solvent separates the analytes within 3.5 minutes.

Introduction

Aldehydes and ketones are important compounds in the chemical industry. One of the most essential aldehydes is formaldehyde because it is used for the production of glued wood and synthetic resin. In addition, formaldehyde is one of the most used disinfectants and preservative agents worldwide. Another relevant aldehyde in the chemical industry is acetaldehyde. This chemical is frequently used as an organic solvent and is an important intermediate product in many industries. For example, acetaldehyde is principally used for the production of acetic acid. In general, aldehydes and ketones with middle carbon chain lengths are used as intermediate products during the production of gum, synthetic resin and plastic products. Therefore, many analytical methods exist for the determination of aldehydes and ketones in different matrices. The majority of these methods use the derivatization with 2,4-dinitrophenylhydrazine yielding the corresponding 2,4-dinitrophenylhydrazone. After that, an HPLC separation with UV detection at 360 nm is then performed.

The introduction of the Agilent 1290 Infinity LC system has improved LC-UV methods in several ways. The pressure of the Agilent 1290 Infinity LC system remains stable as high as 1200 bar at flow rates up to 2 mL/min. This is a significant enhancement in comparison to conventional HPLC systems. The most important advantage of the Agilent 1290 Infinity LC system is the small dwell volume of 125 µL (the volume from the point of mixing solvents A and B up to the column inlet including the autosampler). Because of this very small dwell volume, narrow bore columns can be used to shorten analysis time and reduce organic solvent consumption.

This Application Note focuses on LC method development for the determination of several aldehydes and ketones, as well as the advantages of the Agilent 1290 Infinity LC system.

A commercially available method development software package was used to determine the optimal method parameters. Four basic chromatographic runs were performed to determine the optimal column temperature and solvent gradient. These measurements comprised two linear solvent gradients from 5% to 100% B in 10 and 30 minutes at 20 °C and the same gradients at 40 °C. The measurements were performed on an Agilent ZORBAX StableBond RRHD C18 column (50 mm × 2.1 mm, 1.8 µm) by using acetone as an organic modifier. A method was then developed and experimentally confirmed with high agreement between prediction and experiment.

Experimental

All calculations were performed with Agilent ChemStation software version B.04.02 [65].

LC system

For method development, an Agilent 1290 Infinity LC system was used. The system consists of:

- Agilent 1290 Infinity Binary Pump with integrated degasser (G4220A)
- Agilent 1290 Infinity High Performance Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment SL (G1316B)
- Agilent 1290 Infinity Diode Array Detector (G4212A)

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.

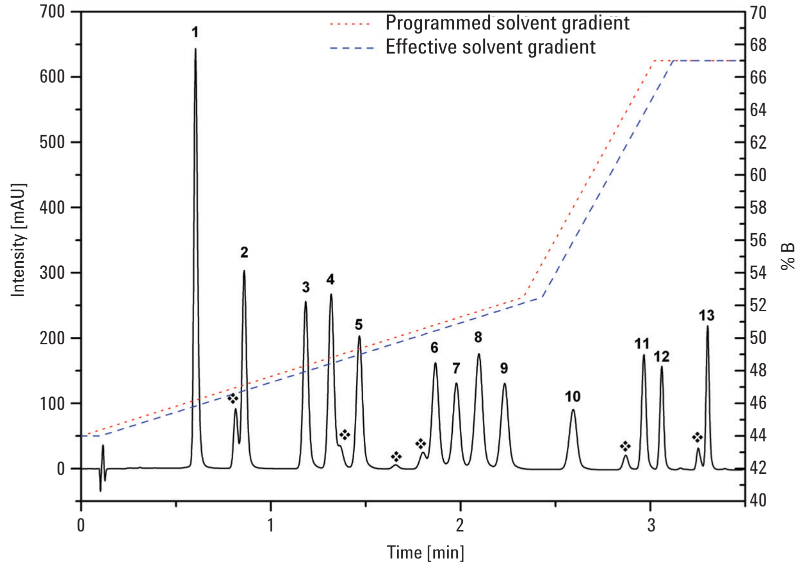
The elution order for all analytes depicted in all figures is:

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

Results and discussion

Figure 1 shows the computer-optimized separation of 13 aldehyde 2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones on an Agilent ZORBAX StableBond RRHD C18 column within 3.5 minutes. Acetone was used as an organic co-solvent. All peaks are baseline separated with a critical resolution of 1.6 between peak pair 6 and 7. The critical resolution was calculated by the tangent method. The impurities, which are present in the reference material and highlighted by stars were not included in the method development. Figure 1 also shows a comparison of the programmed and effective solvent gradient. Due to a very small dwell volume, there is only a minor difference between the programmed and effective solvent gradients compared to a conventional HPLC system, which exhibits a dwell volume of approximately 1000 µL. This means that at a flow rate of 1.2 mL/min, the programmed solvent gradient reaches the column inlet with a delay of 0.83 minutes, so that the elution of the early-eluting analytes occurs under isocratic conditions. In other words, the elution of the early-eluting analytes cannot be affected by the solvent gradient. Using the Agilent 1290 Infinity LC system with a dwell volume of 125 µL at a flow rate of 1.2 mL/min, the programmed solvent gradient reaches the column inlet after 6.25 seconds and enables fast separations within a few minutes.

The chromatogram shown in Figure 1 is a high pressure application. Due to the applied flow rate of 1.2 mL/min and the 1.8 µm particle packed column, a pressure drop of 1100 bar during the solvent gradient can be observed. Figure 2 shows an overlay of ten consecutive chromatograms, demonstrating the robustness and reproducibility of the develped method.



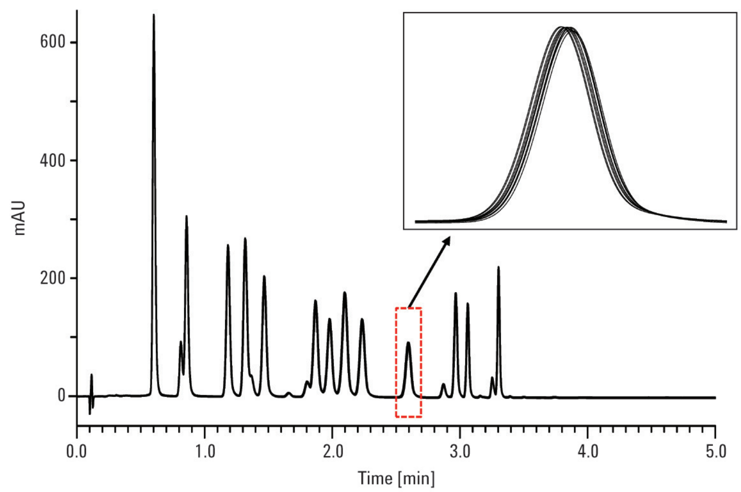
Chromatographic conditions:	
Stationary phase:	Agilent ZORBAX StableBond RRHD C18 (2.1 mm × 50 mm, 1.8 µm)
Mobile phase:	A: deionized water, B: acetone;
Solvent gradient:	0–2.33 min from 44% to 52.5% B, 2.33–3.02 min from 52.5% to 67% B, 3.02–3.50 min isocratic at 67% B;
Flow rate:	1.2 mL/min
Detection:	UV at 360 nm
Injection volume:	1 µL
Temperature:	33.4 °C
Pressure drop:	1100 bar.
Elution order of the analytes:	See experimental section; ❖ impurities

Figure 1
Separation of 13 aldehyde-2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones.

Figure 2 shows that there are virtually no differences among the ten chromatograms. This conclusion is confirmed by the relative standard deviation (RSD) of retention times of the analytes, which ranges between 0.03% and 0.09%.

Conclusion

The Agilent 1290 Infinity LC system is suitable for developing fast HPLC methods. The separation of 13 aldehyde and ketone derivates was completed in around 3.5 minutes, using acetone as an organic modifier in the mobile phase. In addition, the method presented here illustrates that fast HPLC separations are only possible using HPLC systems with small dwell volumes. Finally, we have shown that the Agilent StableBond RRHD C18 column is suitable for separations where the pressure drop is greater than 1100 bar, without loss of separation efficiency.



See Figure 1 for Chromatographic conditions.

Figure 2
Overlay of 10 consecutive chromatograms of the separation of 13 aldehyde-2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones.

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