

Determination of Carbonyl Compounds in Vehicle Cabin Compartments

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Key Words

DNPH Derivatives, Aldehyde and Ketone Compounds Analysis, Environmental Analysis, Air Quality, Air Monitoring, HPLC

Goal

To develop an efficient high-performance liquid chromatography (HPLC) method for the sensitive determination of 2,4-dinitrophenylhydrazine (DNPH)-derivatized carbonyl compounds in vehicle cabin air samples. The target analytes—those specified in California Air Resources Board (CARB) Method No. 1004, International Organization for Standardization (ISO) 16000-3:2011, U.S. Environmental Protection Agency (EPA) Compendium Method TO-11A, and the Chinese HJ/T 400-2007—are formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, methyl ethyl ketone, methacrolein, hexanaldehyde, butyraldehyde, benzaldehyde, valeraldehyde, and *m*-tolualdehyde.

Introduction

Many carbonyl compounds are known to cause adverse effects on human health and thus have been listed by the U.S. EPA in the Health Effects Notebook for Hazardous Air Pollutants.¹ For example, acetaldehyde and formaldehyde are considered probable human carcinogens; inhalation of acrolein may result in upper respiratory tract irritation and congestion; and exposure to high levels of propionaldehyde increases the risk of liver damage and may cause people to choke, pass out, or suffocate.

People are subject to toxic exposure during the manufacture of these carbonyl compounds, which are widely used in large quantities in vehicle manufacturing. Carbonyl compounds can also be hazardous to human health through the direct or indirect release of harmful toxins into vehicle passenger cabin compartments. People in modern society now spend more time in vehicles due to lengthy commutes, long-distance travel, and frequent traffic jams. Therefore, it is important to establish effective methods for the determination of carbonyl compounds found in the air inside vehicle cabins.

Because the carbonyl compounds of interest are volatile, it is preferable to derivatize them into a nonvolatile, stable form. This reduces the possibility of analyte loss and allows a sample to be analyzed later rather than immediately.



The main techniques for the determination of carbonyl compounds are spectrophotometry, gas chromatography (GC), and HPLC. In the spectrophotometric method, hydroxylamine hydrochloride solutions have been used to extract carbonyl compounds from samples via the formation of less-volatile oxime derivatives. The total oxime derivatives were detected at a wavelength of 212 nm and the conjugated diene carbonyl oximes were detected at 272 nm. This method shows limited selectivity and sensitivity.^{2,3}

Compared to the spectrophotometric method, GC and HPLC techniques are more frequently used and some standard methods have been created.⁴⁻¹¹ For example, EPA Methods 554⁴ and 556⁵ contain HPLC/UV and GC/electron-capture detection (ECD) methods using DNPH and *o*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA) as derivatization reagents, respectively, to determine the carbonyl compounds in drinking water. As a derivatization reagent, DNPH has been extensively used with carbonyl compounds due to its high reactivity, selectivity, and stability. It is used for GC with ECD¹² and mass spectrometry (MS),¹³ HPLC with UV,^{6-10,14,15} fluorescence,¹⁶ and MS.^{17,18} Reaction with DNPH yields a chromophore with an absorption maximum of 360 nm. This provides highly sensitive and selective detection of carbonyl compounds that is particularly suitable for HPLC with UV detection.

CARB Method 1004 reports an HPLC/UV method for the determination of 13 carbonyl compounds (structures shown in Figure 1) generated from automotive source samples.⁹ In this method, DNPH-impregnated cartridges are used for sampling. After conversion to the DNPH derivatives, the carbonyl compounds are analyzed by HPLC with UV detection.

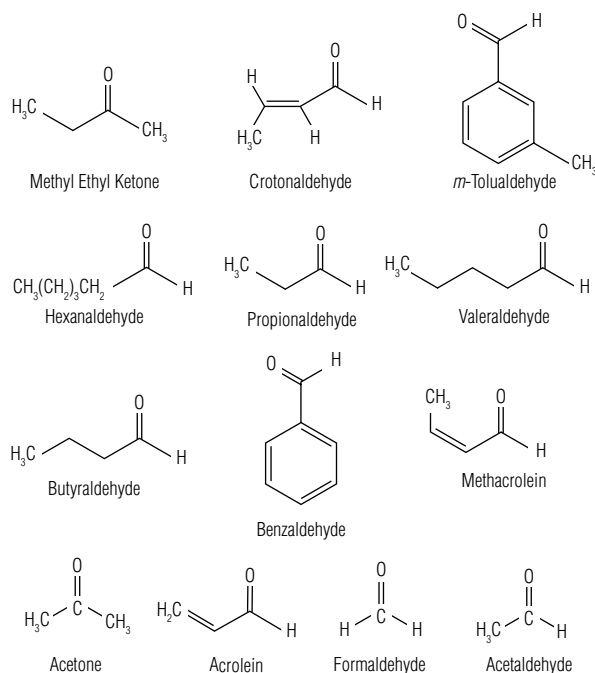


Figure 1. Structures of carbonyl compounds specified in CARB Method 1004.

Equipment

- Two Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC systems were used in this study.*

System 1 included:

- LPG-3400RS Quaternary Pump with SRD-3400 Integrated Solvent and Degasser Rack
- WPS-3000TRS Wellplate Sampler, Thermostatted, with 100 μ L sample loop
- TCC-3000RS Thermostatted Column Compartment
- DAD-3000RS Diode Array Detector with 13 μ L flow cell

System 2 included:

- HPG-3400RS Binary Pump with Solvent Selector Valves and SRD-3400 Integrated Solvent and Degasser Rack
- WPS-3000TRS Wellplate Sampler, Thermostatted, with 25 μ L sample loop
- TCC-3000RS Thermostatted Column Compartment
- DAD-3000RS Diode Array Detector with 2.5 μ L flow cell
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software 6.80 SR9 or higher

*All work can be performed using any UltiMate 3000 system with a compatible sample loop and flow cell.

Reagents and Standards

- Deionized (DI) water, 18.2 M Ω -cm resistivity
- Methanol (CH₃OH), HPLC Grade 99.9% (Fisher Scientific P/N AC610090040)
- Acetonitrile (CH₃CN), HPLC Grade 99.9% (Fisher Scientific P/N AC610010040)
- Tetrahydrofuran (THF), HPLC Grade 99.8% (Fisher Scientific P/N AC 268290040)
- Aldehyde/Ketone-DNPH Stock Standard-13, 13 Components in Acetonitrile for CARB Method 1004, 3 μ g/mL of each component (Cerilliant® P/N ERA-013K)

Consumables

Thermo Scientific™ Target2™ Nylon Syringe Filters, 0.45 μ m, 30 mm (Fisher Scientific P/N F2500-1)

Working Standard Solutions for Calibration

Prepare six working standard solutions for the calibration with 0.03, 0.06, 0.15, 0.30, 0.60, and 1.50 μ g/mL concentrations by adding the proper amount of aldehyde/ketone-DNPH stock standard-13 and diluting with an acetonitrile solution.

Sample Preparation

The preparation for vehicle passenger cabin air samples (including pretreatment, sampling, and elution) was performed by a third-party inspection institution located in Shanghai, China and based on the standard methods enacted by the Chinese government.^{10,19}

Pretreatment

The new vehicles selected for testing came off the production line within 28 ± 5 days and contained no additional interior decoration. For pretreatment the vehicles were moved to an environmental chamber, the doors and windows of the vehicles were opened, and the sampling system was configured. The height of the sampling point was similar to that of a driver's mouth. After at least 8 h, the temperature (25 °C), pressure (1 atm), humidity (50%), and air velocity (0.2 m/s) in the environmental chamber were recorded. The car doors were then closed and the same measurements were again recorded after 16 h.

Sampling

A schematic diagram of the sampling system is shown in Figure 2. Collect the cabin air in a sampling tube impregnated with Cleanert DNPH-Silica (Bonna-Agela Technologies, China) for 30 min using an AirChek 2000 Air Sampling Pump (SKC Inc., USA) at a flow rate of 400 mL/min. The derivatization reactions for carbonyl compounds using DNPH are shown in Figure 3. The permissible flow rate error between beginning and end is <5%, calibrated using a DC-Lite Flowmeter (SKC Inc., USA). After collection, seal the sampling tube with aluminum foil, store at 4 °C or lower, and deliver to the lab for analysis.

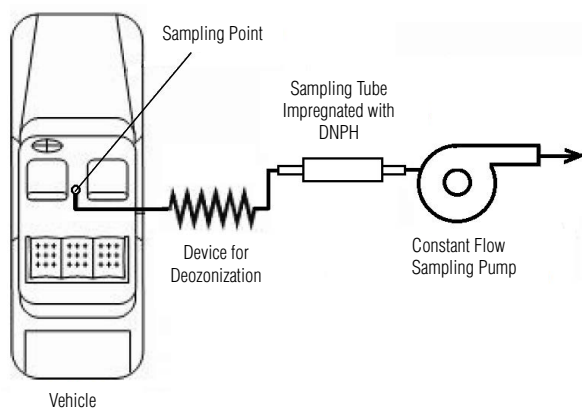


Figure 2. Sampling system for cabin air tests.

Elution

Elute the sampling tube with 5 mL acetonitrile. After elution, bring the eluate to a volume of 5 mL with acetonitrile and filter through a nylon syringe filter.

Chromatographic Conditions

System 1

Column:	Thermo Scientific™ Acclaim™ 120, C18, 3 μm Analytical, 4.6 × 150 mm (P/N 059133)
Mobile Phase:	A. DI water; B. CH ₃ CN/THF (60:40, v/v)
Gradient:	B. 0–8 min, 55%; 20 min, 70%; 21–22 min, 55%
Flow Rate:	1.0 mL/min
Inj. Volume:	10 μL
Temperature:	30 °C
Detection:	UV, 360 nm

System 2

Column:	Thermo Scientific™ Synchronis™ C18, 1.7 μm, 2.1 × 100 mm (P/N 97102-102130)
Mobile Phase:	A. DI water; B. CH ₃ CN/THF (60:40, v/v)
Gradient:	B. 0–2.5 min, 55%; 9 min, 70%; 9.1–10 min, 55%
Flow Rate:	0.35 mL/min
Inj. Volume:	1.0 μL
Temperature:	35 °C
Detection:	UV, 360 nm

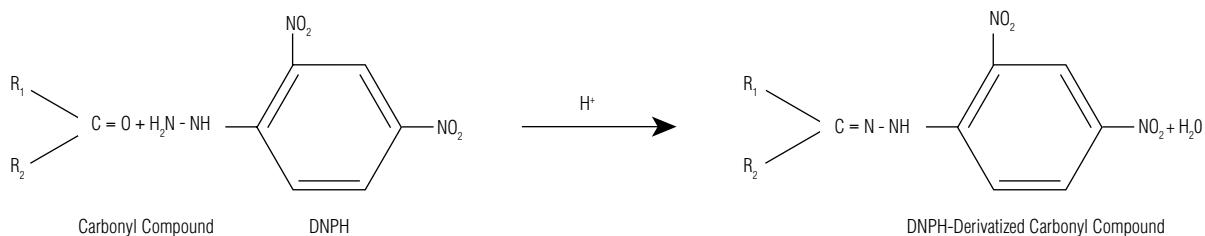


Figure 3. Derivatization reaction of carbonyl compounds using DNPH.

Results and Discussion

Separation of DNPH-Derivatized Carbonyl Compounds Using Different Columns

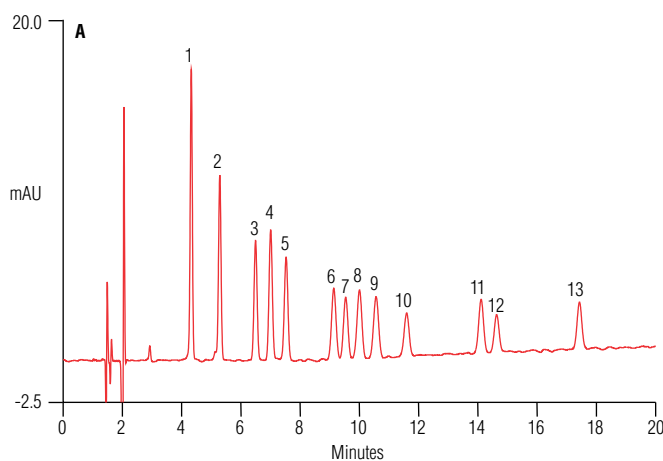
The HPLC methods for carbonyl compounds in CARB Method 10049 and HJ/T 400-200710 use C18 stationary phases. In CARB Method 1004, two systems are required because the DNPH derivatives of methyl ethyl ketone and butyraldehyde do not completely resolve and sometimes coelute on the primary system when using octadecylsilyl-silica gel columns. Resolution requires a complementary step on the secondary system using other equivalent columns to focus solely on the separation of the two peaks.

Likewise, using HJ/T 400-2007 on conventional C18 columns with acetonitrile/water mobile phases, it is difficult to differentiate DNPH derivatives of acetone and acrolein because of their similar size and hydrophobicity. It is possible to optimize chromatographic conditions with a flow rate of 1.2 mL/min, a column temperature of 32 °C, and a mobile phase composition of acetonitrile/water/THF = 34/52.8/13.2.²⁰ However, the mobile phase must be controlled to the tenth of a percent to achieve baseline resolution of the DNPH derivatives of acetone and acrolein, thus demonstrating that the method is not rugged with respect to mobile phase composition.

In the work shown here, two reversed-phase columns—the Acclaim 120 C18 and the Synchronis C18 columns—were evaluated for the separation of the DNPH-derivatized carbonyl compounds. As shown in Figure 4, the separation problem of the DNPH derivatives of acetone (Peak 3) and acrolein (Peak 4) found in HJ/T 400-2007 was resolved using either one of these two columns, as was the problem of coelution of DNPH-derivatized methyl ethyl ketone (Peak 7) and butyraldehyde (Peak 8) found in CARB Method 1004. Moreover, method robustness was improved due to the use of easy-to-prepare mobile phases and easily executed mobile phase gradients. Table 1 lists the calculated peak resolutions (*R*s) between the DNPH derivatives discussed above, showing that satisfactory results can be achieved using either of the two evaluated columns.

Table 1. Peak resolution of critical compound pairs on the evaluated columns.

Analyte Pair	<i>R</i> s	
	Acclaim 120 C18 Column	Synchronis C18 Column
Acetone/Acrolein	3.2	3.1
Methyl Ethyl Ketone/Butyraldehyde	2.7	2.6



A
 Column: Acclaim 120 C18, 3 μ m (4.6 \times 150 mm)
 Mobile Phase: A. DI water
 B. CH₃CN/THF (60:40, v/v)
 Gradient: B. 0–8 min, 55%; 20 min, 70%; 21–22 min, 55%
 Flow Rate: 1.0 mL/min
 Inj. Volume: 10 μ L
 Temperature: 30 °C
 Detection: UV absorbance at 360 nm

B
 Column: Synchronis C18, 1.7 μ m (2.1 \times 100 mm)
 Mobile Phase: A. DI water
 B. CH₃CN/THF (60:40, v/v)
 Gradient: B. 0–2.5 min, 55%; 9 min, 70%; 9.1–10 min, 55%
 Flow Rate: 0.35 mL/min
 Inj. Volume: 1.0 μ L
 Temperature: 30 °C
 Detection: UV absorbance at 360 nm

Peaks:

1. Formaldehyde
2. Acetaldehyde
3. Acetone
4. Acrolein
5. Propionaldehyde
6. Crotonaldehyde
7. Methyl Ethyl Ketone
8. Butyraldehyde
9. Methacrolein
10. Benzaldehyde
11. Valeraldehyde
12. *m*-Tolualdehyde
13. Hexanaldehyde

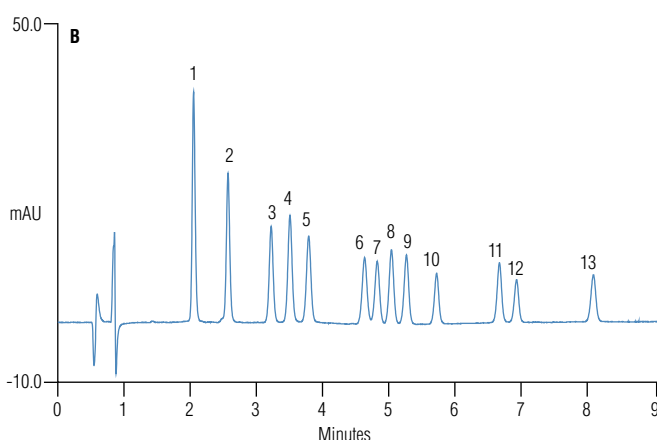


Figure 4. DNPH-derivatized carbonyl compound standards.

Reproducibility, Linearity, and Detection Limits

Method precision was estimated by making five consecutive injections of a calibration standard with a concentration of 0.15 µg/mL for each compound. The retention time and peak area reproducibilities using each of the two columns show good precision and are summarized in Table 2.

Calibration linearity of the DNPH derivatives of carbonyl compounds was investigated by making three consecutive injections of a mixed standard prepared at six different concentrations (i.e., 18 total injections). The external standard method was used to establish the calibration curve and quantify these DNPH derivatives of carbonyl compounds in the cabin air samples. Excellent linearity was observed from 0.03 to 1.5 µg/mL when plotting the concentration versus the peak area, and the coefficients of determination were ≥ 0.9940 for all (Table 3).

Table 2. Reproducibility for peak retention time and area on the evaluated columns (n = 5).

Analyte (DNPH Derivative)	Acclaim 120 C18 Column		Synchronis C18 Column	
	Retention Time RSD	Peak Area RSD	Retention Time RSD	Peak Area RSD
Formaldehyde	0.09	1.94	0.09	1.81
Acetaldehyde	0.10	1.87	0.13	0.28
Acetone	0.11	1.35	0.11	0.28
Acrolein	0.12	1.62	0.08	0.73
Propionaldehyde	0.13	0.99	0.07	2.82
Crotonaldehyde	0.13	1.13	0.06	3.61
Methyl Ethyl Ketone	0.12	1.65	0.06	1.95
Butyraldehyde	0.15	1.43	0.11	1.37
Methacrolein	0.15	1.23	0.08	0.32
Benzaldehyde	0.15	1.88	0.08	1.68
Valeraldehyde	0.16	1.23	0.08	2.68
<i>m</i> -Tolualdehyde	0.17	1.04	0.02	1.32
Hexanaldehyde	0.13	1.32	0.06	2.63

Table 3. Method linearity data.

Analyte (DNPH Derivative)	Acclaim 120 C18 Column		Synchronis C18 Column	
	Regression Equation	r^2	Regression Equation	r^2
Formaldehyde	$A = 6.0813c + 0.5178$	0.9986	$A = 1.2455c + 0.0954$	0.9996
Acetaldehyde	$A = 4.7338c + 0.3147$	0.9981	$A = 0.9805c + 0.0522$	0.9992
Acetone	$A = 3.4175c + 0.3236$	0.9983	$A = 0.7080c + 0.0547$	0.9995
Acrolein	$A = 4.2679c + 0.3537$	0.9972	$A = 0.8478c + 0.0639$	0.9985
Propionaldehyde	$A = 3.5101c + 0.2831$	0.9989	$A = 0.7218c + 0.0478$	0.9995
Crotonaldehyde	$A = 2.9575c + 0.2652$	0.9971	$A = 0.6152c + 0.0425$	0.9990
Methyl Ethyl Ketone	$A = 2.7160c + 0.1859$	0.9975	$A = 0.5751c + 0.0267$	0.9994
Butyraldehyde	$A = 3.0933c + 0.3250$	0.9956	$A = 0.6502c + 0.0491$	0.9987
Methacrolein	$A = 2.8892c + 0.2341$	0.9977	$A = 0.6111c + 0.0313$	0.9997
Benzaldehyde	$A = 1.9963c + 0.2014$	0.9973	$A = 0.4084c + 0.0332$	0.9993
Valeraldehyde	$A = 2.4258c + 0.1934$	0.9984	$A = 0.5070c + 0.0280$	0.9983
<i>m</i> -Tolualdehyde	$A = 1.6818c + 0.1682$	0.9979	$A = 0.3566c + 0.0218$	0.9994
Hexanaldehyde	$A = 2.1026c + 0.1859$	0.9976	$A = 0.4325c + 0.0272$	0.9982

Figure 5 shows a carbonyl compound calibration standard with a concentration of 0.03 $\mu\text{g/mL}$ for each DNPH derivative separated on the Synchronis C18 column. The calculated signal-to-noise (S/N) ratios are summarized in Table 4. The method detection limit (MDL) of each DNPH derivative was measured using UV detection and calculated using $S/N = 3$; all MDLs were $\leq 5 \mu\text{g/L}$, showing good method sensitivity.

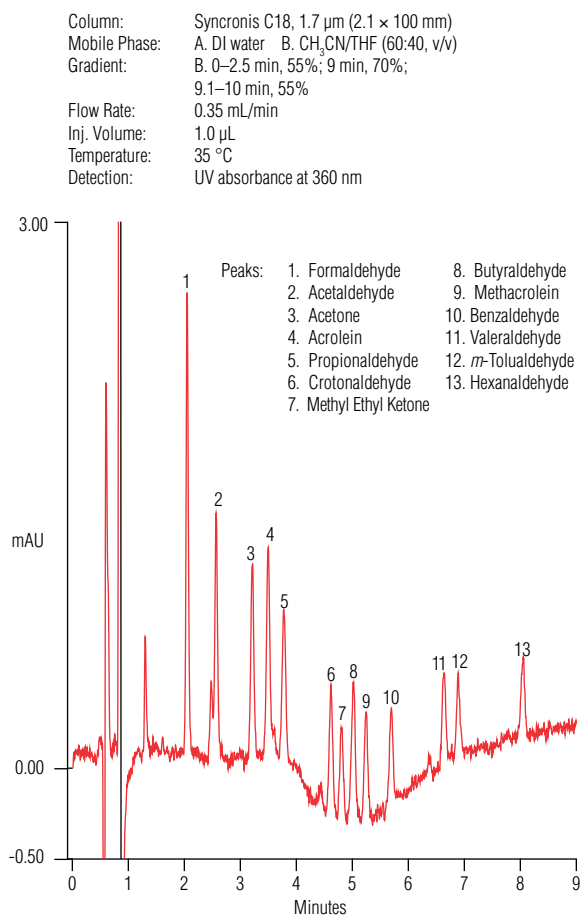


Figure 5. DNPH-derivatized carbonyl compound standards (0.03 $\mu\text{g/mL}$ each).

Table 4. S/N of DNPH derivatives (concentrations of 0.03 $\mu\text{g/mL}$ each) obtained using a Synchronis C18 column.

Peak Name (DNPH Derivative)	S/N
Formaldehyde	41.3
Acetaldehyde	22.0
Acetone	17.4
Acrolein	17.6
Propionaldehyde	13.5
Crotonaldehyde	11.4
Methyl Ethyl Ketone	8.2
Methacrolein	11.9
Butyraldehyde	9.6
Benzaldehyde	8.2
Valeraldehyde	7.1
<i>m</i> -Tolualdehyde	7.3
Hexaldehyde	6.6

Sample Analysis

Figures 6 and 7 show two DNPH-derivatized cabin air samples, each analyzed on one of the two evaluated columns. Formaldehyde and acetone were found in both samples, whereas methyl ethyl ketone was found only in Cabin Air Sample 1, demonstrating the presence of aldehyde and ketone compounds in cabin air. The detected concentration of formaldehyde in Cabin Air Sample 1 was 238 $\mu\text{g/m}^3$, higher than the level permitted in the Standardization Administration of China (SAC) GB/T 27630-2011 (100 $\mu\text{g/m}^3$),¹⁹ thereby disqualifying the vehicle that produced Cabin Air Sample 1. Recoveries for the three detected DNPH derivatives of carbonyl compound standards in the sample were nearly 100% using the Acclaim 120 C18 column, demonstrating good accuracy of the HPLC method. The analysis results summarized in Table 5 validate that successful sample analysis is possible using either of the two evaluated columns.

Column: Acclaim 120 C18, 3 μm (4.6 \times 150 mm)
 Mobile Phase: A. DI water B. $\text{CH}_2\text{CN/THF}$ (60:40, v/v)
 Gradient: B. 0–8 min, 55%; 20 min, 70%;
 21–22 min, 55%
 Flow Rate: 1.0 mL/min
 Inj. Volume: 10 μL
 Temperature: 30 $^\circ\text{C}$
 Detection: UV absorbance at 360 nm
 Samples: A. DNPH-derivatized Cabin Air Sample 1
 B. A spiked with DNPH-derivatized carbonyl compound mixed standard (0.15 $\mu\text{g/mL}$ each)

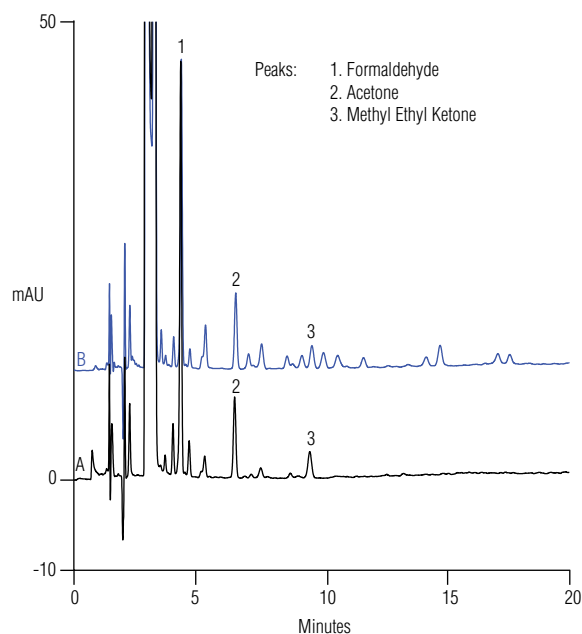


Figure 6. DNPH-derivatized Cabin Air Sample 1 (A) and the same sample spiked with a DNPH-derivatized carbonyl compound mixed standard (B).

Cabin Air Sample 1 Using Acclaim 120 C18 Column					Cabin Air Sample 2 Using Synchronis C18 Column	Permitted in GB/T 27630-2011 ($\mu\text{g}/\text{m}^3$)
Analyte	Detected ($\mu\text{g}/\text{m}^3$)	Added ($\mu\text{g}/\text{mL}$)	Found ($\mu\text{g}/\text{mL}$)	Recovery (%)	Detected ($\mu\text{g}/\text{m}^3$)	
Formaldehyde	238	0.15	0.151	101	70	100
Acetone	114		0.149	99	137	—
Methyl Ethyl Ketone	76		0.152	101	—	—

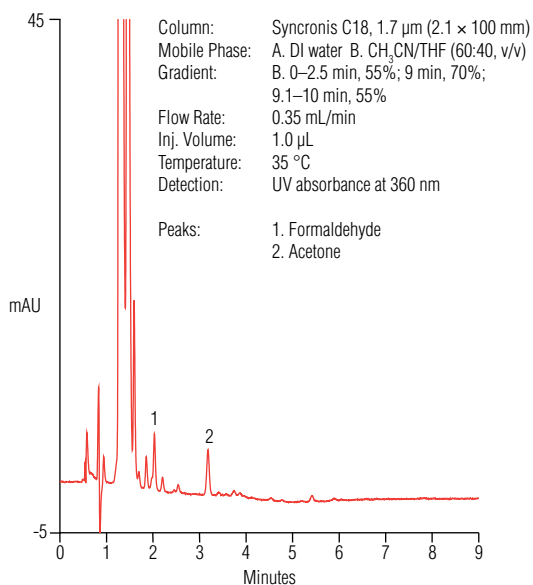


Figure 7. DNPH-derivatized Cabin Air Sample 2.

Conclusion

The work shown here describes efficient HPLC methods combined with UV detection for the determination of DNPH-derivatized carbonyl compounds in cabin air samples. All 13 carbonyl compounds listed in CARB Method 1004, ISO 16000-3, U.S. EPA Compendium Method TO-11A, and the Chinese HJ/T 400-2007 are well separated on the Acclaim 120 C18 and Synchronis C18 columns, and the separation takes <10 min using the Synchronis C18 column.

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