Journal of Chromatography, 640 (1993) 271–277
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2658

Determination of sulphite and ascorbic acid by high-performance liquid chromatography with electrochemical detection

R. Leubolt

Austrian Beverage Institute, Michaelerstrasse 25, A-1182 Vienna (Austria)

H. Klein*

Quality Assurance and Development, Österreichische Brau AG, Poschacherstrasse 35, A-4020 Linz (Austria)

ABSTRACT

A rapid HPLC method with electrochemical detection for the determination of free and total sulphite and ascorbic acid in beer and other beverages is presented. Interferences of these compounds are discussed, in addition to the behaviour in buffer solutions of different pH. Only a dilution step is required before injecting the sample into the chromatographic system. To obtain better specificity for these compounds, two different working electrodes (platinum for sulphite and carbon glass for ascorbic acid) with distinct potentials are used.

INTRODUCTION

Sulphur compounds such as cysteine and glutathione and disulphide bridges in peptides and proteins, occurring naturally in the raw materials used during the brewing process, may be oxidized to sulphite [1]. Yeast such as *Saccharomyces carlsbergenis* could reduce sulphate to sulphite. These redox processes lead to sulphite contents up to 30 mg/l in finished beers. Ascorbic acid is a powerful antioxidant in beer, preventing colour changes and alterations of aroma and flavour and extending the storage time of the bottled beverage. Normally between 30 and 50 mg/l of ascorbic acid are incorporated in the product.

The sulphite content in foods is often analysed determined the Monier-Williams method [2], which is time consuming and subject to interferences at low sulphite levels. Some alternative techniques for the measurement of sulphite in food were summarized by Kim *et al.* [3]; their paper was concerned with a method using ion-exclusion chromatography with electrochemical detection. Other workers have reported comparison studies for the determination of sulphite in food [4]. Most published methods are time consuming because of the sample preparation required [1,5]. Official methods for the European brewing industry are published by the European Brewery Convention (EBC) [6] and the Mitteleuropäische Brautechnische Analysenkommission (MEBAK) [7].

The accepted method of the AOAC for the determination of ascorbic acid is visual titration using 2,6-dichloroindophenol [8], which works poorly with foods because of an ill-defined end-point. Other methods using isotachophoresis [9], differential-pulse polarography [10] and HPLC with fluorimetric [11], electrochemical [12–15] and UV detection [16–18] have been reported. Chromatographic

* Corresponding author.

0021-9673/93/$06.00 © 1993 Elsevier Science Publishers B.V. All rights reserved

methods with derivatization steps have also been published [19,20]. None of the reported HPLC methods considered that sulphite and ascorbic acid elute close together from the column and that this tends to cause serious problems in the subsequent determination of these two compounds even when specific detectors such as electrochemical systems are used (see Fig. 1a and b). Using a pulsed amperometric detector combined with another ion-exclusion column, some negative effects can be avoided [21].

In this paper, the conditions for the determination of free and total sulphite and ascorbic acid in beer by HPLC with electrochemical detection (ED) are discussed.

EXPERIMENTAL

Reagents

Water for the preparation of the mobile phase and the standards of sulphite and ascorbic acid must be freshly distilled and degassed with helium to prevent oxidation. Before use, oxygen and carbon dioxide were removed in an ultrasonic bath. Ascorbic acid, sodium metabisulphite, metaphosphoric acid and sulphuric acid were of analytical-reagent grade from Merck (Darmstadt, Germany) and mannitol was obtained from Sigma (St. Louis, MO, USA). Standard solutions were prepared freshly before use.

Apparatus

The chromatographic system consisted of a Model 64 HPLC pump from Knauer (Berlin, Germany), a Rheodyne (Cotati, CA, USA) Model 7110 injection valve and an Amor electrochemical detector from Spark (Emmen, Netherlands) with a glassy carbon and a platinum electrode. The column used for ion-exchange chromatography was a "Fast Acid" (100 × 7.8 mm I.D.) with a Cation H guard column (30 × 4.6 mm I.D.) or Aminex HPX 87H (300 × 7.8 mm I.D.), from Bio-Rad Labs. (Richmond, CA, USA).

Chromatographic conditions

The eluent was 0.005 *M* sulphuric acid containing 0.001 *M* chloride (NaCl as used) at a flow-rate of 1 ml/min. The temperature was ambient and the sample volume was 20 μl.

The detector conditions were as follows: for sulphite, platinum working electrode, Ag/AgCl reference electrode, potential +0.4 V; and for ascorbic acid, glassy carbon working electrode, Ag/AgCl reference electrode, potential +0.6 V.

Procedure

Buffer and stock standard solutions for the determination of sulphite. Buffer 1 for free sulphite was 0.005 *M* sulphuric acid–0.01 *M* mannitol (pH 2.3) and buffer 2 for total sulphite was 0.02 *M* Na_2HPO_4–0.01 *M* mannitol, (pH 8.9). A stock standard solution containing 7.4 g of $Na_2S_2O_5$ + 37.5 mg of EDTA in 1 l of degassed water was prepared. A working standard solution (5 ppm) was prepared by dilution of the stock standard solution 1:1000 with distilled water and adding 37.5 mg/l of EDTA. The latter solution must be prepared freshly every day.

Stock standard solution of ascorbic acid. A 15-g amount of metaphosphoric acid was dissolved in 1 l of cold, degassed water in a volumetric flask (stabilizing the ascorbic acid), then 50 mg of ascorbic acid were weighed and dissolved in the acidic solution. This solution must be prepared freshly every day.

Sample preparation for free sulphite. A 2-ml volume of a cold beer sample (2°C) was transferred into a 25-ml volumetric flask containing about 20 ml of buffer 1 and diluted to volume with buffer 1.

Sample preparation for total sulphite. The same procedure as for the determination of free sulphite was used except that buffer 2 was used instead of buffer 1.

Sample preparation for ascorbic acid. A 2-ml of a cold beer sample (2°C) was transfered into a 25-ml volumetric flask containing 20 ml of 1.5% metaphosphoric acid. The air above the solution was removed by flushing with helium. The solution was then diluted to volume with 1.5% metaphosphoric acid. This solution must be analysed immediately.

Enzymatic reactions. For sulphite, Boehringer Mannheim Sulfite-Kit, Order No. 725854, and, for ascorbic acid, Boehringer Mannheim L-Ascorbic-Kit, Order No. 409677, were employed.

Samples. The following were used: 1 = lager beer; 2 = export beer 1; 3 = export beer 2; 4 = dark beer; 5 = pilsner 1; 6 = pilsner 2; 7 = pilsner 3; 8 = light beer; 9 = non-alcoholic beer; 10 = "weizen" beer.

RESULTS AND DISCUSSION

Different pH values allow the measurement of free and total sulphite using HPLC with electrochemical detection. At pH 8 sulphite, which is bound to various aldehydes, is set free and then measured as free sulphite. At pH 2 it is possible to detect only free sulphite. For stabilizing sulphite it is essential to add mannitol [3] to the buffers.

If both ascorbic acid and sulphite are present in the beer sample, difficulties arise when using the "Fast Acid" column. Both have very similar retention times (Fig. 1a and b), and they are electrochemically active. Fig. 2a–c show the detector response for sulphite and ascorbic acid for various pH values and different working electrodes of the electrochemical detector. The dilutions in pH 2 buffer show considerable responses at all potentials for free sulphite and ascorbic acid using the platinum working electrode (Fig. 2a). This means that it is impossible to determine free sulphite in the presence of ascorbic acid in the same run, because ascorbic acid is fairly stable at this pH (see Fig. 3a). At pH 8 ascorbic acid is destroyed within a short time (15 min), whereas sulphite is relatively stable. There is no interference by ascorbic acid (Fig. 3b). For the determination of free sulphite in food and beverages the content of ascorbic acid must be known and the integrator counts corrected, otherwise too high results are obtained.

The calibration graphs obtained using external standards (correlation coefficient 0.999) are linear within a wide range up to 50 mg/l, but the slopes are different. Separate calibrations for free and total sulphite must be used. The highest response for sulphite was found at +0.4 V. This is the potential for the lowest response for ascorbic acid. The detection limit for sulphite is about 0.1 mg/l.

To show the efficiency and the recovery of the method, different amount of sodium metabisulphite were added to a beer containing ascorbic acid. Table I shows the results of these experiments. The determination of ascorbic acid suffers minor problems, because sulphite shows no response at the carbon glass electrode below +0.6 V. Above this potential, *e.g.*, +0.8 V, as described in the literature [13], a higher response for ascorbic acid may be founds because of interference by sulphite. As ascorbic acid is also unstable in acidic solution, it

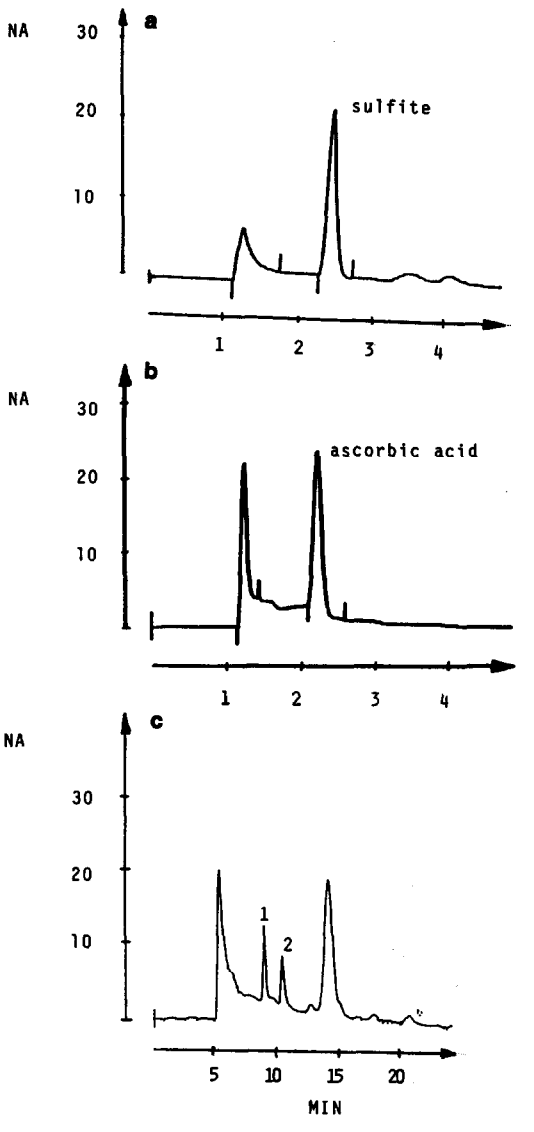

Fig. 1. (a) Chromatogram of a beer sample containing 1.5 mg/l SO_2. Column, "Fast Acid"; eluent, 0.005 *M* sulphuric acid–0.001 *M* chloride (NaCl); flow-rate, 1 ml/min; temperature, ambient; sample volume, 20 µl, detector conditions, Pt working electrode, Ag/AgCl reference electrode, potential +0.4 V; sample preparation, beer was diluted 2:25 in buffer (0.02 *M* Na_2HPO_4–0.01 *M* mannitol, pH 8.9). (b) Chromatogram of a beer sample containing 20 mg/l ascorbic acid. HPLC conditions as in (a); detector conditions, glassy carbon working electrode, Ag/AgCl reference electrode, potential +0.6 V; sample preparation, beer was diluted 2:25 with 1.5% metaphosphoric acid. (c) Chromatogram of a beer sample spiked with ascorbic acid and SO_2. Column, Aminex HPX 87H (300 × 7.8 mm I.D.), other HPLC conditions as in (a); detector conditions, Pt working electrode, Ag/AgCl reference electrode, potential +0.56 V; sample preparation, beer was diluted 2:25 in buffer (0.005 *M* sulphuric acid–0.01 *M* mannitol, pH 2.3). Peaks: 1 = ascorbic acid; 2 = SO_2.

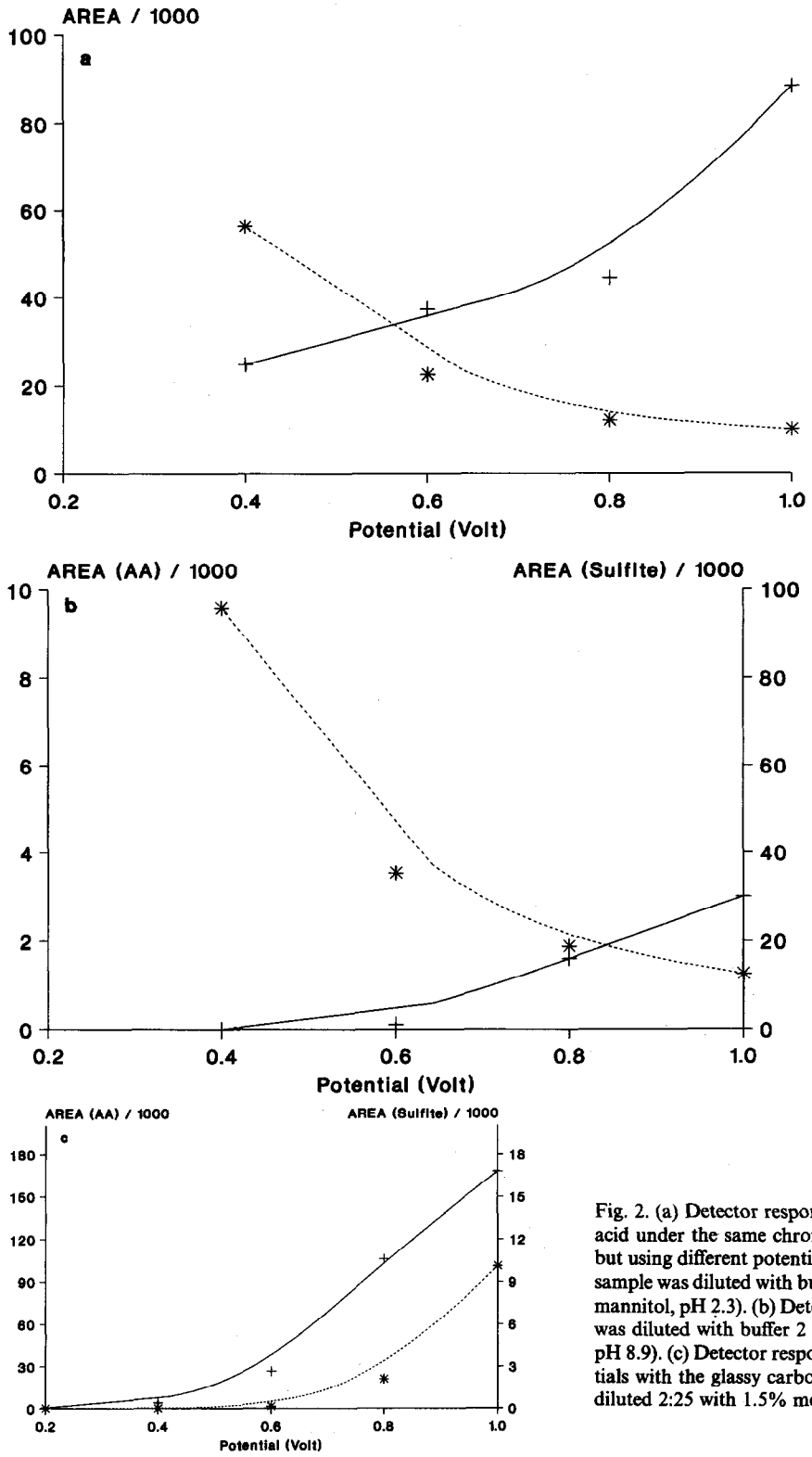

Fig. 2. (a) Detector response for (*) sulphite and (+) ascorbic acid under the same chromatographic conditions as in Fig. 1a, but using different potentials with the Pt working electrode. The sample was diluted with buffer 1 (0.005 *M* sulphuric acid–0.01 *M* mannitol, pH 2.3). (b) Detector response as in (a), but the sample was diluted with buffer 2 (0.02 *M* Na_2HPO_4–0.01 *M* mannitol, pH 8.9). (c) Detector response as in (b) but using different potentials with the glassy carbon working electrode. The sample was diluted 2:25 with 1.5% metaphosphoric acid.

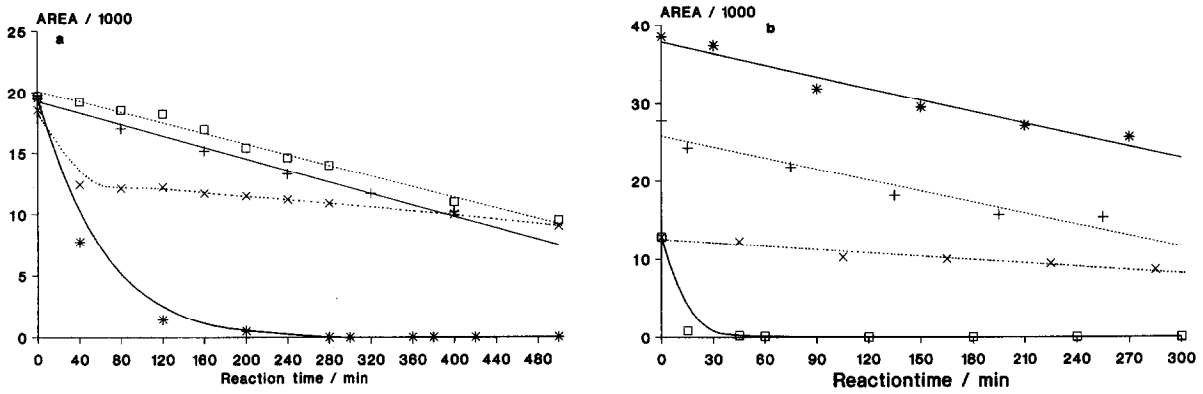

Fig. 3. (a) Different responses of ascorbic acid with the glassy carbon electrode with a potential of +0.6 V. The sample was prepared in different buffer solutions: ✳ = buffer 2 (0.02 *M* Na_2HPO_4–0.01 *M* mannitol, pH 8.9); + = buffer 1 (0.005 *M* sulphuric acid–0.01 *M* mannitol, pH 2.3); × = 0.1% metaphosphoric acid; □ = 1.5% metaphosphoric acid. (b) Different responses of ascorbic acid and sulphite under the same chromatographic conditions as in (a). □ = Response for ascorbic acid in buffer 2; × = response for ascorbic acid in buffer 1; + = response for sulphite in buffer 1; ✳ = response for sulphite in buffer 2.

should be determined immediately. The detection limit for ascorbic acid is about 0.5 mg/l.

Fig. 3a shows the decrease in ascorbic acid in various solutions. This should be kept in mind when working with automatic sample devices. The calibration graph with external standards is linear up to 50 mg/l (correlation coefficient 0.998). The recovery for this measurement is 97–100%. The results obtained with the developed method for ascorbic acid are in good agreement with those given by another HPLC method [12] and with the enzymatic test. The values correspond within ± 1 mg/l at a level of 20 mg/l (see Table II).

The separation of ascorbic acid and sulphite can be achieved under similar chromatographic conditions by using a longer ion-exchange column filled with the same resin as "Fast Acid" [*e.g*, Aminex HPX 87H (300 × 7.8 mm I.D.); Fig. 1c]. The main differences are the flow-rate (0.6 ml/min) and the detector voltage (0.56 V for a Pt working electrode or 0.8 V for a glassy carbon working electrode). Details can be seen in Fig. 2a and b. These condi-

TABLE I

RECOVERY FOR MEASUREMENT OF TOTAL AND FREE SULPHITE AND ASCORBIC ACID

Sample	Total sulphite		Free sulphite		Ascorbic acid	
	Found (ppm)	Recovery (%)	Found (ppm)	Recovery (%)	Found (ppm)	Recovery (%)
Beer	6.6	–	1.0	–		
Beer + 10 ppm sulphite	18.2	110	12.0	111		
Beer + 20 ppm sulphite	28.6	108	22.0	105		
Beer + 30 ppm sulphite	37.4	102	31.5	102		
Beer					18.0	–
Beer + 2 ppm ascorbic acid					19.6	98
Beer + 4 ppm ascorbic acid					22.0	100
Beer + 10 ppm ascorbic acid					27.6	99
Beer + 20 ppm ascorbic acid					36.8	97
Beer + 40 ppm ascorbic acid					58.1	100

TABLE II

COMPARISON OF DIFFERENT METHODS FOR THE DETERMINATION OF ASCORBIC ACID IN DIFFERENT TYPES OF BEER

Beer No.	Ascorbic acid (mg/ml)		
	HPLC–ED on HPX column 87H	HPLC–ED on RP-18 column [12]	Enzymatic test
1	21	19	20
2	20	21	18
3	19	20	19
4	17	15	17
5	43	33	34
6	17	19	32
7	28	25	24
8	10	12	–
9	33	33	–
10	21	21	19

tions give the best sensitivity for both compounds.

The application of this column is limited to the simultanous determination of ascorbic acid and free sulphite. Because of the degradation of ascorbic acid under basic conditions the determination of total sulphite and ascorbic acid is not possible (Fig. 3b). For total sulphite the "Fast Acid" column (100 × 7.8 mm I.D.) is the best choice, giving a short analysis time and a high detector response (0.4 V, Pt working electrode, no interferences by ascorbic acid).

The reference methods for the European brewing industry [6,7] measure only the content of total sulphite in beer. Table III gives the results for the EBC distillation–titrimetric method No. 9.12.1 [6], the MEBAK photometric rosaniline method No. 7.24.1 [7] and the enzymatic test from Boehringer (Mannheim, Germany), which also detects only total sulphite. These results indicate that the levels obtained depend on the method used. The highest values are obtained with the photometric rosaniline method. Compared with the other methods, it seems that some other compounds react in addition to sulphite. The problems with the enzymatic test are the high absorbance (2.0), the low absorbance difference (0.1) and non-specific reactions so that extrapolation is necessary.

Comparing our results with established data in the literature [5,21] we found general agreement for the range of values of free and total sulphite in beers. The values for free sulphite are between 0.5 and 1.5 mg/l and those for total sulphite between 1 and 15 mg/l.

TABLE III

COMPARISON OF DIFFERENT METHODS FOR THE DETERMINATION OF SULPHITE IN DIFFERENT TYPES OF BEER

Beer No.	Sulphite (mg/l)			
	HPLC–ED	EBC titrimetric [6]	MEBAK photometric [7]	Enzymatic
1	3.0	1	8	7
2	3.5	1	12	7
3	4.0	1	10	7
4	2.5	1	–	9
5	3.0	1	7	5
6	5.5	1	8	8
7	4.0	1	5	7
8	5.5	1	11	5
9	1.0	1	2	2
10	7.0	1	7	7

REFERENCES

1 C. Borchert, K. Jorge-Nothaft and E. Krüger, *Monatsschr. Brauwiss.*, (1988) 464.
2 *Official Methods of Analysis of the Association of Official Analytical Chemists*, AOAC, Arlington, VA, 14th ed., 1984, Sec. 20.123.
3 H.-J. Kim, G. Y. Park and Y.-K. Kim, *Food Technol.*, 41 (1987) 85.
4 G. Schwedt and A. Bäurle, *Fresenius' Z. Anal. Chem.*, 322 (1985) 350.
5 L. R. Moreno and P. I. de la Vega, *J. Am. Soc. Brew. Chem.*, 47 (1989) 6.
6 *EBC-Analytica*, Brauerei u. Getränkerundschau, Zürich, 4th ed., 1987, Sec. 9.12.1.
7 *Brautechnische Analysenmethoden*, Vol. II, Mitteleuropäische Brautechnische Analysenkommission, MEBAK Verlag, Freising-Weihenstephan, 1979, Sec. 7.24.1.
8 *Official Methods of Analysis of the Association of Official Analytical Chemists*, AOAC, Arlington, VA, 14th ed., 1984, Sec. 43.064.
9 H. Roben, H. Evers, E. Krüger and K. Rubach, *Monatsschr. Brauwiss.*, 1984 425.
10 A. G. Fogg and A. M. Summam, *Analyst*, 108 (1983) 691.
11 A. J. Speek, J. Schrijver and W. H. P. Schreurs, *J. Agric. Food Chem.*, 32 (1983) 352.

12 E. J. Knudsen and K. H. Siebert, *J. Am. Soc. Brew. Chem.*, 45 (1987) 33.
13 B. Luckas, *Fresenius' Z. Anal. Chem.*, 323 (1986) 496.
14 L. A. Pachla and P. T. Kissinger, *Methods Enzymol.*, 62 (1979) 15.
15 N. Moll and J. P. Joly, *J. Chromatogr.*, 405 (1987) 347.
16 R. C. Rose and D. L. Nahrwold, *Anal. Biochem.*, 114 (1989) 140.
17 S. H. Ashoor, W. C. Monte and J. Welty, *J. Assoc. Off. Anal. Chem.*, 67 (1984) 78.
18 A. Floridi, R. Coli, A. A. Fidanza, C. F. Burgeois and R. A. Wiggins, *Int. J. Vitam. Nutr. Res.*, 52 (1982) 193.
19 J. A. J. S. van Boeckel and C. A. J. M. Meeuwissen, *J. Chromatogr.*, 261 (1983) 176.
20 B. Kacem, M. R. Marshall, R. F. Matthews and J. F. Gregory, *J. Agric. Food Chem.*, 34 (1986) 271.
21 H. P. Wagner, M. J. McGarrity, *J. Chromatogr.*, 546 (1991) 119.