DETERMINATION OF ALDEHYDES AND KETONES BY DERIVATIZATION AND LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

    A method for determining nanogram amounts of carbonyl compounds was developed. Carbonyl compounds were collected as their 2,4-dinitrophenylhydrazine derivatives, and the derivatives were analyzed by high-performance liquid chromatography–mass spectrometry (HPLC–MS) via a moving-belt interface. The method employed reversed-phase gradient LC on 2 mm I.D. columns and methane chemical ionization. Negative ion mass spectra were recorded to distinguish aldehydes from ketones. The analysis of engine exhaust, vapors from decomposed polymers, and liquid soaps illustrate three applications for LC–MS: confirmation of identifications made by LC, determination of compounds in unresolved chromatographic peaks, and characterization of unidentified LC peaks.

INTRODUCTION

    Formaldehyde and other carbonyl compounds (aldehydes and ketones) have attracted attention as environmental pollutants and as chemicals which may be harmful to man if exposure limits are exceeded[1]. At the same time, new analytical techniques have been developed to detect nanogram quantities of carbonyl compounds[2–7]. Consequently, ppb* concentrations of formaldehyde have been found in workplace atmospheres, indoor and outdoor residential atmospheres, and in common consumer products. Several of the new analytical methods have been based on derivatization and high-performance liquid chromatography (HPLC)[2–7]. Typically, 2,4-dinitrophenylhydrazine (DNPH) has been used as the derivatizing reagent.

    We have used sorbent cartridges coated with DNPH to sample urban atmospheres for formaldehyde[5] and impingers filled with a solution of DNPH reagent to sample automobile exhaust for carbonyl compounds[3]. Unfortunately, many samples that we analyzed contained components which could not be identified by LC. We identified some compounds by collecting LC fractions and analyzing the fractions by direct probe mass spectrometry. However, collection and analysis of fractions was

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    * Throughout this article, the American billion (10^9) is meant.

tedious, time consuming, and often unsuccessful. The limitations of HPLC for qualitative analysis led us to investigate liquid chromatography–mass spectrometry (LC–MS) techniques[8,9].

This report describes a method for characterizing DNPH derivatives of carbonyl compounds by LC–MS via a moving-belt interface. The method employs reversed-phase HPLC on a 2 mm I.D. column, an in-line UV absorbance detector, and nebulization[10] to deposit the LC effluent on the interface belt. Relatively little mass spectral data has been published for DNPH derivatives[7]. We have used negative ion methane chemical ionization to distinguish aldehydes from ketones. Examples in this report also illustrate three types of application for LC–MS: confirmation of identifications made by HPLC, determination of compounds in unresolved chromatographic peaks, and characterization of unidentified LC peaks.

EXPERIMENTAL

Sample preparation

Standards were prepared for LC–MS by dissolving aldehydes and ketones in acetonitrile and adding aliquots of the solutions to the 2,4-dinitrophenylhydrazine reagent and 0.01 N perchloric acid in acetonitrile. Solid DNPH derivatives were prepared for direct probe MS[11].

Three commercial liquid soaps (two shampoos and a liquid bubble bath) were diluted with water and added to an acetonitrile solution of the DNPH reagent and perchloric acid catalyst. The amount of shampoo or bubble bath in the final 5-ml solution ranged from 2 to 80 mg, depending on the product. Formaldehyde standards were prepared in water for the analysis. Samples were allowed to react with the DNPH reagent for at least 45 min before they were analyzed.

A ceramic mat which contained an organic binder was heated in the tube furnace of a DuPont Model 990 thermal analyzer to generate organic vapors. An amount of 300 mg of the material was heated to 500°C for 10 min while the furnace was purged with filtered air flowing at 0.5 l/min. The air from the furnace was bubbled through an impinger containing a 10-ml solution of DNPH reagent and perchloric acid in acetonitrile. A 10-μl aliquot from the impinger was analyzed by LC/MS.

Two engine exhaust samples were collected by bubbling exhaust from an ethanol fueled car and a gasohol fueled car through impingers filled with water. The gasohol contained 20% ethanol and 80% gasoline. A volume of 100 μl of the aqueous sample was added to 5 or 10 ml of reagent and perchloric acid catalyst in acetonitrile, and 10-μl of the resulting solution were analyzed by LC–MS.

Liquid chromatography

LC separations were achieved on a 25 cm × 2 mm I.D. column packed with 5-μm Spherisorb ODS (Alltech Assoc., Deerfield, IL, U.S.A.). Samples were injected with a Valco injection valve (Valco, Houston, TX, U.S.A.) equipped with a 10-μl sample loop. A solvent gradient flowing at 0.2 ml/min was provided by a dual pump system (Model 6000A pumps and Model 680 LC gradient controller, Waters Assoc., Milford, MA, U.S.A.). For the first 9 min of the gradient program, the solvent was acetonitrile–water (60:40). The acetonitrile content increased linearity to 100% during the next 4 min of the program and remained at 100% for the last 5 min of the

18-min analysis. The Kratos Model 773 UV absorbance detector was equipped with a 0.5-μl flow cell and was set to monitor absorbance at 365 nm (Kratos Analytical Instruments, Westwood, NJ, U.S.A.).

Mass spectrometry

A Finnigan-MAT Model 4615 quadrupole mass spectrometer was connected in series with the UV absorbance detector. Bored-through union fittings and a short length of 0.01 in. I.D. stainless-steel tubing were used to connect the moving-belt LC–MS interface with the absorbance detector. The interface was equipped with a nebulizer to deposit the LC effluent on the moving belt as a fine spray[10].

Methane chemical ionization (CI) was used for the LC–MS experiments. The ion source was pressurized to 0.3 torr with methane reagent gas, which was ionized with 70-eV electrons. Typically, solutes were desorbed from the polyimide LC–MS interface belt at 210°C. The ion source temperature was 120°C. Positive and negative ion spectra from m/z 120 to 500 were recorded alternately at 1.5 sec/scan.

Negative ion, methane CI spectra of DNPH standards were obtained by direct probe sample introduction. The ion source pressure and temperature were 0.3 torr and 120°C, respectively. Spectra were recorded from m/z 50 to 400 at 3 sec/scan. The probe was heated to 60°C for aliphatic carbonyl compounds and to 100°C for aromatic compounds.

RESULTS AND DISCUSSION

Determination of formaldehyde in aqueous samples

One type of application for LC–MS is the confirmation of identifications made by HPLC. For example, we confirmed the presence of formaldehyde in commercial shampoo and bubble bath products by LC–MS of the DNPH derivative. Liquid chromatograms of a derivatized shampoo sample are shown in Fig. 1. The shampoo contained only 4 μg of formaldehyde per gram of liquid, and the sample was diluted by a factor of 10 with water, so that only 28 ng of the formaldehyde derivative were injected. A chromatographic peak with the proper retention time for the formaldehyde derivative was visible on both the total negative ion current chromatogram from the mass spectrometer (Fig. 1b) and the absorbance chromatogram (Fig. 1a) from the UV absorbance detector. Computer enhanced spectra for the formaldehyde derivative included a molecular ion, M$^-$, at m/z 210 in the negative ion mass spectrum and a protonated molecular ion, (M + H)$^+$, at m/z 211 in the positive ion spectrum. The negative ion spectrum also contained a characteristic fragment ion at m/z 182. The m/z 182 negative ion is derived from the dinitrophenylhydrazine portion of the DNPH derivatives and is useful for detecting small amounts of DNPH derivatives (Fig. 2). For example, in Fig. 1c the reconstructed mass chromatogram for the negative ion at m/z 182 included a large peak with the proper retention time for the formaldehyde derivative. Acetaldehyde also was detected in the shampoo sample.

Analysis by LC–MS confirmed the presence of formaldehyde in a second shampoo which contained 40 μg of formaldehyde per gram of liquid and in a bubble bath which contained 300 μg/g. Quantitative data were obtained by analyzing external standards with the UV absorbance detector. Unfortunately, surfactants in the shampoo and bubble bath samples caused unpredictable changes in sensitivity for the mass spectrometer, which precluded quantitation by LC–MS.
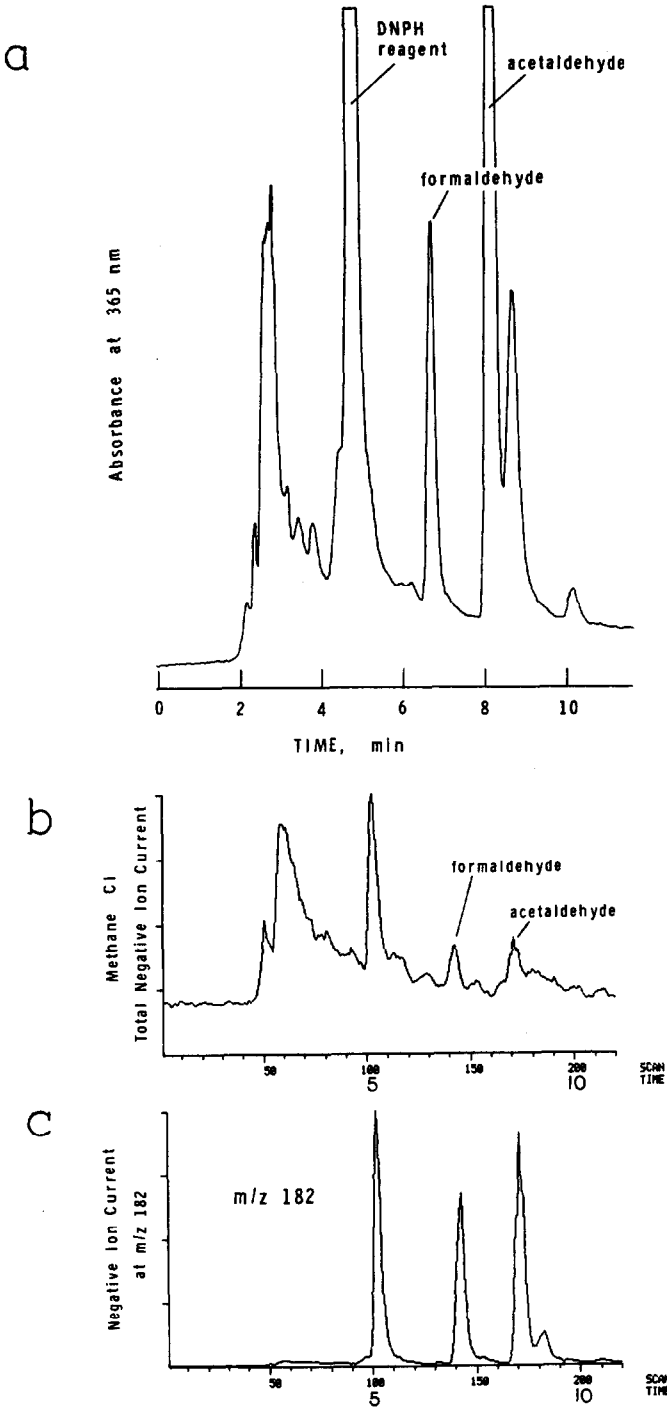
Fig. 1. Liquid chromatograms of DNPH derivatives originating from a shampoo sample. (a) Absorbance at 365 nm. (b) Total negative ion current profile. (c) Reconstructed mass chromatogram of negative ion current at m/z 182.
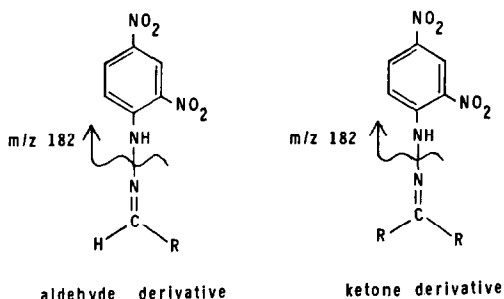
Fig. 2. Origin of the negative ion at m/z 182 in the methane chemical ionization mass spectra of DNPH derivatives. R = Alkyl or aromatic group.

Determination of acrolein and other vapors in a ceramic insulation material

A second type of application for LC–MS is the determination of compounds in unresolved chromatographic peaks. For example, the DNPH derivatives of acrolein and acetone are difficult to separate by HPLC, even with the most efficient columns and optimal chromatographic conditions. Consequently, the two derivatives appear as a single peak on the UV absorbance chromatogram in Fig. 3a and on the total ion current chromatogram from the mass spectrometer in Fig. 3b. Although they are not separated chromatographically, acetone and acrolein can be distinguished from each other by negative ion CI–MS. The acetone derivative produces a molecular ion at m/z 238 while the acrolein derivative produces a molecular ion at m/z 236. To detect the acetone and acrolein derivatives, reconstructed mass chromatograms were plotted for ions from m/z 236 to 240 (Fig. 3c). The peaks at m/z 236 and 238 indicate that the solution contained both acetone and acrolein, even though the DNPH derivatives eluted simultaneously. The areas under the peaks at m/z 236 and 238 can be used for quantitation and to determine the relative amounts of acetone and acrolein in the sample.

The LC–MS technique was used to investigate a materials problem involving acrolein vapor. A ceramic mat, proposed as insulation in the automobile exhaust system, released an irritating odor when initially heated to elevated temperatures. To generate vapor samples in the laboratory, portions of the mat were heated to 500°C in a tube furnace. The furnace was purged with air, which carried the vapors into an impinger filled with a solution of the DNPH reagent. A chromatogram from the LC–MS analysis of the impinger sample is included in Fig. 4. The LC–MS data showed that acrolein was the major carbonyl compound being released from the mat and that significant amounts of acetone were not present. The negative ion mass spectrum for the acrolein peak includes m/z 236 as the molecular ion rather than m/z 238 for acetone (Fig. 4b).

Both the UV absorbance detector and the mass spectrometer were used to quantitate the amount of acrolein released by the heated mat. Quantitation with the UV detector indicated that 970 μg of acrolein vapor were released per gram of mat, and MS quantitation, based on the intensity of the M^- ion at m/z 236, indicated that 900 μg of acrolein were released per gram. About 400 μg of acetaldehyde and 90 μg of formaldehyde were detected. Crotonaldehyde and butyraldehyde were identified by LC–MS as minor components of the evolved vapor (Fig. 4). The aldehyde vapors

a

Absorbance at 365 nm

DNPH
reagent

acetone and
acrolein

propionaldehyde

0   5   10   15

TIME, min

b

Total Negative Ion Current

acetone and
acrolein

propionaldehyde

50   100   150   200   250   300   SCAN
         5   10   15   TIME

c

Negative Ion Current

m/z

—— 240

—— 239

—— 238

—— 237

—— 236

50   100   150   200   250   300   SCAN
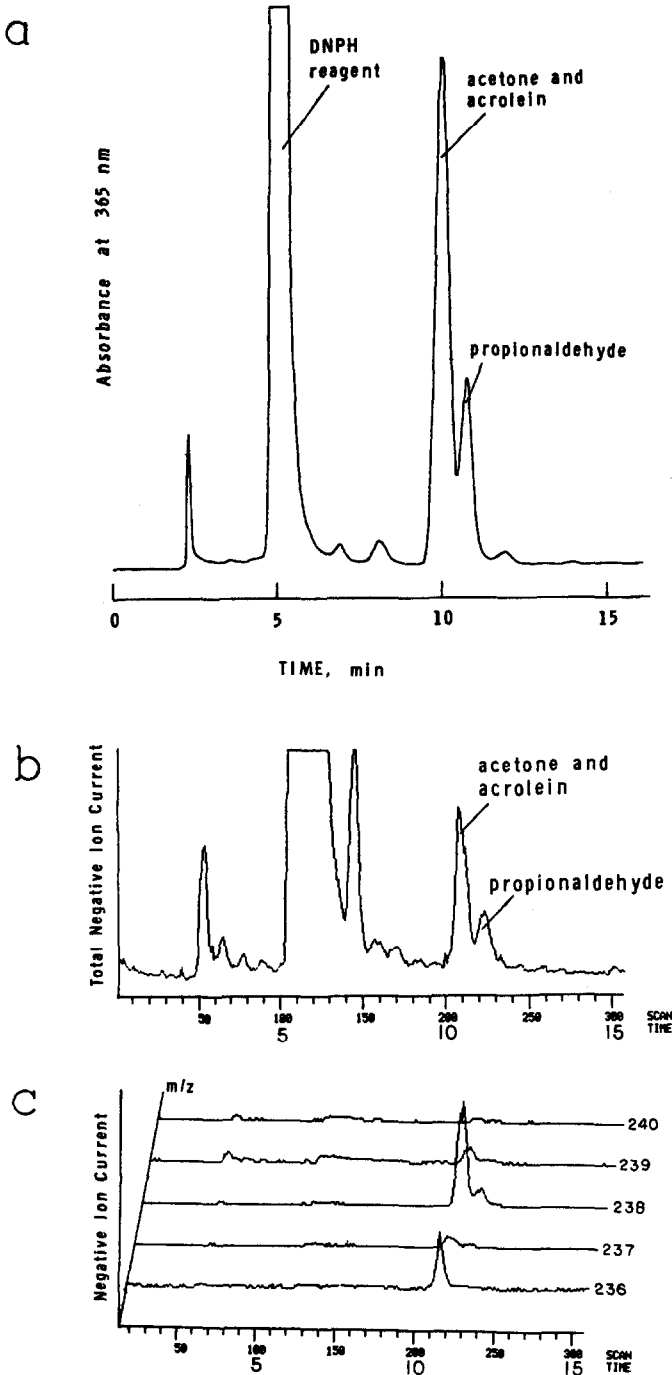   5   10   15   TIME

Fig. 3. Liquid chromatograms for a mixture of three DNPH standards. (a) Absorbance at 365 nm. (b) Total negative ion current profile. (c) Reconstructed mass chromatograms for ions between m/z 236 and 240.
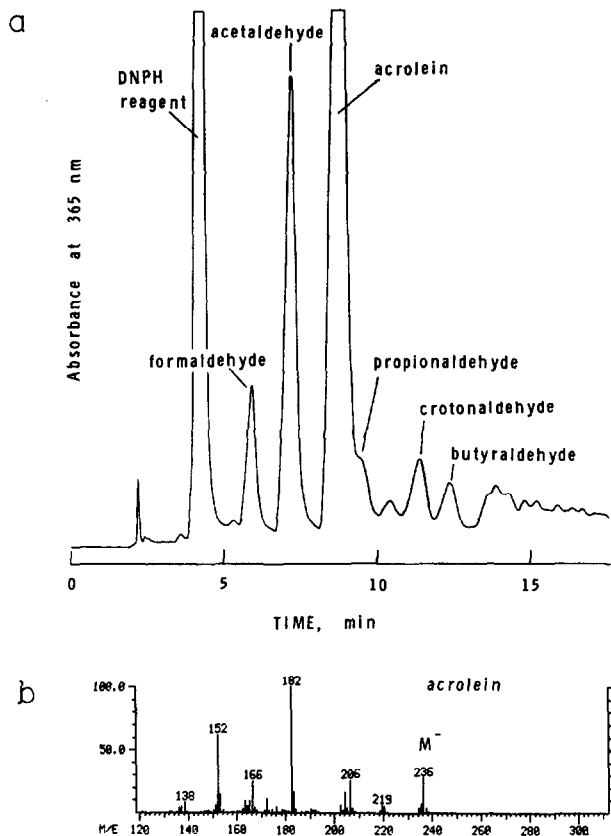
a

b

Fig. 4. (a) Liquid chromatogram of a sample from a liquid impinger. The impinger was used to collect organic vapors that were released from a heated ceramic mat. (b) Negative ion mass spectrum of acrolein derivative which was detected in the impinger by LC–MS.

originated from an organic binder that was incorporated into the mat. The odor problem was resolved when the supplier substituted a new organic binder that did not generate significant amounts of acrolein when heated.

Calibration curves for DNPH derivatives of acrolein, formaldehyde, acetone and benzaldehyde are shown in Fig. 5. Linear curves were obtained by making logarithmic plots for the amount of DNPH derivative *versus* the peak area for its molecular ion. The curves in Fig. 5 show that either the negatively charged molecular ion, M^-, or the positively charged molecular ion species, $(M + H)^+$, can be used for quantitation. Positive ion CI mass spectra of DNPH derivatives are dominated by protonated molecular ions[7]. Except for formaldehyde, better sensitivity was obtained from negative ion CI than positive ion CI.

Identification of aldehydes and ketones in automobile exhaust

A third type of application for LC–MS is the identification of unknown compounds. We have used LC–MS with methane CI to identify DNPH derivatives from a variety of samples, and to distinguish aldehydes from ketones. The method of
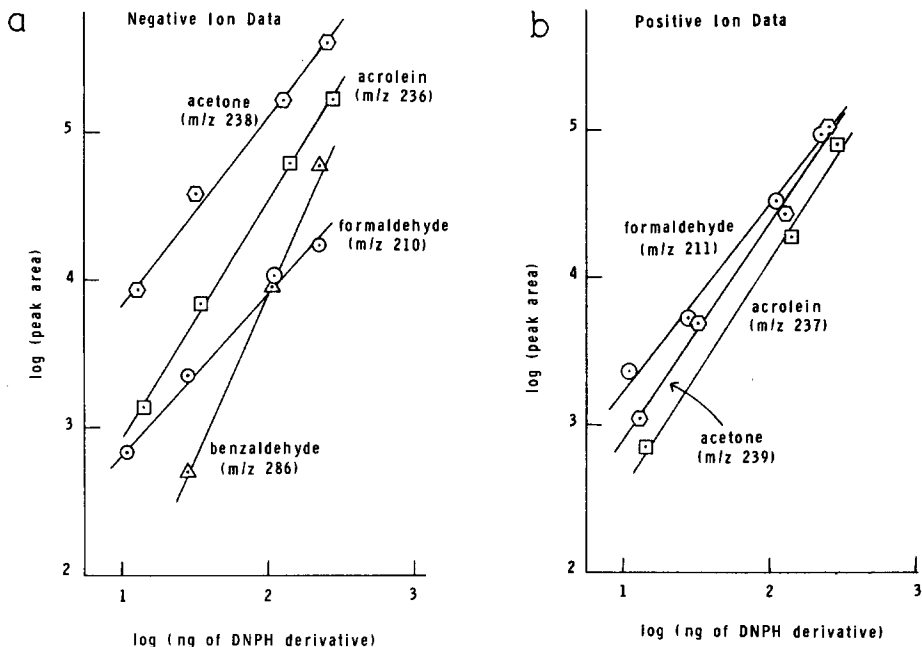
Fig. 5. Calibration curves for DNPH derivatives from LC–MS data. (a) Logarithmic plot of molecular ion intensity, M^-, *versus* sample weight. (b) Logarithmic plot of protonated molecular ion intensity, $(M+H)^+$, *versus* sample weight. Methane chemical ionization was used.
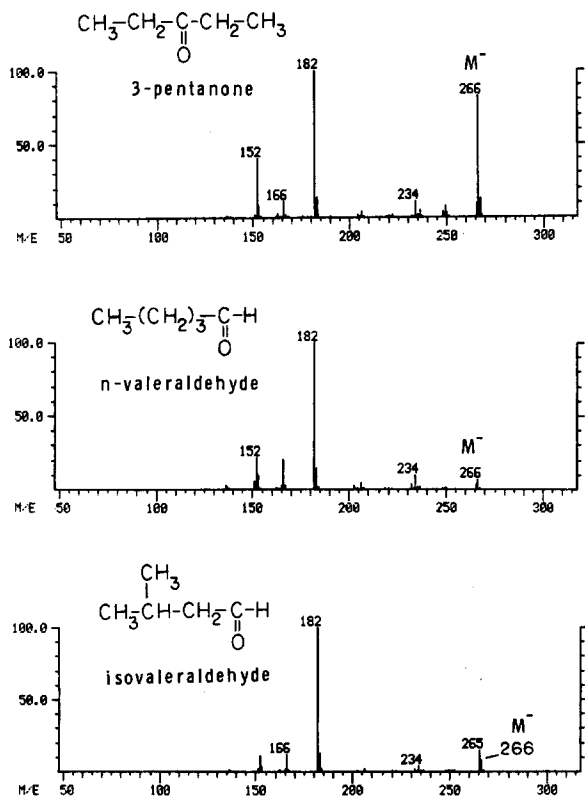

Fig. 6. Negative ion mass spectra of DNPH derivatives which were obtained by direct probe sample introduction and methane chemical ionization.
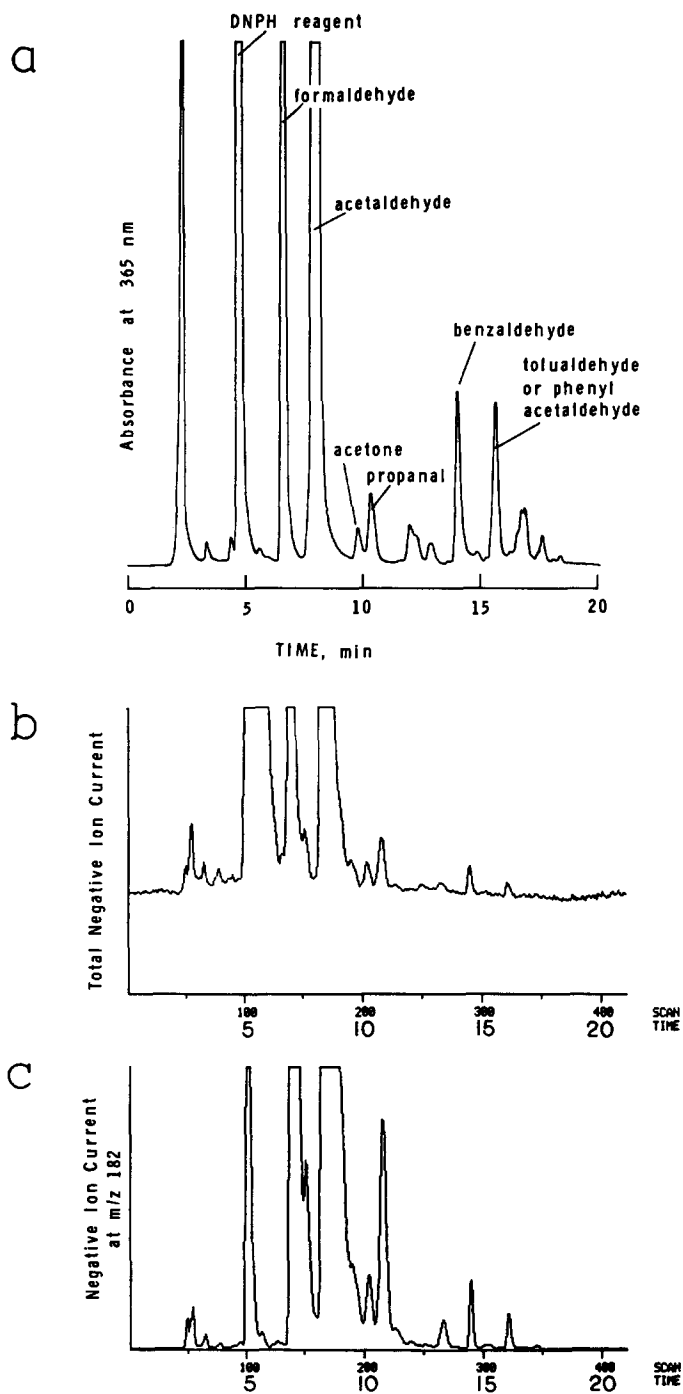
Fig. 7. Liquid chromatograms of DNPH derivatives from an automobile exhaust sample. The automobile was fueled with gasohol. (a) Absorbance at 365 nm. (b) Total negative ion current profile. (c) Reconstructed mass chromatogram of negative ion current at m/z 182.

TABLE I

MASS SPECTRAL DATA FOR DNPH DERIVATIVES OBTAINED BY DIRECT PROBE INTRO-
DUCTION AND METHANE CHEMICAL IONIZATION

DNPH derivative	Molecular weight	Relative intensity of negative ions in methane CI spectra	
		Molecular ion (M^-)	m/z 182
Aliphatic compounds			
Formaldehyde	210	2	100
Acrolein	236	22	100
Propionaldehyde	238	15	100
Acetone	238	62	100
Crotonaldehyde	250	32	100
Butyraldehyde	252	4	100
Isobutyraldehyde	252	9	100
2-Butanone	252	62	100
Valeraldehyde	266	6	100
Isovaleraldehyde	266	8	100
3-Pentanone	266	82	100
Mesityl oxide	278	100	70
Methyl isobutyl ketone	280	100	70
3,3-Dimethyl-2-butanone	280	100	93
4,4-Dimethyl-2-pentanone	294	100	66
Aromatic compounds			
o-Tolualdehyde	300	7	100
p-Tolualdehyde	300	24	100
Phenyl acetaldehyde	300	14	100
Acetophenone	300	100	78
Cinnamaldehyde	312	62	100
Hydrocinnamaldehyde	314	19	100
p-Methyl acetophenone	314	100	42

TABLE II

QUANTITATIVE DETERMINATION OF FORMALDEHYDE AND ACETALDEHYDE IN
AUTOMOBILE EXHAUST SAMPLES

Exhaust sample from test car	Concentration of aldehydes in liquid impinger filled with water ($\mu g/ml$)*			
	Formaldehyde		Acetaldehyde	
	Mass spec. detector**	UV detector***	Mass spec. detector**	UV detector***
Gasohol fuel	18	20	110	120
Ethanol fuel	310	330	120	150

 * The volume of engine exhaust that passed through the impinger was not measured. The aldehydes
were detected as their DNPH derivatives.
  ** Mass spectrometer detector operated in the negative ion mode with methane CI.
  *** UV absorbance detector operated at 365-nm wavelength.

distinguishing aldehydes from ketones is based on the relative intensity of the molecular ion in the negative ion spectrum. In Fig. 6 the spectrum for the DNPH derivative of 3-pentanone can be distinguished from the valeraldehyde spectra because the molecular ion is more prominent for the ketone than for the aldehydes. In some cases, negative ion spectra contain structural information about a specific aldehyde or ketone isomer. For example, the spectra of isovaleraldehyde and valeraldehyde differ in that the $(M-H)^-$ ion at m/z 265 is more intense than the M^- ion at m/z 266 for isovaleraldehyde (Fig. 4). An unusually intense $(M-H)^-$ ion also was observed for isobutyraldehyde.

We have recorded negative ion mass spectra for 22 DNPH derivatives by direct probe sample introduction and methane CI. The spectra confirm the observation that M^- ions are more stable for DNPH derivatives of ketones than aldehydes (Table I).

Two automobile exhaust samples were analyzed for unknown aldehydes and ketones by LC–MS. The exhaust samples were collected in liquid impingers filled with water, and the DNPH derivatives were formed by adding aliquots from the impingers to acetonitrile solutions of the DNPH reagent. One exhaust sample was from a car fueled with gasohol [ethanol–gasoline (20:80)], and the other sample was from a car fueled with ethanol. Formaldehyde and acetaldehyde were the predominant carbonyl compounds detected in the gasohol exhaust (Fig. 7). Acetone, propionaldehyde (propanal), benzaldehyde, and an isomer of tolualdehyde or phenyl acetaldehyde were also identified by MS. Other minor components were tentatively identified as isomers of butyraldehyde and aromatic aldehydes. The large peak appearing at 2.5 min in the absorbance chromatogram was identified as dinitrophenol, a decomposition product of the DNPH reagent.

The only carbonyl compounds detected in the ethanol exhaust sample were formaldehyde and acetaldehyde. The gasohol exhaust sample contained more acetaldehyde than formaldehyde, while the ethanol exhaust sample contained more formaldehyde than acetaldehyde (Table II). Reasonably good agreement was obtained for the MS and the UV absorbance detectors. Pairs of single determinations for formaldehyde and acetaldehyde agreed to within about 10% of each other (Table II).

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