ELSEVIER

Journal of Chromatography A, 694 (1995) 507–514

JOURNAL OF CHROMATOGRAPHY A

Short communication

Micellar electrokinetic capillary chromatographic determination of artificial sweeteners in low-Joule soft drinks and other foods

Catherine O. Thompson, V. Craige Trenerry*, Bridget Kemmery

Australian Government Analytical Laboratories, 338–340 Tapleys Hill Road, Seaton 5023, Australia

First received 13 September 1994; revised manuscript received 9 December 1994; accepted 12 December 1994

Abstract

A rapid method for the determination of artificial sweeteners in low-Joule soft drinks and other foods by micellar electrokinetic capillary chromatography (MEKC) is described. Caffeine, benzoic acid and sorbic acid, which are often added to soft drinks, can also be determined with this procedure. The artificial sweeteners, aspartame, saccharin, acesulfame-K, alitame and dulcin, and the other food additives are well separated in less than 12 min using an uncoated fused-silica capillary column with a buffer consisting of 0.05 *M* sodium deoxycholate, 0.01 *M* potassium dihydrogenorthophosphate, 0.01 *M* sodium borate operating at 20 kV. Dehydroacetic acid was used as the internal standard for the determinations. The levels of artificial sweeteners, preservatives and caffeine were in good agreement with those determined by the high-performance liquid chromatographic (HPLC) procedure currently used in our Laboratory. The MEKC procedure has the same order of repeatability, is faster and less costly to operate than the HPLC method.

1. Introduction

A number of methods for the quantitative determination of artificial sweeteners and preservatives in a variety of foods using high-performance liquid chromatography (HPLC) as the determinative step have been reported in the literature [1–7]. HPLC is currently the method of choice for these determinations because all of the components can be separated in the one run. Micellar electrokinetic capillary chromatography (MEKC) is rapidly gaining acceptance as a rugged analytical tool [8–10]. MEKC procedures exhibit greater resolution than HPLC and have the same order of repeatability. MEKC is faster and less costly to operate than HPLC, and, in some instances, has replaced HPLC as the analytical method of choice [11–13]. We recently reported the quantitative determination of sorbic acid and benzoic acid in a number of foods and beverages using an uncoated fused-silica capillary column with a borate buffer modified with sodium dodecyl sulphate [11]. *l*-Ascorbic acid has been determined in a number of foods with a phosphate–borate buffer containing sodium deoxycholate as the micelle modifier [12,13]. This paper describes the rapid separation and quantitation of aspartame, saccharin, acesulfame-K, benzoic acid, sorbic acid and caffeine in a variety of low-Joule soft drinks and the quantitation of aspartame and saccharin in table-top sweeteners, low-Joule tomato sauce and diet marmalade jam. Two other artificial sweeteners, alitame and dulcin, were also separated from the other com-

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)01224-5

pounds. Dehydroacetic acid was used as the internal standard for these determinations [11].

2. Experimental

2.1. Reagents

Aspartame, sodium saccharin, potassium sorbate, sodium benzoate, sodium deoxycholate, sodium cholate, sodium taurodeoxycholate and sodium dodecyl sulphate were obtained from Sigma, St. Louis, MO, USA. Acesulfame-K and alitame were gifts from Hoechst, Frankfurt, Germany and Pfizer, Groten, CT, USA, respectively. Dulcin was obtained from ICN Biochemicals, Cleveland, OH, USA. Dehydroacetic acid was obtained from BDH, Kilsyth, Australia. Caffeine was obtained from the Curator of Standards, Australian Government Analytical Laboratories, Pymble, Australia. All other chemicals and solvents were analytical-reagent or HPLC grade and used without further purification.

2.2. MEKC buffer

A 2.16-g amount of sodium deoxycholate was dissolved in 100 ml of a 1:1 mixture of 0.02 *M* sodium borate and 0.02 *M* potassium dihydrogenorthophosphate. The pH of the solution was 8.6. The solution was filtered through a 0.8-μm cellulose acetate filter disc before use.

2.3. Apparatus

MEKC

The samples were analysed with an uncoated fused-silica capillary column (75 cm × 75 μm I.D.) with an effective length to the detector of 50 cm purchased from Polymicro Technologies, AZ, USA. An Isco Model 3140 electropherograph (Isco, Lincoln, NE, USA) operating at 20 kV and at 27°C was used for all of the determinations. The capillary was flushed with running buffer for two min between runs. The capillary was cleaned on a weekly basis by washing with 0.1 *M* sodium hydroxide for 10 min followed by deionised water for 10 min before filling with running buffer. The solutions were loaded under vacuum (vacuum level 2, 20 kPa s) and were detected at 220 nm. Aspartame and caffeine were determined quantitatively at 0.005 AUFS, while saccharin, acesulfame-K, sorbic acid and benzoic acid were determined quantitatively at 0.02 AUFS. Electropherograms were recorded with either the ICE data management and control software supplied with the electropherograph or a HP 3350 laboratory data system (Hewlett-Packard, Palo Alto, CA, USA).

HPLC

The analyses were performed with a 501 HPLC pump, 712 WISP, 490 programmable multiwavelength UV detector using a C_{18} μBondapak 10 cm × 8 mm Radial-Pak cartridge equipped with a C_{18} pre-column (Waters Chromatography Division of Millipore, Milford, MA, USA) with a mobile phase consisting of 10% acetonitrile in 0.01 *M* potassium dihydrogenphosphate, adjusted to pH 4.0 with dilute orthophosphoric acid at a flow-rate of 2.0 ml/min. The compounds were detected and quantitated at 220 nm at 0.06 AUFS, except for acesulfame-K, which was determined quantitatively at 254 nm. The chromatograms were displayed on an Omniscribe chart recorder (Houston Instruments, USA). Peak areas obtained from a HP 3350 laboratory data system (Hewlett-Packard) were used in the calculations.

2.4. Samples and standards

MEKC

Samples. The low-Joule soft drinks, cordials, tomato sauce, marmalade jam and table-top sweeteners were purchased from local outlets and analysed within the recommended "use by" dates. Sample solutions were prepared by diluting the products with an appropriate amount of deionised water. Dehydroacetic acid was used as the internal standard at a final concentration of 10 μg/ml. The solutions were filtered through a 0.45-μm cellulose acetate filter disc before analysis.

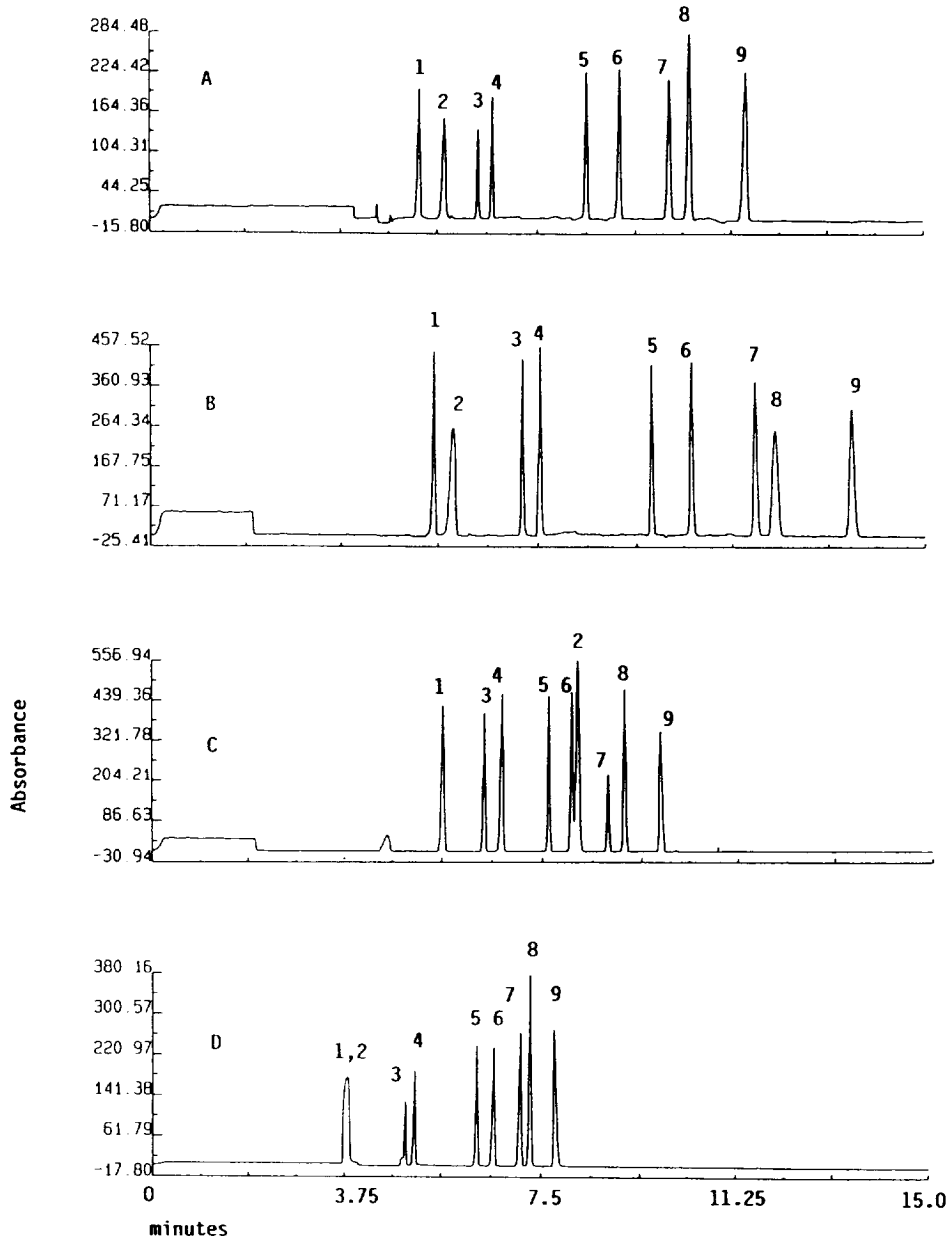

Fig. 1. Electropherograms showing the separation of caffeine (1), dulcin (2), alitame (3), aspartame (4), dehydroacetic acid (5), sorbic acid (6), benzoic acid (7), saccharin (8) and acesulfame-K (9) using (A) a buffer consisting of 0.05 *M* sodium deoxycholate, 0.01 *M* potassium dihydrogenorthophosphate, 0.01 *M* sodium borate pH 8.6, (B) a buffer consisting of 0.05 *M* sodium cholate, 0.01 *M* potassium dihydrogenorthophosphate, 0.01 *M* sodium borate pH 8.6, (C) a buffer consisting of 0.05 *M* sodium dodecyl sulphate, 0.01 *M* potassium dihydrogenorthophosphate, 0.01 *M* sodium borate pH 8.6 and (D) a buffer consisting of 0.01 *M* potassium dihydrogenorthophosphate, 0.01 *M* sodium borate pH 8.6. The *x* axis gives the migration time in minutes.

Standards. Standard solutions were prepared in deionised water with dehydroacetic acid as the internal standard at a concentration of 10 μg/ml. The solutions were filtered through a 0.45-μm cellulose acetate filter disc before analysis.

HPLC

The sample and standard solutions were prepared as described for the MEKC analyses, except that no internal standard solution was used.

3. Results and discussion

Caffeine, dulcin, alitame, aspartame, dehydroacetic acid, sorbic acid, benzoic acid, saccharin and acesulfame-K were well separated using an uncoated fused-silica capillary column with a 0.01 *M* phosphate–0.01 *M* borate buffer containing 0.05 *M* sodium deoxycholate operating at 20 kV (Fig. 1). For the MEKC determinations, the compounds were detected at 220 nm, as this is the most suitable wavelength to determine aspartame, which is the preferred artificial sweetener in the samples analysed. This wavelength was also used for the HPLC determinations, except for acesulfame-K, where 254 nm was the preferred wavelength. The addition of 0.05 *M* sodium deoxycholate to the phosphate–borate buffer was necessary to achieve complete separation of the nine compounds. Caffeine and dulcin co-migrated when the buffer consisted only of the phosphate–borate mixture (Fig. 1). Other micelle modifiers were trialed to see if the separation could be improved or the analysis time shortened. The compounds were well separated when sodium deoxycholate was replaced with either of the related compounds, sodium cholate or sodium taurodeoxycholate [14]. However, in both cases, the run times were longer. Sodium taurodeoxycholate was also unsuitable as a buffer additive as it absorbs at 220 nm, resulting in a very noisy baseline. The noise was eliminated when the detection wavelength was changed to 230 nm. In doing so, the responses for aspartame and alitame were greatly diminished, making this system unsuitable for quantitative work. Replacing sodium deoxycholate with sodium dodecyl sulphate resulted in a slightly shorter run time, but with this system, dulcin and sorbic acid partially coeluted (Fig. 1). Sodium deoxycholate was therefore the modifier of choice. Dehydroacetic acid was used as the internal standard for the quantitation of the food additives, as it is not listed as a food additive in the Australian Food Standards Code and is unlikely to be present in any of the samples to be analysed [15].

Separation of the nine compounds was maintained over 20 repetitive injections. Linearity was established for all of the compounds. Even though all of the compounds were well separated, they could not be all be determined in the one run, as acesulfame-K, saccharin, sorbic acid

Table 1
Comparison of MEKC and HPLC data for standard solutions of varying concentrations showing relative standard deviations (R.S.D.s) between instruments with a sodium deoxycholate-modified buffer

Concentration (μg/ml)	R.S.D. (%)											
	Aspartame		Saccharin		Acesulfame-K		Caffeine		Benzoic acid		Sorbic acid	
	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC
5	2.6	2.0	1.7	0.9	2.5	0.9	1.9	0.9	0.9	2.3	2.0	3.0
50	1.4	1.2	0.5	0.5	1.3	0.8	1.2	1.2	0.7	2.0	1.1	0.7
100	0.6	2.5	–	0.5	1.0	2.2	–	1.6	1.5	2.0	2.9	3.3

and benzoic acid have much greater extinction coefficients than aspartame and caffeine at 220 nm. Therefore aspartame and caffeine were determined at 220 nm and 0.005 AUFS, while the remaining compounds were determined at 0.02 AUFS. Aspartame and caffeine were linear to 200 μg/ml and 50 μg/ml respectively, while acesulfame-K, sorbic acid and benzoic acid were linear to 100 μg/ml and saccharin was linear to 50 μg/ml. The repeatability data (R.S.D.) for seven consecutive injections of a number of standards of different concentrations were comparable to those obtained for the HPLC procedure that is currently used in our laboratory. These data are displayed in Table 1.

A range of soft drinks were then analysed by MEKC and the levels of additives and the instrument repeatability data (R.S.D.) compared with the data obtained from the HPLC procedure. None of the samples analysed had any naturally occurring compounds that comigrated with the internal standard (dehydroacetic acid)

Table 2
Comparison of MEKC and HPLC quantitation of aspartame, benzoic acid and caffeine in low-Joule soft drinks showing R.S.D.s between instruments with a sodium deoxycholate-modified buffer

Sample	Amount (mg/l)					
	Aspartame		Benzoic acid		Caffeine	
	MEKC	HPLC	MEKC	HPLC	MEKC	HPLC
Cola 1	510	530	170	165	140	130
R.S.D. (%)	0.5	3.0	0.9	3.0	0.7	1.0
Recovery (%)	104	87	112	88	112	91
Cola 2	440	405	170	160	100	100
Cola 3	450	430	175	175	90	95
Cola 4	470	450	170	165	85	85
Cola 5[a]	335	335	150	150	60	60
Cola 6[b]	–	–	320	320	80	80
Lemonade 1	405	420	265	260		
R.S.D. (%)	2.1	3.4	0.7	1.1		
Recovery (%)	105	106	103	100		
Lemonade 2	480	470	155	155		
Lemonade 3	395	415	180	175		
Orange 1	450	440	230	235		
R.S.D. (%)	0.5	1.2	1.2	1.3		
Recovery (%)	100	94	104	97		
Orange 2	335	335	170	165		
Lemon 1	395	395	230	230		
R.S.D. (%)	0.5	0.0	1.0	0.5		
Lemon 2[c]	295	295	190	190		

[a] This sample also contained acesulfame-K (MEKC 45 mg/l, HPLC 40 mg/l).
[b] This sample contained saccharin (MEKC 75 mg/l, HPLC 80 mg/l) and cyclamate.
[c] This sample also contained sorbic acid (MEKC 50 mg/l, HPLC 50 mg/l).

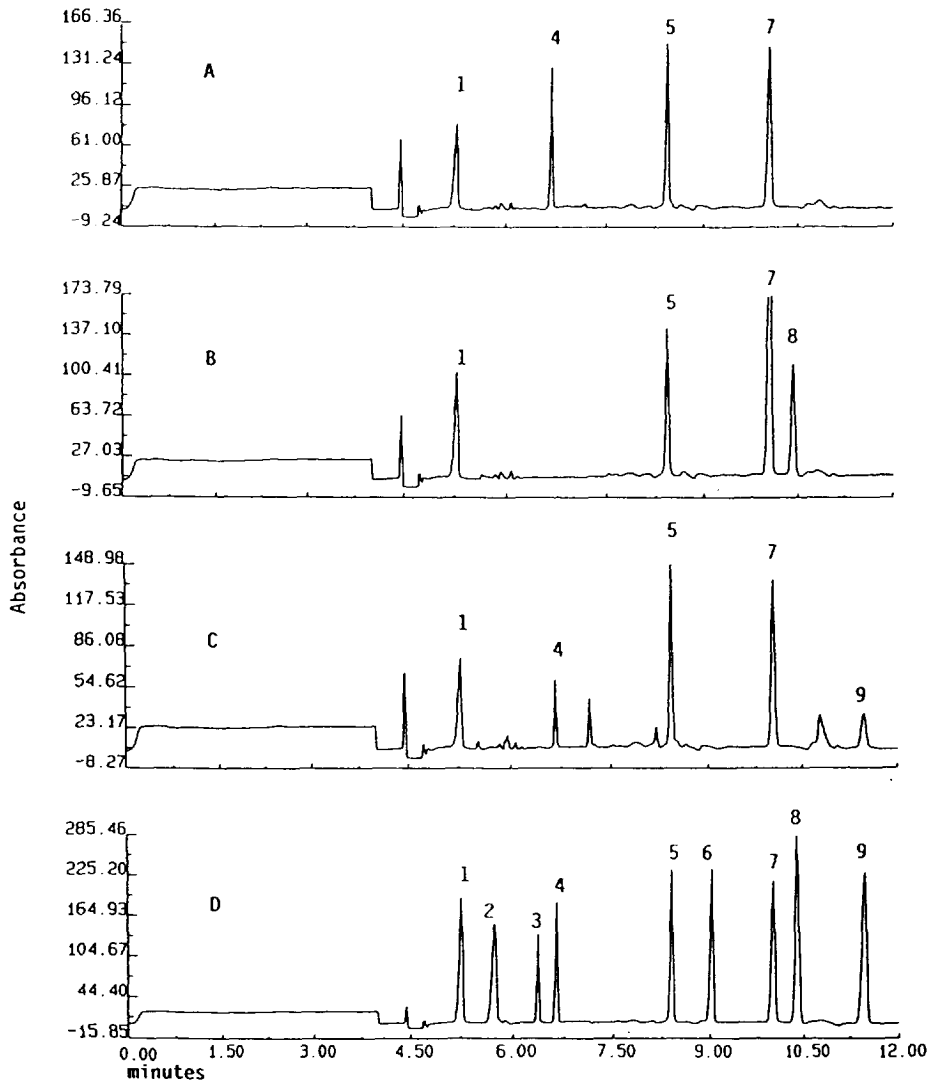

Fig. 2. Electropherograms of (A) low-Joule cola containing caffeine (1), aspartame (4) and benzoic acid (7), (B) low-Joule cola containing caffeine (1), benzoic acid (7) and saccharin (8), (C) low-Joule cola containing caffeine (1), aspartame (4), benzoic acid (7) and acesulfame-K (9) and (D) standard solution with a buffer consisting of 0.05 *M* sodium deoxycholate, 0.01 *M* potassium dihydrogenorthophosphate, 0.01 *M* sodium borate pH 8.6. The *x* axis gives the migration time in minutes. Peak 5 = dehydroacetic acid.

when analysed by MEKC. The levels for aspartame, caffeine and benzoic acid, the R.S.D. data for one sample of each of the soft drinks and recoveries for a number of analytes added to the samples before analysis are listed in Table 2.

One sample of low-Joule cola contained cyclamate as well as saccharin as the artificial sweetener. Cyclamate could not be determined with the MEKC or HPLC procedures as it does not absorb UV light at 220 nm. The determination of cyclamate in various foods by capillary electrophoresis will be reported in a forthcoming article.

Another low-Joule cola contained acesulfame-K as well as aspartame as the artificial sweetener. Acesulfame-K was determined at 254 nm by HPLC, as another compound present in the sample interfered with the quantitation at

220 nm. The levels of additives and the instrument repeatability data (R.S.D.) for all of the samples were in good agreement with the HPLC data. Electropherograms of three colas containing different sweeteners and additives are displayed in Fig. 2, and an electropherogram of a low-Joule lemon drink containing sorbic acid is displayed in Fig. 3.

The levels of saccharin in low-Joule cordials (MEKC 105, HPLC 120 mg/l; MEKC 220, HPLC 225 mg/l; MEKC 350, HPLC 345 mg/l), a low-Joule tomato sauce (MEKC 0.26, HPLC 0.25 g/kg) and a table-top sweetener (MEKC 330, HPLC 315 g/kg) and the levels of aspartame in table-top sweeteners (MEKC 21, HPLC 23 g/kg; MEKC 56, HPLC 56 g/kg) and in a low-Joule marmalade (MEKC 0.32, HPLC 0.31 g/kg) were also in good agreement.

The separation of the standard mixture by HPLC was inferior to the MEKC separation, as dulcin, alitame and sorbic acid coeluted with the HPLC system used for this study. Also, the separation of the nine components by MEKC was much faster than by HPLC. Great care had to be taken when the HPLC mobile phase was prepared, as the elution times for benzoic acid and sorbic acid were very dependent on the pH of the mobile phase. The order of elution of aspartame, saccharin and acesulfame-K was also reversed with HPLC.

4. Conclusions

A rapid method for the separation of aspartame, saccharin, acesulfame-K, alitame, dulcin, caffeine, sorbic acid, benzoic acid and dehydroacetic acid by MEKC is described. The levels of the artificial sweeteners, preservatives and caffeine in a range of soft drinks and the levels of artificial sweeteners in low-Joule tomato sauce, low-Joule cordial, diet marmalade and table-top sweeteners were in good agreement with those determined by the HPLC procedure currently used in our laboratory. The MEKC procedure has the same order of repeatability, is faster and less costly to operate than the HPLC method.

Acknowledgement

The authors wish to thank the Australian Government Analyst, Dr. C.J. Dahl, for his permission to publish this work.

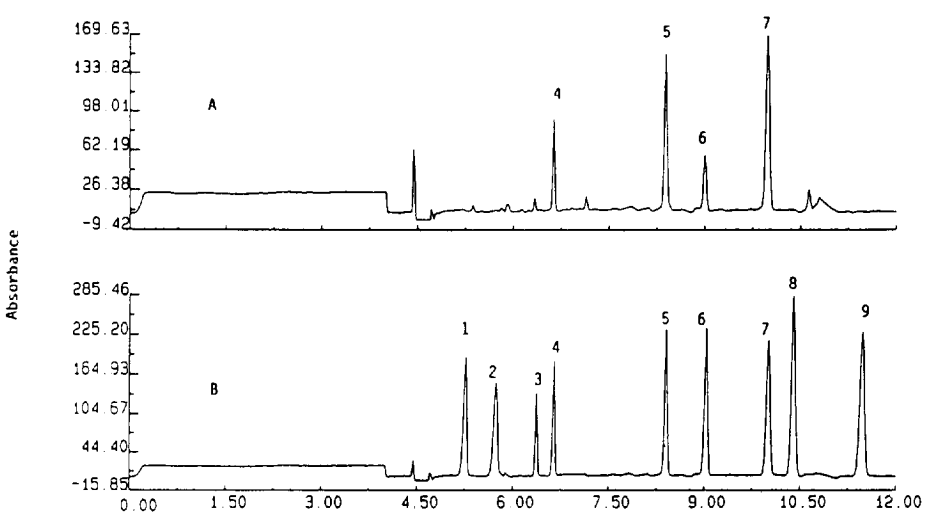

Fig. 3. Electropherograms of (A) low-Joule lemon drink containing aspartame (4), sorbic acid (6) and benzoic acid (7) and (B) standard solution with a buffer consisting of 0.05 *M* sodium deoxycholate, 0.01 *M* potassium dihydrogenorthophosphate, 0.01 *M* sodium borate pH 8.6. The *x* axis gives the migration time in minutes. Peak 5 = dehydroacetic acid.

References

[1] D.S. Smyly, B.B. Woodward and E.C. Conrad, *J. Assoc. Off. Anal. Chem.*, 59 (1976) 14.
[2] B.B. Woodward, G.P. Heffelfinger and D.I. Ruggles, *J. Assoc. Off. Anal. Chem.*, 62 (1979) 1011.
[3] T.A. Tyler, *J. Assoc. Off. Anal. Chem.*, 67 (1984) 745.
[4] M. Veerabhadrarao, M.S. Narayan, O. Kapur and C. Sastry, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 578.
[5] J.F. Lawrence and C.F. Charbonneau, *J. Assoc. Off. Anal. Chem.*, 71 (1988) 934.
[6] J. Prodolliet and M. Bruelhart, *J. Assoc. Off. Anal. Chem. Int.* ,76 (1993) 268.
[7] J. Prodolliet and M. Bruelhart, *J. Assoc. Off. Anal. Chem. Int.* ,76 (1993) 275.
[8] S.F.Y. Li, *Capillary Electrophoresis —Principles, Practice and Applications* (*Journal of Chromatography Library*, Vol. 52), Elsevier, Amsterdam, 1992.
[9] V.C. Trenerry, R.J. Wells and J. Robertson, *J. Chromatogr. Sci.*, 32 (1994) 1.
[10] V.C. Trenerry, R.J. Wells and J. Robertson, *Electrophoresis*, 15 (1994) 103.
[11] I. Pant and V.C. Trenerry, *Food Chem.*, submitted for publication.
[12] P.A. Marshall, C.O. Thompson and V.C. Trenerry, *J. Chromatogr. Sci.*, submitted for publication.
[13] C.O. Thompson and V.C. Trenerry, *Food Chem.*, in press.
[14] R. Weinberger, *Practical Capillary Electrophoresis*, Academic Press, San Diego, CA, 1993.
[15] *Australian Food Standards Code 1994*, Australian Government Publishing Service, Canberra, 1994.