# Analysis of DNPH-derivatized Aldehydes and Ketones using the Agilent 1220 Infinity LC System with Diode Array Detector

Application Note


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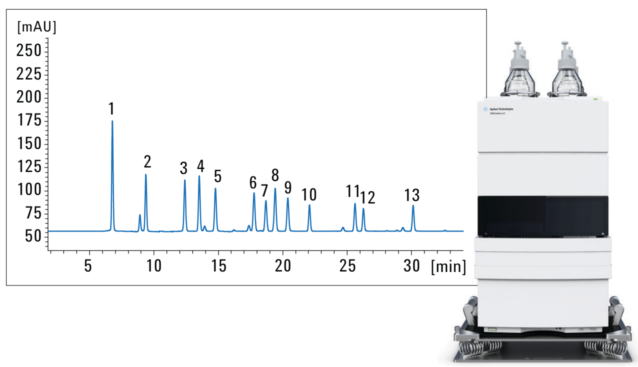



## Abstract

Aldehydes and ketones are important compounds in the chemical industry. However, these compounds can be hazardous when released into the environment. This Application Note describes the successful analysis of 13 DNPH-derivatized aldehydes and ketones using the Agilent 1220 Infinity Gradient LC system with built-in diode-array detector (DAD). In combination with the Agilent 1220 Infinity Mobile Upgrade Kit, the system can be installed in a vehicle and measurements can be performed at different test sites. Using both 1.8-µm and 5-µm columns achieved excellent precision and linearity as well as low limits of detection (LOD) and limits of quantification (LOQ) for all carbonyl-containing compounds.


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## Introduction

The environmental analysis of aldehydes and ketones is important due to the toxic and potentially carcinogenic nature of some of these carbonyl compounds. As combustion products from, for example, automotive exhaust or tobacco smoke, aldehydes and ketones are significant air pollutants and can also be detected in water and soil. Atmospheric carbonyl compounds are also created by thermal degradation of polymers in the plastic processing industry.

Of all the atmospheric carbonyl compounds, acetaldehyde and formaldehyde are the most abundant. In addition, formaldehyde has the largest technical relevance because it an essential precursor for polymers and many other chemical compounds. It is also frequently used as disinfectant and preservative (for example, formalin). Also, acetaldehyde is one of the most important aldehydes because it is highly prevalent in nature and a significant compound in the chemical industry. Many other aldehydes and ketones are also found in the chemical industry, for example, in rubber, synthetic resin, and plastic production.

The determination of aldehydes and ketones in several environmental matrices is mostly conducted with high pressure liquid chromatography (HPLC) with UV detection based on the derivatization of the carbonyl compounds with 2,4-dinitrophenylhydrazine (DNPH).

This Application Note presents the analysis of DNPH-derivatized aldehydes and ketones using the 1220 Infinity Gradient LC system with built-in DAD. As an integrated, binary-gradient liquid chromatography (LC) system with a pressure range of up to 600 bar, 5-µm and 1.8-µm columns could be deployed due to the ultra-HPLC (UHPLC) capabilities of the system. Using the 1220 Infinity LC Mobile Upgrade Kit, the following application can be carried out in a mobile laboratory.

## Experimental

### Instrumentation

The Agilent 1220 Infinity Gradient LC System (G4294B) was equipped with a dual-channel gradient pump with integrated degassing unit, autosampler, column compartment and diode array detector. For transportation, the system was mounted on a transportation plate, Agilent 1220 Infinity Mobile Upgrade Kit (G4292A).

### Software

- Agilent OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.04 [35]
- Agilent OpenLAB CDS 3D UV Add-On Software.

### Sample

The mixture of aldehyde-2,4-dinitrophenylhydrazones and ketone-2,4-dinitrophenylhydrazones was certified reference material from Sigma-Aldrich (Catalog No. 47651-U) diluted in acetonitrile. In the mixture, each analyte had a concentration of 30 µg/mL of carbon. For analysis, the mixture was diluted in dimethyl sulfoxide (DMSO) in a ratio of 1:10. Table 1 shows the elution order for all analytes depicted in all figures. Acetone and DMSO were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22-µm membrane point-of-use cartridge (Millipak).

| 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-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 |

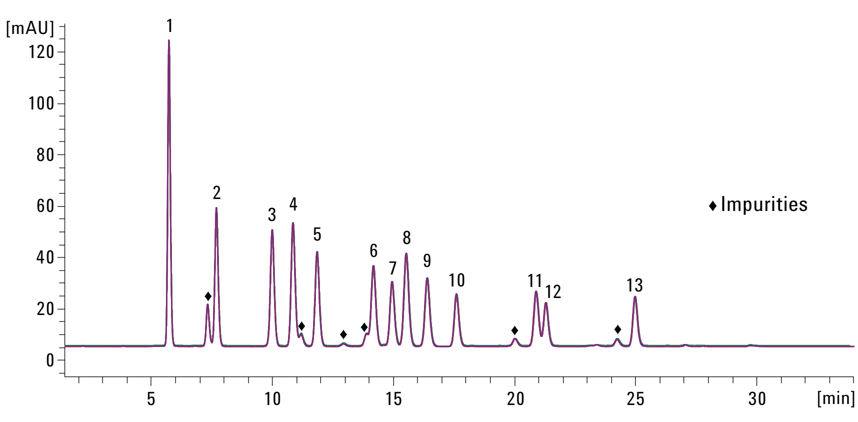
**Table 1**
**Elution order of aldehyde and ketone mixture.**

## Chromatographic conditions

| Parameter | Conditions (4.6 mm id columns) | Conditions (2.1 mm id columns) |
|---|---|---|
| Columns: | Agilent ZORBAX Eclipse Plus C18,<br>4.6 × 150 mm, 5 µm (p/n 959993-902)<br>Agilent ZORBAX RRHT Eclipse Plus C18,<br>4.6 × 150 mm, 1.8 µm (p/n 959994-902) | Agilent ZORBAX RRHD Eclipse Plus C18,<br>2.1 × 150 mm, 1.8 µm (p/n 959759-902) |
| Mobile Phase: | A: Water<br>B: Acetone | A: Water<br>B: Acetone |
| Flow rate: | 1.2 mL/min | 0.25 mL/min |
| Gradient: | 0 minutes: 45 %B<br>12 minutes: 53 %B<br>28 minutes: 67 %B<br>32 minutes: 67 %B<br>33 minutes: 95 %B | 0 minutes: 45 %B<br>12 minutes: 53 %B<br>28 minutes: 67 %B<br>32 minutes: 67 %B<br>33 minutes: 95 %B |
| Stop time: | 34 minutes | 34 minutes |
| Post time: | 20 minutes | 20 minutes |
| Injection volume: | 10 µL | 2.1 µL |
| Temperature: | 45 °C | 45 °C |
| Detection: | 360 nm/10 nm Ref.: off<br>Peak width > 0.025 minutes (0.5 second response time, 10 Hz) | 360 nm/10 nm Ref.: off<br>Peak width > 0.025 minutes (0.5 second response time, 10 Hz) |

# Results and Discussion

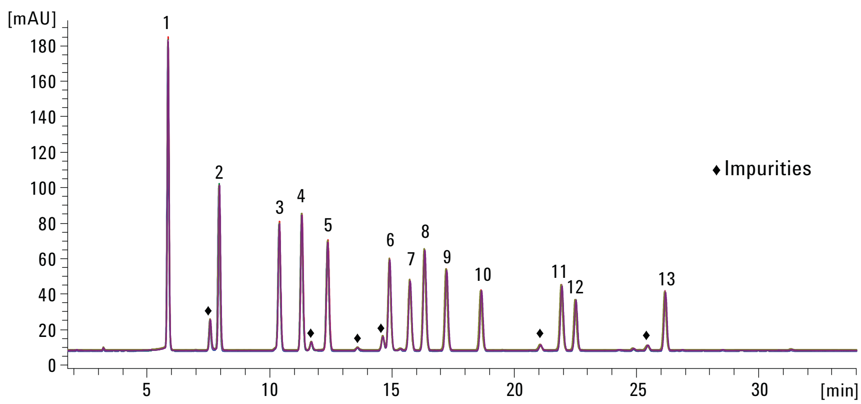
The DNPH-derivatized aldehydes and ketones were separated using an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm column and the relative standard deviation (RSD) of retention times and areas was determined for six consecutive runs, see Figure 1. All RSDs of the retention times were below 0.01 %. The area precision was found to be below 0.4 %. In addition to the 13 carbonyl compounds, some impurities were detected.




| Peak | RSD RT (%) | RSD Area (%) |
|---|---|---|
| 1 | 0.060 | 0.230 |
| 2 | 0.051 | 0.198 |
| 3 | 0.051 | 0.239 |
| 4 | 0.048 | 0.219 |
| 5 | 0.050 | 0.212 |
| 6 | 0.044 | 0.221 |
| 7 | 0.049 | 0.168 |
| 8 | 0.050 | 0.172 |
| 9 | 0.049 | 0.232 |
| 10 | 0.045 | 0.268 |
| 11 | 0.044 | 0.326 |
| 12 | 0.040 | 0.288 |
| 13 | 0.034 | 0.239 |

**Figure 1**
**Six consecutive runs of DNPH-derivatized aldehydes and ketones separated on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm column at 90 bar.**

The same sample was also separated using a ZORBAX RRHT Eclipse Plus C18, 4.6 × 150 mm, 1.8 µm column at a higher pressure of 420 bar. The RSD of retention times and areas was determined for six consecutive runs, see Figure 2.




| Peak | RSD RT (%) | RSD area (%) |
|---|---|---|
| 1 | 0.115 | 0.526 |
| 2 | 0.150 | 0.163 |
| 3 | 0.078 | 0.318 |
| 4 | 0.082 | 0.340 |
| 5 | 0.080 | 0.341 |
| 6 | 0.054 | 0.289 |
| 7 | 0.060 | 0.350 |
| 8 | 0.063 | 0.313 |
| 9 | 0.077 | 0.283 |
| 10 | 0.084 | 0.187 |
| 11 | 0.065 | 0.248 |
| 12 | 0.071 | 0.394 |
| 13 | 0.058 | 0.233 |

**Figure 2**
**Six consecutive runs of DNPH-derivatized aldehydes and ketones separated on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 1.8 µm column at 420 bar.**

The precision of retention times and areas were found similar to those achieved with the 5-µm column. All RSDs of the retention times were below 0.016 %. The area precisions were found to be below 0.4 %. Using sub-two µm columns, an improvement in resolution was visible by a factor of approximately two, especially visible for peak 11 and 12, see Figure 2 and Table 2.

| Peak | Resolution (5-µm columns) | Resolution (1.8-µm columns) |
|---|---|---|
| 1 | – | - |
| 2 | 1.45 | 2.35 |
| 3 | 8.41 | 14.50 |
| 4 | 2.88 | 4.78 |
| 5 | 2.01 | 3.32 |
| 6 | 0.89 | 1.27 |
| 7 | 2.18 | 3.75 |
| 8 | 1.58 | 2.61 |
| 9 | 2.29 | 3.89 |
| 10 | 3.33 | 6.41 |
| 11 | 2.29 | 3.68 |
| 12 | 1.09 | 2.55 |
| 13 | 2.02 | 3.14 |

**Table 2**
**Improvement in resolution from 5-µm to 1.8-µm columns.**

Using the ZORBAX RRHT Eclipse Plus C18 4.6 × 150 mm, 1.8 µm column, the linearity, LOD and LOQ were determined for a dilution series of the DNPH-derivatized aldehydes and ketones from 3 µg/mL down to 4 ng/mL. An excellent correlation was found for all carbonyl-containing compounds. All correlation curves showed very high linearity with correlation factors of 1 or 0.99999, see Table 3.

| Compound | Correlation coefficient |
|---|---|
| Formaldehyde-2,4-dinitrophenylhydrazone | 1 |
| Acetaldehyde-2,4-dinitrophenylhydrazone | 0.99999 |
| Acrolein-2,4-dinitrophenylhydrazone | 1 |
| Acetone-2,4-dinitrophenylhydrazone | 1 |
| Propionaldehyde-2,4-dinitrophenylhydrazone | 1 |
| Crotonaldehyde-2,4-dinitrophenylhydrazone | 1 |
| Methacrolein-2,4-dinitrophenylhydrazone | 1 |
| 2-Butanone-2,4-dinitrophenylhydrazone | 1 |
| Butyraldehyde-2,4-dinitrophenylhydrazone | 1 |
| Benzaldehyde-2,4-dinitrophenylhydrazone | 1 |
| Valeraldehyde-2,4-dinitrophenylhydrazone | 1 |
| *m*-Tolualdehyde-2,4-dinitrophenylhydrazone | 0.99999 |
| Hexaldehyde-2,4-dinitrophenylhydrazone | 1 |

**Table 3**
**Correlation factors of all carbonyl components.**

Figure 3 shows the correlation curves of four of the compounds together with the response factors, which were in the ± 5 % deviation range.

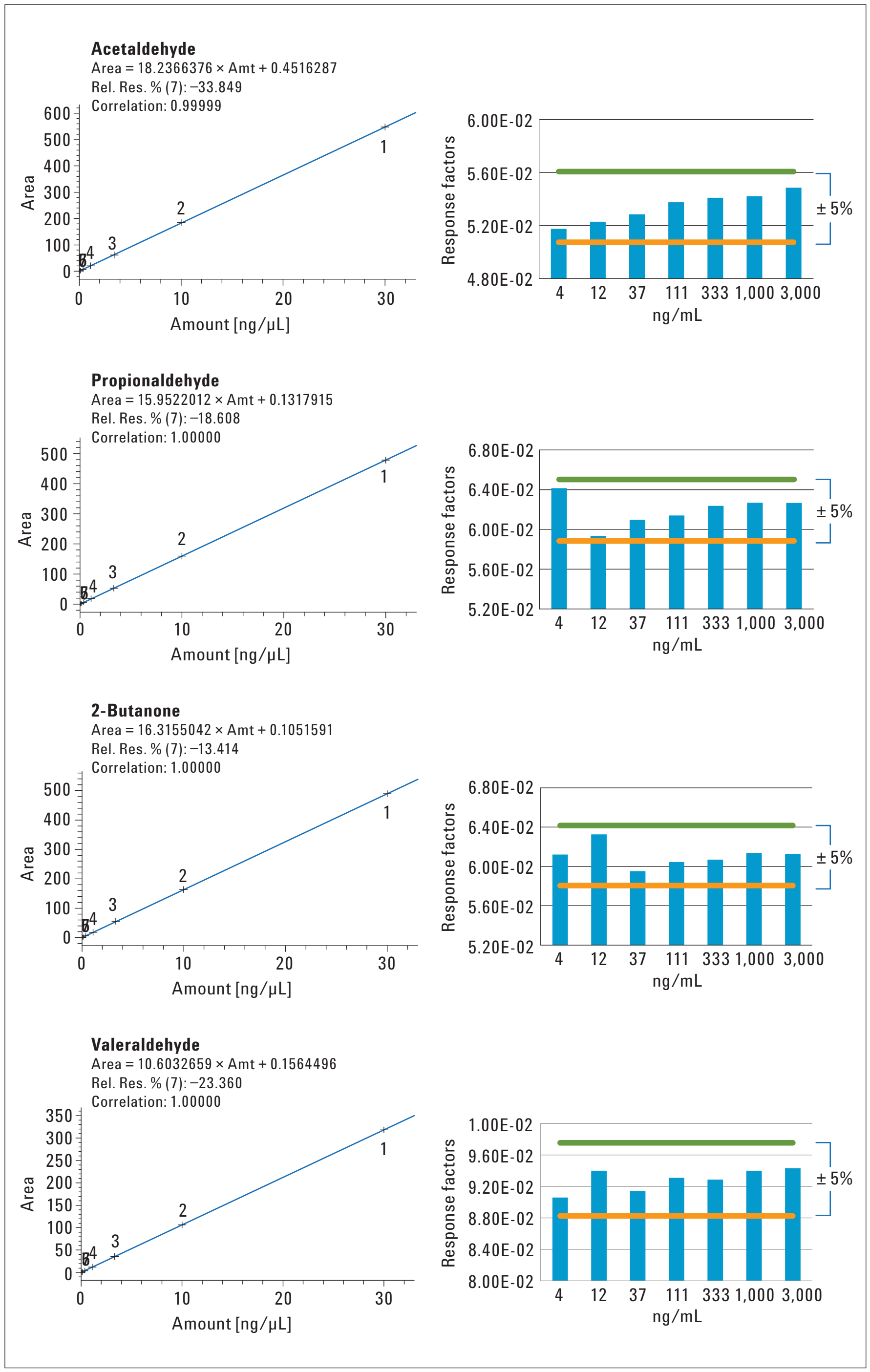



**Figure 3**
**Linearity of four of the carbonyl compounds.**

The LOD and LOQ were evaluated from the concentration of aldehydes and ketones required to give a signal-to-noise ratio of at least 3 and 10, respectively. Table 4 displays the LOD and LOQ for all the components.

Figure 4 shows the separation of the carbonyl compounds on a narrow bore ZORBAX RRHD Eclipse Plus C18, 2.1 x 150 mm, 1.8 µm column. With this setup, it was possible to save approximately 80 % of sample and mobile phase due to the adjustment of injection volume and flow rate to 2.1 id columns.

## Conclusions

DNPH-derivatized aldehydes and ketones were successfully analyzed using the Agilent 1220 Infinity Gradient LC with DAD using 5-µm as well as 1.8-µm columns under UHPLC conditions. Excellent values for precision and linearity were achieved together with low LODs and LOQs for all carbonyl components. Although the 1220 Infinity Gradient LC is designed for 4.6 id columns, applications using 2.1 id columns are possible under nonfast gradient conditions.

| Compound | LOD [pg] | LOQ [pg] |
|---|---|---|
| Formaldehyde-2,4-dinitrophenylhydrazone | 8 | 26 |
| Acetaldehyde-2,4-dinitrophenylhydrazone | 15 | 51 |
| Acrolein-2,4-dinitrophenylhydrazone | 19 | 63 |
| Acetone-2,4-dinitrophenylhydrazone | 18 | 61 |
| Propionaldehyde-2,4-dinitrophenylhydrazone | 24 | 79 |
| Crotonaldehyde-2,4-dinitrophenylhydrazone | 29 | 96 |
| Methacrolein-2,4-dinitrophenylhydrazone | 37 | 124 |
| 2-Butanone-2,4-dinitrophenylhydrazone | 25 | 85 |
| Butyraldehyde-2,4-dinitrophenylhydrazone | 34 | 112 |
| Benzaldehyde-2,4-dinitrophenylhydrazone | 41 | 138 |
| Valeraldehyde-2,4-dinitrophenylhydrazone | 38 | 127 |
| *m*-Tolualdehyde-2,4-dinitrophenylhydrazone | 47 | 156 |
| Hexaldehyde-2,4-dinitrophenylhydrazone | 43 | 145 |

**Table 4**
**LOD and LOQ for all carbonyl components.**

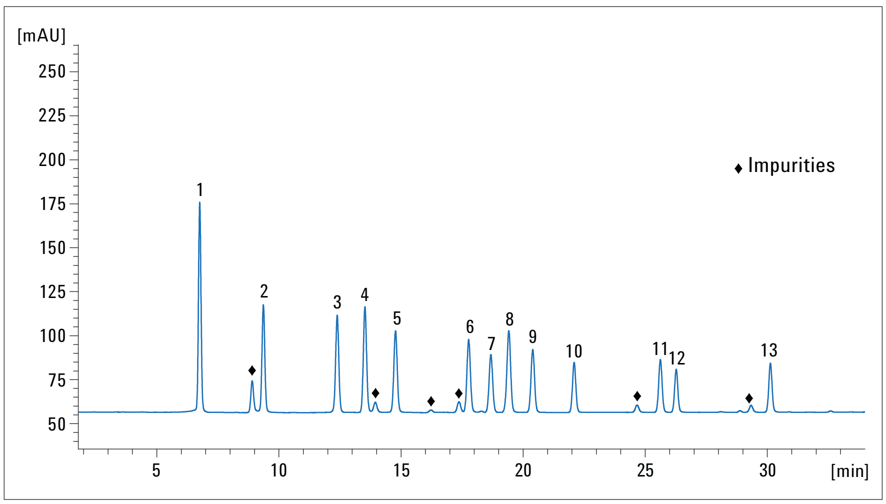



**Figure 4**
**DNPH-derivatized aldehydes and ketones using an Agilent ZORBAX Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm.**

**www.agilent.com/chem/1220**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