

Fig. 2. Scheme for the simultaneous determination of ozone and carbonyls.

period, and 5 mL of eluate was collected. After 30 min of elution, the eluate was analyzed by HPLC.

3. Results and discussion

3.1. HPLC analysis

The spectral profile of the 2PA-2,4-DNPhydrazone derivative revealed that the wavelength of maximum absorption was 378 nm and that the absorption coefficient was large ($3.4 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$) [29]; hence, this derivative could be detected in the presence of other aldehyde derivatives such as formaldehyde and acetaldehyde at a single wavelength of 360 nm. The analysis conditions for 2PA and C1–C3 carbonyl-DNPH derivatives were determined by taking into account the specifications of the HPLC conditions. Fig. 3 shows the chromatogram of a standard mixture containing pyridine-2-aldehyde (2PA), formaldehyde (FA), acetaldehyde (AA), and acetone (AC) 2,4-DNPhydrzones ($100 \mu\text{mol L}^{-1}$).

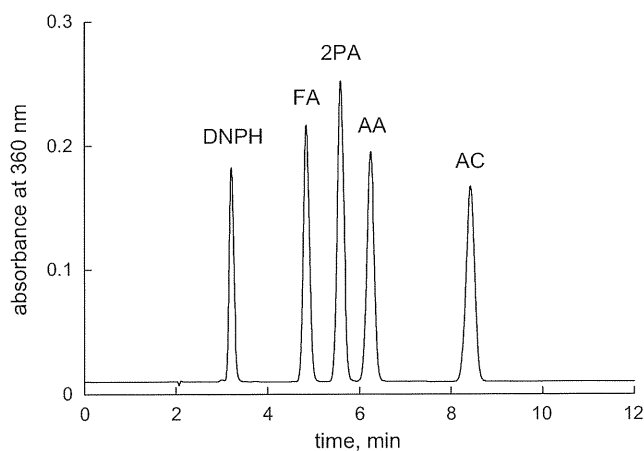


Fig. 3. Chromatogram of pyridine-2-aldehyde and other carbonyl 2,4-DNPhydrzones.

The limit of detection (LOD) and limit of quantitation (LOQ) of DSD-BPE/DNPH-HPLC analysis were calculated using the linear regression theory [30]. A standard mixture ($100 \mu\text{L}$) containing 2PA, FA, AA, and AC 2,4-DNPhydrzones ($100 \mu\text{mol L}^{-1}$) was introduced into the DSD-BPE/DNPH device and analyzed using the analytical conditions described above. LOD and LOQ were calculated as being three times and ten times the standard deviation obtained from the data of 10 replicate measurements, respectively (Table 1). The LOD values of DSD-BPE/DNPH-HPLC method were higher than that of DSD-DNPH-HPLC method [20]. The reason for this discrepancy was due to the fact that the concentration of DNPH in DSD-BPE/DNPH is two times higher than that of DSD-DNPH. HPLC analysis reproducibility was estimated from data of 10 samplers spiked with 5 nmol of 2,4-DNPhydrzones, including 2PA. The relative standard deviations (RSD) for 2PA, formaldehyde and acetaldehyde were 1.2%. RSD for acetone was 1.3%.

3.2. Influence of 2BPE and phosphoric acid contents of DNPH-silica on the reaction with ozone

An ozone generator was operated in the environmental test chamber set at a temperature of 25 °C and a relative humidity of 60%. The ozone concentration reached a constant value of $72 \mu\text{g m}^{-3}$ after 24 h. BPE/DNPH silica particles containing various concentrations of 2BPE, 1% (w/w) DNPH, and 0.85% (v/w) phosphoric acid were packed into the DSD-BPE/DNPH samplers and placed in the environmental test chamber for 24 h. Concurrently, active sampling was performed using the 2BPE/DNPH-cartridge (flow rate: 100 mL min^{-1}). After air sampling, carbonyl compounds including 2PA were analyzed by HPLC. Fig. 4 shows the change in amount of 2PA with the 2BPE content of 2BPE/DNPH-silica.

Table 1
LOD and LOQ of DSD-BPE/DNPH-HPLC method.

Compound	LOD, $\mu\text{mol L}^{-1}$	LOQ, $\mu\text{mol L}^{-1}$
2PA (ozone)	0.056	0.19
Formaldehyde	0.039	0.13
Acetaldehyde	0.054	0.18
Acetone	0.12	0.39

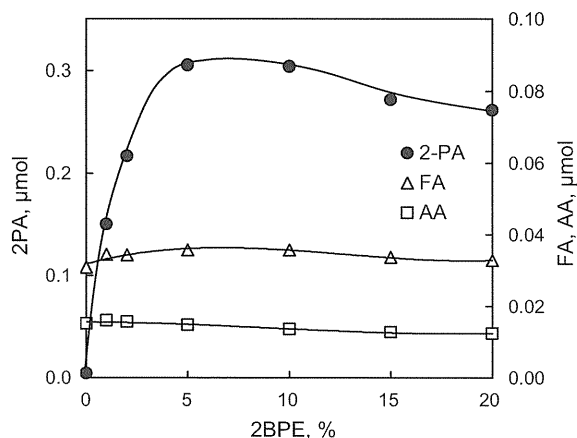


Fig. 4. Change in the amount of 2PA formed with the 2BPE content of 2BPE/DNPH-silica.

As can be seen in Fig. 4, 2PA formation increases with additional amounts of 2BPE until 5% (w/w) and slightly decreases over 10% (w/w) addition. When the 2BPE content exceeds 10% (v/w), precipitation was observed. The preferable 2BPE content for DSD-BPE/DNPH was determined to be 7% (w/w), as 2PA formation was constant in the range 5–10% (w/w).

Carbonyl compounds, including 2PA, react with DNPH to form stable hydrazones. For this reaction, a catalytic amount of acid is required. The ozone generator was operated in an environmental test chamber whose temperature and relative humidity were set to 25 °C and 60%, respectively. The ozone concentration reached a constant value of 72 $\mu\text{g m}^{-3}$ after 24 h. BPE/DNPH silica particles containing 0.01–1% (v/w) phosphoric acid, 7% (w/w) 2BPE, and 1% (w/w) DNPH were packed into the DSD-BPE/DNPH samplers and placed in the environmental test chamber for 24 h. Concurrently, active sampling was performed using the 2BPE/DNPH-cartridge, at the rate of 100 mL min^{-1} . Fig. 5 shows the amount of 2PA formed as a function of the phosphoric acid content of 2BPE/DNPH-silica. 2PA formation reaches a maximum with additional amounts of 0.1% (v/w) of phosphoric acid. When the phosphoric acid content exceeds 0.1% (v/w), pyridine-2-aldehyde phosphate precipitates.

3.3. Simultaneous ozone and carbonyl collection

Seven DSD-BPE/DNPH samplers were placed in the environmental test chamber, which was set to conditions described above. The ozone generator was operated in the environmental test cham-

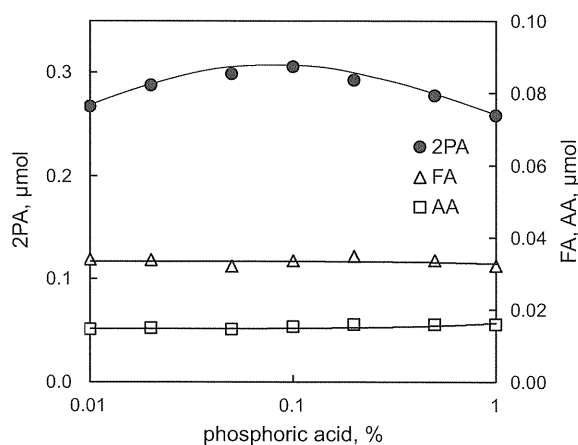


Fig. 5. Change in the amount of 2PA with the phosphoric acid content of 2BPE/DNPH-silica.

ber set to 25 °C and 60% relative humidity. The concentrations of ozone, formaldehyde, and acetaldehyde in the environmental test chamber were 72, 8.3, 8.6 $\mu\text{g m}^{-3}$, respectively. Samplers were recovered separately every 24 h over a period of 1 week. Concurrently, DSD-DNPH samplers were measured for reference. Diurnal concentrations of ozone were calibrated with the BPE/DNPH active sampler. The active samplers were performed for 24 h at the flow rate of 100 mL min^{-1} . Fig. 6 shows the amounts of 2PA (ozone), formaldehyde, and acetaldehyde collected with DSD-BPE/DNPH and DSD-DNPH from 1 to 7 days.

For ozone concentrations measured by the DSD-BPE/DNPH, a linear relationship (slope: 0.158 $\mu\text{mol d}^{-1}$; linear regression coefficient: 0.999) exists between the sampling duration and the ozone concentration for the first 7 days. Cumulative data obtained by using BPE/DNPH active sampling (ACT-BPE/DNPH) with a flow rate of 100 mL min^{-1} and one-day sampling period showed the slope of 0.354 $\mu\text{mol d}^{-1}$. Ozone experimental sampling rate with DSD-BPE/DNPH can be calculated from the slope ratio of the DSD-BPE/DNPH to the ACT-BPE/DNPH in Fig. 6 using the following equation:

$$\text{Ozone sampling rate by DSD-BPE/DNPH} = \frac{0.158}{0.354} \times 100 = 44.6 \text{ mL min}^{-1}$$

Additionally, the theoretical sampling rate of DSD-BPE/DNPH can be calculated from Graham's law of diffusion [20]. According to this law, the diffusion coefficient (D_{gr}) is inversely proportional to the square root of the density (Z) or molecular weight (M) of the gas:

$$D_{\text{gr}} \propto \frac{1}{\sqrt{Z}} \propto \frac{1}{\sqrt{M}} \quad (1)$$

When the diffusion coefficient (D_f) of formaldehyde is given, the diffusion coefficients of various other carbonyl compounds can be calculated from Eq. (2):

$$D_{\text{gr}} = D_f \sqrt{\frac{M_f}{M_d}} \quad (2)$$

where M_f is the molecular weight of formaldehyde, and M_d is the molecular weight of the desired compound. The sampling rates of the carbonyl compounds can be calculated from Eq. (3):

$$R = \frac{D_d}{D_f} R_f \quad (3)$$

where R is the sampling rate of the desired compound, D_d is the diffusion coefficient of the desired compound, and R_f (71.9 mL min^{-1}) is the sampling rate of formaldehyde by DSD-DNPH [20]. The theoretical sampling rate of the DSD-BPE/DNPH can be calculated from Eq. (3) because the diffusion filter is the same as DSD-DNPH. Dashed lines in Fig. 6 represent theoretical values calculated using Graham's law. For ozone, the theoretical sampling rate with DSD-BPE/DNPH is 56.9 mL min^{-1} and the experimental sampling rate is approximately 80% of the theoretical value (44.6 mL min^{-1}). This suggests that ozone decomposes while passing through the diffusion filter of DSD-BPE/DNPH. However, the ozone concentration can be obtained by using the experimental sampling rate because a linear relationship (regression coefficient: 0.999) is observed between the sampling duration and the ozone concentration.

For formaldehyde measured by the DSD-BPE/DNPH, an approximately linear relationship (slope: 0.0280 $\mu\text{mol d}^{-1}$) is observed between the sampling duration and the ozone concentration until 5 days of sampling. Amounts collected with DSD-DNPH agreed with DSD-BPE/DNPH until 5 days; however, when sampling duration

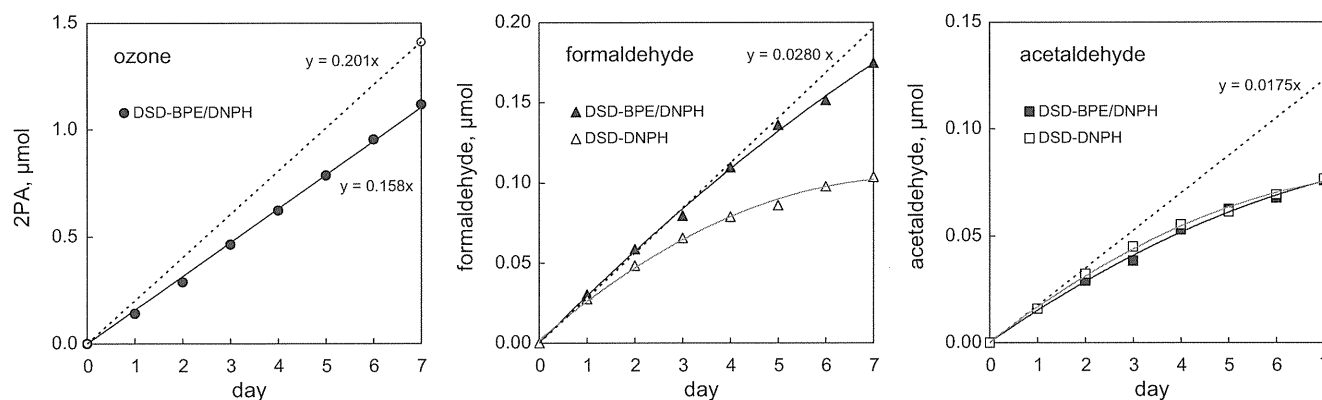


Fig. 6. Relationship between exposure time and collected amount by using DSD-BPE/DNPH and DSD-DNPH diffusive samplers. Air sampling was performed continuously for several days. Dashed lines represent theoretical values calculated from Graham's law of effusion.

was more than 6 days, the agreement gradually decreased. Experimental DSD-BPE/DNPH sampling rate can be calculated in the same way of ozone, as follows:

$$\text{Sampling rate of formaldehyde by DSD-BPE/DNPH} = \frac{0.0280}{0.0389}$$

$$\times 100 = 72.0 \text{ mL min}^{-1}$$

In a previous report, the DSD-DNPH sampling rate was determined to be 71.9 mL min^{-1} at a sampling duration of 24 h [20]; this value was very close to the sampling rate of DSD-BPE/DNPH. However, over 2 days, the DSD-DNPH sampling rate decreased with the sampling term. This suggested that ozone decomposes the formaldehyde hydrazone derivative [31,32] collected by DSD-DNPH and that 2BPE in DSD-BPE/DNPH acts as an ozone scrubber.

For acetaldehyde measured by the DSD-BPE/DNPH diffusive sampler (DSD-BPE/DNPH), an approximately linear relationship (slope: $0.0175 \mu\text{mol d}^{-1}$) exists between the sampling duration and the ozone concentration until 2 days of sampling. Amounts collected with DSD-DNPH agreed with DSD-BPE/DNPH values until 2 days of sampling; however, when sampling duration was more than 3 days, this agreement gradually decreased. The DSD-BPE/DNPH experimental sampling rate for acetaldehyde can be calculated again as follows:

$$\text{Sampling rate of acetaldehyde by DSD-BPE/DNPH} = \frac{0.0175}{0.0295}$$

$$\times 100 = 59.3 \text{ mL min}^{-1}$$

In a previous report, the DSD-DNPH sampling rate was determined to be 59.4 mL min^{-1} at a sampling duration of 24 h [20]; this value was very close to the DSD-BPE/DNPH sampling rate. However, over 3 days, the DSD-DNPH and DSD-BPE/DNPH sampling rates significantly decreased with an increase in the sampling term. This suggested that ozone decomposes the acetaldehyde hydrazone derivative [31,32] collected by DSD-DNPH and DSD-BPE/DNPH. The high molecular weight carbonyls react slowly with DNPH and seemed to decompose with high concentration ozone. DSD-BPE/DNPH method is possible to measure formaldehyde and acetaldehyde under the condition of relatively high concentration of ozone ($72 \mu\text{g m}^{-3}$) for 24 h.

3.4. High concentration and long-term ozone sampling

An ozone generator was operated at $136 \mu\text{g m}^{-3}$ in the environmental test chamber, which was set to 25°C and 60% relative

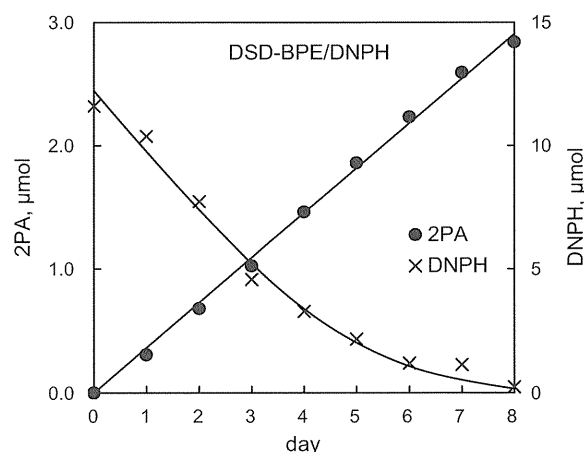


Fig. 7. Change in the amount of 2PA formed with sampling duration.

humidity. Ten DSD-BPE/DNPH samplers were placed in the environmental test chamber and recovered separately every 24 h over a period of 10 days. After air sampling, all the samplers were analyzed by HPLC. Fig. 7 shows 2PA (ozone) amounts collected with DSD-BPE/DNPH over a sampling duration of 1–8 days.

All DNPH in the DSD-BPE/DNPH was eliminated by reacting with ozone at 8 days. Until DNPH was eliminated, 2PA formation was proportional to sampling duration. During long-term sampling, 2PA, formed from ozone and 2BPE, is likely to be oxidized by excess ozone, forming pyridine-2-carboxylic acid (picolinic acid). However, this phenomenon does not occur in the DSD-BPE/DNPH method, because 2PA reacts immediately with coexistent DNPH to form the hydrazone derivative.

3.5. Measurement of indoor and outdoor air

Indoor and outdoor air was collected over the period October 19–26, 2010, using DSD-BPE/DNPH. In case of outdoor measurement, the DSD-BPE/DNPH samplers were placed on the rooftop of Chiba City's Air Monitoring Station in Japan. In case of indoor measurement, the DSD-BPE/DNPH samplers were placed in the living room of a house near by Chiba City's Air Monitoring Station. Collections were performed for 24 h; the ozone auto-analyzer at the Air Monitoring Station records every 1-h mean value obtained from every 1-min data. Fig. 8 shows carbonyl profiles of indoor and outdoor air measured during the period October 21–22, 2010. All the peaks were well separated and baseline-resolved.

Table 2

Ozone and carbonyl concentrations measured in indoor air (I) and outdoor air (O) collected by DSD-BPE/DNPH samplers. Concentration units are in $\mu\text{g m}^{-3}$. Values in parentheses indicate data measured by the auto-analyzer at the Air Monitoring Station.

Date (2010)	Weather conditions			Ozone		Formaldehyde		Acetaldehyde		Acetone	
	WS (m^{-1})	Temp ($^{\circ}\text{C}$)	RH (%)	I	O	I	O	I	O	I	O
Oct, 19–20	(2.7)	(18)	(82)	3.2	42 (43)	72	3.5	25	2.6	22	3.4
Oct, 20–21	(2.2)	(18)	(93)	2.4	14 (17)	82	3.8	27	2.5	24	3.2
Oct, 21–22	(3.7)	(18)	(80)	4.4	61 (59)	61	2.8	21	2.1	19	3.3
Oct, 22–23	(2.7)	(16)	(70)	3.4	57 (57)	55	2.4	22	2.2	20	3.3
Oct, 23–24	(1.6)	(15)	(81)	3.8	43 (40)	54	3.4	21	2.2	21	3.5
Oct, 24–25	(1.4)	(16)	(94)	2.4	17 (19)	63	3.7	24	3.9	24	2.5
Oct, 25–26	(2.3)	(17)	(94)	2.0	11 (12)	86	2.5	26	2.5	18	2.2

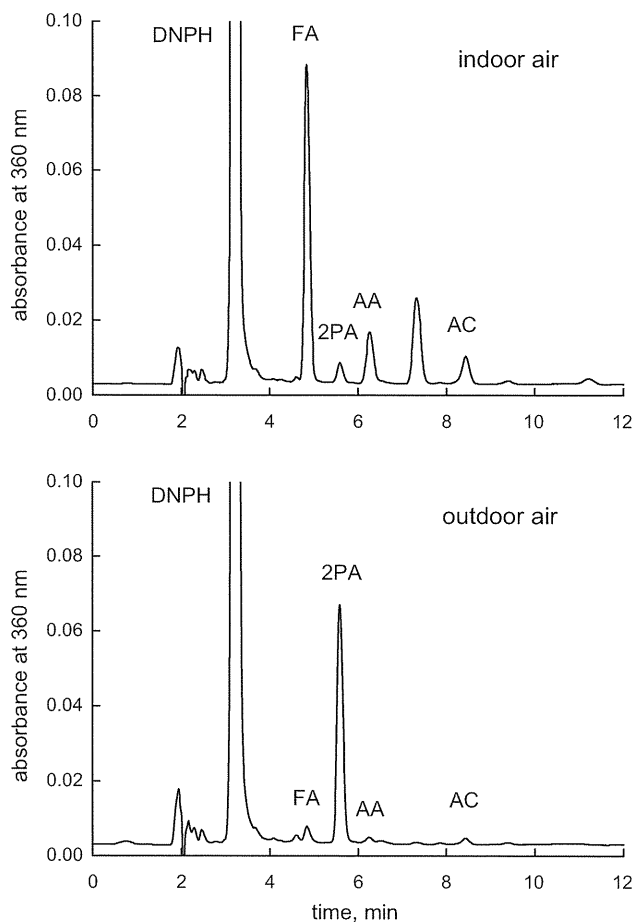


Fig. 8. Comparative carbonyl 2,4-DNPhydrazone profiles of indoor air (upper chromatogram) and outdoor air (lower chromatogram).

Measured ozone and carbonyl concentrations are listed in Table 2.

Ozone concentrations in outdoor air measured by the DSD-BPE/DNPH method were very similar to those obtained by the auto-analyzer at the Air Monitoring Station, and are approximately 10 times higher than in indoor air. A negative correlation between ozone and formaldehyde concentrations was found in indoor air, with a correlation coefficient of -0.736 . Formaldehyde in indoor air may be decomposed by ozone.

4. Conclusions

We developed a new diffusive sampler (DSD-BPE/DNPH) for the simultaneous determination of ozone and carbonyl in air, by using 2BPE and DNPH. The carbonyls in air react with DNPH in the absorbent to form hydrazone derivatives. Concurrently,

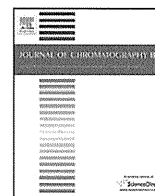
ozone in the air reacts with 2BPE to form 2PA, which reacts immediately with DNPH to form a 2PA-hydrazone derivative. All the hydrazones derived from airborne carbonyls and 2PA (formed from ozone) are completely separated and analyzed by HPLC. The DSD-BPE/DNPH sampling rates for the carbonyls agree well with those for commercially available DSD-DNPH. The DSD-BPE/DNPH sampling rate for ozone is determined to be 44.6 mL min^{-1} by comparison with that obtained in an active sampling method. The DSD-BPE/DNPH method is advantageous because it is simple and allows for the simultaneous analysis of ozone and carbonyls.

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Review

Derivatization of carbonyl compounds with 2,4-dinitrophenylhydrazine and their subsequent determination by high-performance liquid chromatography[☆]

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ABSTRACT

Derivatization of carbonyl compounds with 2,4-dinitrophenylhydrazine (DNPH) is one of the most widely used analytical methods. In this article, we highlight recent advances using DNPH provided by our studies over past seven years. DNPH reacts with carbonyls to form corresponding stable 2,4-DNPhydrazone derivatives (DNPhyrazones). This method may result in analytical error because DNPhyrazones have both *E*- and *Z*-stereoisomers caused by the C=N double bond. Purified aldehyde-2,4-DNPhydrazone demonstrated only the *E*-isomer, but under UV irradiation and the addition of acid, both *E*- and *Z*-isomers were seen. In order to resolve the isometric problem, a method for transforming the C=N double bond of carbonyl-2,4-DNPhydrazone into a C–N single bond, by reductive amination using 2-picoline borane, has been developed. The amination reactions of C1–C10 aldehyde DNPhyrazones are completely converted into the reduced forms and can be analyzed with high-performance liquid chromatography. As a new application using DNPH derivatization, the simultaneous measurement of carbonyls with carboxylic acids or ozone is described in this review.

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1. Introduction

Carbonyl compounds such as aldehydes and ketones have received much attention as hazardous substances in studies of environmental and biological chemistry. Long-term exposure to relatively high levels of formaldehyde is known to increase the risk to human [1–4]. In 2004, the International Agency for Research on Cancer (IARC) reclassified formaldehyde as a human carcinogen that causes nasopharyngeal cancer and also concluded that there is a “strong but not sufficient evidence for

a causal association between leukemia and occupational exposure to formaldehyde” [5]. It is a great public health problem whether there is an association between formaldehyde exposure and leukemia. IARC classification of formaldehyde has started controversial discussions. Recently, some new epidemiological reports including meta-analysis were published [1,3,6–10]. The two studies [7,8] found an elevated mortality rate from myeloid leukemia in individuals occupationally exposed to formaldehyde. In addition, formaldehyde affects indoor air quality, and is known to trigger acute adverse health effects such as skin, eye, nose, and throat irritation. It has been reported that formaldehyde is significantly associated with a higher risk of the “Sick Building Syndrome (SBS)” [11–13]. Acetaldehyde, an analogue of formaldehyde, is listed as possibly carcinogenic to humans (Group 2B) by IARC. Ethanol in alcoholic drinks is mainly oxidized in the liver by alcohol dehydro-

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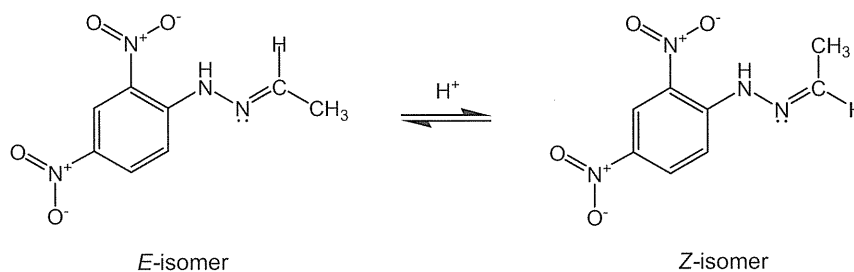


Fig. 2. Chemical structures of *E*- and *Z*-stereoisomers of acetaldehyde DNPhhydrazone.

at 364 nm, the isomer ratios were increased beyond this constant ratio and reached 0.55 and 0.33, respectively. Zero-order rates for decreases of aldehyde derivatives were observed under UV irradiation (364 nm). However, the decreases in concentration were not observed in phosphoric acid solutions.

Similar to alkanals, purified alkenal-2,4-DNPhhydrazone derivatives comprise only the *E*-isomer. However, partial isomerization to the *Z*-isomer occurs upon the addition of acid to attain an equilibrium isomer ratio [57]. The UV–visible spectral properties of the isomers differ; the *Z*-isomer exhibits a 6–10 nm lower absorption maximum wavelength compared to the *E*-isomer. Alkenal-2,4-DNPhhydrazones having a C=C double bond at the 2- or 3-position of the alkenal exhibited similar absorption maximum wavelengths with an equilibrium isomer ratio (0.035) that was much lower than those of other alkenals. The isomer ratio of alkenal-2,4-DNPhhydrazones is listed in Table 1. The C=C double bond at the 3-position migrates to a position of conjugation with the C=N double bond during hydrazone synthesis to form

a stabilized molecular structure. Alkenal-2,4-DNPhhydrazones having a double bond at the 4-position or greater exhibited similar absorption maximum wavelengths and equilibrium isomer ratio (0.14) to alkenal-2,4-DNPhhydrazones. The quantitative analysis of carbonyl compounds using DNPH is usually conducted in the presence of an acid catalyst. Consequently, the solution of the direct extract prepared for HPLC or GC analysis contains both *E*- and *Z*-isomers.

In the case of ketones, purified ketone-2,4-DNPhhydrazones were present only as the *E*-isomer [58]. When acid was added, both *E*- and *Z*-isomers were seen. The isomer ratios of ketone-2,4-DNPhhydrazones are listed in Table 1. In the case of 2-butanone-, 2-pentanone- and 2-hexanone-2,4-DNPhhydrazone, the equilibrium *Z/E* isomer ratios were 0.20, 0.21 and 0.22, respectively. In addition, when trace water was added to the hydrazone derivatives in acetonitrile solution, the concentrations of ketone derivatives were seen to decrease and the concentration of free DNPH was seen to increase. The decomposition rate of 2-butanone-2,4-DNPhhydrazone was dependent on the concentration of acid-catalyst and reached an equilibrium state – carbonyl, DNPH, hydrazone-derivative and H₂O – within 10 h at 0.1 mol/L phosphoric acid solution. The equilibrium constants of ketone-2,4-DNPhhydrazones, $[\text{carbonyl}][\text{DNPH}]/[\text{hydrazone}][\text{H}_2\text{O}]$, were relatively large and ranged from 0.74×10^{-4} to 5.9×10^{-4} . Hydrazone derivatives formed from 2-ketones such as 2-pentanone,

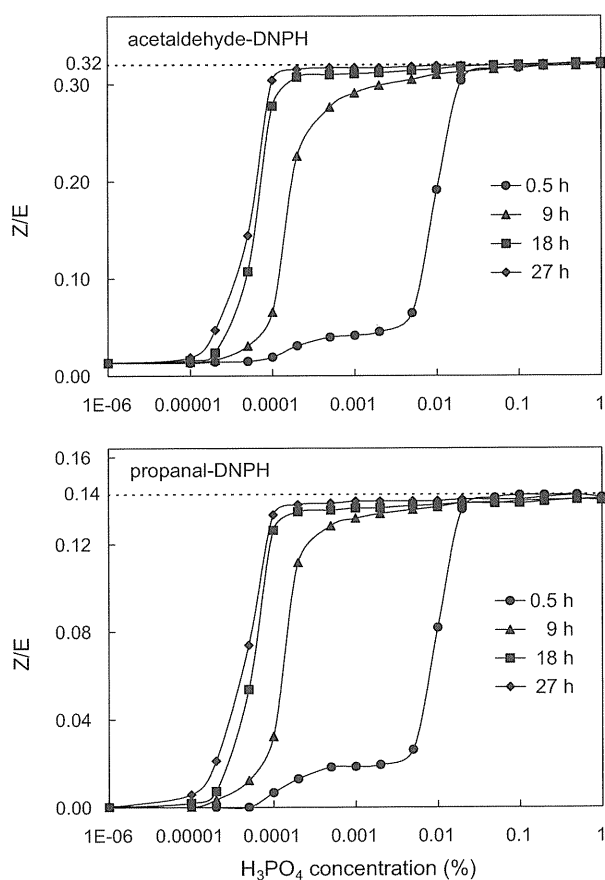


Fig. 3. The changes in the isomer ratios of acetaldehyde and propanal DNPhhydrazone with phosphoric acid. Reproduced with permission from Fig. 5 in Ref. [56].

Table 1

The isomer ratio and maximum absorption wavelengths of (*E*- and (*Z*-)isomers of DNPhhydrazone derivatives at 50/50 (v/v) acetonitrile/water.

Carbonyls	Isomer ratio <i>Z/E</i>	λ_{max} (nm)	
		<i>Z</i> -isomer	<i>E</i> -isomer
Alkanals			
Formaldehyde	n.a.	356	
Acetaldehyde	0.32	360	365
Propanal	0.14	358	366
Butanal	0.15	358	365
Pentanal	0.15	358	365
Hexanal	0.16	358	365
Heptanal	0.15	358	365
Octanal	0.15	358	364
Nonanal	0.15	358	364
Decanal	0.16	358	364
Alkenals			
2-Propenal	0.018	367	374
<i>trans</i> -2-Butenal	0.035	373	383
<i>trans</i> -2-Pentenal	0.035	373	383
<i>trans</i> -2-Hexenal	0.035	373	383
<i>trans</i> -2-Heptenal	0.035	373	383
<i>trans</i> -2-Octenal	0.035	373	383
<i>trans</i> -2-Nonenal	0.036	373	383
<i>trans</i> -2-Decenal	0.036	373	383
Ketones			
2-Propanone	n.a.	369	
2-Butanone	0.20	367	369
2-Pentanone	0.21	367	368
2-Hexanone	0.22	367	370

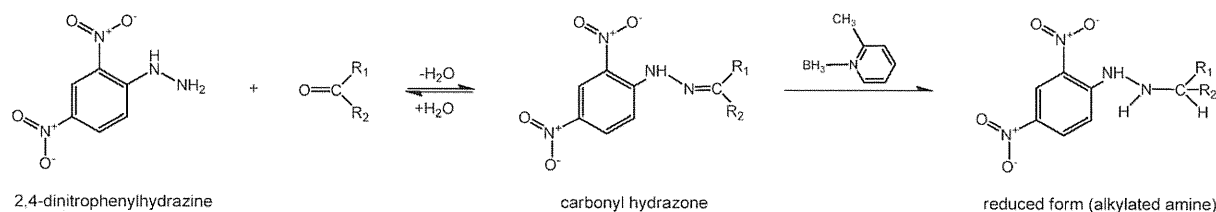


Fig. 4. Scheme of the reductive amination of carbonyl 2,4-DNPhydrazones with 2-picoline borane.

2-hexanone and 4-methyl-2-pentanone showed lower equilibrium constants than corresponding 3-ketones. Consequently, only a minimum concentration of catalytic acid must be added. The better method for the determination of ketone-2,4-DNPhydrazones by HPLC or GC is to add phosphoric acid to both the standard reference solution and samples, forming a 0.001 mol/L acid solution, and analyzing after 27 h.

3. Reductive amination of aldehyde 2,4-DNPhydrazones

As mentioned above, the traditional method for the measurement of carbonyl compounds, using DNPH to form the corresponding 2,4-DNPhydrazones derivatives, is subject to analytical errors because DNPhydrazones form both *E*- and *Z*-stereoisomers as a result of the C=N double bond. In order to resolve the isometric problem, it is necessary to transform the C=N double bond to a C-N single bond through use of a reducing agent. Various kinds of reducing agents, such as sodium cyanohydridoborate (NaBH₃CN) [59,60], sodium triacetoxyborohydride (Na(OAc)₃BH) [61–65], pyridine-borane (pyr-BH₃) [66–68], titanium(IV) isopropoxide/sodium borohydride (Ti(Oi-Pr)₄/NaBH₄) [69–72], borohydride exchange resin [73], zinc borohydride/silica gel (Zn(BH₄)₂/SiO₂) [74], and phenylsilane/dibutyltin dichloride (PhSiH₄/Bu₂SnCl₂) [75] have been developed for this conversion. The choice of the reducing agent is very critical to the success of the reaction, since the reducing agent must reduce imines selectively. Pyridine-borane has been widely used as a reductive amination reagent for aldehydes and ketones [68]. However, this reagent is quite unstable to heat and attempted distillation of the liquid residue at reduced pressures sometimes results in violent decompositions [76–78]. Thus, extreme care must be used if this reagent is handled in large quantities. Sato et al. [79] have developed an expeditious, easy-to-handle and environmentally friendly approach to the synthesis of a variety of amines through a three-component one-pot reaction of carbonyl compounds, amines, and 2-picoline borane. The later is a thermally stable transparent solid that be stored on a shelf for months without appreciable loss of the reduction capability. The use of 2-picoline borane eliminates the problems encountered with the use of other less stable reducing agents such as pyridine borane.

Recently, we developed a method for transforming the C=N double bond into a C-N single bond, using reductive amination of DNPhydrazones derivatives with 2-picoline borane [80]. Reductive amination of aldehyde DNPhydrazones is achieved by adding 2-picoline borane to the acetonitrile solution used to elute the DNPH-cartridge. Fig. 4 shows a scheme of the reductive amination of carbonyl 2,4-DNPhydrazones with 2-picoline borane. Aldehyde DNPhydrazones (C1–C10) are completely converted into their reduced forms within 40 min in the presence of 1 mmol/L 2-picoline borane and 20 mmol phosphoric acid. Fig. 5 shows the chromatograms at the state of coexistent aldehyde DNPhydrazones and their reduced forms. Before the addition of 2-picoline borane, only *E*- and *Z*-DNPhydrazones isomers are detected (upper panel). After the addition of 2-picoline borane, peaks of the reduced

forms began to appear between the *Z*- and *E*-isomer peaks of the corresponding DNPhydrazones. Twenty minutes after the addition of 2-picoline borane solution, reductive amination proceeds to 46–50% (middle panel). Sixty minutes later (80 min total), all DNPhydrazones derivatives, including *Z*- and *E*-isomers, are completely converted to their respective reduced forms (lower panel). These reduced forms are very stable and do not change when stored for two weeks at room temperature. The absorption maximum wavelengths of the reduced forms from C1 to C10 aldehyde DNPhydrazones were 351–352 nm, which shifted 6–7 nm towards shorter wavelengths when compared to the corresponding

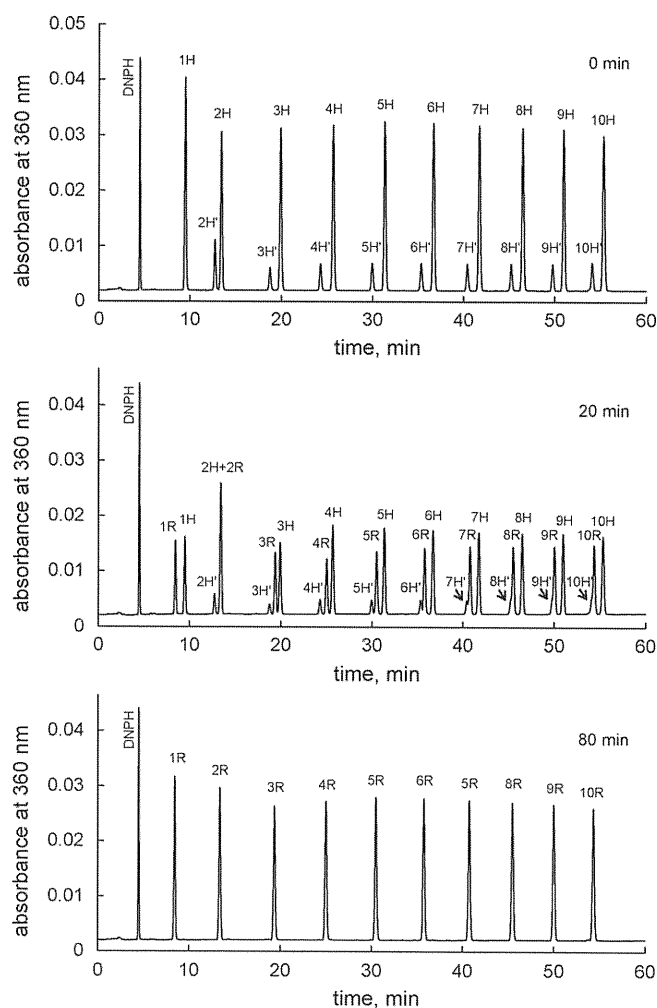


Fig. 5. Chromatographic profiles of DNPhydrazones and their reduced forms changing with reaction time. Number of peak name indicates carbon number of precursor aldehyde (1: formaldehyde, 2: acetaldehyde, 3: propanal, 4: butanal, 5: pentanal, 6: hexanal, 7: heptanal, 8: octanal, 9: nonanal, and 10: decanal), "H" indicates DNPhydrazones derivative and "R" indicates reduced form of DNPhydrazones derivative. Prime sign indicates *Z*-isomer of DNPhydrazones derivative. Reproduced with permission from Fig. 3 in Ref. [80].

DNPhydrazones. The molar absorption coefficients were 1.5×10^4 (C1)– 2.2×10^4 L/mol/cm (C10). Complete separation between C1 and C10 aldehyde DNPhydrazones and the corresponding reduced forms can be achieved by operating the HPLC in gradient mode using an Ascentis RP-Amide column (150 mm \times 4.6 mm i.d.). The RSDs of DNPhydrazone (*Z+E*) peak areas ranged from 0.40 to 0.66 and those of the corresponding reduced forms ranged from 0.26 to 0.41. This demonstrates that the reductive amination method gave improved HPLC analytical precision because of the absence of stereoisomers.

4. Derivatization of phthalaldehydes

Glutaraldehyde is a powerful biocide that was first introduced in 1963. Until relatively recently it has been the only widely available disinfectant for the reprocessing of flexible endoscopes and other heat-sensitive equipment. Orthophthalaldehyde (OPA) was introduced in 1999 as a safer alternative to glutaraldehyde, even though there was little evidence available to support such claims. OPA is a potential dermal and respiratory sensitizer and irritates the skin and respiratory tract [81]. Various analysis methods for difunctional glutaraldehyde have been developed. For the most part, they are based on solid substrate sampling and involve the use of derivatizing agents [82–86]. When derivatized with DNPH, OPA was collected using a silica gel cartridge impregnated with acidified 2,4-dinitrophenylhydrazine (DNPH-cartridge) and derivatives were analyzed by HPLC. The derivatization was examined by comparing the process with three phthalaldehyde isomers (ortho-, iso- and tere-) [87]. Fig. 6 shows chromatograms of OPA-DNPhydrazone, isophthalaldehyde (IPA) - DNPhydrazone and terephthalaldehyde (TPA) - DNPhydrazone synthesized with a fourfold molar excess of DNPH and with a fourfold molar excess of aldehyde. Chromatograms resulting from the use of excess aldehyde or excess DNPH are designated with the suffix “-A” or “-D” respectively. Only one peak is observed in OPA-DNPhydrazone, and two peaks are observed in IPA-DNPhydrazone and TPA-DNPhydrazone. In the early eluting peaks, peak areas of IPA-A and TPA-A are much larger than those of corresponding IPA-D and TPA-D. In the late eluting peaks, peak areas of IPA-D and TPA-D are much larger than those of corresponding IPA-A and TPA-A. Dialdehydes such as phthalaldehydes may give two types of derivatives, namely mono- and bis-DNPhydrazone derivatives. The early eluting peaks are mono-DNPhydrazone derivatives and late eluting peaks are bis-DNPhydrazone derivatives. In the case of iso- and terephthalaldehyde, derivatives synthesized with excess aldehyde consisted mainly of mono-derivatives and derivatives synthesized with excess DNPH consist mainly of the bis-derivative. In the case of OPA, only the bis-derivative was detected and the mono-derivative was never observed under any conditions. OPA is completely retained by the DNPH-cartridge. The derivatization reaction was incomplete and unreacted OPA was flushed from the cartridge during the subsequent solvent extraction process. Unreacted OPA and DNPH react in the extraction solvent solution. Immediately after solvent extraction, both mono- and bis-DNPhydrazone derivatives of OPA are present in the solution. Over time, the mono-derivative decreased and the bis-derivative increased in concentration until only the bis-derivative remained; allowing accurate determination of the OPA concentration. The transformation of mono-derivative to bis-derivative was faster in polar aprotic solvents such as acetonitrile, dimethyl sulfoxide and ethyl acetate. Transformation is found to occur most quickly in acetonitrile solvent and is completed within 4 h. It is suggested that the reaction of OPA and DNPH proceeded in polar aprotic solvents and mono-derivative was completely transformed to bis-derivative according to the reaction of Fig. 7. It is possible to measure OPA as

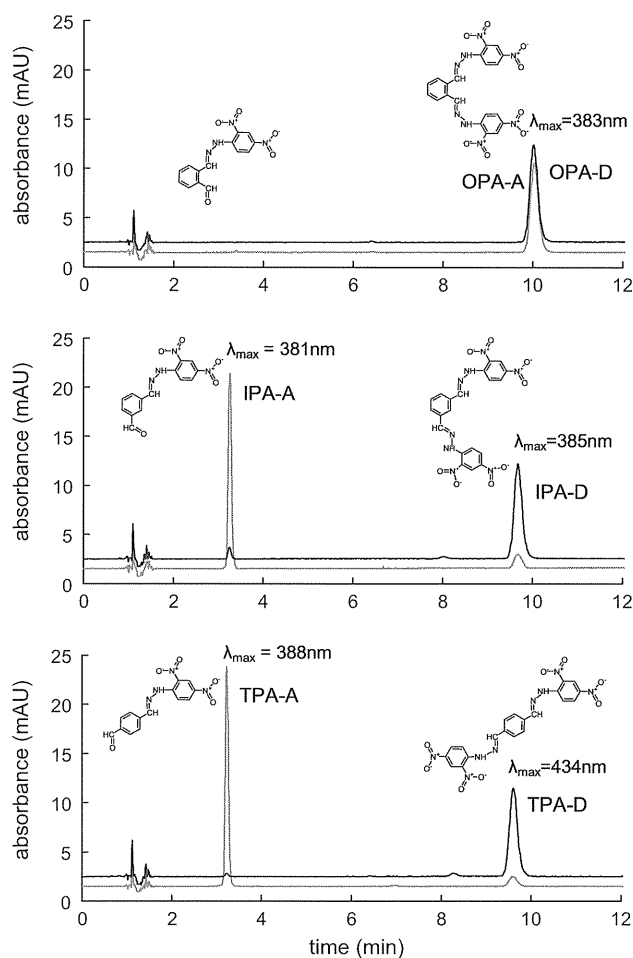


Fig. 6. HPLC chromatograms of OPA-DNPhydrazone (upper), IPA-DNPhydrazone (middle) and TPA-DNPhydrazone (lower) at maximum wavelengths by photo diode array detector. Light-colored chromatograms indicate the derivatives synthesized with excess of aldehyde and dark-colored chromatograms indicate the derivatives synthesized with excess of DNPH. The concentration was 2 mg/L. Reproduced with permission from Fig. 1 in Ref. [87].

the bis-derivative using a DNPH impregnated silica cartridge and HPLC analysis.

5. Application of DNPH derivatization to new analytical methods

5.1. Simultaneous determination of carboxylic acids and carbonyls

It has been recognized that DNPH only reacts with the carbonyl functional groups in aldehydes and ketones and not with those in compounds such as carboxylic acids, esters and amides. However in our experiments, we have found that carboxylic acids such as formic acid and acetic acid react with DNPH to form the corresponding carboxylic-2,4-dinitrophenylhydrazides under specific conditions [88]. A DNPH-cartridge saturated with formic acid vapor becomes gradually discolored and completely changes to light yellow in 6 h at 25 °C. The HPLC chromatogram of the eluant from this DNPH-cartridge indicates complete consumption of DNPH accompanied with formation of formic-2,4-dinitrophenylhydrazide (formic-DNPhydrazone). Fig. 8 shows the peak area changes with time of DNPH and formic-DNPhydrazone at wavelength 360 nm. Acetic acid, propionic acid and butyric acid exhibit similar behavior with longer reaction time in order of

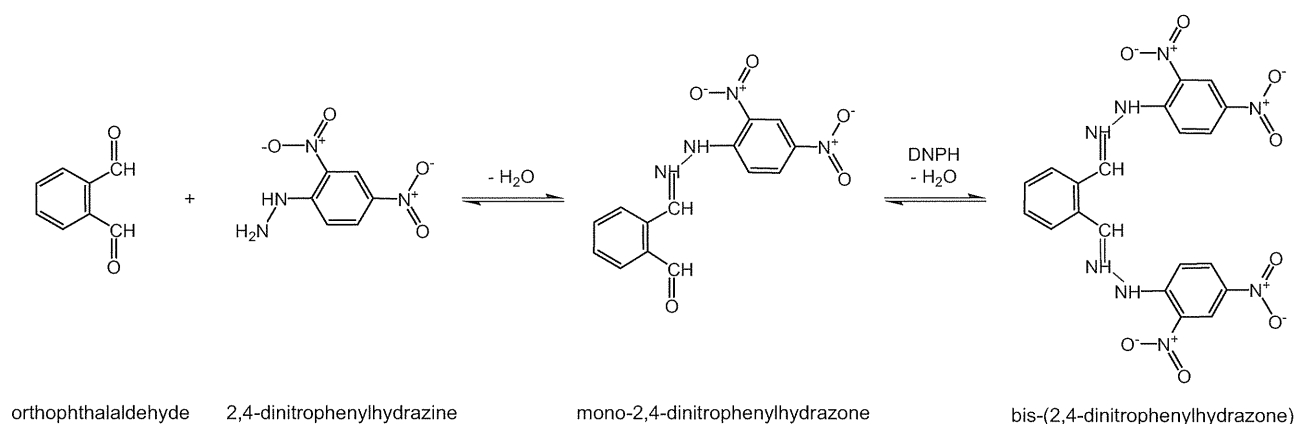


Fig. 7. Scheme of the derivatization reaction of DNPH with orthophthalaldehyde.

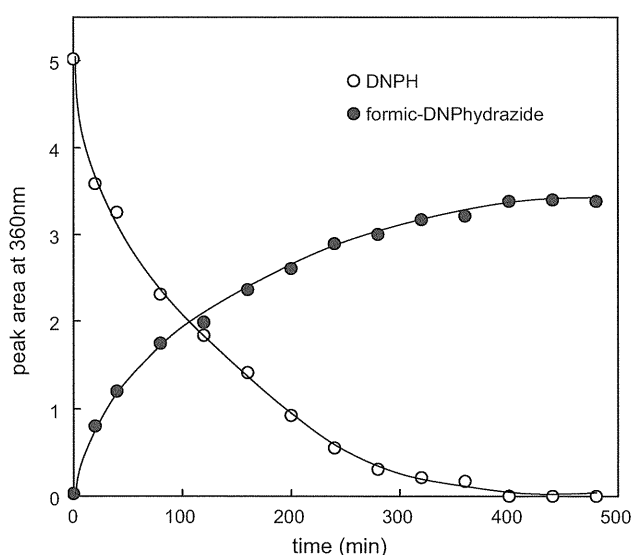


Fig. 8. The reaction of adsorbed formic acid and DNPH with time. ($\lambda = 360\text{ nm}$). Reproduced with permission from Fig. 1 in Ref. [88].

increasing carbon number. Fig. 9 shows the derivatization reaction of DNPH with carboxylic acid. It is suggested that carboxylic acids react with DNPH to initially form corresponding hydrazone derivatives, which then isomerize to hydrazides by keto-enol tautomerization. These hydrazide derivatives have excellent thermal stability with melting points higher than those of the corresponding hydrazones by 32–50 °C. They exhibit maximum absorption wavelengths of 331–334 nm and molar absorption coefficients of $1.4 \times 10^4 \text{ L/mol/cm}$. In reversed-phase HPLC analysis, the separations of hydrazide and hydrazones derivatives may be incomplete. The retention times of DNPhydrazone peaks vary with mobile phase

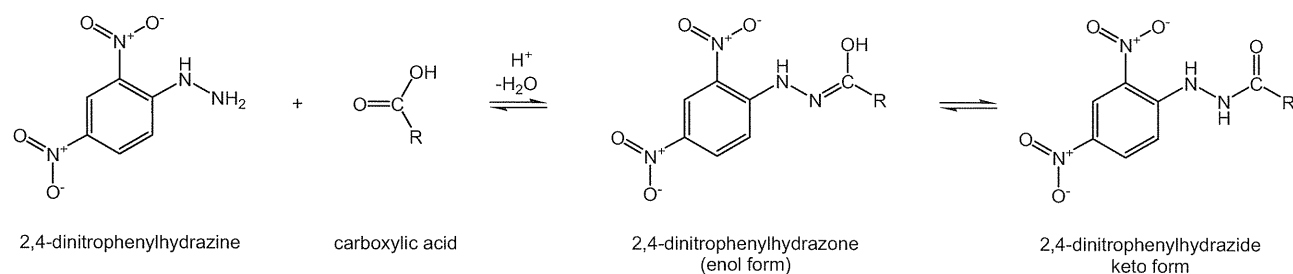


Fig. 9. Scheme of the derivatization reaction of DNPH with carboxylic acid.

pH. The addition of base to the mobile phase shortens the retention times of C1–C6 DNPhydrazone peaks and shifts the UV/vis spectrum profiles to longer wavelengths. Under the conditions of 0, 0.1, and 1.0 mmol/L dibasic potassium phosphate, the spectra of formic DNPhydrazone are unimodal with a maximum wavelength of 339 nm, bimodal with a maximum wavelength of 339 and 423 nm, respectively, and unimodal with a maximum wavelength of 423 nm, respectively. The DNPhydrazone derivatives of carboxylic acids exist in equilibrium with their enol tautomer and exhibit an isosbestic point at 370 nm. Complete separation of C1–C6 carboxylic acids and aldehydes was achieved on an RP-Amide column with the use of ACN–H₂O (40:60) containing dibasic potassium phosphate (0.1 mmol/L) as the mobile phase and UV detection at 370 nm. Fig. 10 shows chromatogram of C1–C6 hydrazide and hydrazone derivatives using an RP-Amide C16 column. The derivatization reaction to completion for the DNPH-cartridges containing 0.2–1% (v/w) phosphoric acid. The best condition for the simultaneous measurement of carboxylic acids and aldehydes is 1% (v/w) phosphoric acid because acidic conditions are needed for the measurement of aldehydes. Cartridges packed with DNPH-coated silica particles (DNPH-cartridge) are used for sampling formic acid and aldehydes. Formic acid is physically adsorbed on the silica particles as the first step of the sampling mechanism. A gradual reaction with DNPH follows. Formic acid reacts very slowly with DNPH at room temperature (20 °C), but reacts completely at 80 °C over 4 h.

5.2. Simultaneous determination of ozone and carbonyls

A new method for the simultaneous determination of ozone and carbonyls in air using a two-bed cartridge system has been developed [89,90]. Each bed consists of reagent-impregnated silica particles. The first contains *trans*-1,2-bis-(2-pyridyl)ethylene (2BPE) while the second contains 2,4-dinitrophenylhydrazine (DNPH). Fig. 11 shows the reaction pathways for the simulta-

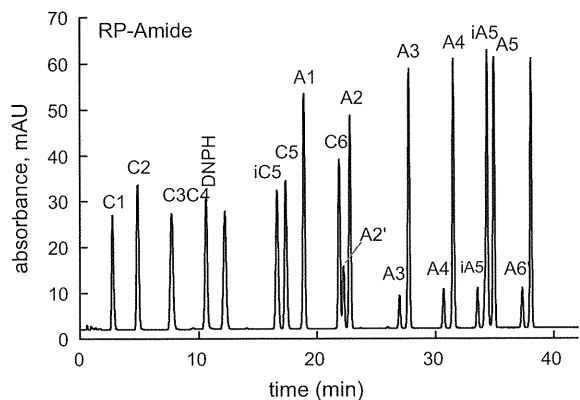


Fig. 10. Chromatographic profiles of C1–C6 carboxylic-DNPhydrazides and aldehyde-2,4-DNPhydrazones on an Ascentis RP-Amide column (100 $\mu\text{mol/L}$) at maximum absorption wavelengths between 300 nm and 500 nm. A prime sign indicates the *Z*-isomer C1, formic acid DNPhydrazide; C2, acetic acid DNPhydrazide; C3, propionic acid DNPhydrazide; C4, butyric acid DNPhydrazide; i-C5, *i*-pentanoic acid DNPhydrazide; n-C5, *n*-pentanoic acid DNPhydrazide; C6, hexanoic acid DNPhydrazide; A1, formaldehyde DNPhydrazide; A2, acetaldehyde DNPhydrazide; A3, propionaldehyde DNPhydrazide; A4, butyraldehyde DNPhydrazide; i-A5, *i*-pentanal DNPhydrazide; n-A5, *n*-pentanal, DNPhydrazide; A6, hexanal DNPhydrazide.

neous determination of ozone and carbonyls. Air samples are drawn through the cartridge first through the 2BPE bed and then through the DNPH. Ozone in the air sample is trapped in the first bed by the 2BPE-coated silica particles to produce pyridine-2-aldehyde. Airborne carbonyls pass unimpeded through the 2BPE and are trapped in the second bed by the DNPH-coated silica particles. They produce carbonyl DNPhydrazones. Fig. 12 shows the chromatographic profiles of 2PA (derived from ozone) and carbonyl DNPhydrazones. DNPH and carbonyl 2,4-DNPhydrazones are not influenced by ozone because of effective trapping by the 2BPE. Extraction is performed in the direction reverse to air sampling. When solvent is eluted through the 2BPE/DNPH-cartridge, excess DNPH is washed into the 2BPE bed where it reacts with pyridine-2-aldehyde and forms the corresponding hydrazone derivative. The use of a 2BPE/DNPH-cartridge has made possible the simultaneous determination of ozone

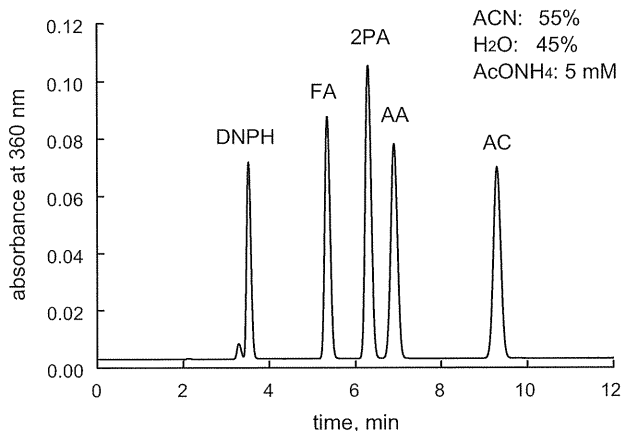


Fig. 12. Chromatogram of pyridine-2-aldehyde and other carbonyl 2,4-DNPhydrazones. Reproduced with permission from Fig. 2 in Ref. [90].

and carbonyls. A separate ozone scrubber is not necessary with the 2BPE/DNPH cartridge because the 2BPE portion of the sampler serves this function. Initially, *trans*-1,2-bis-(4-pyridyl)ethylene (4BPE) was used for the BPE/DNPH-cartridge [89]. However, the method suffered from long reaction times in the eluate, low solubility of the DNPH derivative and a strong dependence on atmospheric moisture. These problems could be overcome using *trans*-1,2-bis-(2-pyridyl)ethylene (2BPE) in place of 4BPE [90]. The efficiency of the reaction of ozone with 2BPE to form pyridine-2-aldehyde (2PA) is higher than the corresponding reaction with 4-BPE. Under the optimized elution conditions, the reaction times of 2PA and 4PA with DNPH are within 15 min and 120 min, respectively. During elution from the sampling cartridge, 2PA formed from 2-BPE and ozone is easier to dissolve in the elution solvent. A stronger influence of humidity was observed in ozone recovery by the 4-BPE/DNPH method. 2BPE exhibits a maximum reaction efficiency of 84% at 32% R.H., while 49% R.H. is required for 4BPE to attain a maximum reaction efficiency of 82%. Humidity has much less influence on the reaction of 2-BPE with ozone. Above 18% R.H., the reaction efficiency of 2-BPE with ozone is in the range 80–84%. Thus, 2-BPE is the more useful reagent for ozone analysis. The measured concentrations of ozone and carbonyls by the improved 2-BPE/DNPH

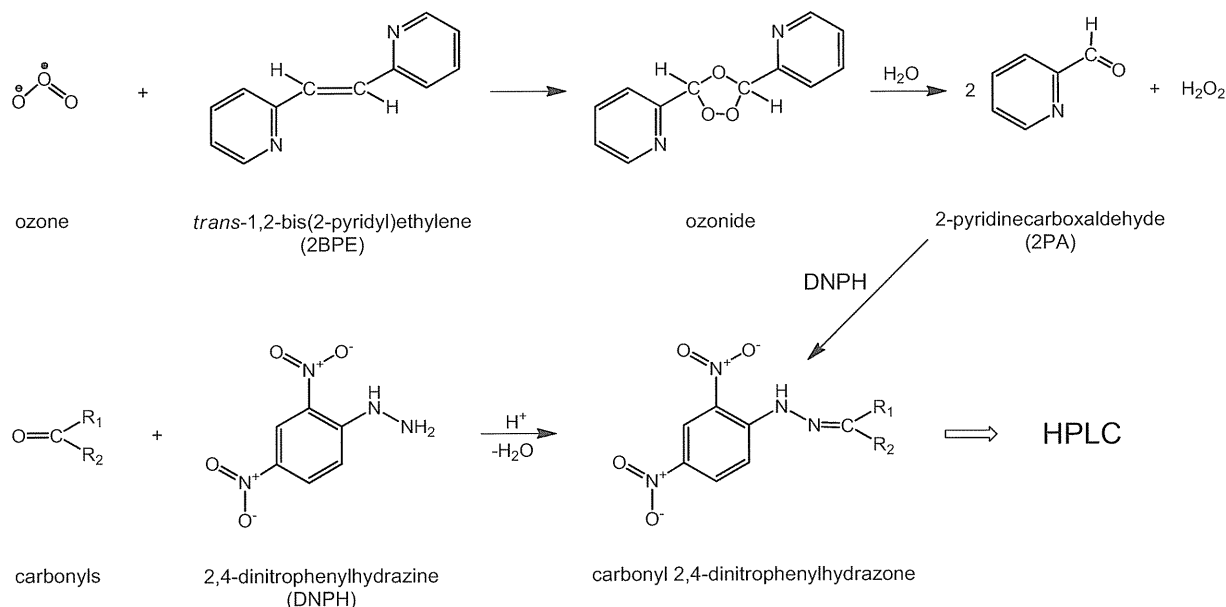


Fig. 11. Scheme of the simultaneous determination of ozone and carbonyls.

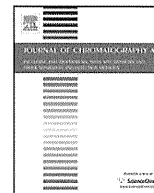
method corresponded with the values obtained using an ozone auto analyzer and a DNPH cartridge coupled with a KI-ozone scrubbing cartridge.

6. Conclusions

The specific reaction of carbonyl compounds with DNPH forming the corresponding DNPhhydrazones is one of the most important qualitative and quantitative methods in analytical chemistry. In this review, basic research such as isomerizations of DNPhhydrazones and reductive amination of aldehyde 2,4-DNPhhydrazones were described. Moreover, applications of new analytical methods, such as the analyses of carboxylic acids and ozone, were introduced. We expect that the traditional DNPH derivatization method will be more useful to analyze carbonyls or other compounds.

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Short communication

Ozone removal in the collection of carbonyl compounds in air

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ABSTRACT

The most widely used method for measuring carbonyl compounds in air is 2,4-dinitrophenylhydrazine (DNPH) derivatization followed by high-performance liquid chromatography (HPLC). However, substantial negative interference caused by the presence of ozone in air has been reported. To avoid the influences of ozone, a potassium iodide scrubber (KI-scrubber) is commonly used. However, when air sampling using a DNPH-cartridge and a KI-scrubber is performed under conditions of high humidity, moist potassium iodide in the KI-scrubber traps carbonyls before they reach the DNPH-cartridge. Moreover, wet KI reacts with I_2 to form KI_3 and this oxidative reagent moves to the DNPH-cartridge and destroys the DNPH and DNPhydrazone derivatives. In order to alleviate these problems, new ozone scrubbers (BPE-scrubber, HQ-scrubber) have been developed. BPE-scrubber and HQ-scrubber consist of silica gel particles impregnated with *trans*-1,2-bis-(2-pyridyl) ethylene (BPE) and hydroquinone (HQ), respectively. BPE reacts with ozone to form pyridine aldehyde and HQ reacts with ozone to form benzoquinone. The amounts of reducing agent in silica gel (130 mg) for ozone scrubber are 1% (w/w) for BPE-cartridge; 0.2% (w/w) for HQ-scrubber. These scrubbers can be used in air containing $140 \mu\text{g}/\text{m}^3$ of ozone for 24 h at a flow rate of 200 mL/min. When the relative humidity exceeded 80%, KI in the KI-scrubber was gradually moistened and changed to yellow in color. Peak abundance of formaldehyde, acetaldehyde and acetone DNPhydrazones was diminished to 25%, 15%, and 2%, respectively, compared with the BPE-scrubber or HQ-scrubber. When using a BPE-scrubber or HQ-scrubber, decomposition of DNPH and DNPhydrazones was not observed at a wide range of relative humidities (3–97%).

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1. Introduction

Formaldehyde, acetaldehyde and other carbonyl compounds are ubiquitous pollutants that are formed through oxidation of hydrocarbons by ozone in the troposphere [1–3] and by the reaction between ozone and terpenoid in indoor air [4–6]. Long-term exposure to relatively high levels of carbonyl compounds such as formaldehyde and acetaldehyde is known to increase the risk of asthma [7] and cancer [8]. Accurate aldehyde measurements are therefore important both for determining the formation mechanism of aldehydes and for evaluating the implications for human health.

The most widely used method for qualitative and quantitative analyses of carbonyl compounds is 2,4-dinitrophenylhydrazine (DNPH) derivatization followed by high-performance liquid chromatography (HPLC). Sampling can be performed using acidic solutions of DNPH in impingers or with acidic DNPH-coated solid sorbents in a cartridge. At the present time, a number of cartridge devices packed with DNPH-coated silica gel particles are

commercially available for sampling aldehydes in air. Due to the importance of the method, it has been introduced as a standard procedure by several national and international standardization bodies.

While the derivatization reaction at first glance appears straightforward, substantial negative interference caused by the presence of ozone in the air sample has been reported [9–11]. Ozone decomposes DNPH and DNPhydrazone derivatives to form 2,4-dinitrophenol, 2,4-dinitroaniline and 1,3-dinitrobenzene [12]. Additionally, Rodier and Birks reported that sampling atmospheres containing isoprene and ozone lead to the formation of artifact carbonyl peaks in a system using DNPH or dansylhydrazine-coated C18 cartridges [13]. The peaks were purportedly due to a reaction of isoprene with ozone on the cartridge surface, which led to positive artifacts for a number of compounds including formaldehyde.

To avoid the influences of ozone, a potassium iodide scrubber (KI-scrubber) can be used to destroy ozone before sampling the carbonyl compounds. In this case the air sample is first drawn over a surface on which solid KI is adsorbed. Ozone reacts with KI to form iodine and potassium hydroxide (Fig. 1). At the present time, KI-scrubbers are commercially available from many suppliers and are widely used. However, KI-scrubbers have two disadvantages. When air sampling using a DNPH-cartridge with a KI-scrubber is performed at high humidity, moist potassium iodide in the

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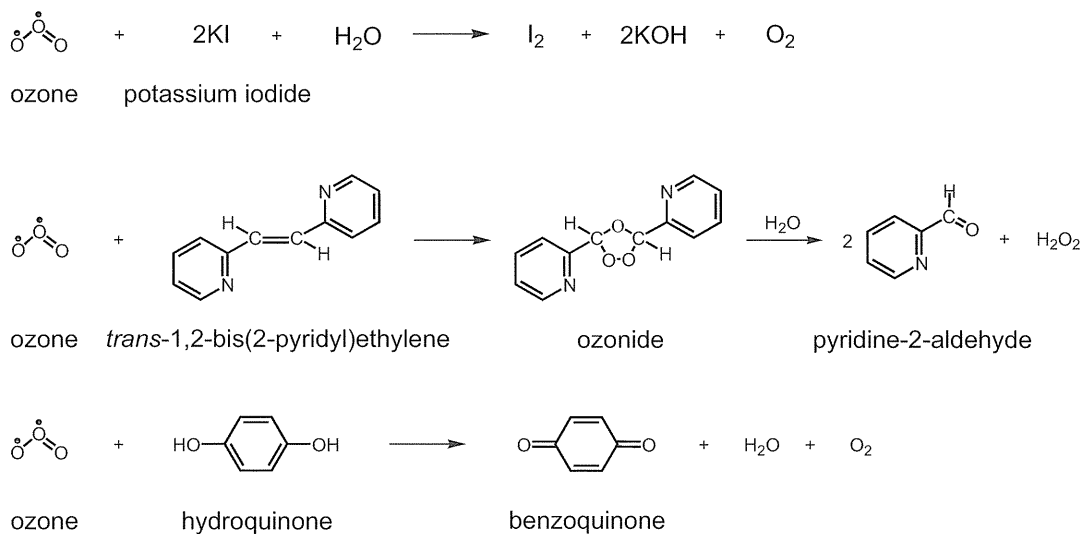


Fig. 1. Reactions of ozone with reducing agents.

KI-scrubber traps carbonyl compounds before they can reach the DNPH-cartridge. Moreover, wet KI reacts with I₂ to form KI₃ and this oxidative reagent moves to the DNPH-cartridge and destroys the DNPH and DNPhydrazone derivatives.

We have previously developed a method for the simultaneous determination of ozone and carbonyls in air using a two-bed cartridge system [14,15]. Each bed consists of reagent-impregnated silica particles. The first contains *trans*-1,2-bis-(pyridyl) ethylene (BPE), while the second contains 2,4-dinitrophenylhydrazine (DNPH). Air samples are drawn through the cartridge first through the BPE and then through the DNPH. Ozone in the air sample is trapped in the first bed by the BPE-coated silica particles and produce pyridine aldehyde (Fig. 1). In this method, BPE acts as an ozone scrubber. We have also developed a method for the determination of acrolein and other carbonyls in cigarette smoke using a dual cartridge system [16]. Each cartridge consists of reagent-impregnated silica particles. The first contains hydroquinone (HQ) for the inhibition of acrolein polymerization, while the second contains DNPH for the derivatization of carbonyls. HQ is a radical and ozone-trapping reagent and is used to inhibit acrolein radical polymerization and to remove ozone. Ozone reacts with HQ to form benzoquinone (Fig. 1). Thus, both BPE and HQ can function as ozone scrubbers. In this study, the effectiveness of KI-scrubber, BPE-scrubber and HQ-scrubber as ozone removers was investigated.

2. Experimental

2.1. Apparatus and reagents

The HPLC system (Shimadzu, Kyoto, Japan) used included two LC-20AD pumps, an SIL-20AC autosampler and an SPD M20A photodiode array detector. The analytical column was an Ascentis Express RP-Amide, 2.7 μm particle size, 150 mm × 4.6 mm i.d. column (Supelco Inc, Bellefonte, PA, USA). Solution A of the mobile phase mixture was acetonitrile/water (45/55, v/v) containing 5 mmol/L ammonium acetate and solution B was acetonitrile/water (75/25, v/v). HPLC elution was carried out with 100% A for 5 min, followed by a linear gradient from 100% A to 100% B in 50 min and then held for 10 min. The flow rate of the mobile phase was 0.7 mL/min. The column temperature was 40 °C, the autosampler temperature was 25 °C and the injection volume was 10 μL.

The environmental test chamber, supplied by Ohnishi Netsugaku Co., Ltd., Tokyo, Japan, was used for the sampler exposure tests.

The test chamber had a volume of 34.8 m³ (4.2 m × 3.6 m × 2.3 m) and was equipped with an adjustable constant temperature and humidity controller. Ozone gas was generated using an Ozone Generator (TGO-1, Funatech Ltd., Tokyo, Japan). Air pumps (MP-Σ30N, Shibata Scientific Technology Ltd., Tokyo, Japan) and wet gas meters (WS D-1A; Shinagawa Co., Tokyo, Japan) were used for air sample collection. The humidity and temperature of standard ozone gas were recorded using a TR-72U data logger (T&D Corporation, Japan).

Water used for HPLC and sample preparation was deionized and purified using a Milli-Q Water System equipped with a UV lamp (Millipore, Bedford, MA, USA). 2,4-Dinitrophenylhydrazine hydrochloride (>98%) and *trans*-1,2-bis(2-pyridyl)ethylene (BPE, >97%) were purchased from Tokyo Kasei Co. Ltd. (Tokyo, Japan). Acetonitrile (HPLC grade, >99.9%), hydroquinone (HQ, >99%), 2-pyridinecarboxaldehyde (pyridine-2-aldehyde, 99%), phosphoric acid (85% solution in water), hydrochloric acid (37%), and ammonium acetate (99.999%) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Rezorian Ozone Scrubbers and LpDNPH Rezorian cartridges were obtained from Supelco Inc. Silica gel (spherical, 60/80 mesh, 120 Å mean pore size) was obtained from AGC Si-Tech. Co., Ltd. (Fukuoka, Japan). Pyridine-2-aldehyde 2,4-DNPhydrazone was synthesized according to previously reported methods [15].

2.2. Preparation of a *trans*-1,2-bis(2-pyridyl)ethylene-impregnated silica cartridge (BPE-scrubber) and a hydroquinone-impregnated silica cartridge (HQ-scrubber)

Silica gel (50 g) was washed with water (3 × 500 mL), methanol (2 × 500 mL), and lastly acetonitrile (2 × 500 mL). The solvent was then completely evaporated to dryness at 100 °C for 30 min under vacuum on a rotary evaporator. After cooling to room temperature, acetonitrile (200 mL) was added to the washed silica gel. BPE (0.5 g) or HQ (0.1 g) was dissolved in 50 mL acetonitrile. This solution was added to the washed silica gel, the mixture was stirred and the solvent was evaporated to dryness at 40 °C under vacuum on a rotary evaporator. BPE-impregnated silica (130 mg) or HQ-impregnated silica (130 mg) was packed into polyethylene cartridges (Rezorian tube, 1 mL, Supelco Inc, Bellefonte, PA) and stored in a refrigerator at 4 °C.

The commercially available ozone scrubbers used in this study contained 1.5 g of potassium iodide.

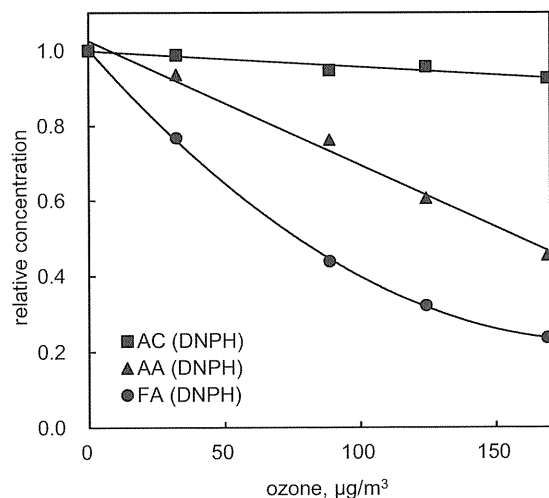


Fig. 2. Changes in measured carbonyl concentrations with the coexistent ozone concentration. DNPH-cartridges were used without an ozone scrubber. FA: formaldehyde; AA: acetaldehyde; AC: acetone.

2.3. Air sampling and analysis

Prior to air sample collection, each KI-scrubber, BPE-scrubber or HQ-scrubber was connected to a DNPH-cartridge to construct a dual-cartridge sampling train (KI-DNPH, BPE-DNPH and HQ-DNPH). Air was drawn through a coupled cartridge pair from the ozone scrubber to the DNPH-cartridge at a flow rate of 200 mL/min. After collection, the coupled cartridges were extracted. In the case of KI-DNPH, the KI-cartridge was discarded and the DNPH-cartridge was eluted with acetonitrile to a final volume of 5 mL. With BPE-DNPH, elution was performed in the reverse direction to air sampling. An intact, coupled cartridge pair was eluted with 30% dimethyl sulfoxide in acetonitrile solution containing 0.085% (v/v) phosphoric acid to a final volume of 5 mL. The HQ-DNPH cartridge pair was also eluted intact and in the reverse direction to air sampling. Acetonitrile was used as the elution solvent to a final volume of 5 mL. After 30 min of elution, the eluates were analyzed by HPLC.

3. Results and discussion

3.1. Decomposition of DNPhydrozones by ozone

An ozone generator was operated in the environmental test chamber set at a temperature of 25 °C and a relative humidity of 50%. The concentrations of formaldehyde, acetaldehyde and acetone in the environmental test chamber were 14, 15 and 8.0 µg/m³, respectively. Air sampling was performed by using a DNPH-cartridge without an ozone scrubber for 24 h at a flow rate of 200 mL/min. After collection, DNPH-cartridges were eluted with acetonitrile and analyzed by HPLC. Fig. 2 shows changes in the concentrations of carbonyl compounds with changes in ozone concentration. Carbonyl concentrations are expressed as relative concentrations in Fig. 2. Concentrations of formaldehyde and acetaldehyde decreased dramatically with increased ozone concentration. When the concentration of ozone is 170 µg/m³, the measured concentrations of formaldehyde and acetaldehyde are 20% and 45%, respectively, of the concentrations measured when no ozone is present. Alternatively, the measured concentration of acetone remained within 5% of the concentration when no ozone was present.

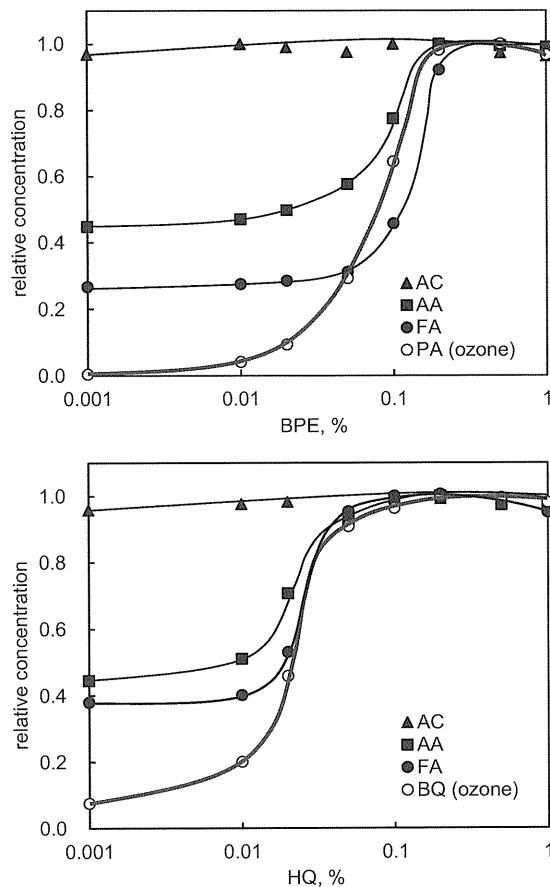


Fig. 3. Changes in the concentrations of carbonyl compounds with the contents of BPE (upper panel) and HQ (lower panel) in the ozone scrubber at a temperature of 25 °C and a relative humidity of 50%. FA: formaldehyde; AA: acetaldehyde; AC: acetone; PA: pyridine-2-aldehyde; BQ: benzoquinone.

3.2. Preferable contents of BPE and HQ in silica gel

An ozone generator was operated in the environmental test chamber set at a temperature of 25 °C and a relative humidity of 50%. The ozone concentration reached a constant value of 140 µg/m³ after 24 h. The concentrations of formaldehyde, acetaldehyde and acetone in the environmental test chamber were 13, 11 and 8.0 µg/m³, respectively. BPE-scrubbers containing 0–1% (0–7.1 µmol) of BPE and HQ-scrubbers containing 0–1% (0–12 µmol) of HQ were connected to DNPH-cartridges. Air sampling was performed for 24 h at a flow rate of 200 mL/min. After collection, BPE-DNPH-cartridges were eluted with 25% dimethyl sulfoxide in acetonitrile solution containing 0.085% (v/v) phosphoric acid and HQ-DNPH-cartridges were eluted with acetonitrile. After 30 min following elution, the eluate was analyzed by HPLC. Fig. 3 shows changes in the relative concentrations of carbonyl compounds with the loading of BPE (upper panel) and loading of HQ (lower panel) in the ozone scrubber.

In the case of BPE-DNPH cartridge, concentrations of formaldehyde, acetaldehyde, acetone, and pyridine-2-aldehyde increased with increasing BPE concentration, and reached a maximum value when the BPE concentration exceeded 0.5% (3.6 µmol). HQ-DNPH cartridges exhibited similar behavior. Measured concentrations of carbonyls reached a plateau when the HQ concentration exceeded 0.1% (1.2 µmol). HQ may react more efficiently with ozone than BPE. Based on the data presented in Fig. 3, appropriate ozone scrubbers with 130 mg silica support packing should contain 1% BPE-cartridge and 0.2% HQ-cartridge.

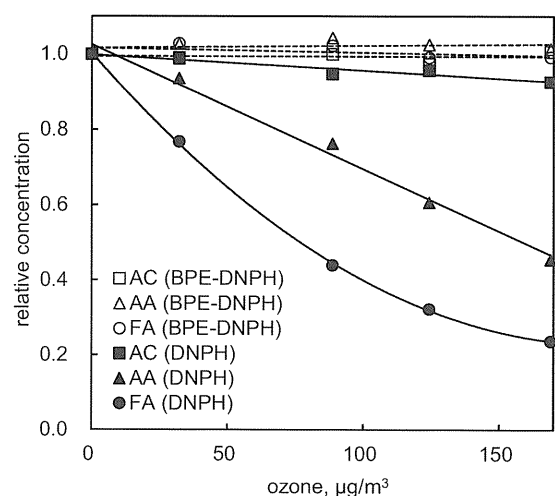


Fig. 4. Changes in measured carbonyl concentrations with the coexistent ozone concentration. DNPH-cartridges were used with BPE-scrubber and without an ozone scrubber. FA: formaldehyde; AA: acetaldehyde; AC: acetone.

Under the same conditions described in Fig. 2, KI-DNPH, BPE-DNPH and HQ-DNPH methods showed good performance in ozone removal and analysis of carbonyls. Broken lines of Fig. 4 show changes in measured carbonyl concentrations with the coexistent ozone concentration by using BPE-DNPH. Decreases of formaldehyde, acetaldehyde and acetone were not observed at a wide range of ozone concentrations (0–170 $\mu\text{g}/\text{m}^3$). HQ-DNPH and KI-DNPH exhibited similar behavior to BPE-DNPH.

3.3. Influence of humidity on the ozone scrubbers

The environmental test chamber was set to a temperature of 25 °C and relative humidity was varied from 3% to 97%. The concentrations of ozone, formaldehyde, acetaldehyde and acetone in the environmental test chamber were 70, 40, 12 and 9.0 $\mu\text{g}/\text{m}^3$, respectively. Air sampling was performed by using KI-DNPH, BPE-DNPH and HQ-DNPH for 24 h at a flow rate of 200 mL/min. Fig. 5 shows the chromatographic profiles of the eluates eluted from KI-DNPH, BPE-DNPH and HQ-DNPH cartridges. In the case of KI-DNPH, when the relative humidity exceeded 80%, KI in the KI-scrubber was gradually wetted and changed to yellow in color (KI_3). The liquefied, wet KI migrated into the DNPH-cartridge where the DNPH-silica was discolored to brown. DNPH was decomposed by the wet KI and the DNPH peak was not detected in the chromatogram. Peak abundance of FA-D, AA-D and AC-D was diminished to 25%, 15%, 2%, respectively, relative to the same peaks when BPE-DNPH or HQ-DNPH was used. It is suggested that carbonyl compounds dissolve in the wet KI because carbonyl compounds are polar, hydrophilic and water-soluble. In the case of BPE-DNPH and HQ-DNPH, decomposition of DNPH was not observed and large unreacted DNPH peaks were detected. Peak abundance of FA-D, AA-D and AC-D is of the same order of magnitude as

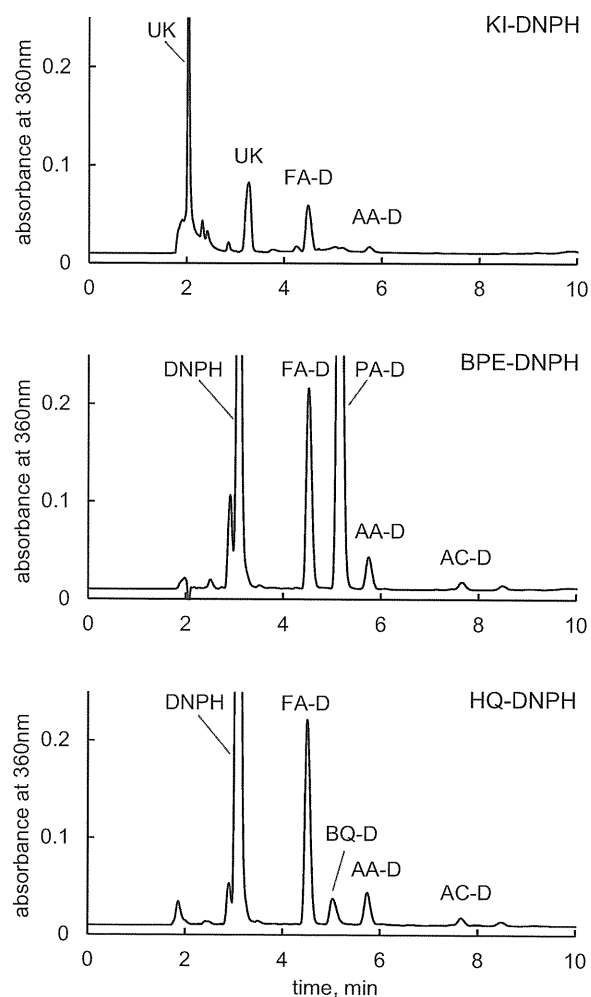


Fig. 5. Chromatographic profiles of DNPhydrazone derivatives obtained from KI-DNPH (upper panel), BPE-DNPH (middle panel) and HQ-DNPH (lower panel) methods. FA-D: formaldehyde DNPhydrazone; AA-D: acetaldehyde DNPhydrazone; AC-D: acetone DNPhydrazone; PA-D: pyridine-2-aldehyde DNPhydrazone; BQ-D: benzoquinone DNPhydrazone; UK: unknown compound.

those measured by both BPE-DNPH and HQ-DNPH under dry conditions.

The large PA-D peak in the BPE-DNPH chromatogram is the derivative derived from PA and DNPH. PA is formed by the reaction of BPE with ozone [15]. Therefore, it is possible to determine ozone concentration by measuring PA concentration quantitatively [15]. By the same token, BQ-D peak in the HQ-DNPH is the derivative derived from BQ and DNPH (Fig. 6). Ozone reacts with HQ completely to form BQ, however, partial subsequent reaction with DNPH also occurs. Ozone concentration can be determined by summing BQ-D and underivatized BQ concentrations.

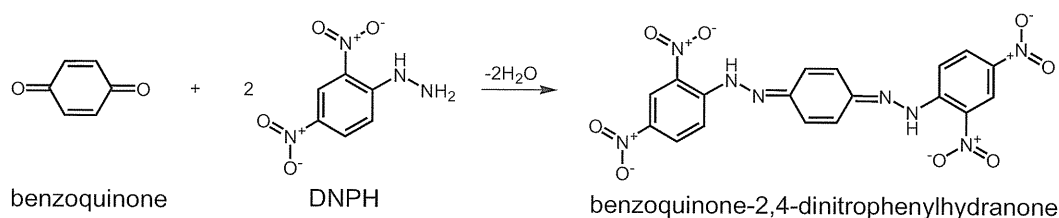


Fig. 6. Reaction of benzoquinone with DNPH.

4. Conclusions

For measuring carbonyl compounds using DNPH-cartridge, a potassium iodide has been widely used as ozone scrubber. However, when air sampling is performed under conditions of high humidity, moist potassium iodide in the KI-scrubber traps carbonyls before they reach the DNPH-cartridge. Moreover, wet KI reacts with I₂ to form KI₃ and this oxidative reagent moves to the DNPH cartridge and destroys the DNPH and DNPhydrazone derivatives. BPE-DNPH and HQ-DNPH methods have the advantage of air sampling under high humidity conditions without the problems associated with the hygroscopic nature of potassium iodide, and besides, these methods allow the simultaneous measurement of ozone and carbonyl compounds.

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報 文

ハイドロキノンと 2,4-ジニトロフェニルヒドラジンを含浸させた二連シリカカートリッジを用いる電子タバコから発生するカルボニル化合物の分析

太田 和司¹, 内山 茂久², 稲葉 洋平², 中込 秀樹¹, 櫻田 尚樹²

電子タバコから発生する煙の成分をハイドロキノン (HQ) と 2,4-ジニトロフェニルヒドラジン (DNPH) を用いた二連カートリッジ法 (HQ-DNPH 法) で分析し, 発生する化学物質, 生成メカニズム等の検討を行った. 市販されている電子タバコの煙を分析した結果, ホルムアルデヒド, アセトアルデヒド, アクロレイン, グリオキサール, メチルグリオキサール等, 多くの有害なカルボニル化合物が高濃度で検出された. 電子タバコ専用カートリッジに含まれる液体を分析した結果, 主成分はグリセロールやグリコール類であった. そこで, 様々なグリコール類をコイル状のニクロム線に塗布した模擬電子タバコを作製し, 一定の電圧を印加し, そこから発生する気体を分析した. その結果, 3 V 以上の電圧を印加すると, エチレングリコールからグリオキサールが, プロピレングリコールからメチルグリオキサールが, グリセロールからアクロレインが発生することが明らかになった.

1 緒 言

電子タバコは, 香港に所在する北京 SBT 如煙科技發展有限公司が 2003 年に世界で初めて開発したとされ, 現在, 世界各国に普及している. それはバッテリー, 蒸発ユニット, 液体カートリッジで構成され「煙草」に模した気体吸引機であり, 実際に吸引することで液体カートリッジに入れられた液体が蒸発ユニットを通過するとき霧状となりそれを吸引する仕組みである. しかし, 世界保健機関 WHO は, 2008 年 9 月に海外で販売されているニコチン入り電子タバコの利用について, 「安全性は確認されておらず, 禁煙療法とは考えられない」と警鐘を鳴らし, 2009 年に Technical Report Series 955¹⁾ を発行している. またアメリカ食品医薬品局 FDA は, 2009 年 7 月, 米国で販売されている電子タバコから不凍液成分であるジエチルグリコールや発がん物質であるニトロサミン類が検出されたと報告している²⁾. 日本国内では, 国民生活センターの調べで, 個人輸入されている海外販売のニコチン含有電子タバコだけでなく, 国内販売している商品からもニコチンがわずかながら検出され問題となった (平成 22 年 8 月 18 日). しかし, 電子タバコから発生する気体の化学分析が困難であったため, 現在までに十分に報告されていない.

ホルムアルデヒドやアセトアルデヒドなどのカルボニル化合物は人体に有害な物質である. 比較的高濃度のホルムアルデヒドやアセトアルデヒドの長期間暴露は, 喘息³⁾ やガン^{4)~6)} のリスクを高めることも知られている. また, アクロレインやクロトンアルデヒドなどの α,β -不飽和アルデヒドは反応性が高く, 人への影響が懸念されている物質である. 2006 年に Feng らは, 煙草煙中のアクロレインが肺がんのリスクを増加させることを報告している⁷⁾. したがって, カルボニル化合物の人に対する健康影響や生成機構を解明することは重要である.

2010 年に Uchiyama らは, α,β -不飽和アルデヒドを含む広範囲のカルボニル化合物の分析方法として, ハイドロキノン (HQ) 含浸シリカと 2,4-ジニトロフェニルヒドラジン (DNPH)⁸⁾ 含浸シリカを用いた HQ-DNPH cartridge 法を報告した⁹⁾. これは, ラジカル捕捉剤である HQ により DNPH シリカ中のアクロレインのヒドラゾン誘導体の分解や重合を防ぎ, α,β -不飽和アルデヒドを含む広範囲のカルボニル化合物の分析を可能とする方法である.

本研究では HQ-DNPH cartridge 法を用いて, 電子タバコから発生する気体に含まれる有害なカルボニル化合物の分析を行い, その発生量や発生メカニズムを検討した.

2 実 験

2.1 装置と試薬

高速液体クロマトグラフィー (HPLC) は, LC-20AD 送液ポンプを二台, SPD M20A フォトダイオードアレー検出

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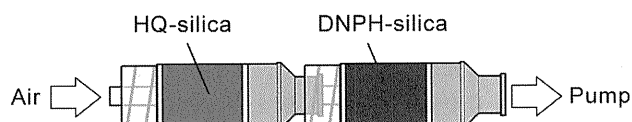


Fig. 1 Schematic drawing of the HQ-DNP cartridge

器を備えた島津製作所製 Prominence LC-20 を使用した。分離カラムは Ascentis Express RP-Amide (2.7 μm particle size, 150 mm \times 4.6 mm i.d., Supelco 製) を用い、カラムオープン温度を 40 $^{\circ}\text{C}$ 、注入量を 10 μL とした。グラジェントモードの HPLC 分析には、移動相にアセトニトリル (40%) と水 (60%) の混合溶液 (A 溶液) と、5 mmol/L の酢酸アンモニウムを含むアセトニトリル (75%) と水 (25%) 混合溶液 (B 溶液) を用いた。カラム流量 0.7 mL/min で A 溶液 100% を 8 分間保ち、37 分間で B 溶液 100% にした後、15 分間 B 溶液 100% を保った。

ガスクロマトグラフィー質量分析 (GC/MS) は、ヒューレットパッカード製 5890/5972 型を使用し、カラムはスペルコ製 SLB-5ms (0.25 mm i.d. \times 30 m, 0.25 μm) を用いた。分析条件は、カラム温度 40 $^{\circ}\text{C}$ (5 分保持) 40 $^{\circ}\text{C}$ ~ 300 $^{\circ}\text{C}$ (昇温速度 10 $^{\circ}\text{C}/\text{min}$), 300 $^{\circ}\text{C}$ (26 分保持) とした。質量分析条件は m/z 40 ~ 350 のスキャン測定、GC と MS のインターフェイス温度は 250 $^{\circ}\text{C}$ 、イオン源温度 280 $^{\circ}\text{C}$ 、電子イオン化 (EI) 電圧は 70 eV、注入条件は高圧スプリットレス法 (2 min) とした。

自動喫煙装置は Borgwaldt Technik GmbH 製 (model LMI/PLUS) を使用し、HCI (Health Canada Intensive) 法¹⁰⁾に準拠して、一回の吸煙量を 55 mL、吸煙時間を 2 秒、吸煙間隔を 28 秒、吸煙回数を 10 回に設定した。

HPLC 及び試料調整用の純水は Millipore 製 Milli-Q システムを使用した。2,4-ジニトロフェニルヒドラジン塩酸塩は東京化成工業製、ヒドロキノン、リン酸 (85%), エタノール (99.5%), グリセロール (99%), エチレングリコール (99.5%), プロピレングリコール (99%) は和光純薬工業製を使用した。シリカゲルは AGC SI-Tech 製の 60/80 mesh, 120 \AA を使用した。電子タバコは、日本国内で一般に市販されている 10 銘柄 (A, B, C, D, E, F, G, H, I, J) について検討を行った。

2.2 DNP cartridge および HQ cartridge の作製

DNP cartridge : 高濃度測定用と低濃度測定用の二種類の DNP cartridge を作製した。シリカゲル 50 g を純水 500 mL で 3 回洗浄する。次に 500 mL のメタノールで 2 回洗浄した後、500 mL のアセトニトリルで 2 回洗浄する。2,4-ジニトロフェニルヒドラジン塩酸塩を、低濃度測定用の場合は 0.25 g、高濃度測定用の場合は 1 g、及びリン酸 1 mL をアセトニトリル 300 mL に溶かす。この溶液を洗浄

したシリカに添加した後、40 $^{\circ}\text{C}$ に設定したロータリーエバポレーターでアセトニトリルを留去した。この DNP-silica を 280 mg 秤量し、1 mL のレゾリアンチューブに充填して DNP cartridge とした。

HQ cartridge : ヒドロキノンを 0.05 g 秤量し、50 mL のアセトニトリルに溶かす。この溶液を洗浄シリカ 50 g に添加した後、40 $^{\circ}\text{C}$ に設定したロータリーエバポレーターでアセトニトリルを留去した。この HQ-silica を 280 mg 秤量し、1 mL のレゾリアンチューブに充填して HQ cartridge とした。

2.3 電子タバコから発生するカルボニル化合物の捕集と分析

DNP cartridge の前段に HQ cartridge を接続し HQ-DNP cartridge (Fig. 1) を作製する。主流煙を測定するときは、電子タバコのカートリッジ (吸引口) に HQ-DNP cartridge の HQ cartridge 側を接続し、DNP cartridge 側を自動喫煙装置の試料口に接続する。HCI 法で捕集した後、直ちに抽出、分析を行わない場合は再び HQ-DNP cartridge を DNP cartridge と HQ cartridge に分割し、冷暗所に保管した。捕集終了後、HQ-DNP cartridge の DNP cartridge 側から、リン酸を 1% 含むアセトニトリル溶液 4 mL で溶出し、誘導体化のため 10 分間放置した後、過剰な付加反応を抑制するために、エタノールを 1 mL 添加した。この溶液 10 μL を HPLC に導入し、各種のカルボニル化合物 DNP 誘導体を分析した。

3 結果と考察

3.1 電子タバコから発生する気体に含まれるカルボニル化合物

国内で市販されている 10 銘柄の電子タバコ (A~J) から発生する煙に含まれるカルボニル化合物を HQ-DNP 法で測定した。代表的なクロマトグラムを Fig. 2 に示す。未反応の DNP のほかに、ホルムアルデヒド、アセトアルデヒド、アセトン、アクロレイン、プロパナール、クロトンアルデヒド、ブタナール、グリオキサール、メチルグリオキサール、ヘキサナールの誘導体のピークが検出されたが、特にホルムアルデヒド、アセトアルデヒド、アクロレイン、プロパナールグリオキサール、メチルグリオキサールなどの、炭素数が 1~3 のアルデヒド類が高濃度で発生することが明らかになった。なお、これらのピークは、合成した各 DNP 誘導体の保持時間から同定した。WHO が策定したホルムアルデヒドの室内指針値¹¹⁾は 0.1 mg/m^3 であるが、A~J のホルムアルデヒド発生量の平均値は、それぞれ 11, 13, 4.6, 0.3, 1.6, 20, 8.1, 2.8, 6.9, 0.5 mg/m^3 であり、いずれの銘柄も指針値以上の濃度を示した。ホルムアルデヒド以外のアセトアルデヒド、アクロレイン、グリオ

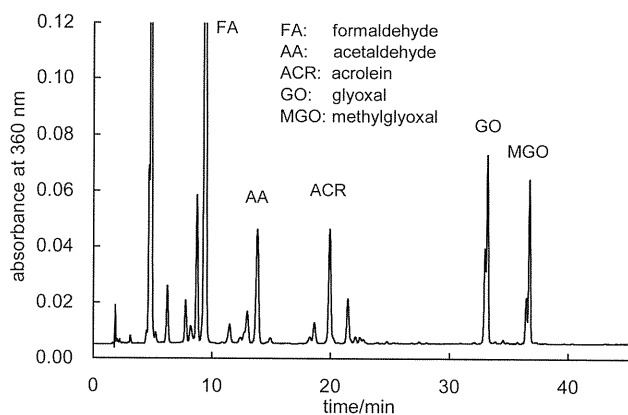


Fig. 2 Representative chromatogram of carbonyl-DNPH derivatives generated from electronic cigarette

キサール, メチルグリオキサールなどの物質についても, 非常に高濃度で発生した. しかし, それらの発生量は同一銘柄でも大きく変動していた. 例えば, 銘柄 F では, ホルムアルデヒド発生量の平均値は 20 mg/m^3 であるが, 最大値は 260 mg/m^3 , 最小値は 0.1 mg/m^3 であり, 変動幅が非常に大きい. それぞれの銘柄間でも大きな変動が認められ, 中には, D, E, J 銘柄のようにカルボニル化合物発生量が全体的に低い電子タバコも存在した. また, カルボニル化合物を多く発生した変霧器は, 捕集 (喫煙) 後, ニクロム線の周囲が黒褐色に変色する傾向が認められた.

次に, A, B, C, D, E の銘柄について, それぞれ 10 サンプルロットずつ購入し, 1 サンプルロットにつき 3 回ずつの測定を行った. Fig. 3 に各銘柄における, ホルムアルデヒド, アクロレイン, グリオキサール, メチルグリオキサールの発生量を示す. 測定したすべての電子タバコから様々なカルボニル化合物が発生したが, 銘柄ごとに発生量, 物

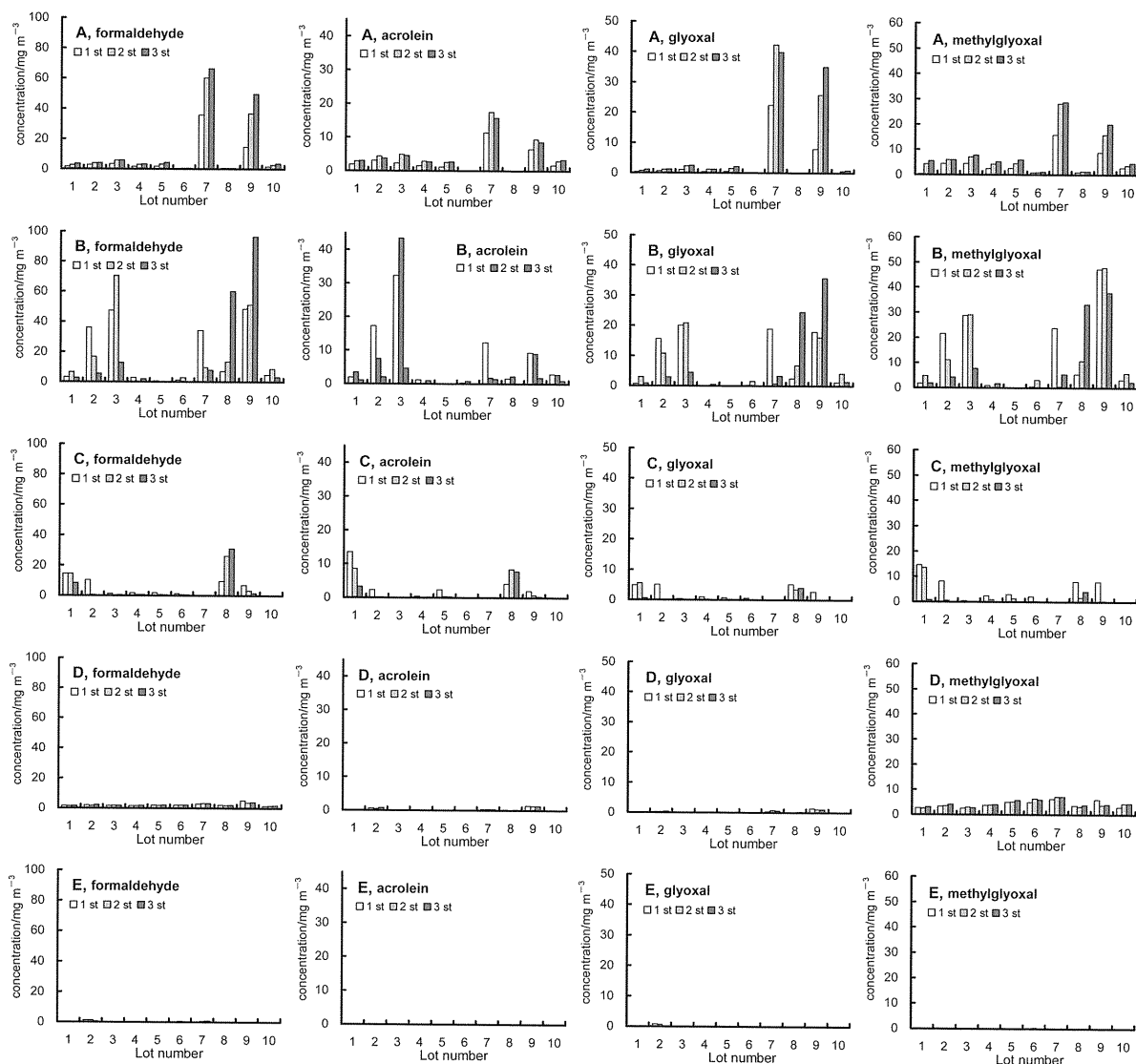


Fig. 3 Concentrations of formaldehyde, acrolein, glyoxal and methylglyoxal generated from various brands of electronic cigarettes