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Note

Determination of diacetyl in beer by gas chromatography with flame-ionization detection

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Diacetyl is one of the carbonyl compounds that has a great influence on the flavour of beer owing to its low threshold, which varies between 0.05 and 0.4 ppm. As its concentration in normal beer is usually between 0.1 and 0.3 ppm, a small increase causes the beer immediately to acquire a typical unpleasant diacetyl taste.

Several methods have been described for the determination of diacetyl in beer, including spectrophotometry[1–4] and chromatography with an electron-capture detector (ECD)[5,6]. As a rule, the spectrophotometric methods determine the total amount of vicinal diketones, mainly diacetyl and pentanedione-2,3, whereas the chromatographic methods have a smaller risk of interferences. When the ethanol is present in a much higher proportion than diacetyl, the tail of the ethanol peak interferes heavily, and the determination of small amounts of diacetyl becomes impossible. The means of avoiding such an interference is through the use of an ECD because, as diacetyl has a much higher electron affinity than ethanol, it should have a sensitivity of the order of 40,000 times higher[7]. Another means of solving the problem would be to use mass fragmentography, which, through the registration of the molecular ion of diacetyl, could give a selective signal for this compound, avoiding completely the interference of ethanol.

In this paper we propose the use of the selective retention of ethanol by utilizing a post-column containing boric acid[8–10], which permits the detection of small amounts of diacetyl without the interference of the ethanol peak, making use of a conventional flame-ionization detector (FID). In order to be able to detect 10 ppb of diacetyl in beer, recourse was made to a technique of previous concentration, similar to that proposed by Bertran[8].

EXPERIMENTAL

Gas chromatography

All gas chromatographic separations were performed with a Perkin-Elmer Model 990 chromatograph equipped with two FIDs. The column packing was Gas-

Chrom Q (80–100 mesh) coated with 8% Carbowax 20M. Stainless-steel columns (2 m × 3 mm I.D.) were used. The oven temperature was 60°, the injector 150° and the detector 175°. Nitrogen was used as the carrier gas at a flow-rate of 15 ml/min.

The post-column (30 cm × 3.2 mm I.D.) was coated with 8% Carbowax 20M on Chromosorb P-AW plus 3% of boric acid. In order to prepare the packing of the post-column, the stationary phase was first deposited on the support; then 3% of boric acid, thoroughly powdered, was added, as well as light petroleum in a sufficient amount to cover all of the solid; the mixture was thoroughly stirred and allowed to stand overnight, then the solvent was vaporized in a Rotavapor.

Mass fragmentography

The work was carried out with a Hitachi-Perkin-Elmer Model RM-60 mass spectrograph coupled to the chromatograph, manually focusing the molecular ion of the diacetyl ($m/e = 86$).

Concentration system

Fig. 1 shows the apparatus used in order to obtain a solution enriched in the volatile fraction of beer. The optimal conditions were thermostat temperature, 35°; nitrogen flow-rate, 200 ml/min; sweep time, 1 h; volume of beer to be extracted, 400 ml; antifoaming agent, 2 drops of SAG-470.

For the chromatographic analysis, use was made of the fraction collected on dry-ice and acetone, as it contains the same components as that collected on ice and common salt, but with greater enrichment.

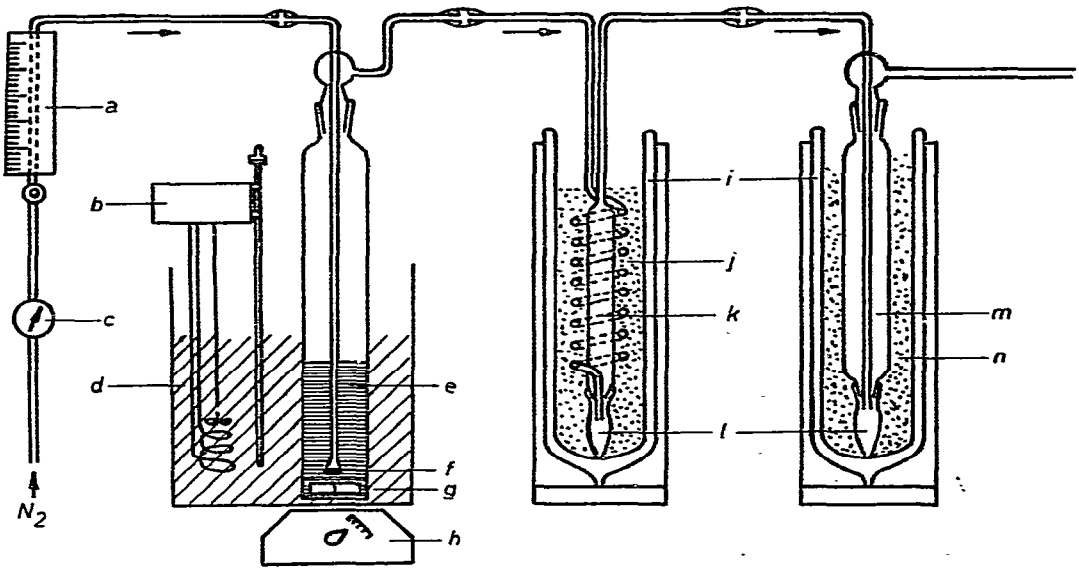


Fig. 1. Apparatus used in order to obtain a solution enriched in the volatile fraction of beer: (a) Rotameter; (b) thermostat; (c) manometer; (d) water-bath; (e) beer; (f) porous plate; (g,h) magnetic stirrer; (i) Dewarflask, (j) ice and common salt, (k) helical condenser; (l) collectors; (m) condenser; (n) dry-ice and acetone.

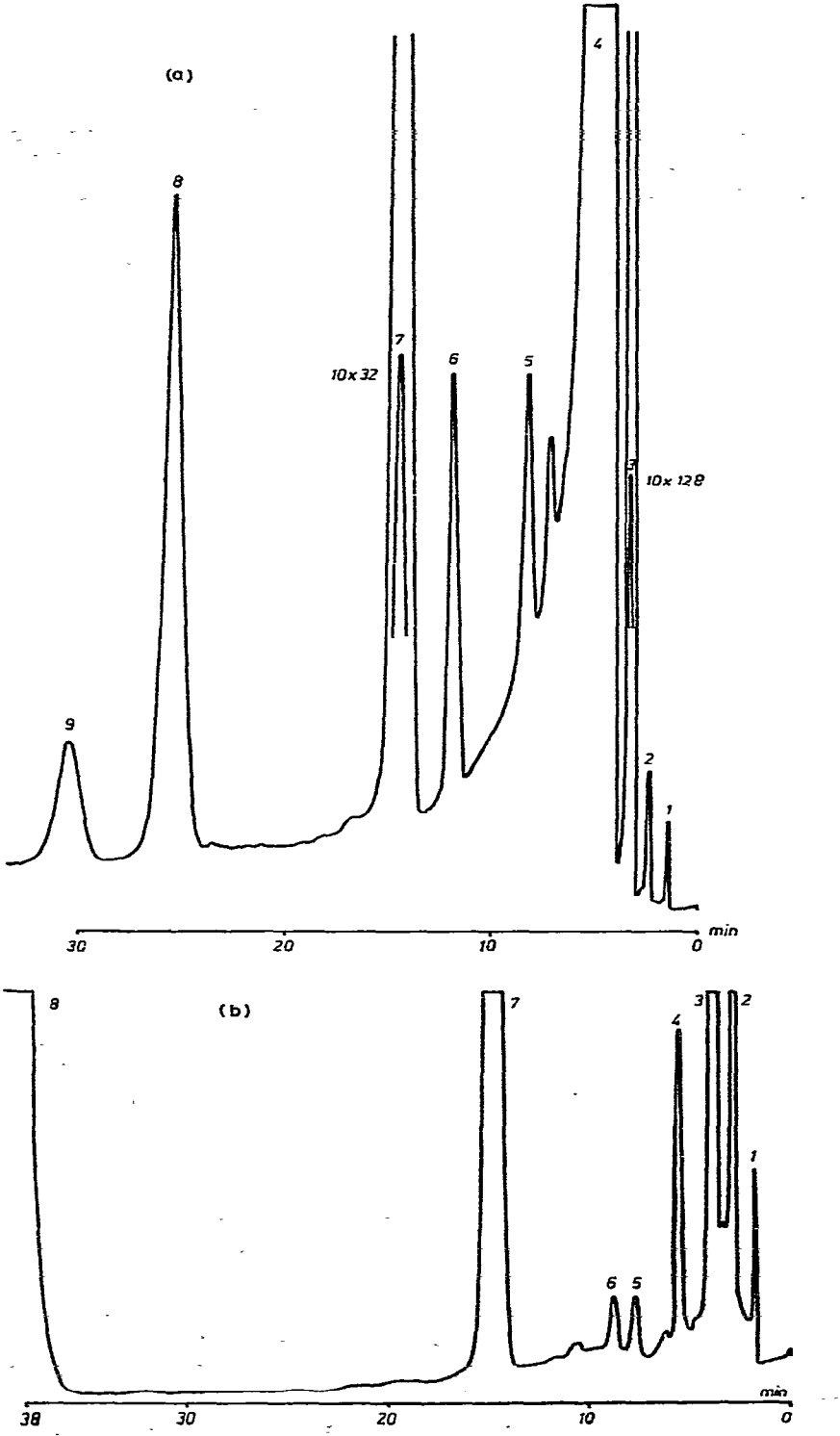


Fig. 2. (A) Chromatogram of a sweep without a post-column: (1) acetyldehyde; (2) acetone; (3) ethyl acetate; (4) ethanol; (5) propanol; (6) isobutanol; (7) isoamyl acetate; (8) isoamyl alcohol; (9) unknown. (b) Chromatogram of a sweep with a post-column; (1) acetaldehyde; (2) acetone; (3) ethyl acetate; (4) diacetyl; (5) and (6) unknown; (7) isoamyl acetate; (8) ethanol.

RESULTS AND DISCUSSION

Fig. 2a shows the chromatogram of a fraction of beer volatiles in which can clearly be seen the strong interference of the ethanol peak, which disappears when the boric acid post-column is used (Fig. 2b); in this instance, the retention time of the ethanol was increased eight-fold, which permits peaks 4, 5 and 6, which were totally masked in the chromatogram in Fig. 2a, to be seen. The identification of peak 4 as diacetyl was checked by combined gas chromatography–mass spectrometry (Fig. 3); in addition, from a comparison of Figs. 2a and 2b it can be concluded that the post-column did not affect the retention times of acetaldehyde, acetone, ethyl acetate and isoamyl acetate.

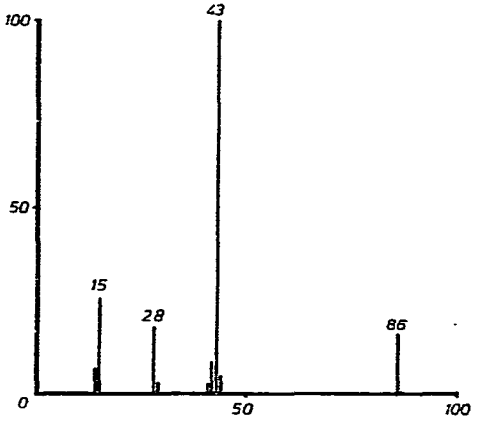
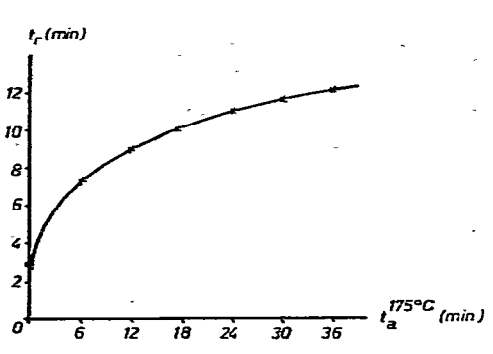


Fig. 3. Mass spectrum of diacetyl.

There are two reasons why boric acid should be used in a post-column instead of in a pre-column. Firstly, if a pre-column is used, the boric acid appears to pass into the column, as the latter is slowly degraded. The second reason lies in the dehydrating action of boric acid on tertiary and unsaturated alcohols, giving rise to the corresponding olefins; thus, when using boric acid in a pre-column, a tertiary alcohol would be eluted as if it were an olefin with a much shorter retention time, whereas if it is used in a post-column, the same tertiary alcohol will have its own retention time, although what is really detected may be the peak of the corresponding olefin.

It has been observed that the efficiency of the post-column on the increment in the retention time of ethanol increases with the conditioning time at 175° (Fig. 4), passes through a maximum and then gradually loses efficiency. Despite this effect, the post-column is very useful, as it permits about 200 analyses, on average, to be carried out. On the other hand, this post-column should not be used at temperatures above 70°, as it loses much of its efficiency as it retains the ethanol to a lesser extent.

It is not possible to use a substance containing an alcohol group as an internal standard; methyl isoamyl ketone is eluted just after isoamyl acetate and does not interfere with the other peaks of interest. Fig. 5 shows a calibration line for the determination of diacetyl between 0.1 and 1 ppm using this ketone as an internal standard.



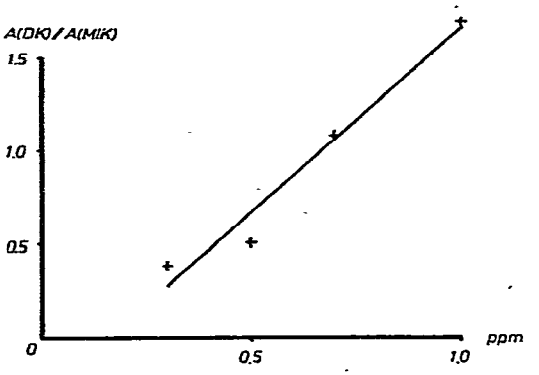
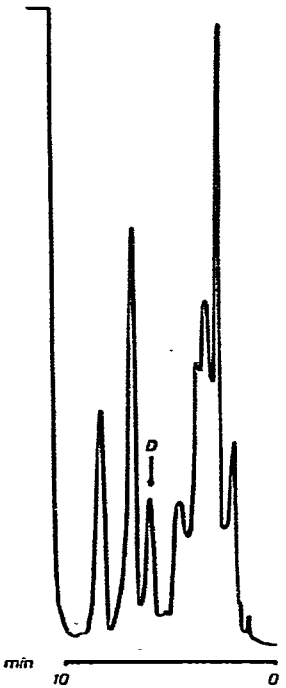


Fig. 4. Efficiency of the post-column *versus* conditioning time.

Fig. 5. Calibration line. DK = diketone (diacetyl), MIK = methyl isoamyl ketone.

We found that it is important that the chromatographic analysis should be carried out immediately after the sweeping, as otherwise erroneous results would be obtained with a considerable decrease in the measured values of diacetyl and acetone. Following the technique proposed here, we have succeeded in detecting 10 ppb of diacetyl in a solution of ethanol, as can be seen in Fig. 6.



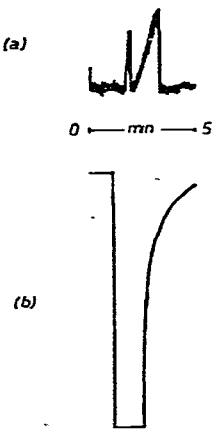


Fig. 6. Chromatogram of 10 ppb of diacetyl (D).

Fig. 7. Single ion monitoring of 0.05 ng of diacetyl (ethanol solution): (a) chromatogram: (b) monitogram.

Single ion monitoring

The aim of this experiment was to study the possibility of quantifying the diacetyl without the need for a previous enrichment, even when it is being eluted jointly with ethanol; such a measurement seems possible, on the one hand because the mass spectrometer can react selectively to the diacetyl, and on the other because the electron multiplier of the mass spectrometer has a sufficient sensitivity to be able to detect the absolute amount that reaches it by direct injection (*ca.* 0.1 ng).

From the mass spectrum of diacetyl (Fig. 3), it is possible to select the fragment of *m/e* 43 or of *m/e* 86; however, as the former is a very small fragment that occurs very frequently with a large number of organic compounds, it was considered to be more useful working with the fragment of *m/e* 86, sacrificing some of the sensitivity for the sake of selectivity. Fig. 7 shows the mass spectrometric results following the injection of 0.5 μl of a 0.1 ppm solution of diacetyl in ethanol; two peaks occur very close to each other, one of them perfectly symmetrical, corresponding to diacetyl, and the other with an asymmetrical shape. On making several consecutive injections of 0.5 μl of this solution, it was seen that the diacetyl signal was not reproducible, oscillations of up to $\pm 100\%$ on the mean signal being observed. However, when a 0.1 ppm solution of diacetyl in isopropanol was employed, the reproducibility of the diacetyl signal was good, showing a maximum oscillation of 10%. In this instance, we also obtained a peak with an asymmetrical shape almost identical with that obtained with the solution in ethanol (Fig. 8).

From the above results, the conclusion was drawn that both the ethanol and the isopropanol always gave results corresponding to *m/e* 86 although, from their molecular weights, they should not give such a fragment; on the other hand, when

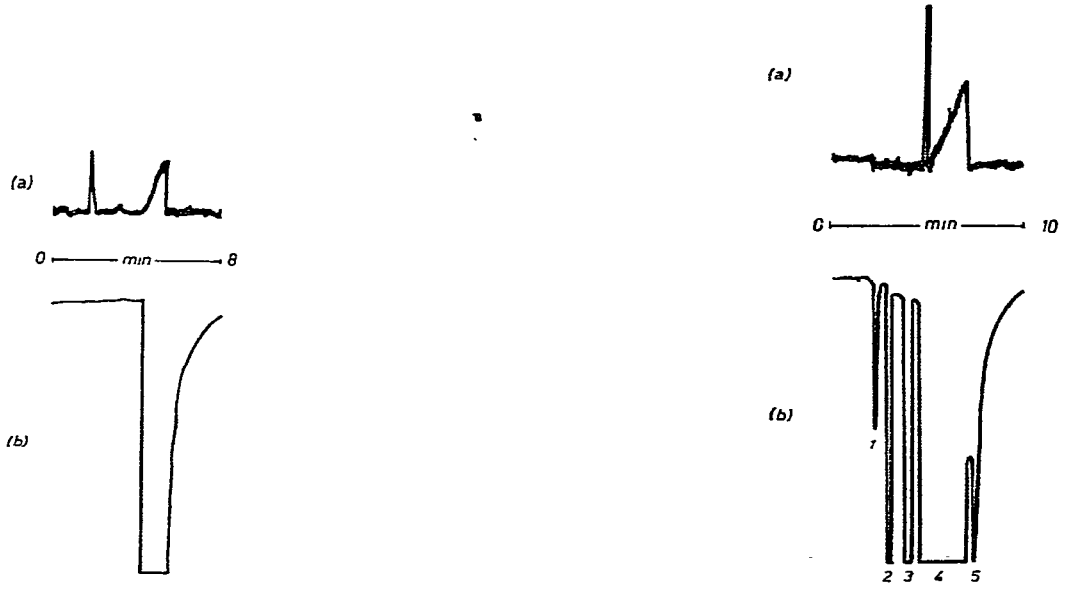


Fig. 8. Single ion monitoring of 0.05 ng of diacetyl (isopropanol solution): (a) chromatogram; (b) monitogram.

Fig. 9. Single ion monitoring of beer (*m/e* 86): (a) chromatogram; (b) monitogram.

we selected the fragment of *m/e* 80 and *m/e* 88, the diacetyl signal disappeared, but the other one remained unaltered. Hence the signal given by both the isopropanol and the ethanol does not represent real fragments, but rather a variation of the background signal of the electron multiplier of the detector in the mass spectrometer, as a result of the vacuum loss upon the entry into it of a large amount of alcohol. This vacuum loss is responsible for the non-linearity of the response to diacetyl when a solution of diacetyl in ethanol is injected into the chromatograph because the diacetyl and the ethanol enter the molecular separator together and, upon the vacuum loss, the jet separator loses efficiency, as some of the diacetyl may go to the vacuum pump. However, this does not take place when using a solution in isopropanol because, as the two compounds are already chromatographically separated, the efficiency of the separator decreases when the diacetyl has already reached the interior of the mass spectrometer; this is the reason why in this second instance we obtain a repeatable signal for the diacetyl.

It can be concluded that mass fragmentometry suffers from serious difficulties, particularly in the determination of diacetyl in beer, although it becomes applicable if a boric acid post-column is used to retain the ethanol. Thus, when both techniques were combined, the results in Fig. 9 were obtained, and the detection of diacetyl did not present any problems on direct injection. Nevertheless, in order to determine diacetyl in beer, we believe that the technique proposed here, including a post-column containing boric acid, is adequate and need be combined with mass fragmentography only when it is necessary to determine the diacetyl at a level below 1 ppb.

ACKNOWLEDGEMENTS

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