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# Automated solid-phase extraction and coupled-column reversed-phase liquid chromatography for the trace-level determination of low-molecular-mass carbonyl compounds in air

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## Abstract

This study reports the development of a coupled-column RPLC method for the trace level determination of several volatile aldehydes in ambient air. Carbonyls in air are sampled using a ozone scrubber and SPE C<sub>18</sub> cartridges which are pretreated with 2,4-dinitrophenylhydrazine. The method is used for the separation of 13 different aldehydes and ketones. All analytes are separated without high blanks or ozone interferences. The analytical detection limits for the most important compounds formaldehyde, acetaldehyde, 2-propenal (acrolein), 2-butenal (crotonaldehyde) and benzaldehyde, range from 50 to 150 ng/m<sup>3</sup> (ppt) or 0.5–1.0 pmol/l, with relative standard deviations of 6%. The sampled air volume was 200 l. Due to the high sensitivity of the method, high purity solvents must be used. The analytical method as described below, has high recoveries for all analytes (>90%) and is fully automated. This procedure has been applied in a pilot study with 60 air samples.

## 1. Introduction

Carbonyl compounds, aldehydes and ketones, are given increased attention as pollutants and possible key compounds in photochemical reactions [1]. Some aldehydes, particularly formaldehyde, are well known for their impact on human health, and therefore require sensitive and selective methods of analysis [2]. A variety of analytical techniques have been developed, most of them focused on formaldehyde. Formaldehyde is a common industrial chemical which is used for example in the manufacturing of glues

and resins. In homes, a major source of formaldehyde is particle board.

The derivatisation with 2,4-dinitrophenylhydrazine (DNPH) prior to high-performance liquid chromatography (HPLC) has received widespread acceptance because of its selectivity. Early field applications of the method involves sampling with impingers which contain a DNPH solution [3,4]. Currently, most sampling is done on small DNPH coated C<sub>18</sub> cartridges as described by Kuwata et al. [5]. However, the drawbacks of this widely used and accepted method are high blanks, ozone interference and long collection times to achieve sub-ppb detection limits [6]. Ozone, usually present in urban air, degrades the hydrazone derivatives [7].

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Some of the problems are the cartridge background levels of formaldehyde, acetaldehyde and acetone which are about 1.1–2.3 nmol (56–70 ng) [8]. Levels of 6  $\mu\text{mol}$  (180  $\mu\text{g}$ ) formaldehyde are also reported on uncoated  $\text{C}_{18}$  cartridges. The actual cause of this background is, however, not known [9].

During the development of the HPLC method, several problems had to be dealt with. The first goal was to improve selectivity and sensitivity for formaldehyde, acetaldehyde, 2-propenal (acrolein), 2-butenal (crotonaldehyde) and benzaldehyde, in order to analyse air samples with a carbonyl concentration of  $\pm 1 \text{ pmol/l}$  ( $\pm 50 \text{ ng/m}^3$ ). Secondly, the method must be automated due to the expected high number of samples to be analysed.

Large volume injection on a small precolumn seems to be a possibility for improving sensitivity but unfortunately the excess of unreacted DNPH reagent is difficult to separate from early eluting compounds. Coupled-column reversed-phase liquid chromatography (RPLC) appeared to be very useful for trace-level determination of various compounds in water. Using two highly efficient separation columns, very strong polar analytes such as ethylenethiourea, chloroallyl alcohol and methylisocyanate in aqueous samples can be analysed directly to a level of 1  $\mu\text{g/l}$  (ppb) [10,11]. Therefore, this method was adopted for the analysis of aldehydes.

The described procedure involves off-line sampling on two DNPH-coated  $\text{C}_{18}$  cartridges coupled to an easy to build and effective ozone scrubber and coupled-column RPLC for the automated highly efficient clean-up and analysis of the samples. Due to the sensitivity of the described method, unforeseen impurities in the solvents used caused additional problems.

## 2. Materials and methods

### 2.1. Reagents

Bakerbond SPE columns  $\text{C}_{18}$  (3 ml LD, 200 mg, 40  $\mu\text{m}$ ), phosphoric acid, methanol HPLC-grade, acetonitrile HPLC-grade and 'Baker ana-

lysed', HPLC-grade water, were purchased from J.T. Baker (Deventer, Netherlands). 2,4-Dinitrophenylhydrazine (DNPH) and potassium iodide were purchased from Fluka Chemie (Buchs, Switzerland). Stock solution of aldehydes, ThetaKit TK-151, and ketones and ThetaKit TK-155 were purchased from Rochrom (Rotterdam, Netherlands). HPLC columns (100  $\times$  4.6 mm I.D.) packed with 3  $\mu\text{m}$  MicroSphere  $\text{C}_{18}$  were obtained from Chrompack (Bergen op Zoom, Netherlands). LC-grade water was obtained by purifying demineralised water with a Milli-Q system (Millipore, Bedford, MA, USA). Ozone was produced with a highly efficiency Hg-lamp in a laboratory made ozone generator. SPE columns were first washed twice with 2 ml acetonitrile ('Baker analysed') and once with 2 ml DNPH solution (1 mg DNPH per ml acetonitrile 'Baker analysed', 2% phosphoric acid). The columns were dried for 20 min under a stream of nitrogen (purity 5.0, HoekLoos, Schiedam, Netherlands). The coated columns, with air tight caps on both sides, were stored in a dark and cool place and used within 10 days [11]. Luer caps were purchased from Omnilabo (Breda, Netherlands). Caps for the other side (I.D. 9 mm) of the SPE columns were purchased from Gilson (Villiers-le-Bel, France). A saturated solution of potassium iodide was put in a copper tube (1 m  $\times$  4.6 mm I.D.) for 10 min and dried under a stream of nitrogen (200 ml/min) for 3 h. This tube was used as an ozone scrubber.

### 2.2. Apparatus

The high-pressure gradient LC-system (Fig. 1) consisted of two Gynkotec (Germening, Germany) dual piston isocratic LC pumps (P-2), a Model 480 programmable and a Model 300 slave pump. A Waters (Millipore, Bedford, MA, USA) Model 6000 solvent delivery system (P-1) was used to condition the first analytical column. All LC solvents were degassed with a Separations GT-103 degasser (Separations, H.I. Ambacht, Netherlands). An ASPEC (Automated Sample Preparation with Extraction Columns, Gilson) system equipped with a Rheodyne (Berkeley, CA, USA) 7010 high-pressure switching

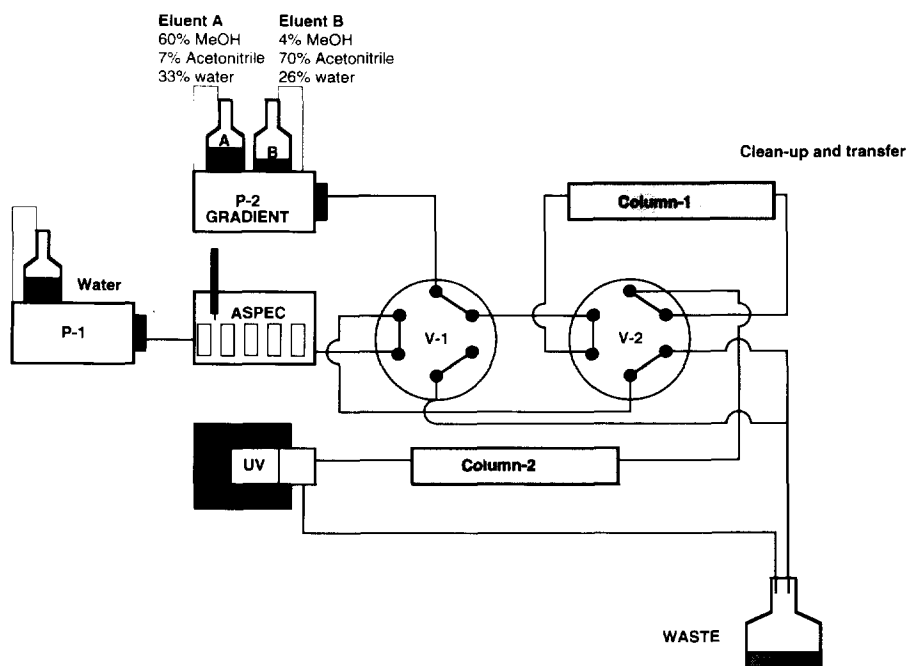


Fig. 1. Schematic setup of the coupled-column HPLC system for the analysis of aldehydes and ketones. ASPEC = Automated sample preparation with extraction columns; V-1 and V-2 = valves; P-1 = isocratic LC pump; P-2 = gradient LC pump; UV = UV-Vis detector.

valve is used for the automated elution and injection of the samples. A multiport stream-switch, MUST (Spark-Holland, Emmen, Netherlands) with two automated Rheodyne 7010 high-pressure six-port switching valves was used for coupled-column chromatography. An ABI 757 UV detector (Applied Biosystems, Foster City, CA, USA) was used at 360 nm. Data were acquired on an HP 3365 Series II ChemStation, running on a HP Vectra personal computer (Hewlett-Packard, Rockville, MD, USA).

### 2.3. Sampling

Sampling took place with two SPE columns, connected to each other with a polypropylene sealing cap (Gilson) and the ozone scrubber placed before the cartridges. A 200-l air sample was taken with a GAST vacuum pump type DOA-V112-BN (MFG, Benton Harbor, MI, USA) at a flow-rate of 2 l/min. After sampling,

SPE columns were capped and stored dark in a refrigerator (5°C).

### 2.4. HPLC analysis

Mobile phase A consisted of methanol–acetonitrile–water (60:7:33, v/v/v). Mobile phase B consisted of methanol–acetonitrile–water (4:70:26, v/v/v). All flow-rates were 1 ml/min. Before each injection the first column, column-1, was conditioned with water for 10 min.

Cartridges were extracted with 3 ml acetonitrile which was diluted with 7 ml water. From this solution, 2 ml were injected on column-1. After injection, valve V-1 was switched to the gradient LC system (P-2) for a linear gradient from 100% mobile phase A to 100% mobile phase B in 30 min. A clean-up volume of 4 ml was used to separate the first eluting analyte from early eluting interferences, and after 4 min, column-1 and column-2 were coupled by switch-

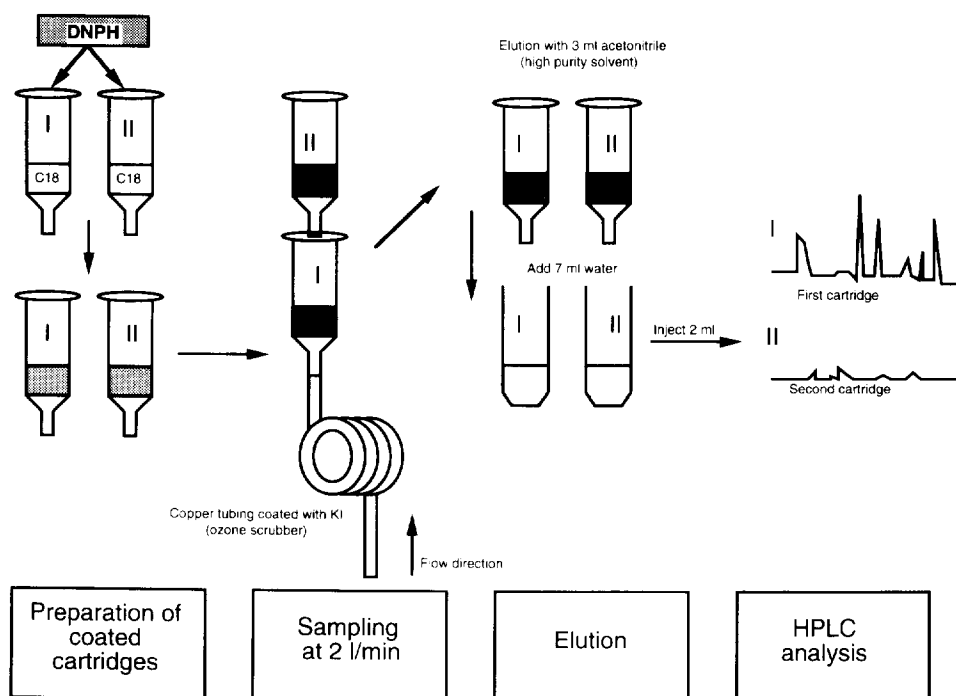


Fig. 2. Procedure for the cartridge preparation, sampling and analysis of carbonyl compounds in air. For details see text.

ing valve V-2 (see also Fig. 1). Compounds were identified by elution. During the method development, on-column derivatisation of the aldehydes and ketones, was checked with FTIR. Blank levels were first obtained by sampling 200 l of nitrogen gas from a cylinder. Afterwards blanks were obtained by eluting an unused DNPH-treated cartridge with acetonitrile.

An ASPEC system was used for the complete automated elution, dilution and injection of the samples.

The procedure is summarised in Fig. 2. Extraction and analysis of the samples must take place within 10 days.

### 3. Results and discussion

The major problem encountered in the determination of the DNPH-derivatives of both aldehydes and ketones, is the presence of unused DNPH reagent which causes, particularly in the first part of the chromatogram, a large back-

ground interference. In order to reduce this interference, the possibility of precolumn switching was investigated. Since  $C_{18}$  cartridges are eluted with 3 ml acetonitrile, dilution is necessary in order to increase retention on another  $C_{18}$  precolumn. The use of a short precolumn (specificity) did not provide the required selectivity. Unfortunately the excess of unreacted DNPH reagent was difficult to separate from the early eluting compounds (data not shown). Better results were obtained when the precolumn was replaced by an analytical column. As has been shown in other applications by Hogendoorn et al. [10], the use of a first column with high separation power provides more efficient separation of the large excess of the early eluting interferences and the first eluting analytes. In this case dilution with water is also necessary to increase retention on the first RPLC column. The dilution was therefore optimised. Fig. 3 shows the results of the analysis with and without the coupled-column switching technique. As can be seen, early eluting compounds such as form-

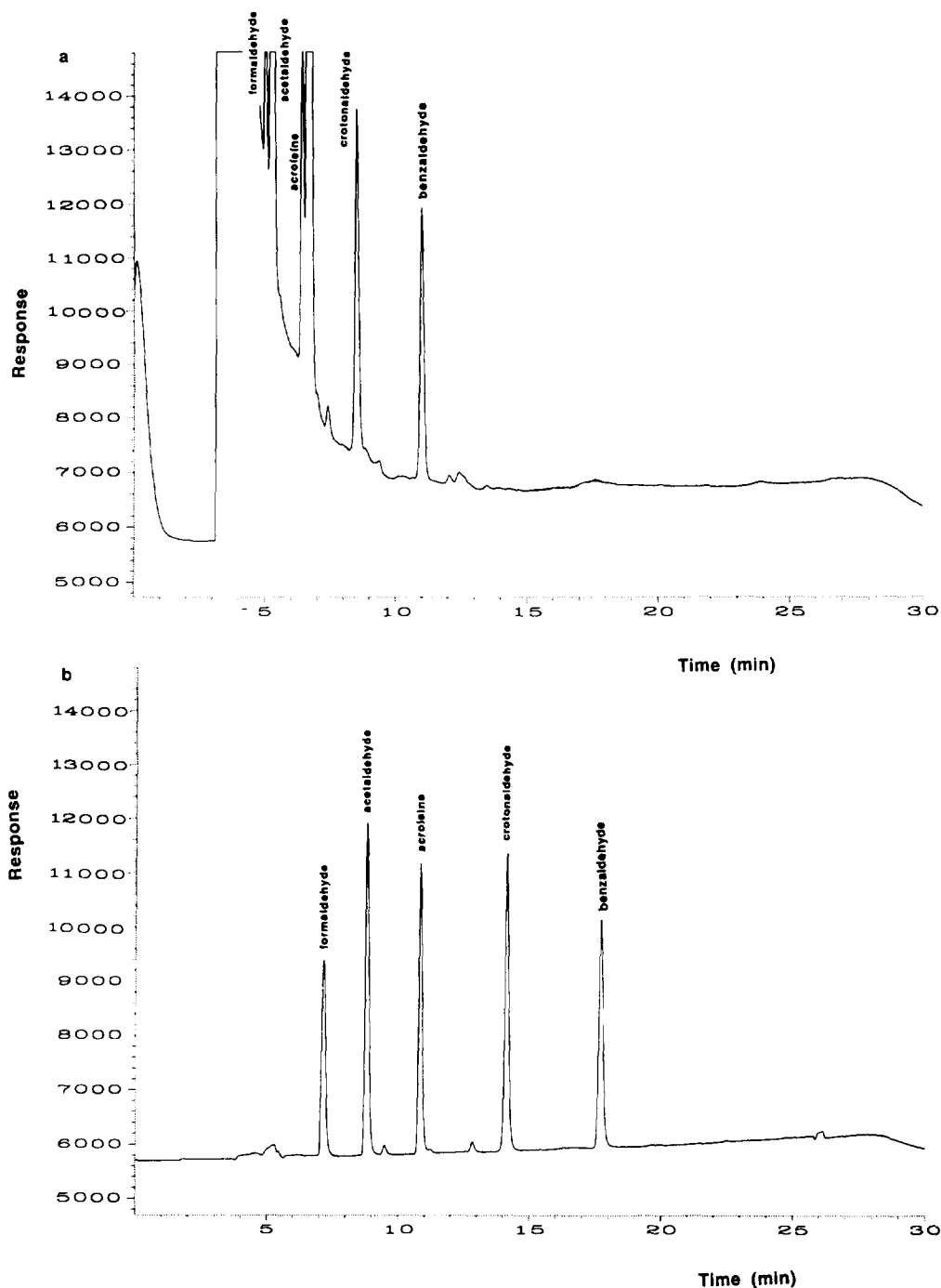


Fig. 3. Results of the clean-up method. Analysis of an air sample (a) with and (b) without column switching. Analytical conditions as described in the text. During the analysis without column-switching, valve-2 in Fig. 1 was not switched. Response of detector in counts.

aldehyde are separated from the large excess of DNPH.

A large air volume with known concentrations of the carbonyl compounds, is very difficult to realise. As the best approach for recovery experiments, standard mixtures were added to an SPE column. All compounds had recoveries above 90%. The minimal detectable amounts range from 0.6 to 3.0 ng (20–40 pmol) carbonyl injected. This means, for a 200 l sample with an injection volume of 2 ml from an extract of 10 ml, a detection limit of 0.5 pmol/l can be realised. This theoretical value has been tested by spiking a DNPH-treated SPE cartridge with ca. 1.0 ng of aldehydes. The cartridge was connected to another, unused, cartridge to check the breakthrough of the aldehydes on the first cartridge. Then, 200 l of nitrogen from a cylinder was led through both cartridges, followed by elution and analysis.

As these levels were easy to analyse (signal-to-noise better than 3, with a relative standard deviation of 6%), the calculated values can be used in practice. From the results from analysed (real) samples, it became clear that the calculated detection limits were realisable. Even lower limits seem possible with larger air volumes.

Concentrations of formaldehyde in the second cartridge are dependent on the relative humidity and ranged from 0.1 to 2.0  $\mu\text{g}/\text{m}^3$  (3–66 pmol/l) in real air samples.

Because the method should be used for monitoring purposes, the expected number of samples will be high. The method therefore needs to be automated, especially when each air sample produces two cartridges to be analysed. Experiments indicated that extracts of the cartridges cannot be stored for long in an autosampler vial at room temperature. Extracts can be stored at least for one year if stored in the dark and refrigerated [12]. With these restrictions, a procedure was developed for a Gilson ASPEC system which subsequently eluted one cartridge, diluted the extract and injected an amount of 2 ml onto the HPLC system.

Quantification of aldehydes and ketones from ambient air samples is difficult due to ozone

interferences. Under chromatographic conditions commonly reported for the aldehydes by HPLC, two of the peaks from the ozone-DNPH decomposition products can readily co-elute with the formaldehyde hydrazone. Smith et al. [13] succeeded in separating ozone DNPH artifacts from formaldehyde. Removal of ozone before its possible reaction with DNPH, is another approach and good results were reported by Dye and Oehme [14]. This latter method was adopted and Fig. 4a shows the results of a 200-l air sample in the presence of 400 ppb ozone with and without using the ozone scrubber. Fig. 4b also shows the compounds one can typically expect in air.

The purity of the acetonitrile used for eluting the hydrazones from the SPE column may cause some unexpected problems, because acetone is a major contamination which will react with the excess DNPH reagent which, in turn, will result in high blanks. Several brands of HPLC-grade acetonitrile were tested. Only high-purity acetonitrile can be used for the elution of the cartridges. One should realise that different batches may contain various concentrations of acetone. Therefore every batch of acetonitrile has to be tested before being used in the procedure. Because acetone is frequently used in a laboratory, high levels can be found in ambient laboratory air which may also cause contamination. These problems can be solved by working in a fume hood, and a laboratory from which acetone is banned.

The method was used for the analysis of formaldehyde, acetaldehyde, 2-propenal (acrolein), 2-butenal (crotonaldehyde) and benzaldehyde in ambient air. The results of these studies will be reported elsewhere. The procedure may be adapted for the separation of additional aldehydes and ketones. Fig. 5 shows the chromatogram of all the aldehydes and ketones which have been separated with the described method. The method proved to be very satisfactory for series of more than 200 air samples. From the analysed air samples it became clear that the calculated detection limits are attainable. The method has also been adapted for the analyses of aldehydes and

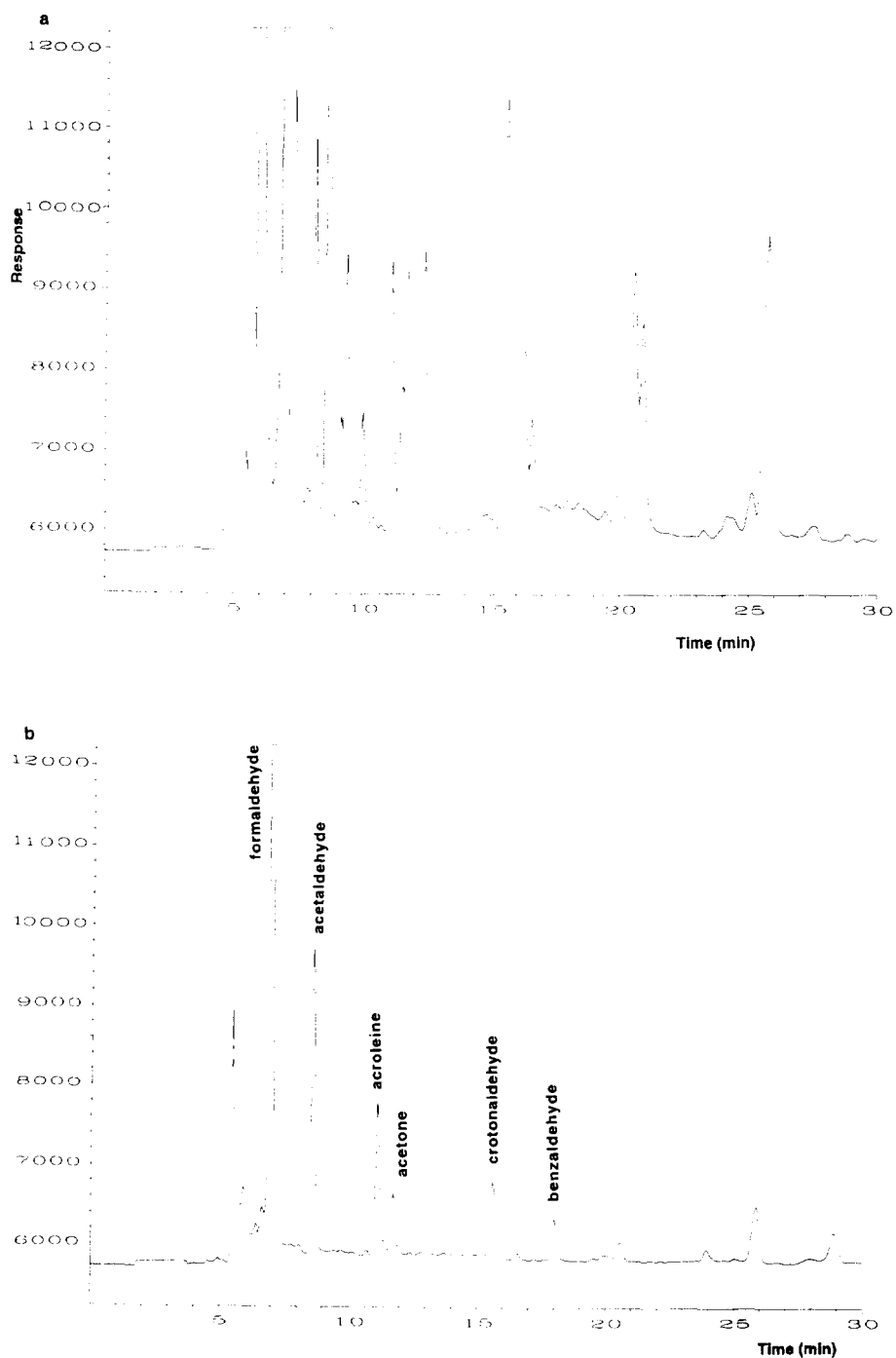


Fig. 4. The effect of an ozone-scrubber on the analysis of carbonyl compounds. Sampling was done in the presence of high concentrations of ozone (400 ppb), (a) without ozone scrubber, (b) with ozone-scrubber. Analytical conditions as described in the text. Response of detector in counts.

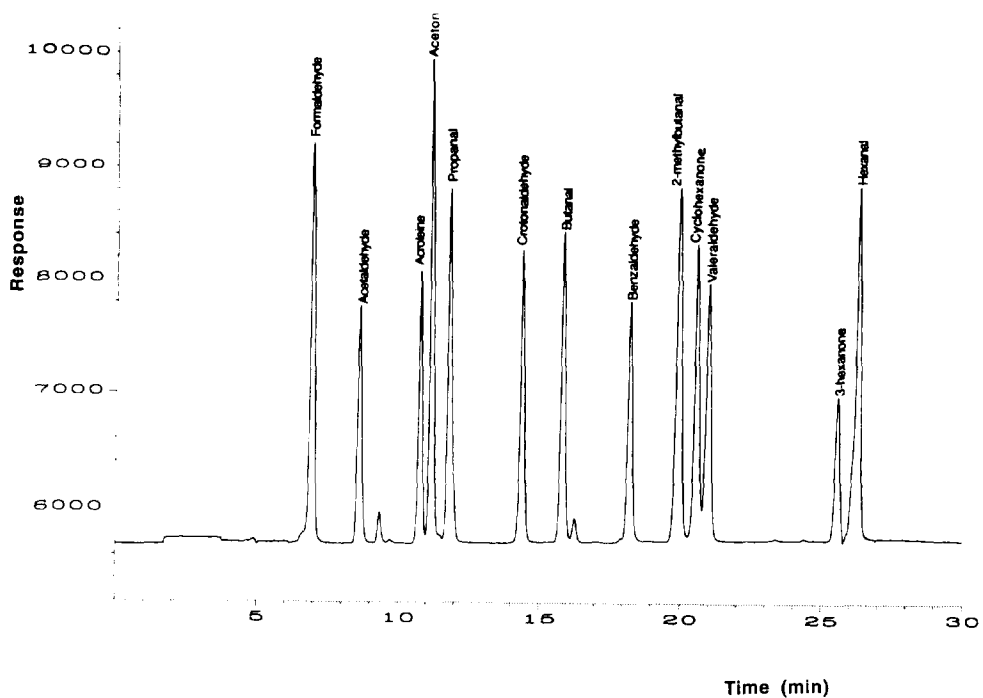


Fig. 5. Analysis of a mixture of 13 different compounds. Analytical conditions as described in the text.

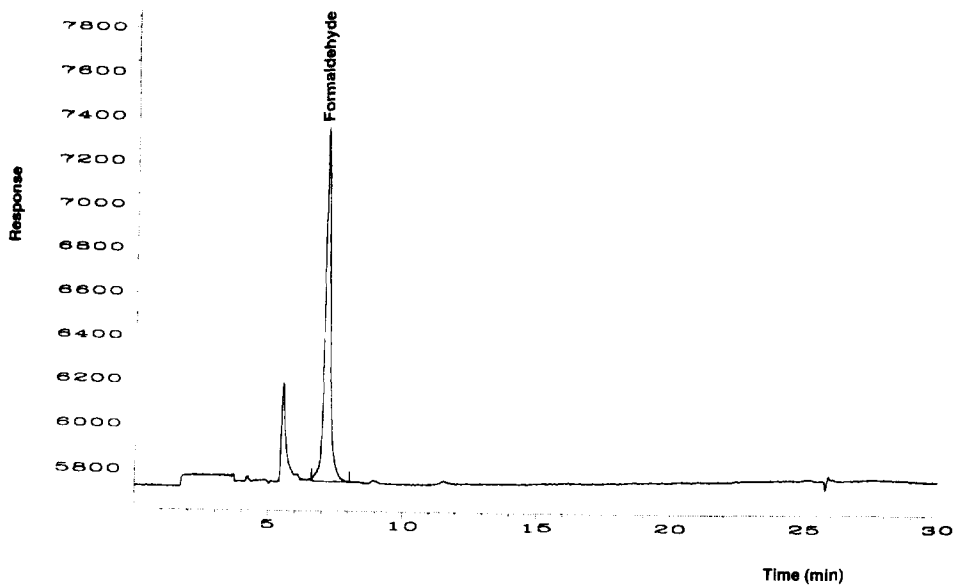


Fig. 6. Analysis of a 100 ml tap water sample pre-concentrated on a DNPH-treated SPE-column. Analytical conditions as described in the text.



ketones in water samples. About 100 ml of water can be preconcentrated on the DNPH-pretreated SPE columns. A chromatogram of tap water is shown in Fig. 6. Further extension to other types of water samples are in progress.

#### 4. Conclusions

The combination of chemical derivatisation, selective detection and coupled column RPLC can be used to attain maximum sensitivity and selectivity in the determination of atmospheric carbonyls. This method does not have the usual drawbacks of high blanks and ozone interference. The contamination of acetone in solvents may cause high blanks.

Coupled-column reversed-phase liquid chromatography in combination with chemical derivatisation and selective UV-detection, solves the major problem in the determination of the DNPH derivatives of both aldehydes and ketones. The background system peak, caused by the unused DNPH reagent, is separated from the compounds of interest. Consequently low detection limits of upto 0.5–1.0 pmol/l (50 to 150 ng/m<sup>3</sup>) were established.

The use of high-purity solvents is necessary. The purity of acetonitrile used for elution of the derivatives from the SPE column is essential. Because of the excess DNPH reagent, aldehyde and ketone contaminants in acetonitrile will result in high blanks. A major impurity is acetone. The method can easily be adapted for the

analysis of aldehydes and ketones in water samples.

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