# Progress in the Gas Chromatographic Determinations of Carbonyls and Other Volatiles in Beer[1]

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## ABSTRACT

This paper is the fifth in a series covering the formation, isolation, and identification of 2-nonenal and is mainly concerned with reporting some of the difficulties encountered in developing a quantitative method of analysis for carbonyls in beer. Included in the paper are observations on direct injection, purging, sample and solvent purification, artifact formation and aldehyde instability. The results from these observations are incorporated in a refined procedure which offers simplification of the apparatus, the use of a more suitable internal standard (2-octanone), and improvements in the method of recovery. Several compounds have been identified by gas chromatography/mass spectrometry, including two which are peculiar to a physically and organoleptically unstable beer.


Key words: *2-Butanone, Carbonyls, Gas chromatography, 4-Methyl-2-pentanone, Volatiles.*

This paper is the fifth (3,4,5,6) in a series covering the formation, isolation, and identification of 2-nonenal, a compound contributing to cardboard flavor in beer. It is mainly concerned with reporting some of the difficulties encountered in developing a quantitative method of analysis for this and related compounds.

Wang and Siebert (8) developed a method to quantitate 2-nonenal, and our objective has been to include this compound in a carbonyls profile.

In our work, we have concentrated mainly on solvent extraction and the formation of dinitrophenylhydrazones (DNPHones) of the carbonyls and their subsequent regeneration and separation by flash exchange gas chromatography. We have also evaluated other techniques, including stripping and trapping and direct injection for the analyses of both carbonyls and other volatiles. This we consider essential, as carbonyls analysis is, in fact, only a part of the analysis of beer volatiles.

Much of our effort was spent on experiments concerned with obtaining reliable gas chromatographic (GC) data, selecting an internal standard, simplifying derivatization, and improving purification of the derivatives.

## EXPERIMENTAL GAS CHROMATOGRAPHY

When prospective internal standards were analyzed at concentrations of 10 to 100 mg/l. in 5% aqueous ethanol, great difficulties were encountered in obtaining reproducible data. When the GC effluent was monitored with a mass spectrometer, it was found that, while the main portion of the water in the sample eluted as a severely skewed peak overlapping the ethanol peak, a decreasing amount continued to come off slowly all through the chromatogram. As the water is not completely eluted before the end of the chromatogram, it affects the behavior of the next sample. This is manifested as a continual buildup of water which apparently affected the response of the detector (2) to the carbonyls. By maintaining the column at 200° C overnight, the water was almost completely eliminated, but by the next day the problem of water buildup would be reencountered. This effect was due to the water retention characteristics of the diatomaceous earth used as the GC solid support. To circumvent this, glass and Teflon beads were evaluated. These proved to be unsuitable, particularly since the liquid phase loading is too low for the amount of sample required for the detection of trace components. Use of a suitable sample size caused stripping of the liquid phase. Hydrophobic porous polymers solved the water problem, but were too retentive for high boiling polar compounds. However, the addition of 10% Carbowax 20 M to Chromosorb 101[2] (7) gave satisfactory results.

The use of glass tubing instead of stainless-steel tubing gave rise to chromatograms with less tailing of peaks and improved qualitative linearity at lower concentrations, which suggests reduced column reactivity.

On the basis of these experimental results, a direct injection method of analysis was devised, allowing completion of the evaluation of potential internal standards. It also produced good results when used for the analyses of beer volatiles which, in turn, permitted evaluation of the extraction step in the carbonyls procedure by comparing the volatiles before and after extraction.

This method also allowed an appraisal of the removal of volatiles by nitrogen and carbon dioxide flushing at different temperatures (25°–40° C) for various periods of time (1 hr to 7 days), with and without ammonium chloride saturation. The data obtained showed that enrichment (volatiles trapped at −80° C) was approximately 600-fold but that removal varied from 0.1 to 5%, depending on the type of compound, its original concentration, and the matrix (1). This means the technique is not suitable for the quantitation of beer volatiles.

## STABILITY OF REFERENCE COMPOUNDS

By determining the response factors of 2-undecenal and its DNPHone, we were able to calculate that only 6% of the 20 μg/l. being added as internal standard was being recovered. Another series of experiments showed that the added undecenal was

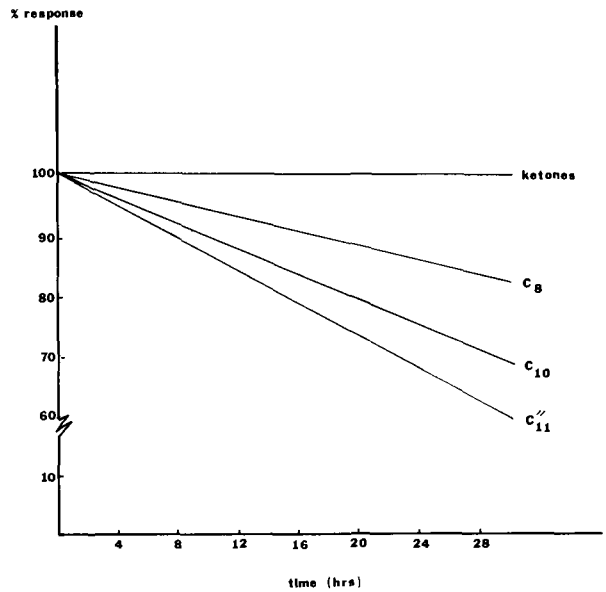


Fig. 1. Stability in 5% aqueous ethanol of 10 mg/l. each of 2-octanone, 2-decanone, 2-undecanone (ketones), n-octanal ($C_8$), n-decanal ($C_{10}$), and n-undecenal ($C''_{11}$).

[1]Presented at the 42nd Annual Meeting, Milwaukee, May 1976.

[2]This was suggested by S. B. Dave of Johns-Manville, Denver, Colo.

probably undergoing decomposition. Mixtures of aldehydes at 10 mg/l. in hexane, ethanol, or 5% aqueous ethanol were analyzed by direct injection after standing for various periods of time at room temperature. It was found that the aldehydes are stable in hexane, fairly stable in ethanol, and unstable in 5% aqueous ethanol. Under the same conditions, ketones were found to be stable in all three media (see Fig. 1) and 2-octanone was selected as the reference compound (internal standard).

## DERIVATIZATION

When 20 μg/l. of 2-octanone was added to ale or to synthetic beers, an artifact formation was observed, due probably to the interaction between some of the extracted compounds and the dinitrophenylhydrazine-impregnated alumina in the reactor. Consequently, the reactor was eliminated. This was done by adding dinitrophenylhydrazine (DNPHine) and trichloroacetic acid (TCA) to a reservoir equipped with a special still head (Fig. 2) to admit the extract to the bottom of the reservoir.

By adding 10 mg of 2-octanone to 1 liter of ale and comparing the DNPHone recovery to that of the same ale with no addition, a complete recovery of the 2-octanone was found. The control ale yielded 10.2 mg of hydrazones, which is equivalent to approximately 3 mg/l. of total carbonyls.

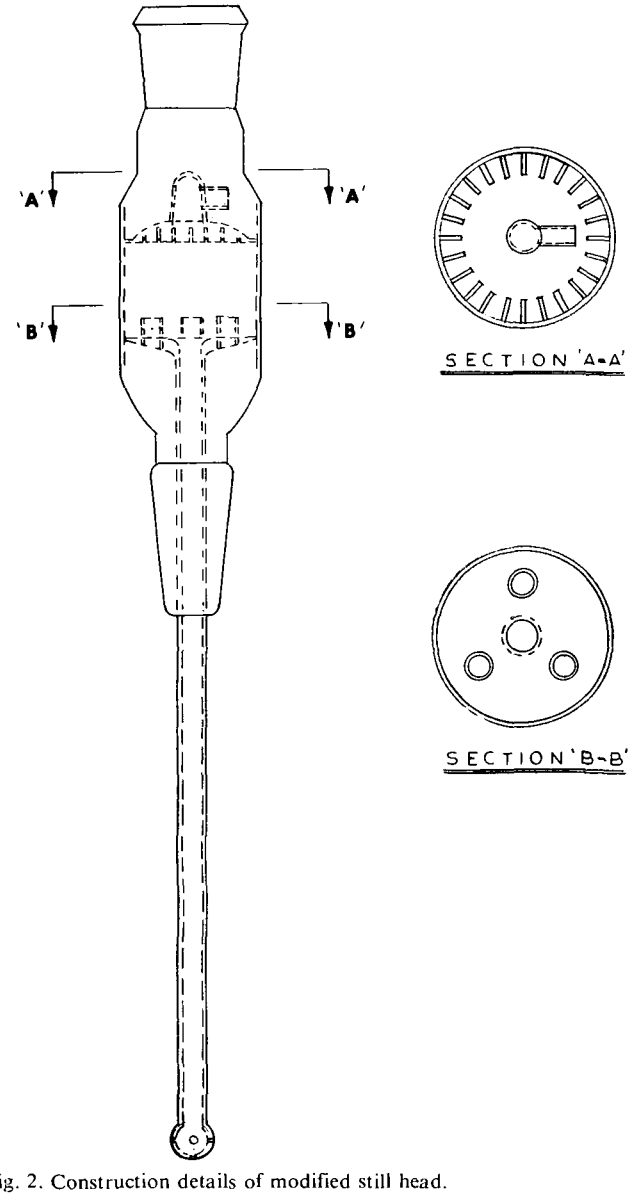


Fig. 2. Construction details of modified still head.

## PURIFICATION

A major part of the interfering peaks found in the chromatogram proved to arise from impurities in the hexane used for extraction. This problem was partially alleviated by eliminating the concentration of the extract prior to recovery of the DNPHones. Further improvements were realized by passing the hexane through a column of basic alumina before use. Another part of the interference was due to the use of excessive amounts of α-ketoglutaric acid caused by false DNPHone recovery weights. These false weights were the result of the presence of isoamyl and phenylethyl alcohols. The following evaluation of various solvents and adsorbants for the removal of these compounds has resulted in a great reduction of this source of error.

### Silica Gel

Various types and grades of silica gel and a wide selection of eluants were tried, but no combination was found which would separate the DNPHones from the alcohols.

### Magnesium Oxide/Celite

Partial separation was achieved, but the capacity of this type of column was too low for the amount of isoamyl and phenylethyl alcohols present.

### Acid Alumina

Alumina of various activities and several solvent systems were used, but an insufficient separation of the alcohols from the DNPHones was obtained.

### Neutral Alumina

No significant difference was found compared to acid alumina.

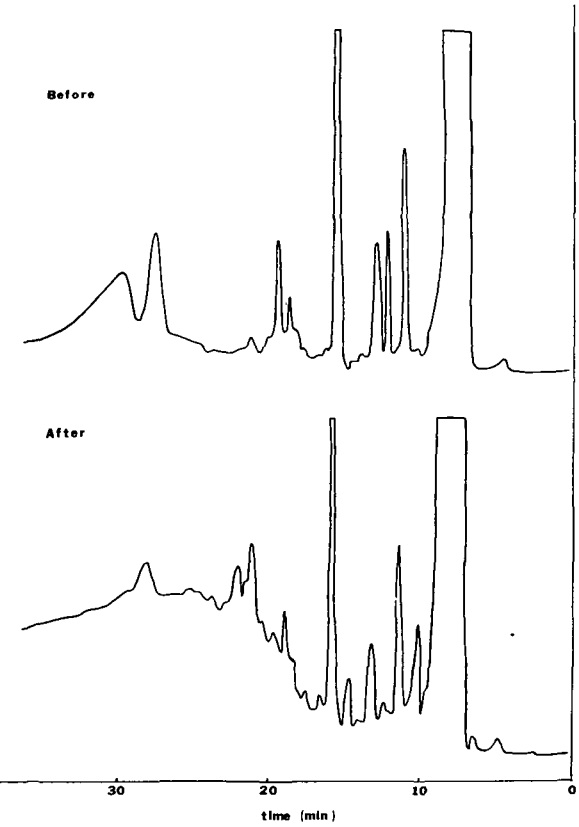


Fig. 3. Removal of volatiles from ale by purging with nitrogen. The upper chromatogram shows the results of direct injection analysis of 1 liter of ale saturated with ammonium chloride at 40°C before purging for 24 hr at 30 ml/min, and the lower chromatogram shows the result after purging.

**Basic Alumina**

Satisfactory resolution and capacity were achieved when the moisture content of the basic alumina was between 1 and 2% and freshly distilled chloroform was used as the eluant.

**Determination of Carbonyls in Beer**

The method for the determination of carbonyls in beer (5) has now been refined as follows:

**Reagents**

Basic alumina—Woelm Activity I (moisture 1–2%);

Hexane, purified—practical grade hexane is passed through a column of basic alumina;

2,4-Dinitrophenylhydrazine (DNPHine) — reagent grade;

Trichloroacetic acid (TCA)—reagent grade;

Chloroform—reagent grade chloroform is glass distilled just prior to use and the last 10% collected is discarded;

2-Octanone—reagent grade;

2-Octanone stock solution—1% reagent grade in absolute ethanol, store under refrigeration;

2-Octanone internal standard—5 ml stock solution diluted to 50 ml with absolute ethanol (prepare weekly, store under refrigeration); and

α-Ketoglutaric acid—mp 115°–116°C.

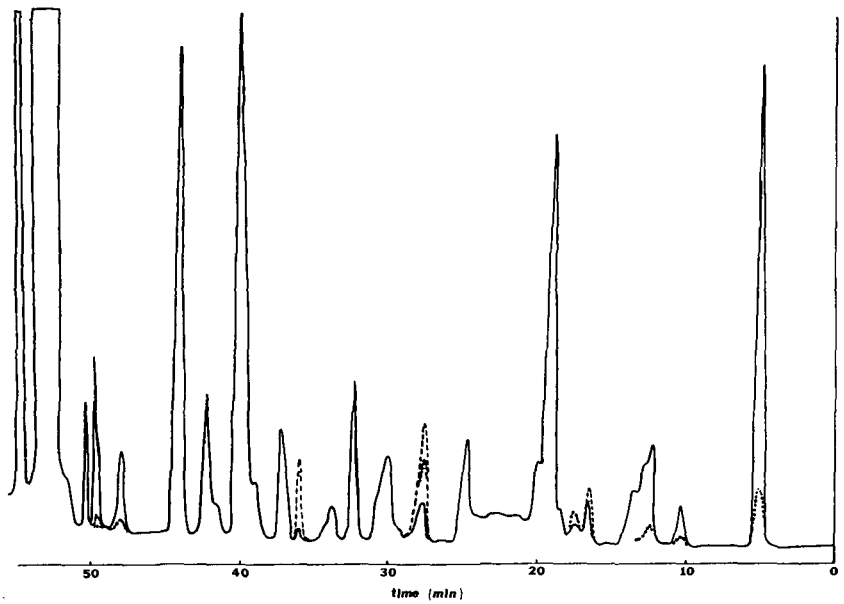


Fig. 4. A chromatogram of regenerated carbonyls from pasteurized ale showing the effect of thermal degradation (24 hr at 60°C)- - - - -, and oxidation damage (high air content, rotated end-over-end at 10 rpm for 3 days) . . . . . .

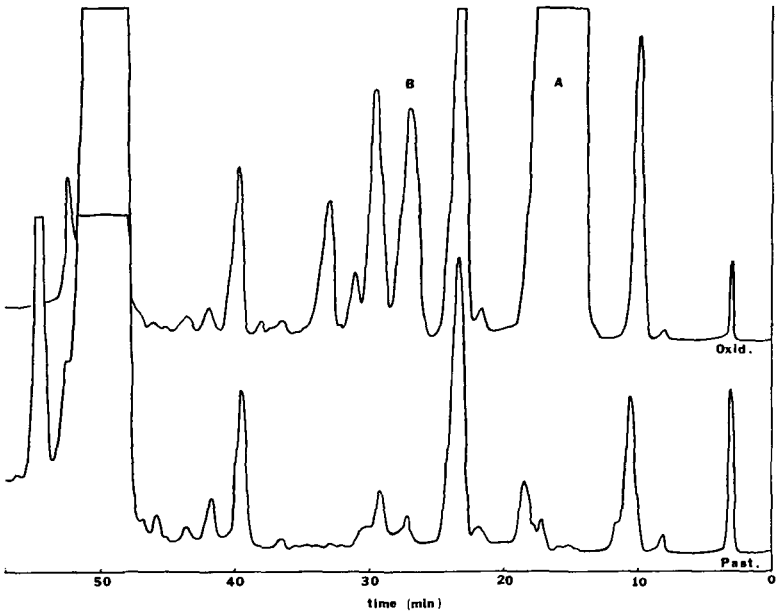


Fig. 5. Changes due to oxidation damage on an unstable ale. The lower chromatogram shows the result obtained from the regenerated carbonyls from the pasteurized ale, and the upper trace is from the same ale after severe oxidation. Peak A is 2-butanone and peak B is 4-methyl-2-pentanone.

**TABLE I**
**Identification of Various Peaks in the Chromatogram from Regenerated Carbonyls. Identification is by Comparison of the Retention Time and Mass Spectrum of the Unknown Compared to Those of Reference Compounds**

| Peak No. | Retention Time | Compound (GC + MS) |
|---|---|---|
| 1 | 3.2 | Ethanal (acetaldehyde) |
| 2 | 8.4 | ... |
| 3 | 10.6 | 2-Propanone (acetone) |
| 4 | 11.7 | ... |
| 5 | 15.3 | ... |
| 6 | 16.3 | ... |
| 7 | 17.6 | n-Butanal |
| 8 | 18.3 | ... |
| 9 | 18.7 | 2-Butanone |
| 10 | 22.4 | 2-Pentanone |
| 11 | 23.7 | Isoamyl alcohols |
| 12 | 27.6 | 4-Methyl-2-pentanone |
| 13 | 29.6 | 3-Hexanone |
| 14 | 31.0 | 2-Hexanone |
| 15 | 32.5 | ... |
| 16 | 33.4 | ... |
| 17 | 35.0 | ... |
| 18 | 37.1 | Furfural (2-furaldehyde) |
| 19 | 40.0 | 2-Octanone (I.S.) |
| 20 | 42.5 | ... |
| 21 | 44.3 | ... |
| 22 | 46.6 | ... |
| 23 | 47.5 | ... |
| 24 | 50.4 | Phenylethyl alcohol + other compounds |
| 25 | 55.1 | ... |

### Apparatus

The apparatus was as previously described (6), except that the reactor was replaced by the still head (Fig. 2) and DNPHine and TCA were added to the reservoir.

### Derivatization

*Procedure.* The reactor/reservoir is prepared containing 0.2 g DNPHine, 0.2 g TCA, and 1 liter hexane. Internal standard stock solution (100 $\mu$l) is added to 1 liter of beer (previously degassed by rapid filtration at 5°C), and the mixture is placed in the extractor. The apparatus is immediately assembled, heat is applied, and the process is allowed to continue. The upper part of the extractor is maintained at 60°C with heating tape. After 20 hr, the reactor/reservoir is removed and the hexane mixture is cooled to 20°C.

*Recovery.* The hexane containing the derivatives is passed through a glass chromatographic column (30 × 2 cm) containing 20 g basic alumina. The DNPHones are then eluted with chloroform and only the first colored band is collected. The chloroform is evaporated in a tared Silli-vial, followed by overnight drying in a vacuum desiccator and subsequent weighing to determine the total amount of DNPHones obtained.

*Regeneration.* The recovered hydrazones are intimately mixed with 10 mg of $\alpha$-ketoglutaric acid in the Silli-vial using a glass rod. Approximately 2 mg of the mixture is taken for GC analysis.

*GC Analysis.*

Apparatus

Perkin-Elmer Model 990 with MS-41 solids sampler as previously described (6).

Conditions

Carrier Gas—Helium 40 ml/min.
Column—10% Carbowax 20 M on Chromosorb 101, 80–100 mesh in 4 ft × 1/8-in. glass column.
Program—Hold 4 min at 50°C, then increase at 3°/min to 200°C.
Detector—F.I.D. at 250°C.
Injection—15 sec hold at 250°C prior to piercing.

## RESULTS AND DISCUSSION

The effect on the volatiles seen by direct injection analyses of ale saturated with ammonium chloride, before and after purging at 40°C for 24 hr using nitrogen is seen in Fig. 3. This shows that not only are very little of the volatiles removed but that some compounds increase in concentration. These increases probably are due to the presence of impurities in the nitrogen and reactions between the ale components and some of these impurities (*e.g.* oxygen).

Carbonyls analyses of different brands gave similar chromatograms; the differences were mainly in relative peak size rather than in the presence or absence of peaks. The analyses were very reproducible.

Several compounds have been found to suffer thermal and oxidation changes. These changes can be increases or decreases where thermal and oxidation damage compete or complement (Fig. 4). Beer found to be physically and organoleptically unstable showed two highly significant changes (Fig. 5), and the compounds responsible for them were identified as 2-butanone and 4-methyl-2-pentanone.

Approximately 25 peaks have been examined and Table I shows identifications confirmed by gas chromatography and mass spectrometry.

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