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The use of pulsed amperometry combined with ion-exclusion chromatography for the simultaneous analysis of ascorbic acid and sulfite

HERBERT P. WAGNER* and MICHAEL J. McGARRITY
John Labatt Limited, Brewing Research Department, London, Ontario N6A 4M3 (Canada)

ABSTRACT

Initial attempts to monitor ascorbic acid and sulfite, in a beer matrix, by combining ion-exclusion chromatography with a pulsed amperometric detector using a single applied voltage to the platinum working electrode, were unsuccessful. Alternatively, good chromatograms for the separation of the two antioxidants were achieved utilizing a standard, amperometric cell. However, remarkably superior results were observed when this standard cell was operated in a pulsed mode and cleaning cycles were continually applied throughout the analysis. The working electrode stability and precision have been examined. Preliminary spike recovery data indicate acceptable accuracy for the method. Comparisons of this method to standard reference methods are currently ongoing.

INTRODUCTION

Several methods are available for detecting the presence of sulfite and ascorbic acid, two antioxidants commonly utilized by food and beverage industries [1–7]. Sulfiting agents, in particular, have received wide-spread attention, in recent years, as a result of their allergenic effect on those individuals who are hypersensitive.

To assay sulfite, the Monier–Williams reference method generally provides accurate results, in most matrices, above 10 mg SO_2/l [1,3]. However, the complexity, time requirements and susceptibility of the method to interferences stimulated the search for suitable alternative analytical methods. In addition, the simultaneous analysis of ascorbic acid has often been a parallel goal.

Amperometry is an extremely sensitive and selective detection method for analytes which are easily oxidized. In combination with ion-exclusion chromatography, this offers the potential of rapid, accurate assays for both ascorbic acid and sulfite [1,2,4–8]. However, normal amperometry is prone to errors resulting from the loss of detector sensitivity which occurs as the working electrode becomes contaminated. In order to minimize these errors, the calibration standards and sample solutions must be analysed sequentially [9]. Pulsed amperometry, which maintains the integrity of the working electrode, has successfully been employed as a means of overcoming these difficulties. Applications include the analysis of carbohydrates using a gold electrode

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[10,11] and the determination of alcohols, glycols, acetaldehyde and formic acid using a platinum electrode [10].

Satisfactory chromatograms for the separation of the two antioxidants in beer matrices were achieved utilizing a standard amperometric cell in combination with ion-exclusion chromatography. However, remarkably superior results were observed when this cell was operated in a pulsed mode and cleaning cycles were continually applied throughout the analysis.

The working electrode stability and precision have been examined. Preliminary standard addition recovery data indicate the method to be accurate. Comparisons of this method to standard reference methods are currently ongoing.

EXPERIMENTAL

Apparatus

A Dionex Model 4000i ion chromatograph, a pulsed amperometric detection (PAD) system and a PAD cell with platinum working electrode and a standard amperometric cell with platinum working electrode *vs.* Ag/AgCl reference electrode, autosampler, HPICE-AS1 column and Spectra-Physics Model 4270 integrator for data handling were employed throughout this study.

Ion-exclusion chromatography

The eluent, 10 m*M* H_2SO_4, prepared with deionized water, was prefiltered using a 0.45-μm membrane. The flow-rate was 1.0 ml/min. The standard amperometric cell was connected to a pulsed detector set at a range of 300 nA full-scale. A measuring potential (E_1) of +0.70 V for 240 ms and cleaning potentials (E_2) of +1.25 V for 60 ms and (E_3) −0.10 V for 240 ms were applied to the platinum working electrode. A 50-μl injection loop was employed and an attenuation of 1024 for the integrator.

Reagents, standards and samples

All reagents were either AnalaR or certified ACS grade. Individual 1000 mg/l standard solutions of both L-ascorbic acid (BDH) and sulfite (Na_2SO_3, Fisher Scientific) were prepared fresh daily in a 20 m*M* phosphate (Na_2HPO_4, BDH)–10 m*M* D-mannitol (BDH) buffer (pH 9). The phosphate was required for the determination of total sulfite in beer samples, whereas, the mannitol was utilized to stabilize the sulfite [1,4,9]. A combined standard solution was prepared for calibration of the instrument by diluting 150 μl and 80 μl of the above standard solutions respectively to 10 ml with pH 9 buffer. The calibration standard was diluted 1:20 with pH 9 buffer immediately prior to injection.

Beer samples were degassed by filtration (Whatman No. 4) and diluted 1:20 with pH 9 buffer immediately prior to injection.

RESULTS AND DISCUSSION

Initial attempts to assay ascorbic acid and sulfite in a beer matrix by ion-exclusion chromatography using a pulsed amperometric detector with a single applied voltage were unsuccessful. The loss of detector sensitivity was extremely rapid and presumably was the result of excessive contamination of the relatively small

surface area of the platinum electrode used. A standard amperometric detector cell employs an electrode with a much larger surface area than that of the pulsed detector. Consequently, a fifty-fold increase in sensitivity has been reported for the use of the standard cell in certain instances [8].

Satisfactory results were obtained for the two antioxidants contained in pH 9 buffer when the standard amperometric cell was combined with ion-exclusion chromatography. However, under the same conditions using beer, the co-elution of beer components was observed with both ascorbic acid and sulfite. The addition of 4% acetonitrile to the eluent combined with a reduced flow-rate eliminated the co-elution difficulties. A typical chromatogram is illustrated in Fig. 1.

This method, incorporating a single applied potential, was anticipated to be subject to errors resulting from the loss of electrode sensitivity observed when using a single applied potential. The introduction of these errors was reported in a collaborative study of sulfites in foods [9] and was attributable to detector sensitivity changes over time when using normal amperometry. This decrease in director sensitivity could be as much as 40% over an 8-hour period [9]. As discussed previously, the sequential analysis of standards and samples would be required to compensate for this loss of detector sensitivity associated with normal single applied potential electrochemical analyses [9]. Pulsing the cell was, therefore, investigated as a means of maintaining the stability of the working electrode.

When the standard amperometric cell was operated in a pulsed mode and cleaning cycles were continually applied, some very interesting effects were observed. Using the standard buffer solution, a noticeable change in the chromatogram was observed, particularly with increased sensitivity being noted, especially at the column void volume response. The co-elution of ascorbic acid with the column void volume

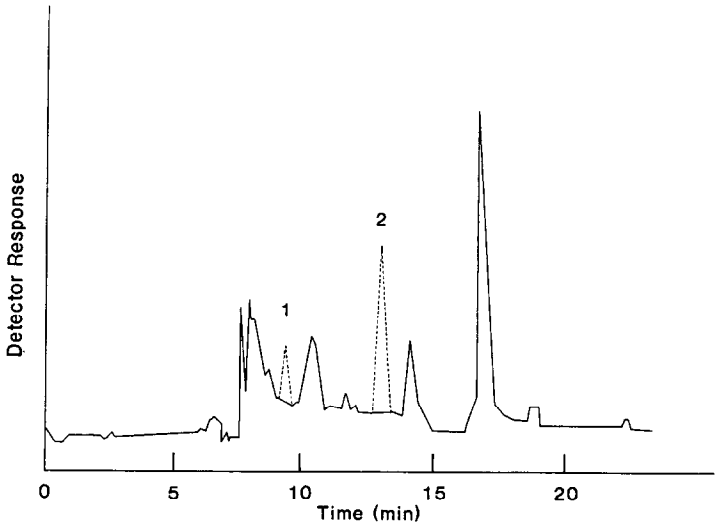

Fig. 1. Chromatogram obtained from a commercial beer (solid line), spiked with 15 mg/l ascorbic acid and sulfite (dotted line), diluted 1:10 with pH 9 buffer. Column, HPICE-AS1. Eluent 4% acetonitrile–10 m*M* H_2SO_4, Flow-rate 0.8 ml/min. PAD with standard amperometric cell and fixed potential of +0.70 V. Attenuation × 1024, range 1000 nA. Peaks: 1 = ascorbic acid; 2 = sulfite.

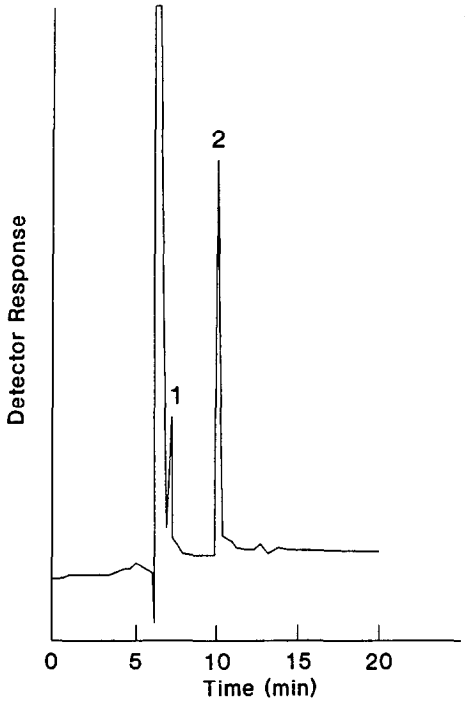

Fig. 2. Chromatogram obtained from 15 mg/l ascorbic acid and 8 mg/l sulfite, diluted 1:20 with pH 9 buffer. For conditions, see text. Attenuation × 1024, range 300 nA. Peaks: 1 = ascorbic acid; 2 = sulfite.

was, in this instance, overcome by eliminating the acetonitrile and marginally increasing the flow-rate. As indicated in Fig. 2, excellent separation of the two antioxidants in pH 9 buffer, was achieved.

To confirm the individual analytes identification, similar additions of 8 mg/l ascorbic acid and 2 mg/l sulfite were made to both the pH 9 buffer and a commercial beer. The spiked beers, when compared to the control beers (no addition) showed recoveries of 99–118% and 95–105% respectively, based on results for the same additions to pH 9 buffer. These recoveries indicated the method to be free of interferences.

A commercial beer, which is known to have added ascorbic acid, and to also contain approximately 2–3 mg/l sulfite (residual from fermentation), was similarly analysed. A typical chromatogram is illustrated in Fig. 3.

The precision of this method was anticipated to be highly dependant on the electrode stability. The susceptibility of sulfite to oxidation was also expected to have a significant effect on the precision of the method. Therefore, in this instance, the combined random and systematic errors were used to establish the precision of the method. Ten samples of the production beer, known to contain both analytes, were analysed consecutively. Individual bottles, from a single purchased lot, were analysed rather than a single bottle repeated ten times. Immediately prior to injection the samples were degassed by filtration and diluted with pH 9 buffer. The samples were

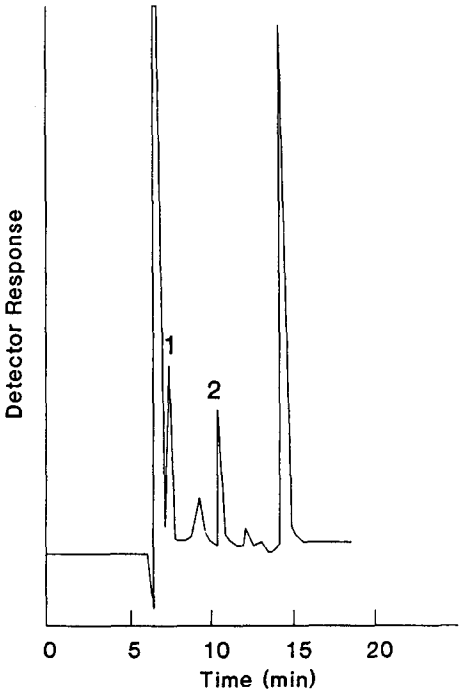

Fig. 3. Chromatogram obtained from a commercial beer known to contain ascorbic acid and sulfite, diluted 1:20 with pH 9 buffer. For conditions, see text. Attenuation × 1024, range 300 nA. Peaks: 1 = ascorbic acid (20.5 mg/l); 2 = sulfite (2.8 mg/l).

TABLE I

DETERMINATION OF METHOD PRECISION

Sample	Ascorbic acid (mg/l)	Sulfite (mg/l)
1	18.93	2.97
2	20.04	3.14
3	19.65	3.10
4	20.23	3.17
5	18.79	2.58
6	18.94	2.66
7	19.29	2.64
8	18.53	2.35
9	19.68	2.55
10	20.23	2.68
Range	18.53–20.23	2.35–3.17
Mean	19.43	2.78
Standard deviation	0.6229	0.2871
R.S.D. (%)	3.2	10.3

injected at exactly 20-min intervals. As illustrated in Table I, relative standard deviations (R.S.D.) of 3.2% for ascorbic acid and 10.3% for sulfite were achieved. Further studies of the accuracy of the method, by comparison to standard reference methods, are currently in progress.

CONCLUSIONS

A novel method for the simultaneous analysis of ascorbic acid and sulfite in beer has been developed and this method can have wide application. The electrode stability and hence the method precision appear acceptable. Excellent recoveries of ascorbic acid and sulfite added to beer were obtained, indicating the method to be free of interferences.

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