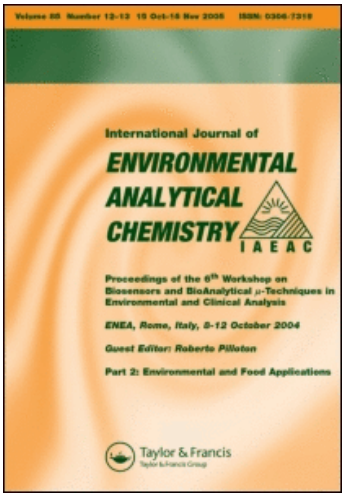

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of Biacetyl in Beer by Liquid Chromatography with Sensitized Phosphorescence Detection

R. A. Baumann[a]; C. Gooijer[a]; N. H. Velthorst[a]; R. W. Frei[a]; J. Strating[b]; L. C. Verhagen[b]; R. C. Veldhuyzen-doorduin[b]
[a] Department of General and Analytical Chemistry, Free University, Amsterdam, HV, The Netherlands
[b] Heineken Technisch Beheer B.V., Zoeterwoude, The Netherlands

To cite this Article Baumann, R. A. , Gooijer, C. , Velthorst, N. H. , Frei, R. W. , Strating, J. , Verhagen, L. C. and Veldhuyzen-doorduin, R. C.(1986) 'Determination of Biacetyl in Beer by Liquid Chromatography with Sensitized Phosphorescence Detection', International Journal of Environmental Analytical Chemistry, 25: 1, 195 — 207
To link to this Article: DOI: 10.1080/03067318608077088
URL: http://dx.doi.org/10.1080/03067318608077088

Intern. J. Environ. Anal. Chem., 1986, Vol. 25, pp. 195–207
0306-7319/86/2503-0195 $18.50/0

Printed in Great Britain

Determination of Biacetyl in Beer by Liquid Chromatography with Sensitized Phosphorescence Detection†

R. A. BAUMANN, C. GOOIJER, N. H. VELTHORST and R. W. FREI

Department of General and Analytical Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

and

J. STRATING, L. C. VERHAGEN and R. C. VELDHUYZEN-DOORDUIN

Heineken Technisch Beheer B.V., P.O. Box 510, 2380 BB Zoeterwoude, The Netherlands

(*Received December 10, 1985*)

A new determination method for biacetyl in beer is developed. After a simple sample preparation method and separation by HPLC, biacetyl is detected by sensitized room temperature phosphorescence. A detection limit of 0.5 ppb biacetyl in beer can be obtained. The linearity of response is 0.5–150 ppb; the relative standard deviation is 4.2% at the 16 ppb level. Prepared samples are stable for at least 20 hours when kept in closed vials. The separation of biacetyl and 2,3-pentanedione, an α-diketone also present in beer, is described. It is found that sensitized phosphorescence detection is about a factor of 30 less sensitive for 2,3-pentanedione. The reliability of the method is demonstrated by comparison with a routine head space gas chromatography method.

INTRODUCTION

During fermentation processes in young beer, pyruvate (2-aceto-2-acetoxypropionic acid-ethyl ester) is converted enzymatically to α-

†Presented at the 2nd Symposium on Handling of Environmental and Biological Samples in Chromatography. October 24–25, 1985, Freiburg, F.R.G.

acetolactate.[1] This compound can decompose spontaneously and biacetyl (2,3-butanedione) and/or acetoin is formed. The decomposition of α-acetolactate to biacetyl is a non-enzymatic oxidative decarboxylation which is accelerated by low pH, elevated temperatures, high oxygen concentration and the presence of catalyzing/oxydizing metalions, especially Fe(III) and Cu(II).[1-3] As long as yeast is present in the fermenting liquid the concentration of biacetyl is near zero due to the high reducing activity of the yeast cells; it is converted to acetoin and 2,3-butanediol. When the yeast activity is decreasing and has finally become zero at the end of the fermentation process the level of biacetyl increases as it is still formed from α-acetolactate but not broken down by the yeast cells any more.

A concentration of more than 150 ppb biacetyl in beer has a negative influence on its taste. Hence, it is important to quantitate biacetyl in final beer for reasons of quality control. If necessary, the brewing process can be adjusted in order to achieve an adequate breakdown of α-acetolactate and biacetyl.

Till now, head space gas chromatography[4-6] and colorimetric techniques[7-9] are the most frequently applied methods for the determination of biacetyl.

Recently we have shown that time-resolved sensitized phosphorescence used as a detection method in liquid chromatography for biacetyl can be a sensitive and simple technique compared to other methods at least for the analysis of standard solutions. The choice of a suitable energy donor in the sensitized RTPL method was discussed in terms of spectroscopic properties. Also the luminescence detector, working in a pulsed source-time resolved phosphorescence mode, was described. It was outlined that optimizing the instrumental parameters of this instrument led to an increased sensitivity compared with conventional detectors. However, analyzing real beer samples, problems were encountered regarding the choice of an effective clean-up procedure.

This paper describes a sample preparation method for beer based on the high volatility of biacetyl. It is liberated from a heated sample by a nitrogen gas stream and collected in a small volume of mobile phase. After a fixed purge time an aliquot of this solution is injected onto the column. In this way a clean chromatogram is obtained and it is possible to detect biacetyl quantitatively in beer down to a concentration of 0.5 ppb. Furtheron it is shown that 2,3-

pentanedione, an α-diketone also present in beer, can be detected by the sensitized RTPL method on the same HPLC system simultaneously with biacetyl. The relative sensitivity of the detection method for the two compounds is discussed. Finally, a comparison is made between a routinely used head space GC method and this new sensitized phosphorescence/HPLC method for the quantitative determination of biacetyl in beer. The GC method employs an Electron Capture Detector (ECD) after separation of a head space sample of beer on a capillary column.

EXPERIMENTAL

Chemicals

All reagents used were analytical grade unless stated otherwise. The source of NaH_2PO_4. H_2O, sodium hydroxide, acetonitrile, 1,5-naphthalene disulfonic acid disodium salt (NDSA), biacetyl and water has been given.[10] Methylglyoxal (2-oxo-propionaldehyde) was obtained from EGA (Steinheim, F.R.G.) as a technical 40% solution in water. 2,3-Pentanedione (80%) was provided by Fluka (Buchs, Switzerland). Beer samples were kept in the original bottles and stored at 4°C.

Apparatus and chromatographic conditions

HPLC A description of the HPLC system has been published.[10] The eluent vessel in this system is described in detail in ref. 11.

The thermostated bath was from Tamson (Zoetermeer, The Netherlands). Separations were performed on a 25 cm × 4.6 mm I.D. 5 μm ODS Spherisorb column with 70/30 (v/v) water/acetonitrile; 5.10^{-3} M phosphate buffer pH 6.5; 2×10^{-4} M NDSA as the mobile phase. The applied detector was the Perkin-Elmer Model LS-2 (Perkin-Elmer, Beaconsfield, U.K.) filter fluorimeter operated in the phosphorescence mode. The delay time was 0.1 msec, the gating time 0.9 msec. For excitation an interference filter with a maximum of 310 nm was used, the emission wavelength was 516 nm. The injection volume was 50 μl and the flow rate 1.0 ml min^{-1}. Analyses were performed at ambient temperature. Batch experiments were carried out as described previously.[10]

GC The column was a 100 metre capillary (0.5 mm I.D.) coated with didecylphthalate. The injector temperature was 125°C and the oven temperature 60°C. As carrier gas nitrogen was used (pressure 1.05 atm) with a flow rate of 13 ml min^{-1}. The ECD temperature was 150°C, the voltage 50 V and the pulse width 5 μs constant current.

Sample preparation

HPLC Sample preparation for biacetyl determination was as follows. Five ml of cold (4°C) fresh beer sample was added to a glass tube with grind joint NS 14.5. The tube was placed in a 50°C water bath and simultaneously stoppered with a PTFE stopper in which two holes (diameter 1/16″) were drilled. In one hole an O.D. 1/16″ stainless steel capillary was placed through which nitrogen gas was led with a flow rate of 60 ml min^{-1} to purge the sample. Volatile components, including biacetyl, were liberated from the sample and taken with the nitrogen stream through a similar 1/16″ capillary placed in the second drilled hole. This capillary was led in a second glass tube filled with 1 ml of mobile phase kept at room temperature. A part of the components coming from the sample were collected in this solution. After 10 min of sample purging 50 μl of the solution was directly injected onto the column. The experimental set-up of the sample purge unit is given in Figure 1.

GC To a vial with septum containing 2 g of cold beer 16 μl of internal standard (1.62 mg 2,3-hexanedione in 5% ethanolic solution) is added. After 30 min equilibration in a waterbath at 30°C, 0.5 ml of the vapour is collected with a gas-tight syringe and injected onto the column.

RESULTS AND DISCUSSION

Biacetyl

Experiments concerning the removal of biacetyl from beer or standard solutions by purging with nitrogen gas were originally carried out at ambient temperature and collecting the volatile components in 1 ml of mobile phase which was cooled in ice at 0°C. Under these conditions the maximum recovery found for biacetyl was about 20%.

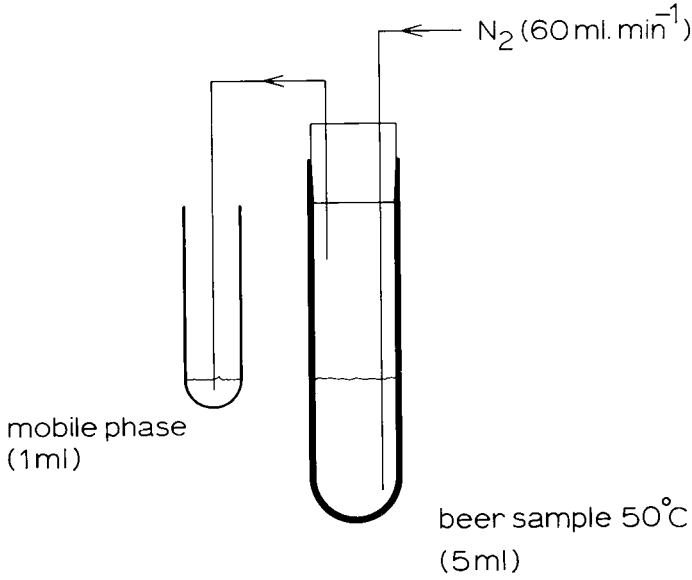

FIGURE 1 Purge system.

This value was reached after 30 min of sample purging with a flow rate of 60 ml min^{-1}. This low recovery is probably due to the slow removal of biacetyl from the sample. The recovery remained the same when the collection solution was kept at room temperature instead of 0°C. By heating the sample in a thermostated bath during purging the purge time could be reduced considerably while the maximum recovery was increased. A plot of the recovery against purge time for a 50 ppb standard biacetyl solution heated in a water bath at 50°C is shown in Figure 2. It is seen that the maximum recovery is reached after a purge time of 10 min. For standard solutions up to a 150 ppb biacetyl concentration as well as for real beer samples the optimal recovery was $32 \pm 1\%$. The decrease in recovery when purge times longer than 10 min are employed is due to loss of biacetyl from the collection solution. As 5 ml sample is used and biacetyl is collected in 1 ml liquid, a concentration with a factor of $5 \times 0.32 = 1.6$ compared to the original concentration in the sample, is achieved.

A chromatogram for a beer sample containing 14 ppb biacetyl is given in Figure 3. Since the sample clean-up procedure as well as the

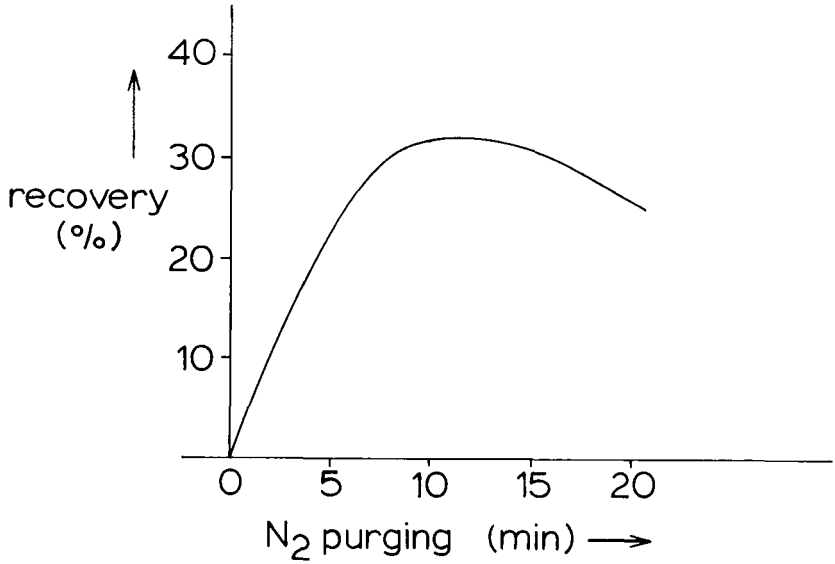

FIGURE 2 Recovery vs. purge time for a 50 ppb biacetyl standard solution.

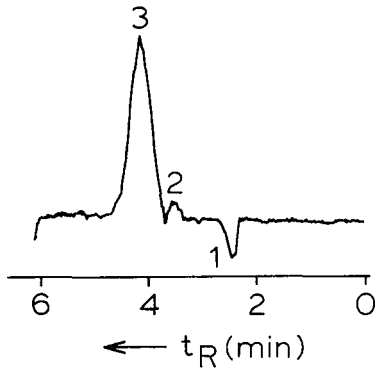

FIGURE 3 HPLC chromatogram of a beer sample containing 14 ppb biacetyl: (1) solvent front; (2) unknown; (3) biacetyl.

detection method are highly selective a very clean chromatogram is obtained. The detection limit calculated from this and similar chromatograms based on a signal to noise ratio of 3 is 0.5 ppb biacetyl in beer. The biacetyl concentration of a sample was cal-

culated by the method of standard addition. Care was taken that the total biacetyl concentration in the beer sample did not exceed 150 ppb because of the limited linear range of the sensitized phosphorescence detection method.[10] A linear calibration curve with a regression coefficient of 0.9997 ($n=6$) was obtained. The reproducibility of the method was investigated by taking four separate 5 ml aliquots of the same beer sample and applying the standard sample handling procedure. The relative standard deviation calculated at the 90% probability level was 4.2%.

Other α-diketones

It is known that apart from biacetyl other α-diketones can also be formed from α-ketomonocarboxylic acids during fermentation processes in beer.[1] Both methylglyoxal (2-oxopropionaldehyde) and 2,3-pentanedione were tested on their ability to exhibit sensitized phosphorescence as observed for biacetyl. In batch experiments, methylglyoxal showed neither direct nor sensitized phosphorescence in the mobile phase used. In addition, from injections made on the HPLC system, no chromatographic peaks were observed up to a concentration of 3.5 ppm methylglyoxal in standard solutions. On the other hand 2,3-pentanedione did show sensitized RTPL. In Figure 4 an emission spectrum is given of a deoxygenated solution containing 0.5 ppm (5.10^{-6} M) 2,3-pentanedione dissolved in the mobile phase. The chromatographic separation of biacetyl and 2,3-pentanedione is presented in Figure 5, in which a chromatogram is given of a standard solution containing 0.6 ppm 2,3-pentanedione and 10 ppb biacetyl. It is clear that biacetyl can be detected more sensitively by a factor of about 30. This difference in sensitivity can be described mainly to the difference in phosphorescence efficiency of the two compounds. In the homologeous series of α-diketones radiationless deactivation of the excited triplet state increases considerably with increasing chain length.[12] The detection limit of 2,3-pentanedione based on a signal to noise ratio of 3 and calculated from an actual chromatogram is 15 ppb ($1.5 * 10^{-7}$ M) in standard solutions. The relative standard deviation is 5.8% at the 50 ppb level and 2.4% at the 0.5 ppm level, both based on 5 repetitive injections. The calibration curve has a linear range from 15 ppb to 0.9 ppm with a regression coefficient of 0.9995 ($n=6$). When the pentanedione

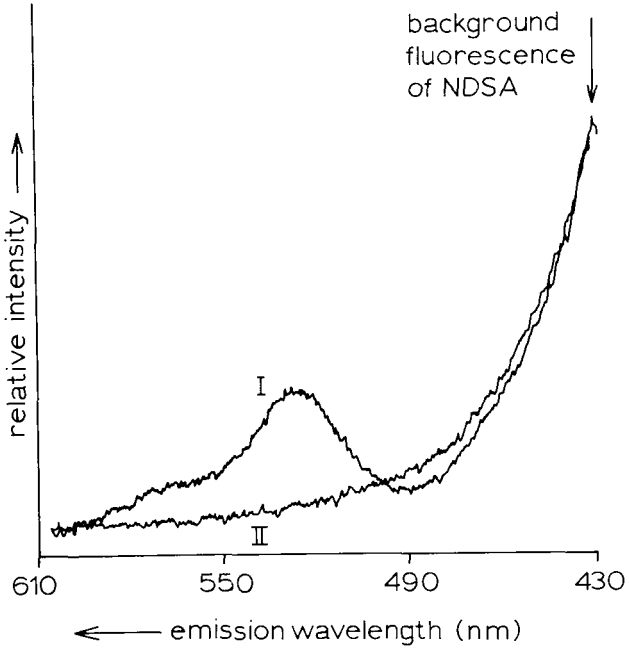

FIGURE 4 Sensitized phosphorescence emission spectrum of 2,3-pentanedione dissolved in a deoxygenated aliquot of mobile phase. I correponds to the emission maximum of pentanedione and II to the emission spectrum recorded without deoxygenation. $\lambda_{ex} = 306$ nm; spectral band-passes, both excitation and emission, were 10 nm.

concentration in the injected sample exceeds 0.9 ppm, a deviation from linear behaviour occurs. This means that the approximation of the efficiency of energy transfer from donor to acceptor, as described in ref. 10, is no longer valid.

2,3-Pentanedione has about the same volatility as biacetyl. This means that for quantitative measurements samples should not be deoxygenated or disturbed otherwise before injection in order to prevent losses of this compound. Figure 6 shows a chromatogram for a beer sample containing both biacetyl and 2,3-pentanedione. The beer sample was prepared as described in the experimental part under sample preparation for the determination of biacetyl. The amount of pentanedione present in this beer sample can hardly be determined quantitatively, but nevertheless an approximation of

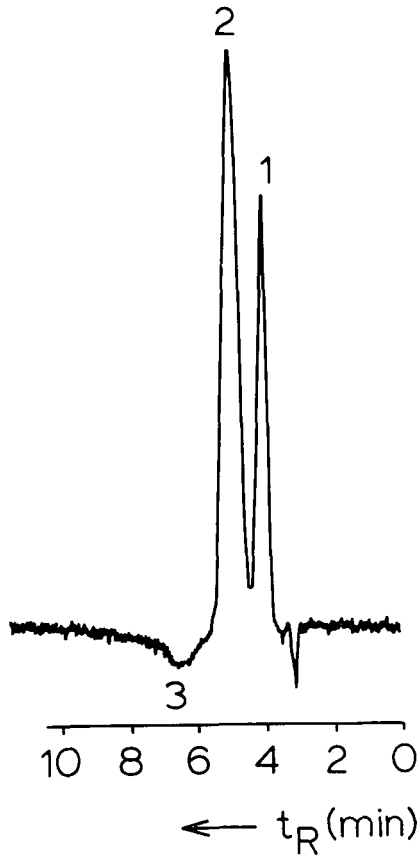

FIGURE 5 HPLC chromatogram of a 10 ppb biacetyl (1) and 0.6 ppm 2,3-pentanedione (2) standard solution; (3) trace of oxygen.

about 25 ppb for the amount present can be given. Notice the absence of the pentanedione peak in the chromatogram given in Figure 3 which deals with a beer sample of different origin prepared identically.

Other α-diketones with longer side chains, as 2,3-hexanedione, were not tested because of their very poor phosphorescence properties which only allows detection in the (sub) ppm range. This concentration range is not relevant for measurements concerning beer samples.

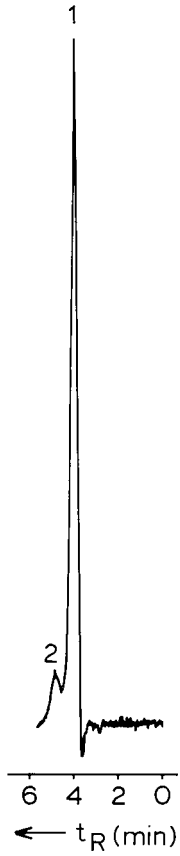

FIGURE 6 HPLC chromatogram of a beer sample containing 13 ppb biacetyl (1) and about 25 ppb pentanedione (2).

HPLC vs. GC

In order to investigate the reliability of the new HPLC method for the determination of biacetyl in beer, a comparison was made between this method and a routine head space GC method. Four different beer samples were analyzed with both methods: the results are summarized in Table I. It can be seen from this table that the figures are in good agreement. In Table II some additional analytical data are compared. A GC and an HPLC chromatogram of a beer

TABLE I

Calculated biacetyl concentrations for four different beer samples analyzed both with GC and HPLC

Beer no.	[biac] (ppb) GC	[biac] (ppb) HPLC
292	22	24
8/98	66	63
8/340	86	90
9/67	26	29

TABLE II

Comparison of analytical data of the GC and HPLC methods

GC	HPLC
Linear range 2–120 ppb	Linear range 0.5–150 ppb
Limit of detection 2 ppb	Limit of detection 0.5 ppb
R.s.d. 1.5% (65 ppb level)	R.s.d. 4.2% (16 ppb level)

sample containing 66 ppb biacetyl are given in Figure 7. It is emphasized that experiments should be carried out in a biacetyl free atmosphere, as contamination is a serious problem in quantitative measurements.

AUTOMATION AND SAMPLE STABILITY

The possibility of automation of the HPLC method was investigated by performing some experiments regarding the stability of the samples. After storage for 20 hours in capped sample vials (Chrompack, Middelburg, The Netherlands) the biacetyl signal decreased slightly ($<10\%$); of course, this is corrected for when using the standard addition calculation method. The reproducibility was not significantly altered compared with the direct analysis of the samples (r.s.d. $= 3.1\%$ vs. 4.2%).

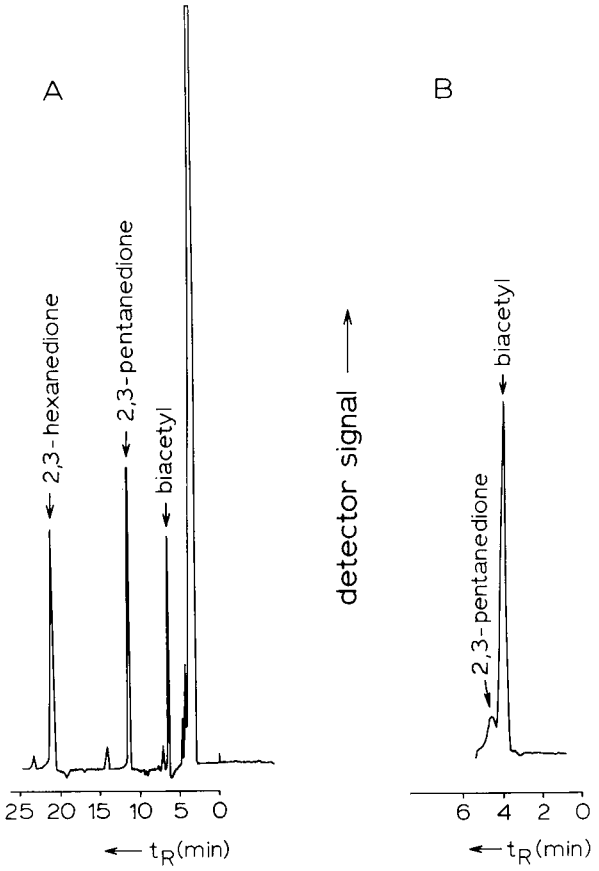

FIGURE 7 (A) GC chromatogram of a beer sample containing 66 ppb biacetyl. (B) HPLC chromatogram of the same beer sample.

CONCLUSIONS

The head space GC and the sensitized phosphorescence/HPLC method are comparable for the quantitative determination of biacetyl in beer. The HPLC method has a more favourable limit of detection (0.5 vs. 2.0 ppb). The GC method can also be used for quantitative 2,3-pentanedione measurements; the HPLC method is not very sensitive towards this compound. When one method is used

for routine analysis, the other can be employed as a reliable reference method. In principle the HPLC method can be automated in combination with an autochanger; the samples are stable for at least 20 hours.

References

1. S. E. Godtfredsen and M. Ottesen, *Carlsberg Res. Commun.* **47,** 93 (1982).
2. J. C. de Man, *Recueil* **78,** 480 (1959).
3. A. D. Haukeli and S. Lie, *J. Inst. Brew.* **84,** 85 (1978).
4. G. A. F. Harrison, W. J. Byrne and E. J. Collins, *J. Inst. Brew.* **71,** 336 (1965).
5. H. Suomalainen and P. Ronkainen, *Nature (London)* **220,** 792 (1968).
6. R. T. Marsili, *J. Chromatogr. Sci.* **19,** 451 (1981).
7. E. A. Prill and B. W. Hammer, *Iowa State College J. Sci.* **12,** 385 (1938).
8. A. G. C. White, L. O. Krampitz and C. H. Werkman, *Arch. Biochem.* **9,** 229 (1946).
9. T. M. Cogan, *J. Dairy Sci.* **55,** 382 (1972).
10. R. A. Baumann, C. Gooijer, N. H. Velthorst and R. W. Frei, *Anal. Chem.* **57,** 1815 (1985).
11. J. J. Donkerbroek, N. J. R. van Eikema Hommes, C. Gooijer, N. H. Velthorst and R. W. Frei, *J. Chromatogr.* **255,** 581 (1983).
12. H. H. Richtol and F. H. Klappmeier, *J. Chem. Phys.* **44,** 1519 (1966).