## Notes

CHROM. 5274

# Quantitative gas chromatographic analysis of micro amounts of volatile carbonyl compounds via their DNPH derivatives

Volatile carbonyl compounds have been found qualitatively to be one of the major constituents in the aroma of fat-containing foodstuffs. They occur, however, in very low concentrations which make them difficult to analyse quantitatively. Methods for precise determination of these minute amounts are therefore of great help in aroma research.

Carbonyls are frequently isolated as 2,4-dinitrophenylhydrazones (DNPHs) and as such have been fractionated by liquid–liquid or liquid–solid chromatography in columns or on thin layers and evaluated by an instrumental method (spectroscopy, densitometry). Much work has been done in order to develop such chromatographic systems, by amongst others, SCHWARTZ *et al.*[1], DHONT AND DEROOY[2] and URBACH[3].

However, a gas chromatographic (GC) method is, in several respects, superior to these techniques. Some of its advantages are: rapid analysis, high reproducibility and good precision. This paper describes a reactor that reproducibly can regenerate the carbonyls from micro quantities (5 $\mu$g) of DNPHs prior to the GC analysis.

*The reactor*

A schematic diagram of the reactor is given in Fig. 1. It consists of a metal rod which has been partly drilled through and which fits into the injection port (A) at one end and into the carrier gas source (E) at the other. There is also an injector (B) connected to the carrier gas stream for conventional injection of fluids and connections for attaching a teflon tube (C).

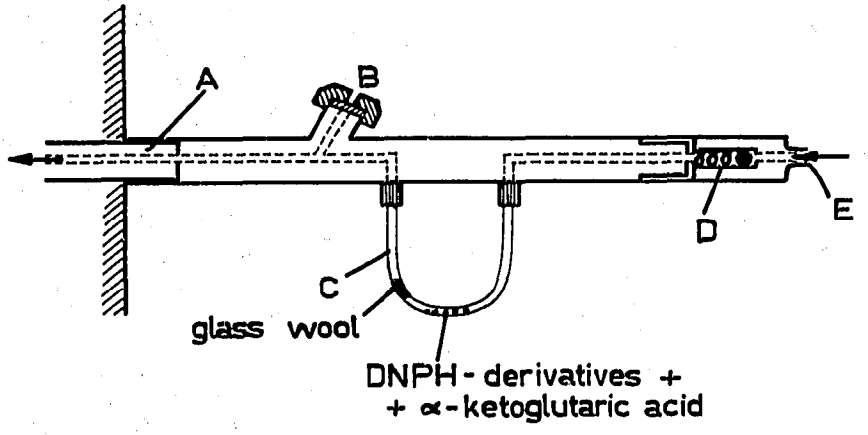



Fig. 1. Schematic diagram of the reactor. A = Injector port (gas chromatograph); B = injector; C = exchangeable teflon tube; D = back pressure valve; E = connection to carrier gas source.

*Regeneration procedure*

Carbonyls can be regenerated from their DNPHs in several ways. We used the exchange reaction with α-ketoglutaric acid to liberate them. A mixture of the DNPHs and α-ketoglutaric acid in the w/w ratio 1:3 is put in the teflon tube (I.D. 2 mm). The carrier gas stream is turned on and the teflon tube is heated at 250° (polyglycol bath) for 5 sec. The liberated carbonyls are now swept into the column by the carrier gas and chromatographed in the usual way.

*Experimental*

The DNPHs of the carbonyl compounds were synthesised by shaking 100 μl of each parent compound with 100 ml of a saturated solution of 2,4-dinitrophenylhydrazine in 2 *M* aq. HCl overnight at room temperature. The derivatives were filtered off, washed with water and dried over $P_2O_5$ in vacuum. Further purification was unnecessary according to the gas chromatograms of the DNPHs.

The gas chromatographic analyses were carried out on a Varian 1400 chromatograph equipped with an FID under the following conditions. Column: Porapak S (molecular sieve), 1.20 m × 1 mm; oven temperature: program 100–180°, 10°/min; injector temperature, 210°; detector temperature, 190°: nitrogen flow, 25 ml/min; hydrogen flow, 25 ml/min.

The detector responses were calculated by a Varian model 475 electronic integrator.

*Results and discussion*

The regeneration technique described above was tested on three different mixtures which contained the DNPHs of 8 saturated aldehydes (A), 3 saturated ketones (B), and 3 unsaturated aldehydes (C), respectively. All of them were aliphatic. Table I below shows the results of 8 GC analyses of the homogeneous mixtures. About 1.5 mg

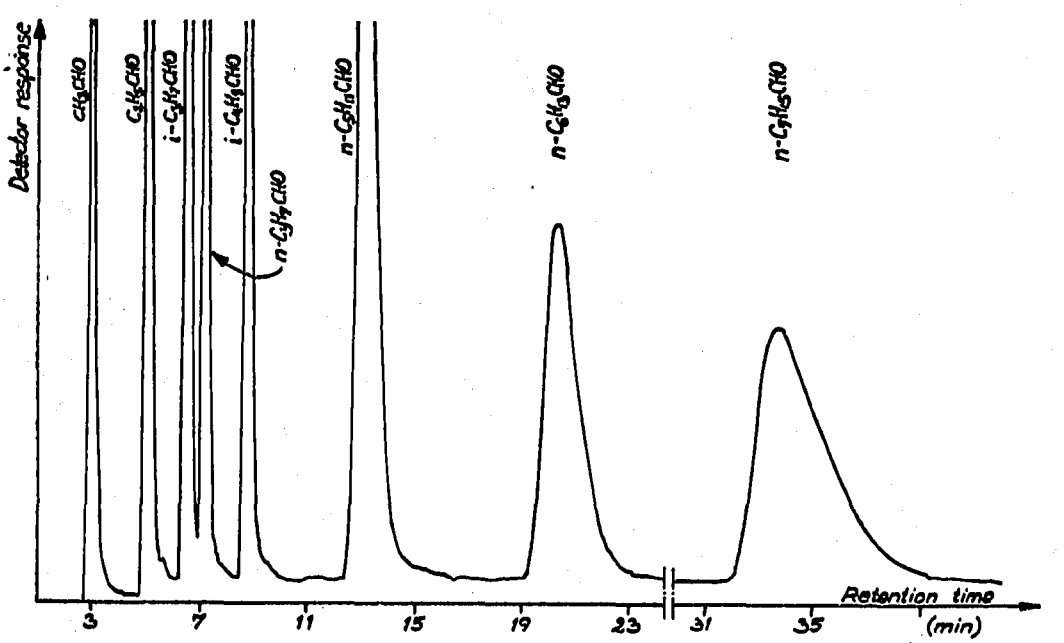
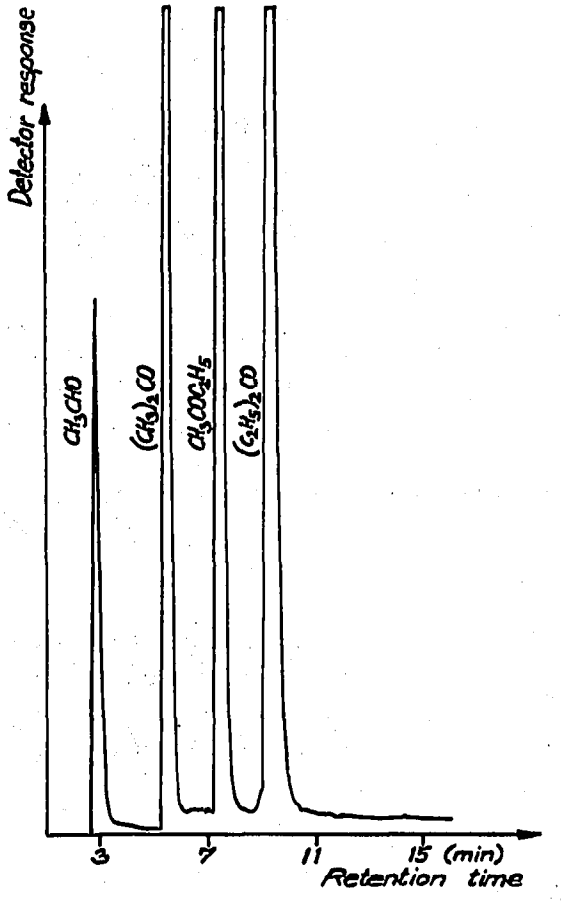



Fig. 2. Gas chromatogram of the regenerated mixture A. Conditions, see text. Range, $10^{-11}$; attenuation, 128.

of the A and B mixtures and about 2.5 mg of the C mixture were regenerated at each analysis. The results are expressed as relative detector response per 0.1 mg DNPH.

The values given in Table I are directly comparable to each other, *i.e.* they are determined or recalculated to be valid under identical conditions. Typical gas chromatograms from the three DNPH mixtures are shown in Fig. 2–4.



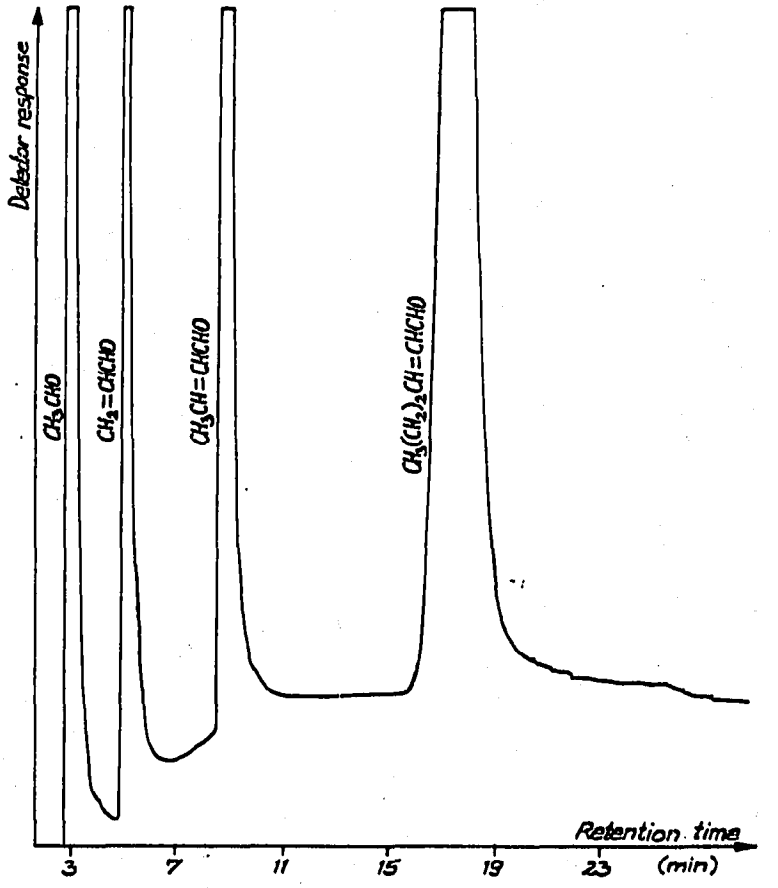




Fig. 3. Gas chromatogram of the regenerated mixture B in relation to $CH_3CHO$. Conditions, see text. Range, $10^{-11}$; attenuation, 128.

Fig. 4. Gas chromatogram of the regenerated mixture C in relation to $CH_3CHO$. Conditions, see text. Range, $10^{-11}$; attenuation, 16.

Is is evident from Table I that the precision of this method is quite satisfactory for quantitative work. The deviations from the mean values are of the same magnitude as those obtained by conventional injection of a standard solution of carbonyl compounds. The fact that it is more difficult to regenerate DNPHs from $\alpha$-$\beta$-unsaturated carbonyls compared to saturated is apparent from Table I. The amounts of the saturated carbonyls liberated are 5–10-fold greater than those from unsaturated DNPHs of comparable chain length.

With this method, it is possible to make accurate determinations of micro amounts of carbonyls with the utilization of a DNPH as an internal standard. We have obtained reproducible detector responses from quantities as small as 3 $\mu$g per DNPH. Is is difficult to handle smaller quantities, but by diluting the DNPH mixture with

pure 2,4-dinitrophenylhydrazine it is probably possible to analyse even smaller quantities. 2,4-Dinitrophenylhydrazine itself does not give any detector response when regenerated with α-ketoglutaric acid.

This reactor can probably be used to analyse DNPHs from other types of carbonyl compounds, *e.g.* dicarbonyls, aromatic aldehydes and ketones and keto acids. It has been claimed[4] that it is not possible to regenerate DNPHs from dicarbonyls. We have, however, succeeded in liberating diacetyl from its bis-DNPH even if the accuracy is not as good as that reported for the monocarbonyls.

TABLE I

RESULTS FROM EIGHT GC ANALYSES OF THE DNPH MIXTURES A, B AND C (see text)

| *Analysed DNPHs from* | *Relative detector response per 0.1 mg DNPH* | |
|---|---|---|
| | *Mean value* | *Confidence limits (95% probability)* |
| $CH_3CHO$ | 3.2 | ± 0.28 |
| $C_2H_5CHO$ | 5.8 | ± 0.25 |
| *i*-$C_3H_7CHO$ | 6.3 | ± 0.45 |
| *n*-$C_3H_7CHO$ | 5.1 | ± 0.21 |
| *i*-$C_4H_9CHO$ | 5.7 | ± 0.17 |
| *n*-$C_5H_{11}CHO$ | 8.8 | ± 0.16 |
| *n*-$C_6H_{13}CHO$ | 8.4 | ± 0.16 |
| *n*-$C_7H_{15}CHO$ | 8.8 | ± 1.17 |
| $(CH_3)_2CO$ | 6.3 | ± 0.09 |
| $CH_3COC_2H_5$ | 8.5 | ± 0.06 |
| $(C_2H_5)_2CO$ | 10.1 | ± 0.10 |
| $CH_2=CHCHO$ | 0.51 | ± 0.042 |
| $CH_3CH=CHCHO$ | 1.46 | ± 0.062 |
| $CH_3(CH_2)_2CH=CHCHO$ | 1.36 | ± 0.055 |

*Further applications*

This reactor can probably be used for the controlled destruction of other kinds of non-volatile substances to volatile compounds, either by heating or by the combined interaction of heat and some chemical substance.

The author is indebted to Mr. J. RITZING for his skilful practical work when the reactor-prototype was under development. Thanks are also due to Mrs. B. BERGER for running the gas chromatograms.

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