ELSEVIER

Journal of Chromatography A, 847 (1999) 369–375

JOURNAL OF CHROMATOGRAPHY A

Simultaneous determination of antioxidants, preservatives and sweeteners permitted as additives in food by mixed micellar electrokinetic chromatography

Mary C. Boyce*

School of Natural Sciences, Edith Cowan University, 2 Bradford Street, Mount Lawley, WA 6050, Australia

Abstract

A micellar electrokinetic chromatography method was developed to simultaneously analyse commonly used food additives. The additive mixture, comprising propyl gallate, octyl gallate, dodecyl gallate, butylated hydroxyanisole, butylated hydroxytoluene, tertiary butylhydroquinone, *p*-hydroxybenzoic acid methyl ester, *p*-hydroxybenzoic acid ethyl ester, benzoic acid, sorbic acid, saccharin, aspartame and acesulfame-K, was not resolved using single surfactant micellar systems consisting of sodium dodecyl sulfate (SDS), sodium cholate (SC) or sodium deoxycholate (SDC). The separation of these additives using mixed micellar systems, involving SDS/SC, SDS/SDC and SC/SDC, was investigated. Organic solvents were added to the mixed micellar phases to optimise the separation. The mixture was successfully separated using a 20 m*M* borate buffer with 35 m*M* SC, 15 m*M* SDS and 10% methanol added at pH 9.3. Additives in cola beverages and low-joule jam were investigated and quantified using this method.

Keywords: Food analysis; Antioxidants; Artificial sweeteners; Sorbic acid; Benzoic acids

1. Introduction

Antioxidants, preservatives and sweeteners are often added to food singly or in combination to extend the shelf life or improve the product. Antioxidants are added to foods to retard lipid oxidation. Many substances with antioxidant activity occur naturally, but a variety of synthetic forms have also been developed. A number are permitted for use as food additives, and those that may be used in Australia include propyl gallate (PG), octyl gallate (OG), dodecyl gallate (DG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*.-butylhydroquinone (BHQ) [1]. Several analytical methods have been developed to determine antioxidants in food, including high-performance liquid chromatography (HPLC) and gas chromatography (GC) [2–5]. The application of capillary electrophoresis to the analysis of antioxidants in food has not been widely reported [6,7].

Preservatives generally inhibit bacterial and fungal growth in foods. Preservatives in common use include sorbic acid and its salts, benzoic acid and its salts, *p*-hydroxybenzoic acid methyl ester (methyl paraben, MP), *p*-hydroxybenzoic acid ethyl ester (ethyl paraben, EP), *p*-hydroxybenzoic acid propyl ester (propyl paraben, PP), *p*-hydroxybenzoic acid butyl ester (butyl paraben, BP) and salicylic acid. The latter three are not listed in the Australian Food Standards Code [1]. Several HPLC methods for the determination of preservatives have been reported in the literature [8,9], and, methods using capillary zone electrophoresis (CZE), cyclodextrin-modified CZE

*Tel.: +61-8-93706328; fax: +61-8-93706103.
E-mail address: m.boyce@cowan.edu.au (M.C. Boyce)

0021-9673/99/$ – see front matter
PII: S0021-9673(98)01019-X

and micellar electrokinetic capillary chromatography (MECC) have also been reported [10–12].

Aspartame, acesulfame-K and saccharin are permitted synthetic sweeteners commonly added to low-joule foods in Australia. HPLC is the common method for their determination, however, reports have indicated that CE is suitable for the analysis of sweeteners in a variety of foods [13,14].

In many instances more than one additive is added to a food. For example, most low-joule soft drinks contain both preservatives and sweeteners. Many fat-based foods, such as salad dressings and crisps, contain both added antioxidants and preservatives. Therefore, analytical methods that simultaneously determine artificial sweeteners, antioxidants and preservatives are advantageous. The large differences in polarity among the preservatives, sweeteners and antioxidants, makes the simultaneous separation of these additives by HPLC quite difficult, although an ion-pair HPLC method has been reported [15]. However, no such CE method has been reported. The high separation capabilities of CE, and ease with which the micellar phase and/or the running buffer can be modified, suggests it may be suitable for this complex determination.

This paper describes a mixed MECC method for the determination of permitted antioxidants, preservatives and sweeteners in food in Australia. The method has been used to detect and quantify additives in jams and beverages.

2. Experimental

2.1. Chemicals

Propyl gallate, octyl gallate, dodecyl gallate, butylated hydroxyanisole, butylated hydroxytoluene, tertiary butylhydroquinone, *p*-hydroxybenzoic acid methyl ester, *p*-hydroxybenzoic acid ethyl ester, *p*-hydroxybenzoic acid butyl ester, benzoic acid, sorbic acid, sodium cholate, sodium deoxycholate, saccharin, aspartame, acesulfame-K and Sudan III were purchased from Sigma and used as received. HPLC-grade acetonitrile, methanol, isopropanol, analytical-reagent grade disodium tetraborate, potassium hydroxide and sodium dodecyl sulfate were purchased from BDH (UK). The food samples were purchased locally.

2.2. Standards and samples

The additives were dissolved in a mixture consisting of acetonitrile–water (80:20) at concentrations of approximately 200 μg/ml. Samples of beverages were filtered and applied directly. Additives were extracted from low-joule jam by sonication: approximately 1 g of jam was mixed with 10 ml of water and sonicated for 10 min. The mixture was made up to volume (15.00 ml) and then filtered through a 0.45-μm filter (Millipore, Australia).

2.3. Apparatus and conditions

MECC separations were carried out using a Waters Quanta 4000 system equipped with a 60-cm (52 cm effective length)×75 μm I.D. fused-silica capillary (Polymicro Technology, Phoenix, AZ, USA). The capillary was conditioned daily by first washing with 0.5 *M* potassium hydroxide (15 min), then water (15 min) and finally with the running buffer (15 min). The samples were injected by hydrodynamic injection for 3 s and with an applied voltage of 18 kV unless otherwise stated. The samples were run at ambient temperatures (25–26°C). The detection wavelength was 214 nm.

2.4. MECC buffers

Running buffer solutions containing one surfactant type were prepared by dissolving 25, 50 or 75 m*M* sodium dodecyl sulfate (SDS), sodium cholate (SC) or sodium deoxycholate (SDC) in a 20 m*M* sodium tetraborate solution. The pH of the buffers was adjusted to 9.5, if necessary, with sodium hydroxide. Buffer solutions containing two surfactant types, SDS and SC, SDS and DSC or SC and DSC were prepared. The total concentration of the surfactant was kept constant at 50 m*M*. The relative concentrations of the two surfactants varied in the following way: 0:50, 15:35, 25:25, 35:15, 50:0. The surfactants were dissolved in 20 m*M* sodium tetraborate buffer and the pH adjusted to 9.5. Running buffers containing SC and SDS and SDS and DSC were prepared in a 20 m*M* sodium tetraborate

solution with 10% organic solvent (acetonitrile, isopropanol or methanol) added and the pH adjusted to 9.5 unless otherwise stated.

3. Results and discussion

3.1. Single micellar systems

Initially single micellar systems, consisting of the surfactant SDS, SC or DSC, were tested for their ability to separate the mixture. Buffers with 25, 50 or 75 m*M* of each surfactant in 20 m*M* borate at pH 9.5 were trialled. Preliminary experiments determined the optimal pH of the buffer was 9.5 as, at lower pH (8.5), OG and BHA coeluted. None of the micellar systems tested resolved all of the components. The non-polar SDS buffer (50 and 75 m*M*) failed to resolve the late eluting and non-polar components DG and BHT (Fig. 1a). Both components were highly solubilised in the micelle and eluted close to the t_{mc}. Using 25 m*M* SDS buffer these components

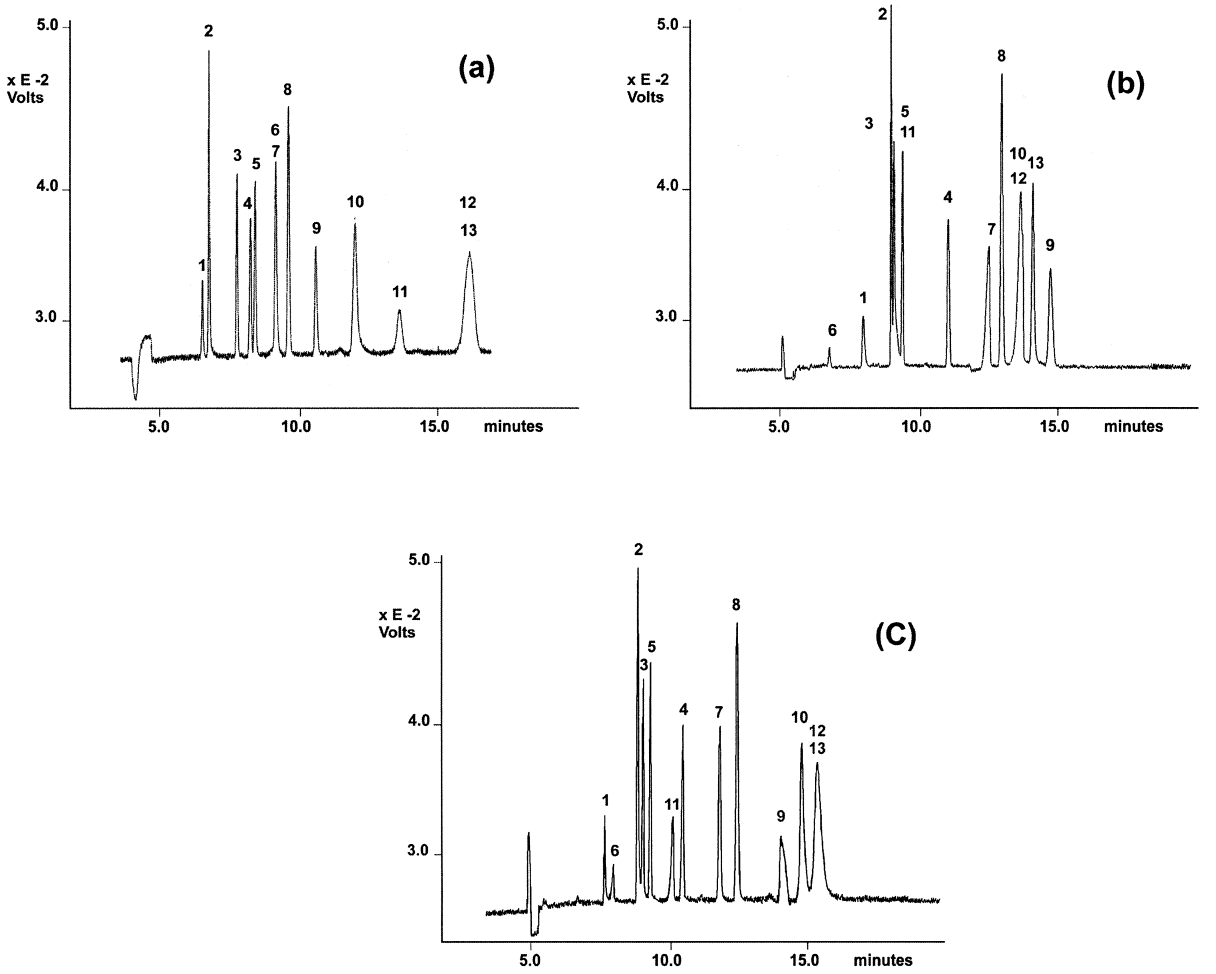

Fig. 1. Electropherograms showing the separation of aspartame (1), propyl gallate (2), methyl paraben (3), sorbic acid (4), ethyl paraben (5), *tert.*-butylhydroquinone (6), benzoic acid (7), saccharin (8), acesulfame-K (9), octyl gallate (10), butylated hydroxyanisole (11), butylated hydroxytoluene (12), dodecyl gallate (13), using (a) a buffer consisting of 50 m*M* sodium dodecyl sulfate (SDS), 20 m*M* borate, pH 9.5, (b) a buffer consisting of 50 m*M* sodium cholate (SC) 20 m*M* borate, pH 9.5, and (c) a buffer consisting of 50 m*M* sodium deoxycholate (SDC), 20 m*M* borate, pH 9.5.

were partially resolved. The addition of methanol to this buffer resolved DG and BHT, but the efficiency of the method was poor and peaks were substantially broadened as a result of micelle polydispersity [7].

Bile salts containing hydroxyl groups are more polar than SDS and their use leads to a general reduction in migration factors in MECC [16]. The more polar micellar phase, sodium cholate, was employed in an attempt to reduce the residence time of the non-polar solutes in the micelle, and to resolve DG and BHT. Sodium cholate was successful in resolving BHT and DG, however, BHT then coeluted with OG (Fig. 1b). The early eluting components were not well resolved, as the polarity of the micellar phase was reduced BHA, BHQ and EP eluted earlier along with PG, MP and aspartame. Similar results were observed when deoxycholic acid was employed as the surfactant (Fig. 1c).

Sodium deoxycholate is less polar than cholic acid, and it was less effective than SC at resolving BHT and DG, but superior to SDS. As for SC, the early eluting peaks were not resolved. The variation in selectivity observed between SC and DSC can presumably be attributed to differences in the number of hydroxyl groups or polarity.

It is clear that while some components are resolved by one surfactant system they coelute in another surfactant system. Combining these surfactants to produce mixed micellar systems might be more effective at simultaneously resolving both the polar and non-polar components.

3.2. Mixed micellar buffer systems

The ability of three mixed micellar systems (SDS/SC, SDS/DSC and SC/DSC) to separate the mixture was investigated. The total concentration of the micellar phase was kept constant, while the relative concentrations of the two surfactants was varied: 0:50, 15:35, 25:25, 35:15, 50:0. The mixed micellar systems were more effective at resolving the mixture. For the SC/SDS system, BHT was increasingly resolved from DG and the early eluting peaks remained at least partially resolved as the concentration of SC increased (or the concentration of SDS decreased) (Fig. 2). As the polarity of the micellar phase is increased, the migration times for BHT, BHA and BHQ was reduced as their solubilisation in

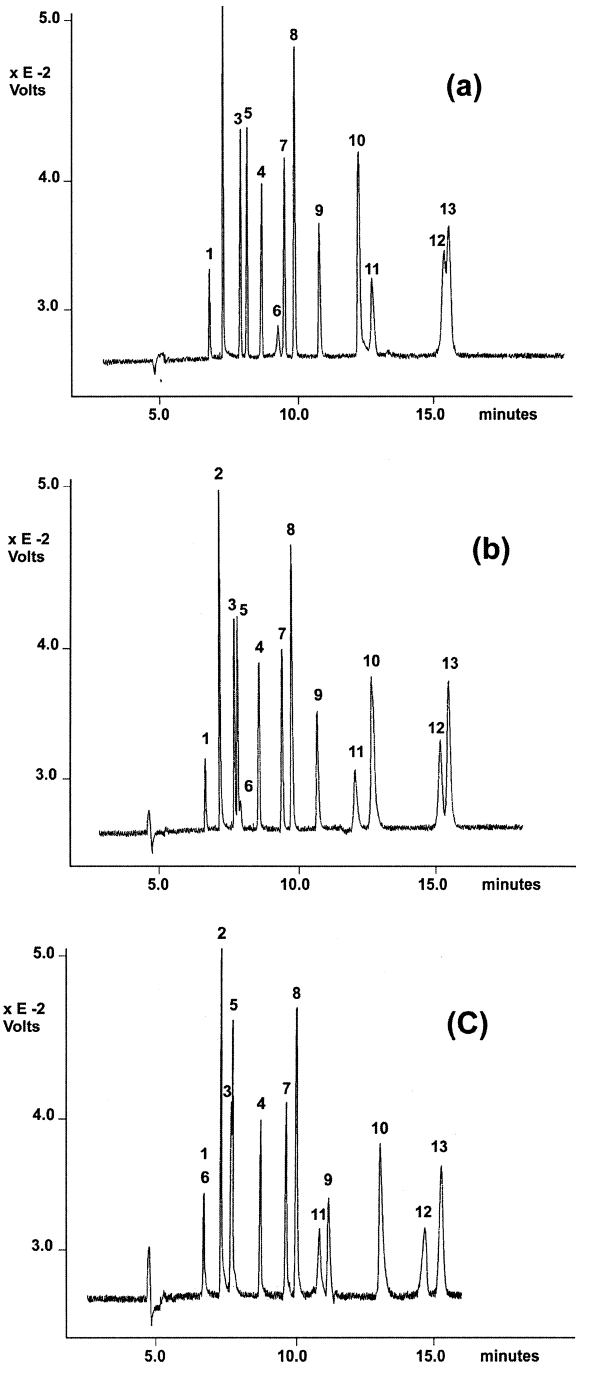

Fig. 2. Electropherograms showing the separation of a mixture of permitted food additives using (A) a buffer consisting of 35 m*M* SDS, 15 m*M* sodium cholate (SC) 20 m*M* borate, pH 9.5; (B) a buffer consisting of 25 m*M* SDS, 25 m*M* SC, 20 m*M* borate, pH 9.5; and (C) a buffer consisting of 15 m*M* SDS, 35 m*M* SC, 20 m*M* borate, pH 9.5. See Fig. 1 for peak identification.

the micellar phase was reduced. The elution order and relative retention times for the early eluting more polar components aspartame and propyl gallate remained relatively constant. These compounds which elute close to t_o partition strongly with the aqueous phase and are, therefore, not significantly influenced by changes in the polarity of the micellar phase.

The SDS/DSC and SC/DSC mixed micellar phase systems also improved separation when compared to the single micellar systems. However, the separating capabilities of the SC/DSC system were not as effective as either SC/SDS or SDS/DSC. The late eluting peaks were not fully resolved for any of the SDC/SC mixed micellar phases investigated.

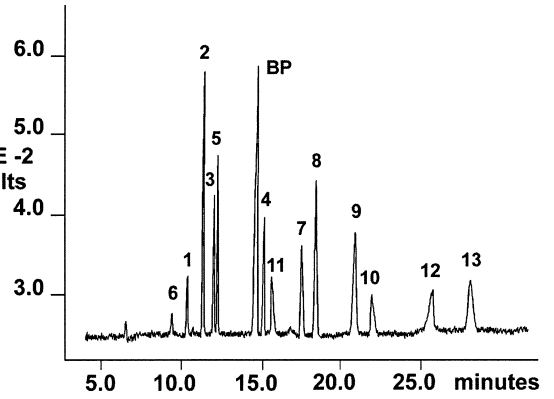

Fig. 3. Electropherogram showing the separation of the additive mixture using a buffer consisting of 35 m*M* SC, 15 m*M* SDS, 20 m*M* borate, 10% methanol, pH 9.3. See Fig. 1 for peak identification. BP is the internal marker butyl paraben.

3.3. Addition of organic solvents to the mixed micellar phases

Organic solvents were added to those mixed micellar buffers that gave the best separation. Adding 10% acetonitrile to a 15 m*M* SDS, 35 m*M* SC, 20 m*M* borate buffer solution resolved all the components in under 30 min. The reproducibility of the method was poor: the percent relative standard deviation (R.S.D.) for the migration times of the components was approximately 1% (over three runs). Adding 10% methanol to the running buffer and adjusting the pH to 9.3 similarly resolved all of the components, however, the run time increased to approximately 35 min. The reproducibility of the method was excellent. The R.S.D. for the migration times of the components was less than 0.1% (over three runs). The run time was decreased to inside 30 min by increasing the applied voltage to 20 kV with no loss in the resolution (Fig. 3).

3.4. Analysing additives from selected food products

The buffer system 35 m*M* SC, 15 m*M* SDS, 20 m*M* sodium borate and 10% methanol was used to determine the additives present in food stuffs. As butyl paraben is not permitted in food, and it does not coelute with any of the components under investigation it was used as an internal marker (Fig. 3). Butyl paraben was not found in any of the food samples analysed.

Sorbic acid and aspartame were extracted from low-joule jam. Spiking the samples identified the additives. Fig. 4a depicts the electropherogram. Spiking was necessary as the retention time of the additives in the real samples did differ from those recorded for the standard mixture. The R.S.D. for the migration times of sorbic acid and aspartame when real samples and the standard solutions were compared was 1.4 and 2.4%, respectively. Table 1 lists the amounts of sorbic acid and aspartame detected in the jam. The R.S.D. for concentrations of these additives was less than 1.5%. The extraction recoveries for sorbic acid and aspartame spiked in the jam ranged from 98.9 to 101. The R.S.D. values for the recoveries were all below 3.3%. These results suggest that this extraction method and the MECC method are suitable for determination of additives in jams.

Aspartame, acesulfame and benzoic acid levels in two cola drinks were measured. The identity of the additives was again confirmed by spiking the samples. Fig. 4b depicts the electropherogram for one of these colas. Table 2 lists the amounts of additives measured in the two colas analysed. In conclusion, this MECC method resolves a complex mixture of additives and the method can be applied to real samples.

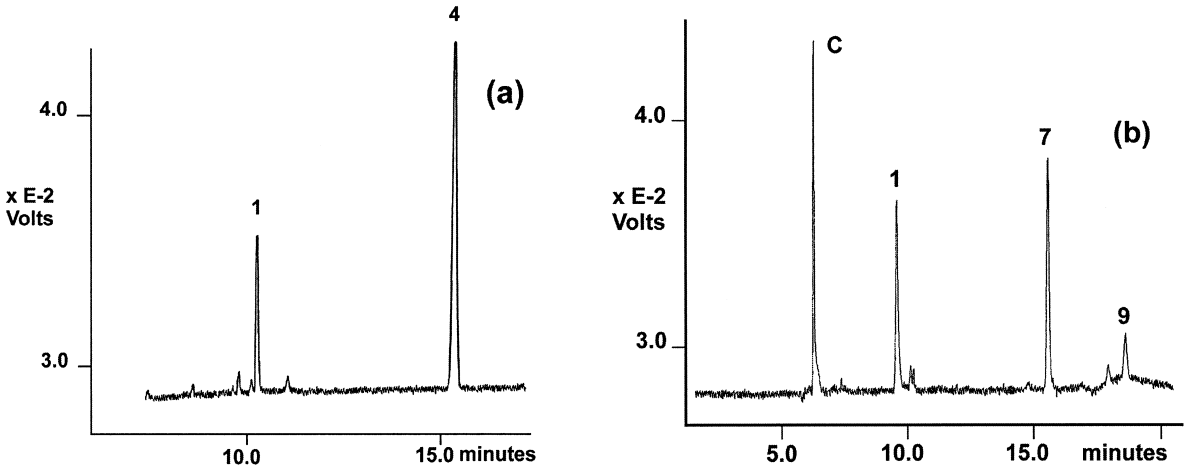

Fig. 4. Electropherograms of (a) a jam and (b) a beverage. The buffer consisted of 35 m*M* SC, 15 m*M* SDS, 20 m*M* borate, 10% methanol, pH 9.3. See Fig. 1 for peak identification. The peak labelled C in (b) is caffeine.

Table 1
Migration time data for the additives and the determination of the amount of additives and their recovery in low-joule jam[a]

Additive	Concentration (mg/g) (R.S.D., %)	Recovery (%) (R.S.D., %)	Migration time (min) (R.S.D., %)
Sorbic acid	1.38 (1.4)	98.94 (0.5)	15.87 (1.5)
Aspartame	1.02 (1.5)	100.86 (3.3)	10.55 (0.91)

[a] $n=3$.

Table 2
Migration time data for the additives and the determination of the amount of additives in selected beverages[a]

Food sample	Additive	Concentration (μg/ml) (R.S.D., %)	Migration time (min) (R.S.D., %)
Beverage 1	Aspartame	422 (0.9)	10.1 (1.6)
	Acesulfame-K	179 (1.25)	21.12 (1.5)
	Benzoic acid	39.3 (1.5)	17.49 (1.7)
Beverage 2	Benzoic acid	287 (1.2)	17.56 (1.5)

[a] $n=3$ determinations.

References

[1] Australian New Zealand Food Authority (ANZFA), Australian Government Publishing Service, Canberra, 1996.
[2] M. Gonzalez, M. Ballesteros, M. Gallego, M. Valcarcel, Anal. Chim. Acta 359 (1998) 47.
[3] C. Grossett, D. Cantin, A. Villet, J. Alary, Talanta 37 (1990) 301.
[4] G. Yentur, N. Ozudogru, A. Bayhan, J. Food Qual. 19 (1996) 343.
[5] Official Methods of Analysis, Method 983.15, Association of Official Analytical Chemists (AOAC), VA, 1993.
[6] C. Hall III, A. Zhu, G. Zeece, J. Agric. Food Chem. 42 (1994) 919.
[7] M. Boyce, E. Spickett, J. Agric. Food Chem. (submitted).
[8] H. Terada, Y. Sakabe, J. Chromatogr. 346 (1985) 333.
[9] N. Brown, L. Hall, H. Sleeman, J. Chromatogr. 166 (1978) 316.
[10] D. Kaniansky, M. Masar, M. Madjova, J. Chromatogr. A 677 (1994) 179.
[11] K. Kuo, Y. Hsieh, J. Chromatogr. A 768 (1997) 334.

[12] I. Pant, V. Trenerry, Food Chem. 53 (1995) 219.
[13] C. Thompson, C. Trenerry, B. Kemmery, J. Chromatogr. A 704 (1995) 203.
[14] C. Thompson, C. Trenerry, B. Kemmery, J. Chromatogr. A 696 (1995) 507.
[15] B. Chen, S. Fu, Chromatographia 41 (1995) 43.
[16] R. Cole, M. Sepaniak, W. Hinze, J. Gorse, K. Oldiges, J. Chromatogr. 557 (1991) 113.