Application Note

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# Carbonyl-DNPH Derivatives in Indoor and In-car Air by UHPLC and Triple Quadrupole LC/MS

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## Abstract

Ultra high performance liquid chromatography (UHPLC) and LC/MS/MS methods determine free carbonyl compounds in air after derivatization with 2,4-dinitrophenylhydrazine. The methods use UHPLC with DAD detection and triple quadrupole LC/MS/MS to identify and quantitate the target analytes. Using an Agilent InfinityLab Poroshell 120 EC-C18 HPLC column, UHPLC separates 14 carbonyl-DNPH components in 12 minutes. LC/MS/MS gives a lower limit of detection than LC alone. Using multiple reaction monitoring, more carbonyl-DNPH can be resolved.

## Introduction

Carbonyl compounds are hazardous substances existing in air, with formaldehyde and acetaldehyde being the most abundant airborne carbonyls. They are classified as probable human carcinogens by the United States Environmental Protection Agency (EPA) and designated carcinogenic to humans by the International Agency for Cancer Research (IARC). The compounds are sources of contamination in indoor living and working environments, and are probably released from multiple sources, including plywood, particle board, furniture, glues, tobacco smoke, textiles, and other building materials. The air inside automobiles is another place where carbonyls are found, and many regulations from EU, Germany, China, and Japan are set to control levels of carbonyl compounds in vehicle air. Because of their highly volatile and reactive nature, carbonyl compounds are often converted to dinitrophenylhydrazine (DNPH) derivatives prior to LC analysis.

This application note developed a UHPLC method for separating 14 carbonyl-DNPH compounds in only 12 minutes. MS/MS detection was used to identify more compounds, because some could not be resolved through UHPLC alone. Both methods were developed on an Agilent InfinityLab Poroshell 120 EC-C18 column. Two samples collected from indoor air and in-vehicle air were analyzed, and some of carbonyl-DNPH compounds were detected and the amounts of them were calculated.

## Materials and methods

The Agilent 1290 Infinity LC System includes a binary pump, a thermostatted column compartment, a high-performance autosampler and a diode array detector. The LC/MS/MS system used a 1290 Infinity LC System and an Agilent 6460 Triple Quadrupole LC/MS.

Stock solutions were purchased from AccuStandard, Inc, New Haven, CT, USA (Table 1). They were diluted to appropriate concentrations with acetonitrile.

Two air samples were prepared as follows:

1. Sample 6 L air using an AirChek 2000 air sampling pump, SKC Inc., Eighty Four, PA, USA, with DNPH-sampling SPE cartridge (silica coated with DNPH) at a flow rate of 200 mL/min in 30 minutes.
2. Wash the cartridges with 5.0 mL acetonitrile.
3. Collect the eluted solution and filter through an Agilent 0.2 µm regenerated cellulose filter (p/n 5064-8221).
4. Transfer the filtered solutions to autosampler vials for analysis.

**Table 1.** The target carbonyl-DNPH compounds analyzed in this application note.

| No. | Name | CAS | Stock concentration (µg/mL) |
|---|---|---|---|
| 1 | Acetaldehyde-DNPH | 1019-57-4 | 2.000 |
| 2 | Acetone-DNPH | 1567-89-1 | 2.000 |
| 3 | Acrolein-DNPH | 888-54-0 | 2.000 |
| 4 | Benzaldehyde-DNPH | 1157-84-2 | 2.000 |
| 5 | 2-Butanone-DNPH | 958-60-1 | 2.000 |
| 6 | Butyraldehyde-DNPH | 1527-98-6 | 2.000 |
| 7 | Crotonaldehyde-DNPH | 1527-96-4 | 2.000 |
| 8 | Cyclohexanone-DNPH | 1589-62-4 | 5.000 |
| 9 | Formaldehyde-DNPH | 1081-15-8 | 4.000 |
| 10 | Hexaldehyde-DNPH | 1527-97-5 | 2.000 |
| 11 | Methacrolein-DNPH | 5077-73-6 | 2.000 |
| 12 | Propionaldehyde-DNPH | 725-00-8 | 2.000 |
| 13 | *p*-Tolualdehyde-DNPH | 2571-00-8 | 2.000 |
| 14 | Valeraldehyde-DNPH | 2057-84-3 | 2.000 |

## Conditions

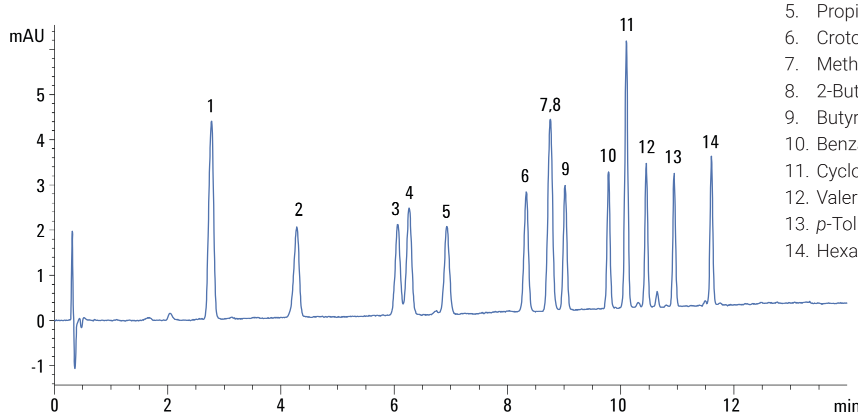
| LC system | |
|---|---|
| Column | Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 75 mm, 2.7 µm (p/n 697975-302) |
| Eluent | A) Water<br>B) ACN |
| Injection volume | 5 µL |
| Flow rate | 1.0 mL/min |
| Gradient | Time (min) %B<br>0 35<br>2 35<br>7 45<br>12 70 |
| Stop time | 14 minutes |
| Post run | 1.5 minutes |
| Temperature | 30 °C |
| Instrument | Agilent 1290 Infinity LC System (installed with a 1290 in-line filter after injector valve, p/n 5067-4638) |
| Detector | UV, 360 nm |
| **LC/MS/MS system** | |
| Column | Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 75 mm, 2.7 µm (p/n 697975-302) |
| Eluent | A) Water<br>B) ACN |
| Injection volume | 5 µL |
| Flow rate | 0.5 mL/min |
| Gradient | Time (min) %B<br>0 35<br>5 55<br>12 70<br>14 70 |
| Temperature | 30 °C |
| Stop time | 14 minutes |
| Post run | 2.0 minutes |
| Instrument | Agilent 1290 LC Infinity (installed with a 1290 in-line filter after injector valve, p/n 5067-4638), and an Agilent 6460 Triple Quadrupole LC/MS |
| **MS conditions** | |
| Gas temperature | 325 °C |
| Gas flow | 10 L/min |
| Nebulizer | 50 psi |
| Sheath gas temperature | 350 °C |
| Sheath gas flow | 11 L/min |
| Capillary | Negative 3, 500 V |

## Results and discussion

The superficially porous particles of a Poroshell 120 have nearly identical efficiency as sub-2 µm totally porous materials and can be used to provide similarly fast and high resolution analyses at a lower pressure. A quick separation of 14 carbonyls in 12 minutes was achieved on the Poroshell 120 EC-C18 column with a gradient method (Figure 1), while a long analyses time of over 30 minutes was necessary to resolve all the compounds in another work[1].

### Optimized MRM conditions

| No. | Compound | Ion source | Ion pair qualitative and quantitative analyses | RT (min) |
|---|---|---|---|---|
| 1 | Formaldehyde | ESI (–) | 208.8 → 163 (7 V), 208.8 → 133 (7 V) | 4.084 |
| 2 | Acetaldehyde | ESI (–) | 223 → 163 (7 V), 223 → 122 (10 V) | 5.203 |
| 3 | Acetone | ESI (–) | 237 → 178 (16 V), 237 → 164 (13 V) | 6.329 |
| 4 | Acrolein | ESI (–) | 235 → 163 (10 V), 235 → 158 (10 V) | 6.399 |
| 5 | Propionaldehyde | ESI (–) | 237 → 163 (10 V), 237 → 152 (12 V) | 6.818 |
| 6 | Crotonaldehyde | ESI (–) | 249 → 202.1 (12 V), 249 → 172 (12 V) | 7.710 |
| 7 | Methacrolein | ESI (–) | 249 → 172 (12 V), 249 → 109 (15 V) | 8.079 |
| 8 | 2-Butanone | ESI (–) | 251 → 178.2 (12 V), 251 → 164 (12 V) | 8.137 |
| 9 | Butyraldehyde | ESI (–) | 251 → 163 (12 V),251 → 122 (15 V) | 8.364 |
| 10 | Benzaldehyde | ESI (–) | 285 → 238 (12 V), 285 → 163 (12 V) | 9.052 |
| 11 | Cyclohexanone | ESI (–) | 277 → 247.2 (10 V), 277 → 231.1 (10 V) | 9.710 |
| 12 | Valeraldehyde | ESI (–) | 265 → 191.1 (11 V), 265 → 163.1 (8 V) | 10.087 |
| 13 | *p*-Tolualdehyde | ESI (–) | 299 → 252.1 (12 V), 299 → 163 (12 V) | 10.729 |
| 14 | Hexaldehyde | ESI (–) | 279 → 163 (10 V), 279 → 205.2 (10 V) | 11.917 |




**Peak identification**

1. Formaldehyde
2. Acetaldehyde
3. Acetone
4. Acrolein
5. Propionaldehyde
6. Crotonaldehyde
7. Methacrolein
8. 2-Butanone
9. Butyraldehyde
10. Benzaldehyde
11. Cyclohexanone
12. Valeraldehyde
13. *p*-Tolualdehyde
14. Hexaldehyde

**Figure 1.** Chromatogram of carbonyl standards on an Agilent InfinityLab Poroshell 120 EC-C18 column fitted to an Agilent 1290 Infinity LC System.

Reasonable resolution was achieved between all the standard components, except methacrolein and 2-butanone. These two compounds could still be identified because of different product ions using the triple quadruple LC/MS system. Reproducibility tests were carried out by seven consecutive injections of 0.5 ng standards. This separation could be easily reproduced even at such low carbonyl levels.

The air samples from indoor and in-vehicle air were measured by the UHPLC method. Figure 3 shows chromatograms of sample 1, sample 2, and the standards. Compounds varied in the different samples. Acetaldehyde, acetone, and acrolein were found in the two samples but additional compounds were detected in sample 2. The amounts of the detected compounds could be measured given the concentration of the standards.

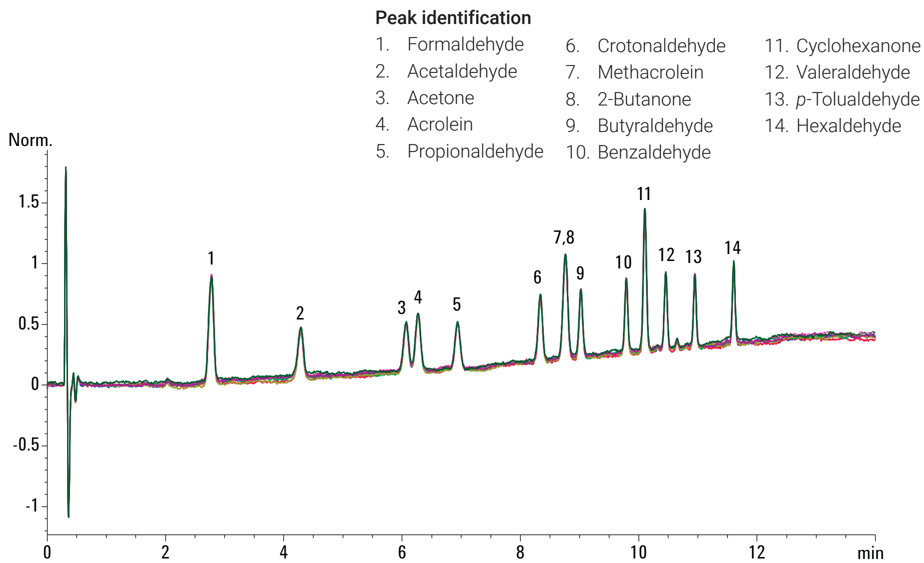



**Figure 2.** Overlay chromatogram of seven consecutive injections of 0.5 ng standards on an Agilent InfinityLab Poroshell 120 EC-C18 column.

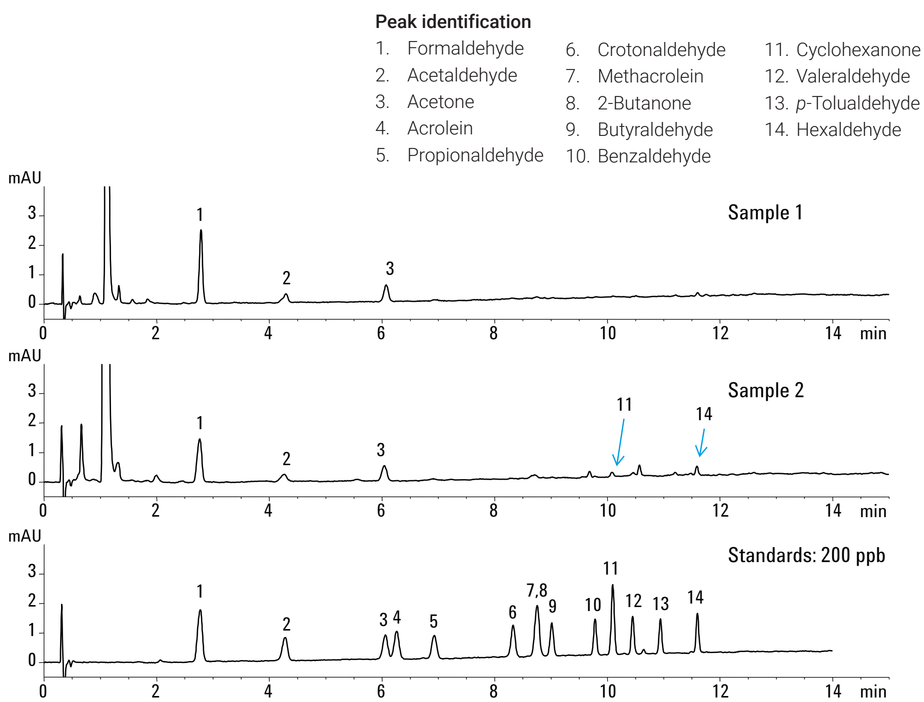



**Figure 3.** Chromatograms of carbonyl samples and standards using an Agilent InfinityLab Poroshell 120 EC-C18 column.

The LC method was then transferred to an LC/MS/MS method because the mobile phase using water/acetonitrile was MS friendly. Compounds such as methacrolein and 2-butanone that could not be resolved by LC could be identified by LC/MS/MS due to the different product ions. Figure 4 shows the MRM (multiple-reaction monitoring) chromatograms of 14 carbonyl-DNPH standards and a sample of in-vehicle air. The calculated limit of detection with triple quadrupole LC/MS was in the range of 0.03 to 0.3 parts per billion (ppb), which was far below that of the UHPLC method of 60 to 180 ppb.

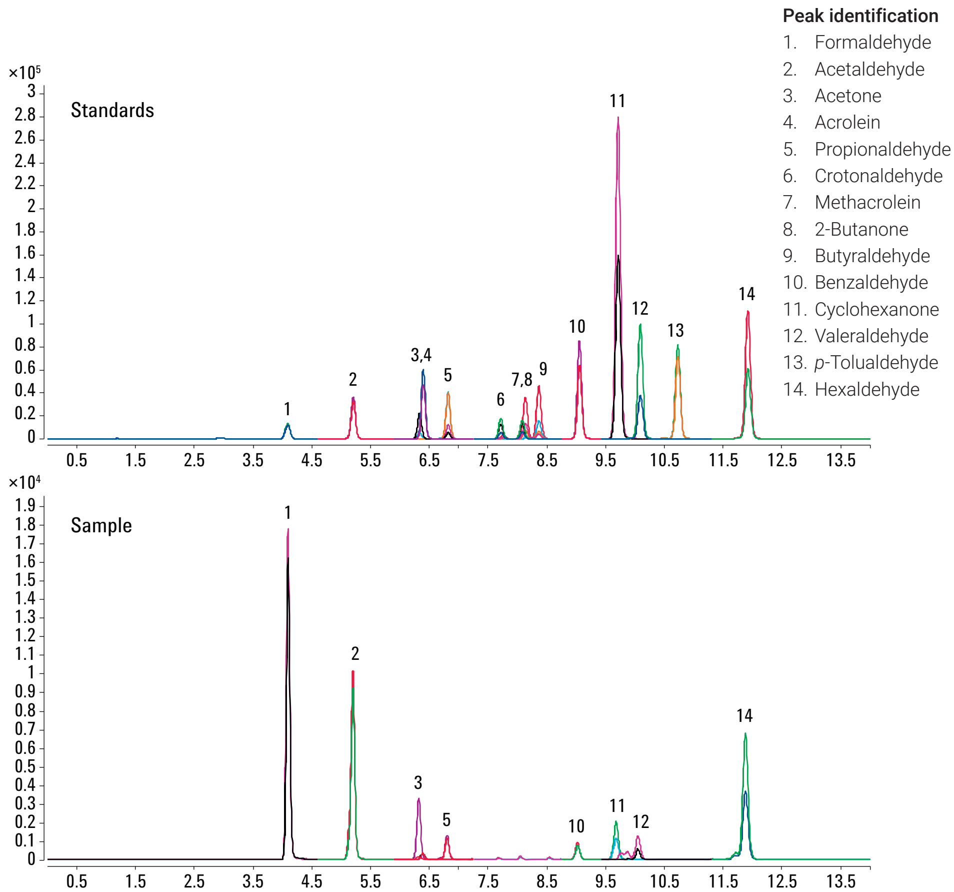



**Figure 4.** MRM chromatograms of carbonyl standards and sample (in-vehicle air) using an Agilent InfinityLab Poroshell 120 EC-C18 column.

## Conclusions

A method was developed for the separation of carbonyl components using the Agilent InfinityLab Poroshell 120 EC-C18 column. The column provided good selectivity for these compounds and good resolution. The method developed on the Agilent 1290 Infinity LC was suitable for fast screening and quantitative analysis of these compounds in air. The LC/MS/MS method enabled co-eluted compounds to be identified and quantified using MRM and was suitable for low-level concentration analysis of carbonyls.

## Reference


1. Anon. Determination of Carbonyl Compounds by High Performance Liquid Chromatography (HPLC), EPA Method 8315a. *Environmental Protection Agency*, Washington, DC, USA (**1996**).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