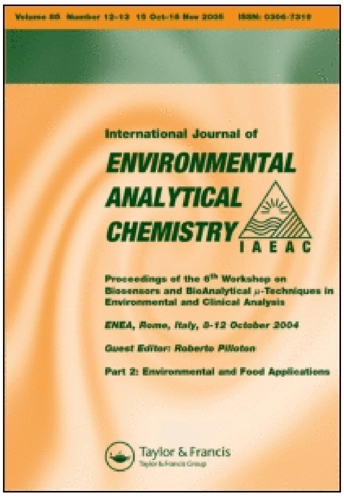

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# Liquid Chromatographic Analysis of $C_1$-$C_{10}$ Carbonyls

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# LIQUID CHROMATOGRAPHIC ANALYSIS OF $C_1$–$C_{10}$ CARBONYLS

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Retention times, response factors, uv-visible spectra and chemical ionization (CI) mass spectra are presented for 2,4-dinitrophenylhyrazine (DNPH) derivatives of $C_1$–$C_{10}$ carbonyls analysed by liquid chromatography (LC) with uv detection, LC-diode array detection and on-line LC-mass spectrometry using $C_{18}$ columns and acetonitrile-water eluent. The forty two carbonyls studied include aliphatic aldehydes (saturated and unsaturated), aliphatic ketones (saturated and unsaturated), aromatic aldehydes, hydroxycarbonyls, oxo-acids and dicarbonyls. A correlation was obtained between response factors ($R_f$ = peak height for 1 μg/mL carbonyl) and retention times ($t_R$), $\log R_f = (-1.33 \pm 0.05) \log t_R + (2.98 \pm 0.05)$, $R = 0.985$, $n = 28$. LC-diode array and LC-MS analysis have been used to show that β-hydroxycarbonyls yield the DNPH derivatives of the corresponding dicarbonyls. The results are of diagnostic value to identify and measure carbonyls at low concentrations in complex environmental mixtures including ambient air.

**KEY WORDS:** Carbonyls, DNPH method, liquid chromatography, diode array, mass spectrometry

## INTRODUCTION

The ability to measure aldehydes and ketones at levels of parts per billion (ppb) is important in many areas of environmental chemistry and toxicology[1]. In the field of air chemistry alone, research and regulatory applications include indoor air quality[1,2], urban pollution[1,3], monitoring of human exposure to toxic air contaminants[4], policy decisions regarding air emissions from oxygenated fuels[5] and laboratory studies of the mechanisms of oxidation of hydrocarbons-both biogenic and anthropogenic-in the atmosphere[6,7].

While several spectroscopic methods are now suitable for measuring formaldehyde in air[8-12], the detection of ppb levels of other carbonyls still relies on more conventional methods. Of these, the classical method which involves the reaction of carbonyls with 2,4-dinitrophenylhydrazine (DNPH) continues to receive much attention[13-20]. Thus, the analysis of carbonyls as their DNPH derivatives by liquid chromatography with uv detection (hereafter DNPH-LC-uv), a method first applied to measuring carbonyls in ambient air some 15 years ago[19,20], is the method currently recommended by the Intersociety Committee and by air pollution control agencies in several countries for measuring carbonyls in ambient air[21-22]. Until direct measurement methods become available for all carbonyls, the DNPH-LC-uv method will continue to be an important analytical tool in environmental chemistry. For example, the U.S. Environmental Protection Agency has specified the use of the DNPH-LC-uv method for measuring airborne carbonyls as part of a new nationwide monitoring program[23]. This program,

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* Corresponding author.

which begins in 1994, will result every year in the collection of thousands of ambient air samples to be anaylzed for carbonyls using the DNPH-LC-uv method[23,24].

We have for a number of years employed the DNPH-LC-uv method to measure carbonyls in outdoor air, indoor air and laboratory studies of atmospheric oxidation mechanisms for hydrocarbons[25–37]. We have reported on the use of a diode array detector for improved analytical selectivity[30], and on the use of chemical ionization mass spectrometry (hereafter CI-MS) for structure confirmation of carbonyls as their DNPH derivatives[27]. In this article, retention times, response factors, uv-visible spectra and CI-MS spectra are presented for the DNPH derivatives of some forty carbonyls. These parameters are of diagnostic value when attempting to identify complex mixtures of carbonyls in environmental samples. The carbonyl-DNPH studied here are DNPH derivatives of $C_1$–$C_{10}$ carbonyls including saturated carbonyls, unsaturated carbonyls, dicarbonyls, hydroxycarbonyls and oxoacids. These carbonyls are important as indoor and outdoor air contaminants and are major oxidation products of alkenes, unsaturated oxygenates, cyclic olefins, isoprene, terpenes and aromatics in the atmosphere[32–37].

## EXPERIMENTAL METHODS

### *Synthesis of 2,4-dinitrophenylhydrazones*

Pinonaldehyde was prepared by bubbling ozone through a small impinger containing a solution of alpha-pinene in acetonitrile[32]. The dicarbonyl 2-oxobutanal, $CH_3CH_2COCHO$, which has been reported previously as a component of cigarette smoke[38], was prepared *in-situ* by the gas phase reaction of ozone with 1-penten-3-one and with 2-ethylacrolein[37]:

$$CH_2 = CHCOCH_2CH_3 + O_3 \rightarrow CH_3CH_2COCHO + HCHO + \text{other products}$$

$$CH_2 = C(C_2H_5)\,CHO + O_3 \rightarrow CH_3CH_2COCHO + HCHO + \text{other products}$$

All other carbonyls were from commercial sources (Aldrich, Sigma, Fluka, PolyScience, highest purity available).

DNPH (Aldrich) was recrystallized twice from hot ethanol, rinsed 2–3 times with ethanol, dried under slight vacuum for 1 min and subsequently for 1–2 hours in a dessicator, and analyzed by liquid chromatography for possible carbonyl impurities[39]. To prepare 2,4-dinitrophenylhydrazones, 20 mL of a solution of the carbonyl in ethanol (0.5 g carbonyl in 95% ethanol for most carbonyls; 1.25 g in 95% ethanol for dicarbonyls, e.g. glyoxal and methylglyoxal) were added to 15 mL of a freshly prepared solution of DNPH. This solution was prepared by adding 2 mL concentrated $H_2SO_4$ to 0.4 g DNPH, and then adding dropwise, while stirring, 3 mL water (*caution:* this solution becomes hot rapidly) followed by 10 mL 95% ethanol. Crystals of the carbonyl-DNPH were filtered on Whatman 41 paper, with no vacuum applied in order to minimize contamination, and were recrystallized from 30 mL hot ethanol (or ethyl acetate for glyoxylic acid, or 20:1 by volume tetrahydrofuran-chloroform for glyoxal and methylglyoxal). The crystals were suction-filtered on glass fiber filters, washed with cold ethanol, dried in a dessicator, and stored in the dark at 2–4°C. All solvents mentioned above were HPLC-grade (Baker, Burdick and Jackson).

Carbonyl-DNPH derivatives prepared as described above were of high purity (as verified by liquid chromatography analysis) with the following exceptions. The β-hydroxycarbonyl 3-hydroxy-2-butanone yielded a large amount of the DNPH derivative of 2,3-butanedione. Another β-hydroxycarbonyl, 1,3-dihydroxyacetone, gave glyoxal-DNPH and methylglyoxal-DNPH as the only products. Glyoxylic acid-DNPH contained another component ($t_R$ ratio = 0.51, peak height ratio = 0.37), perhaps due to isomerization as observed previously[40,41]. The DNPH of the dicarbonyl 2,3-pentadione contained one large and two small unknown impurities. Chromatograms of 1-penten-3-one-DNPH contained many compounds, whose relative abundance varied from batch to batch and also with storage time. Attempts to synthesize the DNPH derivative of 2,4-pentanedione were not successful. The dicarbonyl glutaraldehyde yielded the mono-DNPH derivative (in agreement with earlier work[42]) along with formaldehyde-DNPH as a small impurity (< 10%). The di-DNPH derivative of glutaraldehyde was observed by LC-MS but not by LC-uv.

### *Preparation of carbonyl-DNPH calibration standards*

Calibration standards were prepared by weighing the solid hydrazones and dissolving them in acetonitrile. They were stored at 2–4°C in the dark in glass vials with Teflon-lined screw caps. Each carbonyl-DNPH standard was stored in two vials, one used for calibrations and the other kept as a control to verify possible contamination over time. Acetonitrile solutions of DNPH-carbonyl standards were stable for at least one year for most carbonyls. DNPH derivatives of several unsaturated carbonyls (e.g. 1-penten-3-one) were not stable and decomposed within a few weeks presumably due to hydrolysis.

### *Liquid chromatographic analysis*

Acetonitrile solutions of carbonyl-DNPH standards and of air samples were analyzed by liquid chromatography (LC) with uv detection. The LC system included a Valco injection valve with a 20 μL sample loop, a SSI model 300 pump, a Perkin Elmer model LC 75 uv-visible detector and a Hitachi model D-2000 integrator/recorder. The acetonitrile-water eluent was filtered and degassed under vacuum using a Lazar filtration system, a 0.2 μm pore size Teflon filter and a Barnant Air Cadet pump. The detection wavelength was 360 nm. Two $C_{18}$ analytical columns were employed. Method A involved the use of a Whatman Partisphere column (5 μm, 110 × 4.7 mm) with a Whatman Partisphere $C_{18}$ guard cartridge at an eluent flow rate of 1.0 mL/min (column pressure 1,500–2,000 psi), eluent 55:45 by volume $CH_3CN:H_2O$. Method B involved the use of an Axxiom ODS column (5 μm, 150 × 4.7 mm, Cole Scientific Inc.) with a $C_{18}$ guard cartridge (Brownlee Applied Biosystems) at an eluent flow rate of 1.4 mL/min (column pressure 1,500–2,000 psi), eluent 54:46 by volume $CH_3CN:H_2O$. Under these conditions, the analytical detection limit for formaldehyde was 7 ng carbonyl. Detection limits for other carbonyls are not listed here and can be estimated from the carbonyl/formaldehyde response factor ratios given in the Results and Discussion section.

Absorbance spectra (200–600 nm) of the DNPH reagent and of the carbonyl-DNPH derivatives were recorded using a Hewlett-Packard (HP) model 1090/Series II liquid chromatograph and a HP 1090 diode array detector. The column employed was the Axxiom ODS column described above. The column temperature was 40.0°C, the eluent flow rate was 1.4 mL/min and the injection volume was 20 μL.

*Mass spectrometric analysis*

Chemical ionization mass spectra were recorded using two instruments, a Kratos Scientific Instruments model MS-25 double-focusing magnetic sector instrument and a Hewlett Packard 5989 A mass spectrometer. On the Kratos mass spectrometer, all analyses were carried out directly with small amounts of carbonyl-DNPH (typically 0.1–1.0 microgram) using the instrument solid insertion probe, i.e. no solvents were used. The source temperature was 140–320°C (200°C for most samples), the electron beam energy was 100 eV, the acceleration potential was 2 kV, the reagent gas (methane) pressure was 0.1 Torr and the reagent gas flow rate was 30 mL $min^{-1}$. Details of the experimental protocols have been given previously[43].

On the Hewlett Packard mass spectrometer, all analyses were carried out by LC-MS using the HP 1090 liquid chromatograph described above and a HP 59980-B particle beam LC-MS interface. The mass spectrometer was operated in the chemical ionization mode with methane as the reagent gas. The eluent was 100% $CH_3CN$, the eluent flow rate was 0.4 mL/min, and the instrument's auto sampler was used to inject 20 μL aliquots directly (no analytical column was used) into the particle beam interface.

## RESULTS AND DISCUSSION

*Retention times*

Listed in Table 1 are the retention times, relative to that of formaldehyde-DNPH, of the DNPH derivatives of forty two carbonyls under conditions of isocratic elution on two $C_{18}$ columns. The retention times increase with carbonyl carbon number, i.e. from formaldehyde-DNPH to the DNPH derivatives of $C_9$–$C_{10}$ carbonyls n-nonanal, nopinone, 4-acetyl-1-methylcylcohexene and pinonaldehyde (Figure 1).

For a given carbonyl carbon number, the data in Table 1 and Figure 1 indicate that unsaturated carbonyls elute before their saturated homologues, e.g. methacrolein-DNPH before n-butanal-DNPH. In the same way, ketones elute before the corresponding aldehydes, e.g. 3-methyl-2-butanone-DNPH before n-pentanal-DNPH. Derivatives of oxoacids elute later than those of their monofunctional carbonyl homologues, e.g. pyruvic acid-DNPH elutes well after acetone-DNPH. The reverse is true for hydroxycarbonyls, e.g. 5-hydroxy-2-pentanone-DNPH elutes before 3-pentanone-DNPH and hydroxypyruvic acid-DNPH elutes before pyruvic acid-DNPH. Aromatic aldehydes elute before aliphatic carbonyls that have the same number of carbon atoms, e.g. benzaldehyde-DNPH elutes before n-heptanal-DNPH and m-tolualdehyde-DNPH elutes before n-octanal-DNPH. The DNPH derivatives of dicarbonyls elute much later than those of their monofunctional homologues having the same carbon number, e.g. glyoxal-DNPH elutes well after acetaldehyde-DNPH and methylglyoxal-DNPH elutes well after 2-butanone-DNPH. For hydroxycarbonyls, our limited results for acetonitrile-water eluent are consistent with those we reported earlier using methanol-water eluent[25], i.e. the DNPH derivatives of $C_3$–$C_5$ hydroxycarbonyls elute between the DNPH reagent and formaldehyde-DNPH and/or between formaldehyde-DNPH and acetaldehyde-DNPH. A similar trend has also been observed for dansylhydrazine derivatives of hydroxycarbonyls[44]. For a comprehensive study of hydroxycarbonyl-DNPH derivatives, a more polar column might be more suitable that the $C_{18}$ columns employed in this study. For example, a $C_8$ column has been employed to analyse the DNPH derivatives of polar ketoacids[45].

**Table 1** Retention times of carbonyl-2,4-dinitrophenylhydrazones

| *Carbonyl* | *Retention time*[a] | |
|---|---|---|
| | *Method A* | *Method B* |
| DNPH reagent | 0.70 | 0.67 |
| Aldehydes, saturated: | | |
| Linear: | | |
| formaldehyde | (1.00)[b] | (1.00)[c] |
| acetaldehyde | 1.25 | 1.30 |
| propanal | 1.76 | 1.93 |
| n-butanal | 2.44 | 2.85 |
| n-pentanal | 3.49 | 4.23 |
| n-hexanal | 5.13 | 6.72 |
| n-heptanal | 7.78 | 10.58 |
| n-octanal | 10.6 | 16.33 |
| n-nonanal | 19.5 | 27.21 |
| Branched chain: | | |
| 2-methylpropanal | 2.44 | 2.86 |
| 3-methylbutanal | – | 3.99 |
| 2-methylbutanal | – | 4.10 |
| 2,2-dimethylpropanal | – | 4.05 |
| Aldehydes, unsaturated: | | |
| acrolein | 1.60 | 1.75 |
| crotonaldehyde | 2.30 | 2.41 |
| methacrolein | 2.27 | 2.66 |
| 2-ethylacrolein | 4.34 | 4.15 |
| Ketones, saturated: | | |
| acetone | 1.65 | 1.75 |
| 2-butanone | 2.41 | 2.77 |
| 3-pentanone | 3.40 | – |
| 3-methyl-2-butanone | – | 4.09 |
| Ketones, cyclic: | | |
| cyclohexanone | 3.40 | 4.04 |
| 2-methylcyclohexanone | 5.52 | 7.45 |
| nopinone[d] | 6.36 | 8.78 |
| Ketones, unsaturated: | | |
| methyl vinylketone | 2.25 | – |
| 3-pentene-2-one | 3.20 | 3.52 |
| 1-pentene-3-one | – | 3.92 |
| 4-acetyl-1-methylcyclohexene | 8.43 | 11.92 |
| Hydroxycarbonyls: | | |
| 3-hydroxy-2-butanone | 0.87 | 0.83 |
| 5-hydroxy-2-pentanone | 0.93 | – |
| hydroxypyruvic acid | 0.79 | – |
| Aromatics: | | |
| benzaldehyde | 3.07 | 3.62 |
| m-tolualdehyde | – | 5.49 |
| Dicarbonyls: | | |
| glyoxal | 3.32 | 4.19 |
| methyglyoxal | 5.10 | 6.94 |
| 2,3-butanedione | 8.18 | 10.03 |
| 2-oxobutanal | 8.70 | 10.40 |
| 2,3-pentanedione | – | 17.66 |
| glutaraldehyde[e] | 4.00 | 4.74 |
| pinonaldehyde | 13.90 | – |
| Oxo-acids: | | |
| glyoxylic acid | 2.00 | 2.23 |
| pyruvic acid | 2.75 | 3.34 |

[a] Relative to that of formaldehyde-DNPH.
[b] Retention time = 3.45 ± 0.07 min, n = 20.
[c] Retention time = 3.20 ± 0.16 min, n = 68.
[d] 6,6-dimethylbicyclo [3.1.1]heptan-2-one.
[e] Glutaraldehyde mono-DNPH.

### *Response factors*

Quantitative analysis of carbonyl-DNPH involves the use of external carbonyl-DNPH standards prepared as described in the Experimental Section. Calibration curves are constructed by plotting, at a given detection wavelength (e.g. 360 nm), absorbance (i.e. peak height) vs concentration for five concentrations of the carbonyl-DNPH standard. Examples of calibration curves are shown in Figure 2. Least squares linear regression

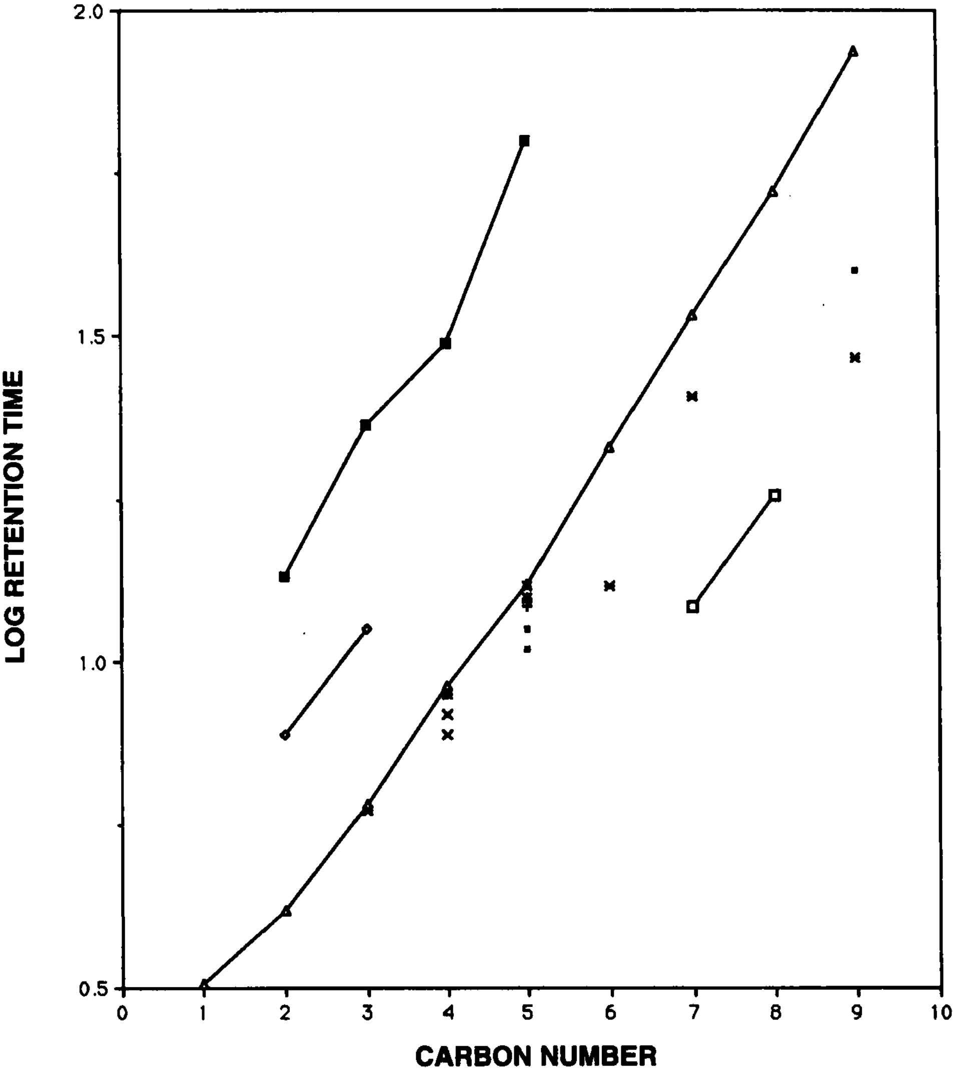


Figure 1 Scatter plot of carbonyl-DNPH retention times vs. carbonyl carbon number. Solid lines connect data points for n-alkanals (△), oxoacids (□), dicarbonyls (■) and aromatic aldehydes (▣). Other data points are for branched-chain saturated aldehydes (+), unsaturated aldehydes (x), saturated ketones (*) and unsaturated ketones (•).

analysis of the data yields a slope, i.e. response factor, for each carbonyl-DNPH. A summary of these response factors is given in Table 2.

Shown in Figure 3 is a scatter plot of response factors vs the corresponding retention times for the DNPH derivatives of 28 carbonyls including saturated aldehydes (linear and branched-chain), unsaturated aldehydes, saturated ketones (linear, branched-chain

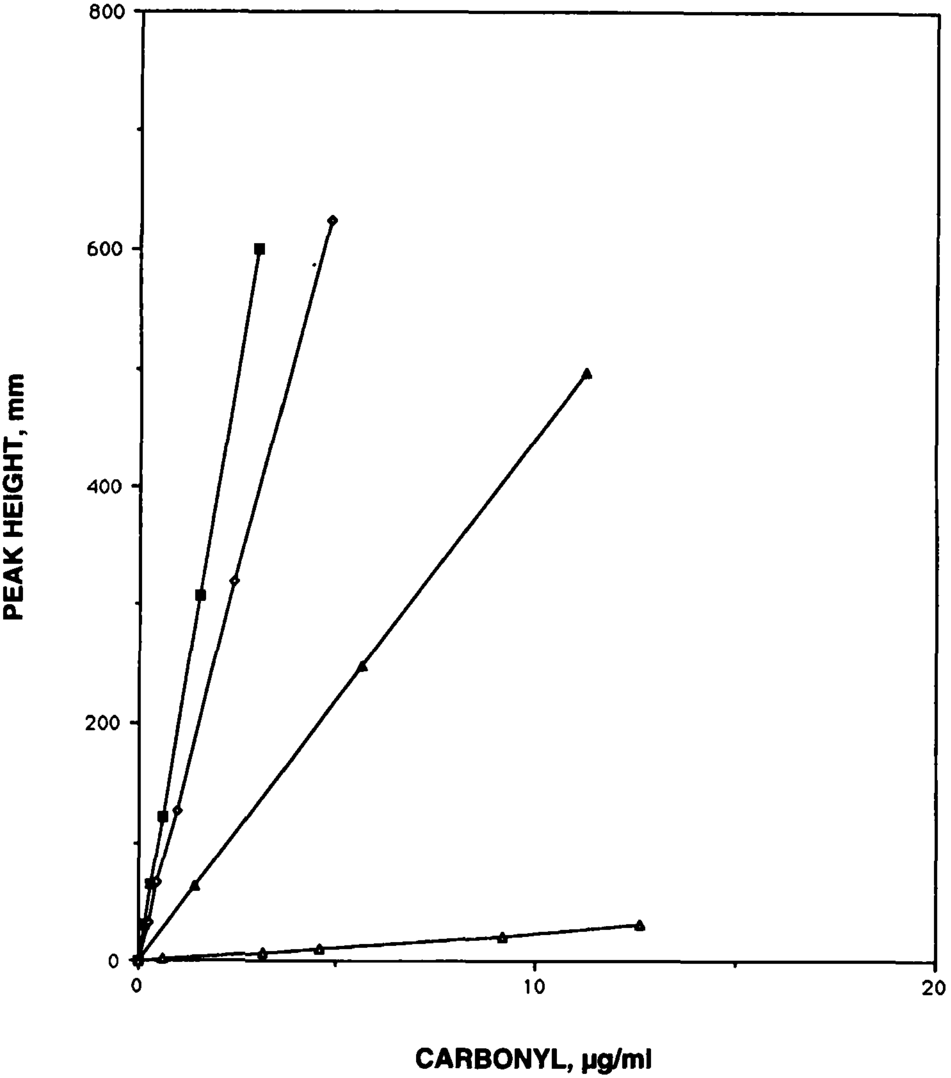


**Figure 2** Calibration curves for the DNPH derivatives of formaldehyde (■), acetaldehyde (◇), cyclohexanone (▲) and methylglyoxal (△, concentrations multiplied by ten for clarity).

and cyclic), unsaturated ketones, aromatic aldehydes and dicarbonyls. Least squares linear regression analysis of the data shown in Figure 3 yielded the following relation:

$$\log R_f = -(1.331 \pm 0.046) \log t_R + (2.979 \pm 0.054), \quad R = 0.985,\ n = 28$$

where retention times ($t_R$) are in minutes and response factors ($R_f$) are in units of mm (peak height on attenuation setting 7) for 1 µg/mL carbonyl. This relation is useful to estimate the concentrations of unknown carbonyls and of known carbonyls for which no carbonyl-DNPH standards are available.

**Table 2** Response factors for carbonyl-2,4-dinitrophenylhydrazones at 360 nm.

| *Carbonyl* | *Response factor*[a] | | *Carbonyl* | *Response factor*[a] | |
|---|---|---|---|---|---|
| | *Method A* | *Method B* | | *Method A* | *Method B* |
| Aldehydes, saturated: | | | Ketones, cyclic: | | |
| Linear: | | | cyclohexanone | 14.0 | 34.6 |
| formaldehyde | 90.5 | 238.1 | nopinone | 13.0 | 6.8 |
| acetaldehyde | 64.3 | 153.3 | | | |
| propanal | 42.8 | 89.3 | Ketones, unsaturated: | | |
| n-butanal | 14.9 | 46.5 | methyl vinylketone | 19.4 | – |
| n-pentanal | 11.8 | 28.1 | 3-pentene-2-one | 15.1 | 37.2 |
| n-hexanal | 8.0 | 16.1 | 4-acetyl-1-methylcyclohexene | 8.7 | 7.5 |
| n-heptanal | 7.3 | 9.6 | | | |
| n-octanal | – | 4.5 | Hydroxycarbonyls: | | |
| n-nonanal | – | 3.0 | 3-hydroxy-2-butanone | 20.8 | – |
| | | | 5-hydroxy-2-pentanone | 14.3 | – |
| Branched chain: | | | | | |
| 2-methylpropanal | 8.0 | 52 | Aromatics: | | |
| 3-methylbutanal | – | 27 | benzaldehyde | 9.4 | 34.1 |
| 2-methylbutanal | – | 26 | m-tolualdehyde | – | 20.8 |
| 2,2-dimethylpropanal | – | 28 | | | |
| | | | Dicarbonyls: | | |
| Aldehydes, unsaturated: | | | glyoxal | 15.9 | 38.8 |
| acrolein | 50.0 | 128.2 | methyglyoxal | 7.7 | 21.8 |
| crotonaldehyde | – | 66.9 | 2,3-butanedione | 2.9 | 13 |
| methacrolein | 32.9 | 47.8 | 2,3-pentanedione | – | 24 |
| Ketones, saturated: | | | Oxo-acids: | | |
| acetone | 33.3 | 101.4 | pyruvic acid | 15.0 | – |
| 2-butanone | 26.8 | 45.0 | | | |
| 3-pentanone | 14.0 | – | | | |
| 3-methyl-2-butanone | – | 28.0 | | | |

[a] Peak height, mm, for 1 μg/mL carbonyl, attenuation setting 7 (Method A) or attenuation setting 5 (Method B), acetonitrile-water eluent.

### *Consistency of method performance for retention times and response factors*

For carbonyl identification and quantitative analysis in complex matrices such as indoor and outdoor air, the use of retention times and response factors as diagnostic tools requires that these parameters be reproducible over time. The consistency of method performance is illustrated by the following examples. Compiled in Table 3 are retention times and response factors for the DNPH derivatives of thirteen carbonyls. The mean values and relative standard deviations listed in Table 3 were calculated from eight sets of calibrations carried out over a period of four months. The relative standard deviations on retention times were less than 0.32%; those on response factors were less than 2.8%. Shown in Table 4 is a comparison of retention times and response factors obtained for seven carbonyl-DNPH over a six-year period. The data in Table 4 show consistency in method performance over time even though several liquid chromatography columns, several batches of calibration standards and many batches of eluent solvents were employed. Listed in Table 5 are the relative standard deviations for retention times and response factors calculated, for twenty two carbonyl-DNPH, from up to sixty eight calibrations performed during a sixteen-month period (Jan. 1993 – April 1994). These

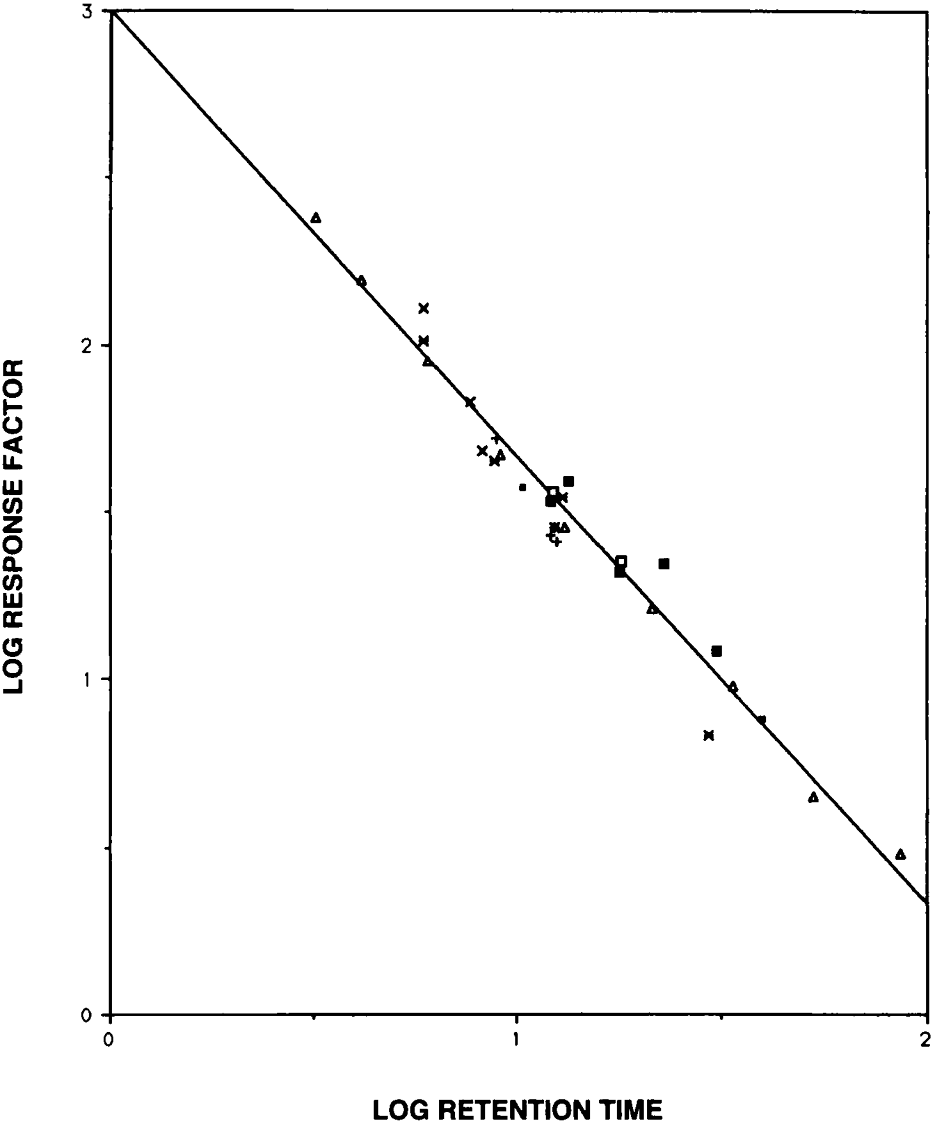


**Figure 3** Scatter plot of carbonyl-DNPH response factors vs carbonyl-DNPH retention times. Symbols are the same as in Figure 1. See text for linear regression parameters.

relative standard deviations were 0.2–15.7 percent for retention times and 0–22.4 percent for response factors.

### *Uv-visible spectra and 430/360 nm absorbance ratios*

Absorbance spectra (200–600 nm) of carbonyl-DNPH in acetonitrile-water eluent have been recorded for about 40 carbonyls using a diode array detector. Examples are given in

**Table 3** Retention times and response factors for carbonyl-DNPH in eight sets of calibrations carried out within A 4 month period.

| | *Retention time, relative to that of formaldehyde-DNPH*[a] | | *Response factor, mm for 1 μg/mL carbonyl, att. 7*[a] | |
|---|---|---|---|---|
| *Carbonyl* | *Mean* | *Relative standard deviation, percent* | *Mean* | *Relative standard deviation, percent* |
| 3-Hydroxy-2-butanone | 0.87 | 0.01 | 20.8 | 0.0 |
| Formaldehyde | 1.00 (reference) | 0.00 | 84.5 | 2.7 |
| Acetaldehyde | 1.24 | 0.00 | 56.0 | 2.7 |
| Acetone | 1.58 | 0.03 | 30.4 | 1.4 |
| Propanal | 1.70 | 0.04 | 37.8 | 2.7 |
| 2-Butanone | 2.28 | 0.04 | 19.0 | 1.5 |
| n-Butanal | 2.28 | 0.06 | 15.5 | 0.8 |
| Pyruvic acid | 2.56 | 0.08 | | |
| Benzaldehyde | 2.81 | 0.11 | | |
| Glyoxal | 2.90 | 0.12 | 22.5 | 1.8 |
| Cyclohexanone | 3.17 | 0.06 | 14.4 | 0.8 |
| Methyl glyoxal | 4.37 | 0.25 | 6.9 | 0.5 |
| 2,3-Butadione | 6.65 | 0.31 | 2.9 | – |

[a]Method A, 55:45 acetonitrile: water eluent.

**Table 4** Comparison of retention times and response factors for carbonyl-DNPH over a six-year period.

| | *Retention time, relative to that of formaldehyde-DNPH*[a] | | | *Response factor, mm for 1 μg/mL carbonyl, att. 7*[a] | | |
|---|---|---|---|---|---|---|
| *Carbonyl* | *1986* | *1989* | *1992* | *1986* | *1989* | *1992* |
| 5-Hydroxy-2-pentanone | 0.90 | – | 0.93 | 12.7 | – | 13.1 |
| Formaldehyde | (1.00) | (1.00) | (1.00) | 81.5 | 84.4 | 90.5 |
| Acetaldehyde | 1.30 | 1.28 | 1.25 | 58.2 | 57.6 | 64.3 |
| Acetone | 1.80 | 1.69 | 1.65 | 34.5 | 34.4 | 33.3 |
| 2-Butanone | 2.80 | 2.56 | 2.41 | 25.2 | 24.7 | 26.8 |
| Benzaldehyde | 3.50 | 3.25 | 3.10 | 10.0 | 11.2 | 9.3 |
| n-Pentanal | 4.30 | 3.80 | 3.50 | 11.2 | 9.7 | 11.8 |

[a]Method A, 55:45 by volume acetonitrile-water eluent.

Figure 4. The corresponding absorption maxima are listed in Table 6. The DNPH derivatives of aliphatic compounds that contain only one carbonyl group exhibit absorption maxima near 360 nm (as compared to 357 nm for DNPH) in acetonitrile: water eluent. As discussed earlier[30], this observation reflects the fact that the carbonyl substituents $R_1$ and $R_2$ ($R_2$ = H for aldehydes) have only a small influence, compared to that of the main chromophore, $2,4\text{-}(NO_2)_2C_6H_3NH\text{-}N = CR_1R_2$, on the overall absorption of the DNPH derivative. The contribution of the carbonyl substituents increases for aromatic carbonyls, resulting in a small bathochromic shift from 360 to about 385 nm.

**Table 5** Relative standard deviations for carbonyl-DNPH retention times and response factors for calibrations carried out over a 16-month period.

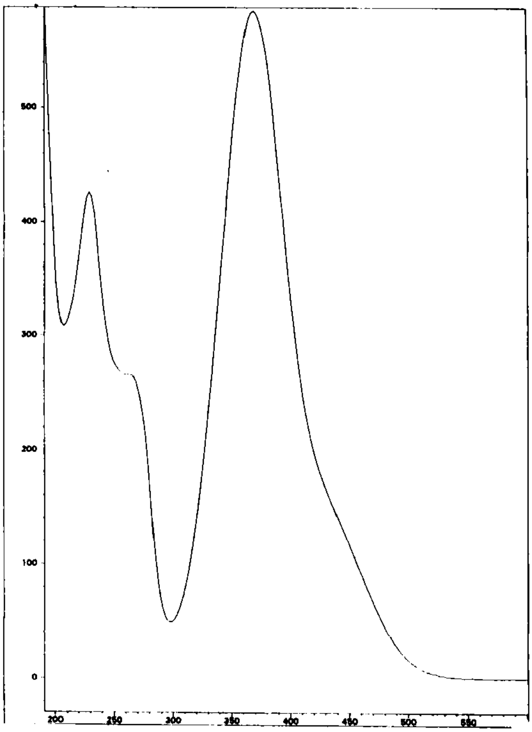
| *Carbonyl*[a] | *Relative standard deviation, percent*[b] | |
|---|---|---|
| | *Retention time* | *Response factor* |
| Formaldehyde | 5.0(68)[c] | 13.7 (68) |
| Acetaldehyde | 5.7 (68) | 11.9 (68) |
| Acetone | 6.7 (38) | 14.3 (40) |
| Acrolein | 7.3 (6) | 4.8 (5) |
| Propanal | 6.0 (29) | 11.1 (30) |
| Glyoxylic acid | 1.4 (3) | – |
| Crotonaldehyde | 3.2 (3) | – |
| 2-Butanone | 7.9 (6) | 2.9 (6) |
| n-Butanal | 9.1 (32) | 12.9 (29) |
| 2-Methylpropanal | 6.2 (6) | 3.1 (6) |
| Pyruvic acid | 10.6 (5) | – |
| 3-Penten-2-one | 0.3 (2) | 0.0 (2) |
| Benzaldehyde | 13.4 (4) | – |
| Cyclohexanone | 10.4 (39) | 12.1 (39) |
| Glyoxal | 12.4 (29) | 7.1 (9) |
| 3-Methyl-2-butanone | 0.2 (2) | – |
| 2-Methylbutanal | 4.9 (2) | – |
| n-Pentanal | 6.7 (21) | 22.4 (19) |
| Methyl glyoxal | 12.2 (12) | 8.7 (3) |
| n-Hexanal | 14.1 (13) | 13.0 (12) |
| n-Heptanal | 15.7 (8) | 12.5 (8) |
| n-Nonanal | 11.0(3) | 10.0 (3) |

[a] listed in order of increasing retention times.
[b] Method B, 53:47 by volume acetonitrile water eluent.
[c] number of calibrations indicated in parentheses.

For dicarbonyls, the di-DNPH chromophore results in a larger bathochromic shift of the absorption maximum, from about 360 nm to about 410–430 nm.

The absorption maxima listed in Table 6 have diagnostic value. A sample cantaining unknown carbonyls can be analyzed at only two wavelengths as a test for aliphatic vs. aromatic substituent (i.e. 385/360 nm absorbance ratio) or as a test for monofunctional carbonyl vs. dicarbonyl (i.e. 430/360 nm absorbance ratio). Measured 430/360 nm absorbance ratios are listed in Table 6 along with the corresponding absorption maxima. The 430/360 ratios are about 10 times larger for dicarbonyls (ratios = 1.7–2.3) than for monofunctional carbonyls (ratios = 0.10–0.41). For monofunctional carbonyls, the 430/360 ratio is about the same for saturated aldehydes and ketones. For n-alkyl-substituted aldehydes, the 430/360 nm absorbance ratio increases with carbonyl carbon number. The 430/360 ratio of unsaturated carbonyls is larger than that of their saturated homologues, and appears to be slightly larger for branched-chain compounds than for their n-alkyl isomers. From the limited data available, it appears that 430/360 ratios for hydroxycarbonyls are similar to those of their alkyl-substituted homologues, e.g. 0.24 for 5-hydroxy-2-pentanone-DNPH vs 0.23 for n-pentanal-DNPH. Those of oxoacids are smaller, e.g. 0.15 for pyruvic acid-DNPH vs 0.23 for propanal-DNPH and 0.25 for acetone-DNPH.

(a)


500
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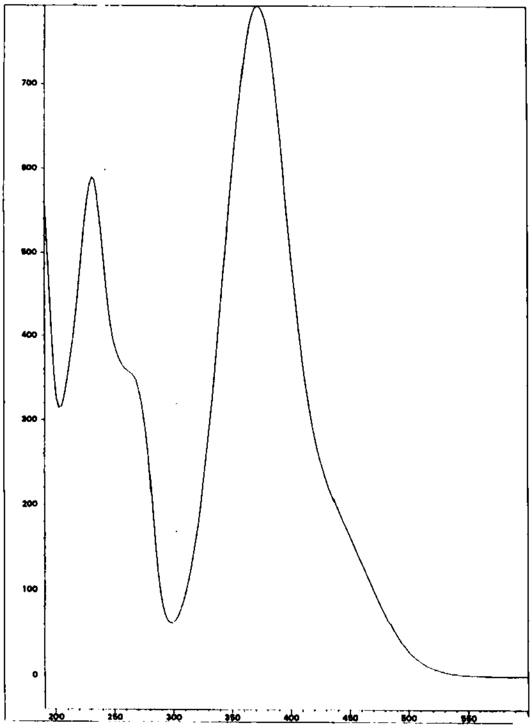
(b)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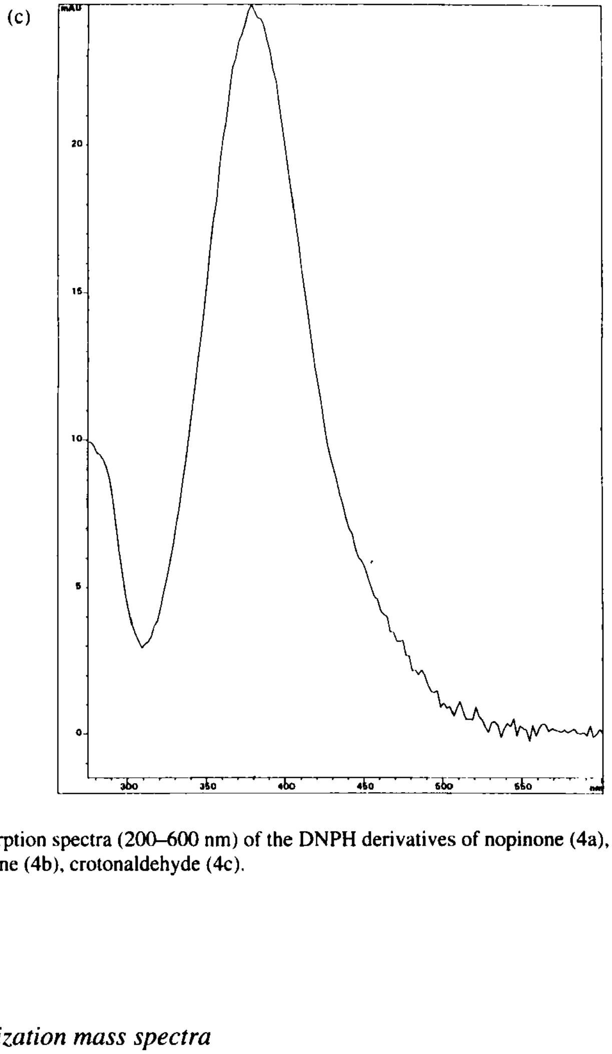

**Figure 4** Absorption spectra (200–600 nm) of the DNPH derivatives of nopinone (4a), 4-acetyl-1-methylcyclohexene (4b), crotonaldehyde (4c).

### *Chemical ionization mass spectra*

Chemical ionization mass spectra have been recorded for a number of carbonyl-DNPH standards. Examples are given in Figure 5. A summary of the major ions recorded is given in Table 7. For all but a few carbonyls, CI mass spectra of the DNPH derivatives indicated little fragmentation: the protonated molecular ion (MH = M +1, where M is the molecular weight of the DNPH derivative) was the most abundant ion (base peak). Other ions, all of diagnostic value, included MH +1 (contribution of $^{13}C$ to the MH ion) and M + 29 (reagent gas adduct, M + $C_2H_5$). Exceptions included the DNPH derivatives of the hydroxycarbonyl and of the two oxoacids. For the two oxoacids, glyoxylic acid and pyruvic acid, the base peak was M + 29 (M + $C_2H_5$ and/or MH + CO). For the hydroxycarbonyl, 5-hydroxy-2-pentanone, the base peak was m/e = 85 and the abundance of the MH ion was only 25% of that of the base peak. This result is consistent with earlier work in which the CI mass spectra of DNPH derivatives of several hydroxycarbonyls have been observed to include $M_cH - H_2O$ as the base peak, where $M_c$ is the molecular weight of the hydroxycarbonyl[27].

**Table 6** Absorption maxima and 430/360 nm absorbance ratios of carbonyl-2, 4-dinitrophenylhydrazones.

| *Carbonyl* | *Absorption maximum, nm*[a] | *430/360 nm peak height ratio*[a] | *Carbonyl* | *Absorption maximum, nm*[a] | *430/360 nm peak height ratio*[a] |
|---|---|---|---|---|---|
| DNPH reagent | 357 | – | Ketones, cyclic: | | |
| | | | cyclohexanone | 371 | 0.28 |
| Aldehydes, saturated: | | | 2-methylcyclohexanone | 371 | 0.33 |
| Linear: | | | nopinone | 372 | 0.34 |
| formaldehyde | 352 | 0.18 | | | |
| acetaldehyde | 362 | 0.22 | Ketones, unsaturated: | | |
| propanal | 365 | 0.23 | methyl vinylketone | 376 | – |
| n-butanal | 363 | 0.22 | 3-pentene-2-one | – | 0.17 |
| n-pentanal | 363 | 0.23 | 4-acetyl-1-methylcyclohexene | 370 | 0.33 |
| n-hexanal | 363 | 0.24 | | | |
| n-heptanal | 359 | 0.21 | Hydroxycarbonyls | | |
| n-octanal | 363 | 0.29 | 3-hydroxy-2-butanone | – | 0.22 |
| n-nonanal | 363 | 0.28 | 5-hydroxy-2-pentanone | 369 | 0.24 |
| Branched chain: | | | Aromatics: | | |
| 2-methylpropanal | 363 | 0.26 | benzaldehyde | 385 | 0.50 |
| 3-methylbutanal | – | 0.27 | m-tolualdehyde | – | 0.49 |
| 2-methylbutanal | – | 0.25 | | | |
| 2,2-dimethylpropanal | – | 0.29 | Dicarbonyls: | | |
| | | | glyoxal | 437 | 2.30 |
| Aldehydes, unsaturated: | | | methyglyoxal | 427 | 2.25 |
| acrolein | 373 | 0.29 | 2,3-butanedione | 412 | 2,13 |
| crotonaldehyde | 376 | 0.41 | 2-oxobutanal | – | 2.27 |
| methacrolein | – | 0.34 | 2,3-pentanedione | 405 | 1.67 |
| 2-ethylacrolein | – | 0.32 | glutaraldehyde[b] | 355 | 0.23 |
| | | | pinonaldehyde | – | 0.54 |
| Ketones, saturated: | | | | | |
| acetone | 367 | 0.25 | Oxo-acids: | | |
| 2-butanone | 367 | 0.29 | glyoxylic acid | 355 | 0.10 |
| 3-pentanone | 365 | 0.28 | pyruvic acid | 369 | 0.15 |
| 3-methyl-2-butanone | – | 0.27 | | | |

[a] In 54:46 by volume acetonitrile-water eluent.

[b] Glutaraldehyde mono-DNPH.

### *Limitation of the method: conversion of β-hydroxycarbonyls to dicarbonyl-DNPH*

Attempts to synthesize the DNPH derivatives of one subset of hydroxycarbonyls, the β-hydroxycarbonyls, have consistently yielded the DNPH derivatives of the corresponding dicarbonyls. This observation, first made by comparing retention times and 430/360 nm absorbance ratios, was subsequently verified by LC-diode array and CI-MS analysis of standards and samples (Table 8). The conversion of β-hydroxycarbonyls to dicarbonyl-DNPH presumably involves acid-catalyzed oxidation during the derivatization step. Attempts to prepare the DNPH derivatives with less acid and without acid were not successful, either in the liquid phase or by collection of ppb levels of hydroxycarbonyls in purified air onto $C_{18}$ cartridges coated with acidic DNPH. This limitation of the method, while not critical when a given hydroxycarbonyl and the corresponding dicarbonyl are not present together, will be investigated in future work.

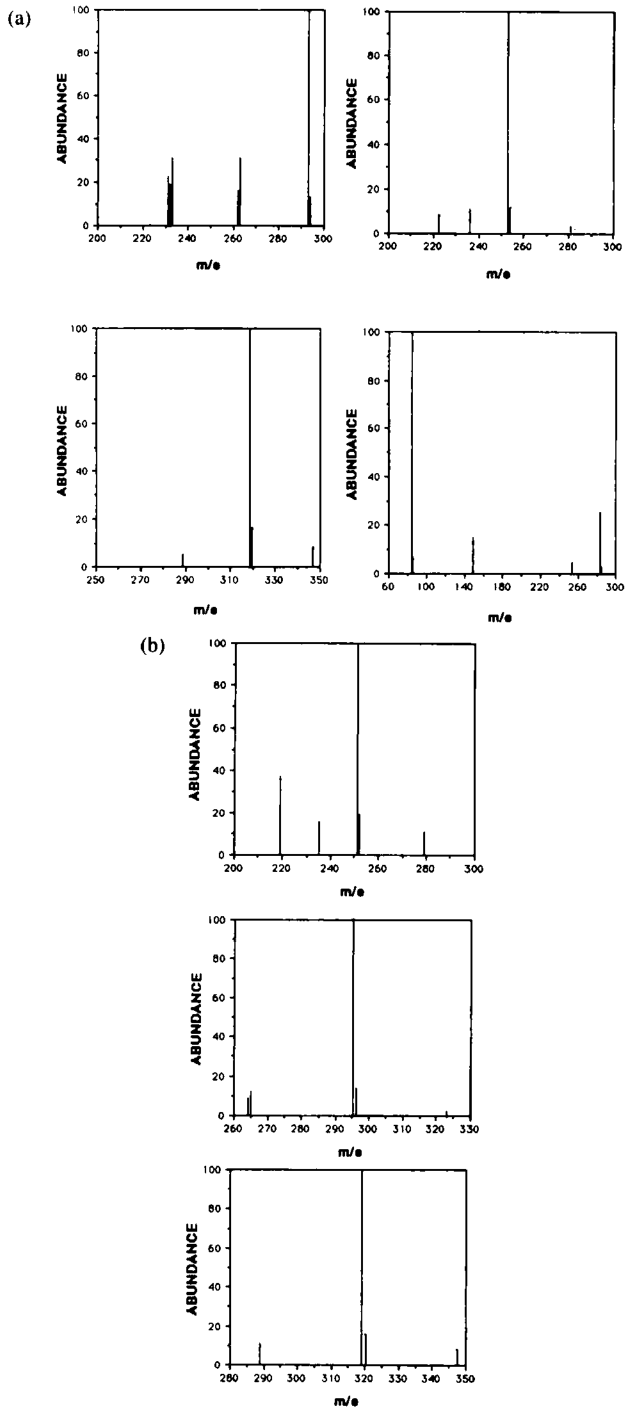

**Figure 5** Chemical ionizaton mass spectra of the DNPH derivatives of 2-methylcyclohexanone (5a, top left), 2-methylpropanal (5a, top right), 4-acetyl-1-methylcyclohexene (5a, bottom left), 5-hydroxypentanone (5a, bottom right), methylvinylketone, (5b, top), n-heptanal (5b, middle) and nopinone (5b, bottom).

**Table 7** Summary of chemical ionization mass spectrometry results for carbonyl-DNPH.

| *Carbonyl* | *Molecular weight of DNPH derivative* | *Base peak (BP)* | *Other abundant ions*[a] | |
|---|---|---|---|---|
| | | | *m/e* | *Percent of BP* |
| Aldehydes, saturated: | | | | |
| formaldehyde | 210 | 211 (MH) | | |
| acetaldehyde | 224 | 225 (MH) | | |
| propanal | 238 | 239 (MH) | | |
| n-butanal | 252 | 253 (MH) | | |
| 2-methylpropanal | 252 | 253 (MH) | 236 (MH-17) | 11 |
| n-pentanal | 266 | 267 (MH) | | |
| n-hexanal | 280 | 281 (MH) | | |
| n-heptanal | 294 | 295 (MH) | 265 (MH-30) | 12 |
| n-octanal | 308 | 309 (MH) | | |
| n-nonanal | 322 | 323 (MH) | | |
| Aldehydes, unsaturated: | | | | |
| acrolein | 236 | 237 (MH) | | |
| methacrolein | 250 | 251 (MH) | | |
| crotonaldehyde | 250 | 251 (MH) | | |
| Ketones, saturated: | | | | |
| acetone | 238 | 239 (MH) | | |
| 2-butanone | 252 | 253 (MH) | | |
| 3-pentanone | 266 | 267 (MH) | | |
| cyclohexanone | 278 | 279 (MH) | | |
| 2-methylcyclohexanone | 292 | 293 (MH) | 263 (MH-30) | 31 |
| nopinone | 318 | 319 (MH) | 302 (MH-17) | 4 |
| | | | 289 (MH-30) | 10 |
| Ketones, unsaturated: | | | | |
| methylvinylketone | 250 | 251 (MH) | 235 (MH-16) | 10 |
| | | | 219 (MH-32) | 15 |
| 4-acetyl-1-methylcylcohexene | 318 | 319 (MH) | 302 (MH-17) | 2 |
| | | | 289 (MH-30) | 12 |
| Dicarbonyls: | | | | |
| glyoxal | 238,418 | 419 (MH) | | |
| methylglyoxal | 252, 432 | 253 (MH) | 433 | 80 |
| 2, 3-butanedione | 266, 460 | 267 (MH) | 250 (MH-17) | 3 |
| | | | 447 | < 2 |
| glutaraldehyde | 280, 460 | 461 (MH) | 427 (MH-34) | 6 |
| | | | 344 (?) | 20 |
| | | | 281 | 8 |
| | | | 263 (281-18) | 60 |
| Oxoacids: | | | | |
| glyoxylic acid | 254 | 283 (M + 29) | | |
| pyruvic acid | 268 | 297 (M + 29) | | |
| Hydroxycarbonyls: | | | | |
| 5-hydroxy-2-pentanone | 282 | 85[b] | 283 (MH) | 25 |
| | | | 253 (MH-30) | 5 |
| Aromatics: | | | | |
| benzaldehyde | 286 | 287 (MH) | | |
| m-tolualdehyde | 300 | 301 (MH) | | |
| Ohers: | | | | |
| DNPH reagent | 198 | 199 | 239[c] | < 1 |

[a] MH + 1 ($^{13}$C isotopic contribution to MH) and M + 29 (M + $C_2H_5$ adduct) are not listed but were present in the spectra of nearly all compounds.

[b] Base peak = $M_cH - H_2O$, where $M_c$ is the carbonyl molecular weight, see text.

[c] From acetone impurity in some batches of acetonitrile solvent.

**Table 8** Formation of dicarbonyl-DNPH derivatives from beta-hydroxycarbonyls: summary of experimental data.

| *Hydroxycarbonyl* | *dicarbonyl-DNPH* | | | | |
|---|---|---|---|---|---|
| | *Dicarbonyl* | *Retention time* | *430/360 nm ratio* | *Diode array spectrum* | *CI mass spectrum* |
| Hydroxyacetaldehyde<br>$CH_2OHCHO$ | Glyoxal<br>CHOCHO | + | + | + | + |
| Hydroxyacetone<br>$CH_3COCH_2OH$ | Methylglyoxal<br>$CH_3COCHO$ | + | + | + | + |
| 3-Hydroxy-2-butanone<br>$CH_3COCHOHCH_3$ | 2,3-Butanedione<br>$CH_3COCOCH_3$ | + | + | + | |
| 2-Hydroxybutanal[a]<br>$CH_3CH_2CHOHCHO$ | 2-Oxobutanal<br>$CH_3CH_2COCHO$ | + | + | + | |
| 1,3-Dihydroxyacetone<br>$CH_2OHCOCH_2OH$ | Glyoxal and methylglyoxal | + | + | | |
| Hydroxypyruvic acid<br>$CH_2OHCOCOOH$ | HC(O)COCOOH? | | | + | |

[a] Studied as a product of the ozone-1-penten-3-ol reaction

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