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Short communication

Trace analysis of carbonyl compounds by liquid chromatography–mass spectrometry after collection as 2,4-dinitrophenylhydrazine derivatives

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Abstract

This study describes the utilization of carbonyl-2,4-dinitrophenylhydrazine (DNPH) derivatives for the determination of a micro amount of carbonyl compounds in air by liquid chromatography–mass spectrometry (LC–MS). After the carbonyl compounds are collected using a Waters Sep-Pak C₁₈ cartridge column with impregnated DNPH on octadecylsilica, they are eluted by acetonitrile as carbonyl–DNPH derivatives. A 20-mm³ aliquot of eluent is injected into the LC–MS system. The four derivatives (formaldehyde-, acetaldehyde-, acrolein- and acetone–DNPH) were eluted within 7 min with acetonitrile–water (60:40, v/v) as the mobile phase. The proposed method offers sub-ppb sensitivity and good reproducibility and was applied to the determination of these carbonyl compounds in actual air samples from store rooms, laboratories and offices. The relative standard deviations for these samples ($n=6$) were 1 to 3%. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Derivatization, LC; Air analysis; Carbonyl compounds; 2,4-Dinitrophenylhydrazine

1. Introduction

A long time has passed since the toxicities of a number of carbonyl compounds in the human body were established. Consequently, various approaches for the development of sampling and analytical methods, which are fast and accurate, have been carried out. Formaldehyde, in particular, which comes from some building materials and wallpaper adhesive, and which is also present in the exhaust fumes of motor vehicles, is considered to be a carcinogen and produces negative effects on the body [1,2]. Therefore, it is recognized as one of the

important polluting substances in indoor air [3]. Recently, the effects of these air-polluting substances have given rise to the term ‘sick building syndrome’, which has been paid some attention.

The analysis of carbonyl compounds in air has involved methods that use various derivatizations after the air is collected by wet sampling with an impinger [4]. Other methods involve impregnating derivative reagents on silica gel or porous polymer beads, followed by easy sampling methods in which formaldehyde is reacted directly with derivative reagents in the adsorption tube and then applied to indoor air samples [5,6]. Of these sampling methods, the 2,4-dinitrophenylhydrazine (DNPH) method provided good stability for the formed derivative, and is

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accepted as the official method for the sampling and analysis of some aldehydes in environmental air [7–10]. There are impinger and cartridge methods for collecting some carbonyl compounds using DNPH as the derivatizing reagent, and analysis of the derivatives has involved the use of a high-performance liquid chromatography (HPLC) system equipped with a UV detector [11–13]. The results obtained by HPLC only involve a retention time, therefore, it is difficult to identify or determine substances that have the same retention time. As a consequence, in the determination of carbonyl compounds using the HPLC system, for example, the separation of acrolein and acetone may often require considerable effort in optimizing the composition of the mobile phase. A liquid chromatography–mass spectrometry (LC–MS) system, in which a mass detector is connected to a HPLC system, can provide structural information from the MS spectra of compounds, and can attain highly sensitive and selective detection. Therefore, it has gained attention as a very good method for the identification and determination of polluting substances in the environment [14,15]. This paper reports an analytical method using a micro amount of carbonyl compounds from indoor air, using a cartridge impregnated with DNPH, and a LC–MS system. We used a dry cartridge because a small amount of reagent is needed, and the simplicity and transportability are far better than with the impinger method [16].

2. Experimental

2.1. Reagents

2.1.1. Purification of DNPH

The purification of DNPH was performed by dissolving 20 g of DNPH (Wako Pure Chemicals) in 167 cm³ of acetonitrile and then adding 500 cm³ of water, and storing the mixture overnight. The precipitate was filtered through No. 5A filter paper (Advantec Toyo) and was then washed with water and ethanol several times. The precipitate was stored in a desiccator after drying.

2.1.2. Preparation of standard carbonyl–DNPH derivatives

A DNPH solution was prepared by adding 10 cm³ of 12 mol dm⁻³ hydrochloric acid to 5 g of the purified DNPH and the mixture was diluted to 500 cm³ with water. It was filtered through No. 5A filter paper after heating and dissolution. The DNPH derivatives were synthesized by reacting DNPH in the filtrate with the corresponding carbonyl compounds. The carbonyl–DNPH derivative was washed with water, and recrystallized several times using ethanol. The crystals, which were obtained by this method, were the standard reference materials. Standard solutions were prepared by dissolving each definite mass of the carbonyl–DNPH derivative in acetonitrile. These prepared solutions were stored in a refrigerator and were prepared fresh every month.

2.1.3. Other reagents

Analytical grade acetonitrile was used (Wako Pure Chemicals). Ultra-pure water was obtained using a Milli-Q SP reagent water system (Millipore). All other reagents were of analytical grade.

2.1.4. Preparation of DNPH-impregnated cartridge

A Waters Sep-pak C₁₈ cartridge was washed with acetonitrile and dried in a flow of nitrogen gas. DNPH was impregnated in the cartridge by the slow passage of 2 cm³ of a solution that contained an acetonitrile solution containing 0.2% DNPH and 1% phosphoric acid. The derivative reaction of carbonyl compounds and DNPH occurs following the addition of acids, which act as a catalyst. Therefore, the effect was examined following the addition of phosphoric acid, hydrochloric acid, nitric acid and oxalic acid to the DNPH solution, in order to determine which acid acted best as a catalyst. The maximum value was obtained with phosphoric acid; the other acids had high blank values and backgrounds, and the peak had broken into two peaks. The cartridge was then dried and stored in a desiccator.

2.2. Instruments

Carbonyl–DNPH derivatives were analyzed using a LC–MS system equipped with atmospheric chemical ionization (APCI; Hitachi LC–MS 1000S). The negative mode was selected for the measurements.

The scan range of the MS was from 0 to 500 for the determination of m/z values. The selective ion monitoring mode (SIM) was used for analysis of the actual samples. A PEGASIL ODS packed column (Sensyu Kagaku; 150×4.6 mm I.D.; particle size, 10 μm) was employed for the separation of the carbonyl–DNPH derivatives. The mobile phase was prepared by mixing acetonitrile and water in a 60:40 (v/v) ratio. The flow-rate was 1.2 $\text{cm}^3 \text{min}^{-1}$. Other conditions were as follows: drift voltage, –25 V; focus voltage, –60 V; vaporization temperature, 180°C; desolvation temperature, 399°C and multiplier voltage, 2000 V.

A HPLC system was used for comparison with the proposed method. This system consisted of an Intelligent HPLC pump (Jasco, 880-PU), a Capcell Pak ODS column (Shiseido; 150×4.6 mm I.D.; particle size, 5 μm) and a UV variable wavelength spectrophotometric detector (Jasco, 870-UV) adjusted to 360 nm. A sample solution (20 mm^3) was injected through a syringe loading sample injector valve (Rheodyne, 7125) into the stream of the mobile phase. The mobile phase was a ternary mixture of acetonitrile–tetrahydrofuran–water(40:20:40, v/v) and the flow-rate was 1.2 $\text{cm}^3 \text{min}^{-1}$.

The air sampling system used a low volume air L-20 sampler (Shibatagakaku).

2.3. Analytical procedure

Air samples were collected by connecting the downstream end of Sep-pack C_{18} cartridges impregnated with DNPH to the low volume air sampler, then the carbonyl–DNPH derivatives were formed by the reaction of the carbonyl compounds in the air sample. The reaction proceeds according to the equation shown in Fig. 1. Sampling was performed at a flow-rate of 1.0 $\text{dm}^3 \text{min}^{-1}$ for 30 min, based on the breakthrough test. After sampling, the DNPH derivatives in each cartridge were slowly eluted with acetonitrile and were diluted to exactly 10 cm^3 by the addition of acetonitrile. A 20- mm^3 aliquot of the eluate was injected into the LC–MS system. The concentrations of the carbonyl compounds were determined by measuring the peak areas and using the peak–area calibration graph drawn using the standard carbonyl solutions.

3. Results and discussion

3.1. Operating conditions

The LC–MS conditions were examined using standard solutions of the carbonyl–DNPH deriva-

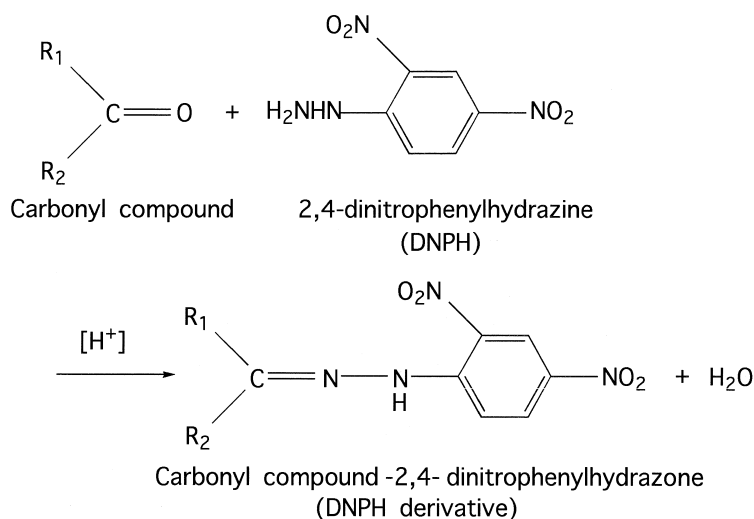


Fig. 1. Schematic representation of the derivatization reaction.

tives, each containing 0.1 ppm as carbonyl compounds. The vaporization temperature was examined first, by varying the temperature from 160 to 240°C. As a result, a good peak and reproducibility were obtained at around 180°C. The desolvation temperature was established at 399°C, which was evaluated in a similar manner. Such a high temperature was needed to obtain desolvation of a sample molecule, but a stable vaporization was performed, it was thought that most of the supplied heat was consumed as the vaporous heat of solvent. The time required to go from spray to ionization of sample molecule was less than 1 ms.

To establish the optimum drift voltage, the effect of the drift voltage was examined over a range from -10 to -50 V. In the case of three of the components, the exception being acrolein, the maximum peak areas and reproducibilities were obtained at -25 V. In the case of acrolein, a maximum value was obtained at -30 V, however, a drift voltage of -25 V was selected for the purpose of simultaneous determination.

3.2. LC-MS separation

The mobile phases were prepared by mixing water, methanol, acetonitrile and tetrahydrofuran. Using these solutions, the elution behaviors and the sensitivities were examined. As a result, both methanol-water and acetonitrile-water could be used to determine the four carbonyl-DNPH derivatives. By comparing two chromatograms, the carbonyl-DNPH derivatives could be determined with acetonitrile-water more sensitively and the reproducibility with acetonitrile-water was better than with methanol-water. Next, the behavior of each derivative in the chromatogram was examined by varying the composition ratio of the acetonitrile-water. The retention time increased on increasing the ratio of water in the mobile phase. On the other hand, any unreacted DNPH existing in the sample provided an obstacle when the carbonyl-DNPH derivatives were determined. For this reason, it was considered important to separate any unreacted DNPH and the carbonyl-DNPH derivatives. Consequently, the composition of the mobile phase was chosen as acetonitrile-water (60:40, v/v), as it was found to be the most effective in achieving a good separation.

3.3. Standard chromatogram and mass spectrum

It is necessary that only the quasi molecular ion is formed, and the formation of the fragment ion and others must be controlled by establishing various conditions. The formation of an ion except for the quasi molecular ion influences the determination of the DNPH derivatives and can cause errors.

The formaldehyde-DNPH derivative was eluted at 3.6 min, acetaldehyde-DNPH eluted at 4.8 min, acrolein-DNPH eluted at 5.3 min and acetone-DNPH was eluted at 5.4 min. The MS spectra of the carbonyl-DNPH derivatives are shown in Fig. 2. Each quasi molecular ion (parent ion) $[M-H]^-$ was also satisfactorily observed. Although the acrolein-DNPH and acetone-DNPH derivatives have the same retention time under the analytical conditions employed, because the acrolein-DNPH derivative has a molecular mass of 235 and the acetone-DNPH

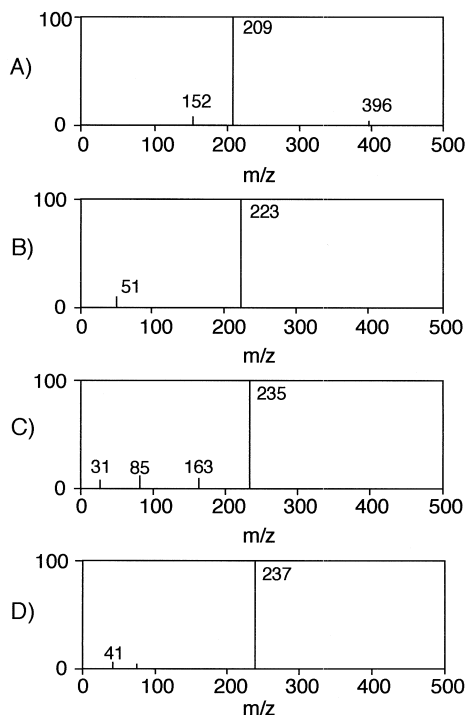


Fig. 2. MS spectra of four carbonyl-DNPH compounds. Mobile phase, acetonitrile-water (60:40, v/v); drift voltage, -25 V; focus voltage, -60 V; vaporization temperature, 180°C. (A) Formaldehyde, (B) acetaldehyde, (C) acrolein and (D) acetone.

derivative has a molecular mass of 237, they were separated in the MS detector. Moreover, propionaldehyde–DNPH and acetone–DNPH have the same molecular mass, but different retention times. Therefore, it was confirmed that propionaldehyde did not affect the determination of acetone.

4. Calibration curves

The calibration curves were obtained from peak areas, which were determined by measuring each quasi molecular ion with the standard derivative solutions of formaldehyde, acetaldehyde, acrolein and acetone. The proposed method gave a linear calibration curve (seven data points) in the concentration range of 0.05 to 10 ppm when measuring each of the quasi molecular ions (formaldehyde: $y=0.708x$; acetaldehyde: $y=0.765x$; acrolein: $y=0.600x$ and acetone: $y=0.776x$). Regression coefficients of these lines were more than 0.999. Relative standard deviations were 1–3%, and the limits of detection for $S/N=3$ were 3 ppb as the concentration for solution on each carbonyl compound. The concentrations of these compounds in the air sample at 27°C according to the proposed procedure were 0.82 ppb for formaldehyde, 0.56 ppb for acetaldehyde, 0.44 ppb for acrolein and 0.42 ppb for acetone.

5. Practical applications

Carbonyl compounds in the air of store rooms, laboratories and offices were determined using the

proposed method. These results are shown in Table 1. A high concentration of acetone was often detected in air samples from the laboratory. A large amount of formaldehyde and acetaldehyde, and a trace amount of acrolein were detected in an office where smoking was not prohibited. To verify the reliability of the proposed method, the results were compared with those obtained using the HPLC method mentioned above (Table 2).

These substances mainly enter the human body through the mouth. Therefore, the relationship between height, that is, the position of the mouth from the floor, and the concentration was examined. Three situations in daily life, sleeping (height, 15 cm; formaldehyde, 5.82 ppb; acetaldehyde, 7.18 ppb; acrolein, not detected and acetone, 16.4 ppb), sitting (height, 85 cm; formaldehyde, 6.01 ppb; acetaldehyde, 5.33 ppb; acrolein, not detected and acetone, 10.2 ppb) and standing (height, 165 cm; formaldehyde, 5.74 ppb; acetaldehyde, 2.62 ppb; acrolein, not detected and acetone, 6.05 ppb) were selected as representative heights. Close to the floor, higher concentrations of acetaldehyde and acetone were detected. The concentration of formaldehyde did not vary, but maintained its average value. These values are suitable because of the relative vapor-density of each substance, and the amount of acrolein was not appreciable.

The proposed method can be applied to the determination of carbonyl compounds in indoor air samples. Furthermore, after they are separated by LC, they are defined by the mass detector. Therefore, the proposed method can determine carbonyl compounds.

Table 1
Analytical results of air sampling

	Store room ^a (ppb)	Laboratory ^b (ppb)	Office ^c (ppb)
Formaldehyde	5.81	4.54	15.8
Acetaldehyde	1.67	1.44	9.02
Acrolein	n.d.	n.d.	0.51
Acetone	10.6	61.9	40.3

^a Sampled on 20/12/1997.

^b Sampled on 10/11/1997.

^c Sampled on 19/1/1998.

n.d., not detected.

Table 2
Recovery of carbonyl compounds^a

	LC–MS (proposed method)			RSD (<i>n</i> =6) (%)	UV–LC Found (ppb)
	Added (ppb)	Found (ppb)	Recovery (%)		
Formaldehyde	–	6.33	–	3.0	6.73
	8.18	14.5	100.0		
	21.8	27.3	96.9		
Acetaldehyde	–	4.47	–	3.2	4.71
	5.56	9.82	97.9		
	14.8	19.0	98.6		
Acrolein	–	n.d	–	1.9	n.d.
	4.36	4.36	100.0		
	11.6	11.6	100.0		
Acetone	–	15.4	–	2.4	15.56
	4.26	19.8	100.9		
	11.2	27.5	105.8		

^a Sampled 2/9/1997.

n.d., not detected (below 0.44 ppb).

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