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Determination of cyclamate in low joule foods by capillary zone electrophoresis with indirect ultraviolet detection

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Abstract

A rapid method for the determination of cyclamate in low joule cordials and other low joule foods by capillary zone electrophoresis (CZE) with indirect ultraviolet (UV) detection at 254 nm is described. Sorbic acid, which is often added to low joule cordials as a preservative, can also be determined with this procedure. Cyclamate and sorbate are well separated from the other components in the foods in less than 5 min using an uncoated fused-silica capillary column with an electrolyte consisting of 1 m*M* hexadecyltrimethylammonium hydroxide, 10 m*M* sodium benzoate operating at a voltage of −20 kV. Alpha-hydroxyisobutyric acid was used as the internal standard. The levels of cyclamate determined by CZE were in good agreement with those determined by the AOAC gravimetric method. The artificial sweeteners aspartame, acesulphame-K, alitame and saccharin can also be separated using this procedure. Saccharin and benzoic acid, which are often added with cyclamate to low joule cordials, and caffeine, which is added to low joule colas containing cyclamate, cannot be determined quantitatively with this system.

1. Introduction

Cyclamate, as the sodium or calcium salt, is added as an artificial sweetener to a variety of low joule foods and beverages available in Australia [1]. A number of methods based on wet chemical and HPLC procedures for the quantitative determination of cyclamate in a variety of low joule foods have been reported in the literature.

Cyclamate can be determined using the gravimetric or colourimetric procedures outlined in the Official Methods of Analysis of the Association of Official Analytical Chemists [2]. Unfortunately, these two procedures are labour intensive and time-consuming. Traditional high-performance liquid chromatography (HPLC) is not suited for the determination of cyclamate as cyclamate does not absorb in the usable UV range (above 200 nm). Herrmann et al. [3] used HPLC with indirect photometry to determine the level of cyclamate in a variety of low joule foods. The artificial sweeteners saccharin, dulcin and aspartame could also be determined with this procedure. Ion chromatography with conductivity detection has also been used to determine cyclamate, saccharin and acesulphame-K in a number of low joule foods [4] and cyclamate and saccharin in table top sweeteners [5]. HPLC has also been used to determine cyclamate after oxidation to cyclohexylamine and with pre-column derivatisation to a fluorescent derivative [6]

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and after conversion to N,N-dichlorcyclohexylamine [7].

Methods based on capillary zone electrophoresis (CZE) are rapidly gaining acceptance as rugged analytical procedures [8,9]. Recently, CZE with indirect UV detection has been used to determine a number of different anions [10] and organic acids in a variety of samples [11]. CZE procedures exhibit greater chromatographic resolution, have the same order of repeatability and are often less costly to operate than HPLC [8,9]. It was of interest to see whether CZE could be used to provide a fast and robust method for the determination of cyclamate in low joule foods.

This paper describes a rapid CZE method for the determination of cyclamate in a variety of low joule foods using indirect UV detection at 254 nm. Alpha-hydroxyisobutyric acid was used as the internal standard. The accuracy of the procedure was evaluated by comparing the levels of cyclamate in the foods with those determined by the gravimetric procedure described in the literature [2]. The artificial sweeteners aspartame, acesulphame-K, alitame and saccharin can also be separated using this procedure.

2. Experimental

2.1. Reagents

Aspartame, sodium saccharin, potassium sorbate, alpha-hydroxyisobutyric acid, sodium cyclamate, hexadecyltrimethylammonium bromide (CTAB) and sodium benzoate were obtained from Sigma (St. Louis, MO, USA). OFM Anion-BT reagent was obtained from Waters Millipore (Milford, MA, USA). Hexadecyltrimethylammonium chloride was obtained from Aldrich (Milwaukee, WI, USA) and hexadecyltrimethylammonium hydroxide hydrate was obtained from Fluka Chemie (Buchs, Switzerland). Acesulfame-K and alitame were gifts from Hoechst (Frankfurt, Germany) and Pfizer (Groten, CT, USA), respectively. Caffeine was obtained from the Curator of Standards, Australian Government Analytical Laboratories, 1 Suakin Street, Pymble, NSW, Australia. All other chemicals and solvents were AR grade or HPLC grade and used without further purification.

2.2. CZE buffer

A 36.5-mg amount of hexadecyltrimethylammonium hydroxide hydrate was dissolved in 100 ml of 10 m*M* sodium benzoate. The solution was filtered through a 0.8-μm cellulose acetate filter disc before use.

2.3. Apparatus

CZE

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), with an electrolyte consisting of 1 m*M* hexadecyltrimethylammonium hydroxide and 10 m*M* sodium benzoate pH 6.6. An Isco Model 3140 electropherograph (Isco, Lincoln, NE, USA) operating at −20 kV and at 28°C was used for all determinations. The solutions were loaded under vacuum (vacuum level 2, 20 kPa s) and were detected at 254 nm with indirect detection and were determined quantitatively at 0.005 AUFS. The capillary was flushed with running buffer for 2 min between analyses. 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. Electropherograms were recorded with the ICE Data Management and Control Software supplied with the electropherograph.

HPLC

The apparatus and conditions for the determination of sorbate in the cordial sample are based on those described by Lawrence and Charbonneau [12] and are detailed in a separate report [13].

2.4. Gravimetric analysis

The level of cyclamate in the cordials, soft drink and jam were determined by the method described in the AOAC Official Methods of Analysis No. 20.180 [2].

2.5. Samples and standards

Samples

The samples were purchased from local outlets and analysed within the recommended "use by" dates. The cordials and soft drinks were diluted with an appropriate amount of deionised water. The jam was blended with deionised water using a commercial food processor, the solution filtered through a Whatman No. 1 filter paper and made to volume with deionised water. The concentration of cyclamate in the final solutions should be less than 100 μg/ml. Alpha-hydroxyisobutyric acid was used as the internal standard at a final concentration of 50 μg/ml. The solutions were filtered through a 0.45-μm cellulose acetate filter disc before analysis.

Standards

Standard solutions were prepared in deionised water with alpha-hydroxyisobutyric acid as the internal standard at a concentration of 50 μg/ml. The solutions were filtered through a 0.45-μm cellulose acetate filter disc before analysis. The detector response for cyclamate was linear to 100 μg/ml.

3. Results and discussion

The analysis of cyclamate by traditional HPLC methods has limited application as cyclamate does not show absorption in the usable UV range (above 200 nm). HPLC with indirect photometry [3] and ion chromatography with conductivity detection [4,5] have been used to determine cyclamate in a number of foods. Recently, CZE using indirect UV detection, has been used to separate and quantify a number of compounds that do not absorb in the usable UV range. These include anions, cations and organic acids [10,11]. To achieve the separation of anions and organic acids, a cationic surfactant is added to the UV absorbing electrolyte causing the electroosmotic flow to move towards the anode, forcing the anions to migrate in the same direction as the electroosmotic flow from the injection end to the detector end of the instrument. This mode of CZE using OFM Anion-BT reagent as the cationic surfactant has been well documented by Jandik and Jones [14]. Anions are well separated using a chromate electrolyte containing OFM Anion-BT reagent, whilst phthalate and benzoate electrolytes containing OFM Anion-BT reagent have been used for the analysis of organic acids [10,11]. These analyses are easy to perform, have reasonable sensitivity (ppm range) and have short run times. Cyclamate (cyclohexane sulphamate) (Fig. 1) which is negatively charged and does not absorb in the usable UV range is well suited for this type of analysis.

Initial experiments with a 10 m*M* sodium chromate electrolyte containing 1 m*M* OFM Anion-BT reagent resulted in poor peak shapes for cyclamate and alpha-hydroxyisobutyric acid (added as an internal standard) even though the migration times were less than 5 min. Replacing sodium chromate in the electrolyte with sodium benzoate resulted in much sharper peaks for cyclamate and alpha-hydroxyisobutyric acid even though the migration times for cyclamate and alpha-hydroxyisobutyric acid remained the same.

The separation of cyclamate and alpha-hydroxyisobutyric acid was maintained over twenty repetitive injections and the detector response for cyclamate was linear to 100 μg/ml. To test the suitability of the system for quantitative purposes, standard solutions of different concentrations were analysed seven times and the peak area repeatability data (C.V., %) for cyclamate determined. The C.V. data were acceptable

Cyclamate

Fig. 1. Structure of cyclamate.

(standard concentration, 5 μg/ml, C.V. 1.8%; 10 μg/ml, C.V. 1.6%; 20 μg/ml, C.V. 1.5%; 50 μg/ml, C.V. 2.3%; 100 μg/ml, C.V. 1.2%). The levels of cyclamate in a number of samples were then determined by CZE. The amounts of cyclamate present in the samples were in good agreement with those determined by the standard AOAC gravimetric procedure [2]. (Cordial 1 CZE 2.5 g/l, AOAC 2.6 g/l; cordial 2 CZE 6.5 g/l, AOAC 6.5 g/l; cordial 3 CZE 3.1 g/l, AOAC 3.2 g/l; cola CZE 0.7 g/l, AOAC 0.7 g/l; jam CZE 8.1 g/kg, AOAC 8.1 g/kg). None of the samples had any naturally occurring compounds that comigrated with the internal standard when analysed by CZE. The recoveries of added cyclamate and the peak area repeatability (C.V., %) data for cyclamate for seven replicate determinations were also excellent (cordial 1, recovery 101%, C.V. 1.2%).

The electropherogram of cordial 2 contained a negative peak which migrated before cyclamate and was assigned as sorbate. The peak appears in the opposite direction to the other peaks in the electropherogram as sorbate absorbs UV light more strongly than the benzoate electrolyte. The separation of sorbate and cyclamate was only marginal with this electrolyte and so other cationic surfactants were trialled in order to improve the separation and therefore the accuracy of the quantitation. Replacing OFM Anion-BT reagent with hexadecyltrimethylammonium chloride had little effect on the separation, whist the separation was only marginally better when OFM Anion-BT reagent was replaced with hexadecyltrimethylammonium bromide. Replacing OFM Anion-BT reagent with hexadecyltrimethylammonium hydroxide gave a greater separation of cyclamate and sorbate. The separation of cyclamate, sorbate and alpha-hydroxyisobutyric acid was maintained over twenty repetitive injections and the detector response for cyclamate was linear to 100 μg/ml, whilst the detector response for sorbate was linear to 5 μg/ml. The electropherograms showing the separation of cyclamate and sorbate with electrolytes containing Anion OFM-BT reagent and hexadecyltrimethylammonium hydroxide as the cationic surfactants are displayed in Fig. 2A,B.

To test the suitability of this system for quantitative purposes, standard solutions of different concentrations were analysed seven times to evaluate the peak area repeatability data (C.V., %) for cyclamate and sorbate. As with the previous system, the data were acceptable (cyclamate, standard concentration 5 μg/ml, C.V. 4.6%; 10 μg/ml, C.V. 1.7%; 20 μg/ml, C.V. 2.1%; 50 μg/ml, C.V. 1.6%; 100 μg/ml, C.V. 2.4%; sorbate, standard concentration 0.2 μg/ml, C.V. 3.9%; 0.5 μg/ml, C.V. 2.5%; 1.0 μg/ml, C.V. 1.7%; 2.0 μg/ml, C.V. 0.4%; 5.0 μg/ml, C.V. 2.1%).

The levels of cyclamate in three samples of low joule cordial and a sample of low joule cola were then determined using this electrolyte, with one cordial (sample 5) being analysed seven times for area repeatability (C.V., %) data. The electropherogram for cordial 5 is displayed in Fig. 2C. The levels of cyclamate in the samples were in good agreement with those determined by the AOAC gravimetric procedure [cordial 3, CZE 3.0 g/l, AOAC 3.2 g/l; cordial 4, CZE 2.2 g/l, AOAC 2.5 g/l; cordial 5, CZE 5.5 g/l (C.V. 1.0%), AOAC 5.5 g/l; cola, CZE 0.7 g/l, AOAC 0.7 g/l]. The sorbate content of cordial 5 compared favourably with the level determined by HPLC [12] (CZE 85 mg/l, HPLC 90 mg/l).

The results indicate that cyclamate can be determined quantitatively by CZE with either OFM Anion-BT reagent or hexadecyltrimethylammonium hydroxide as the cationic surfactant. However, a better separation of sorbate and cyclamate is seen with hexadecyltrimethylammonium hydroxide as the cationic surfactant. This would result in a more accurate quantitation, and is therefore the preferred electrolyte for the analysis.

Other food additives could also be separated with this CZE procedure. The artificial sweeteners, aspartame, alitame, acesulphame-K and saccharin also migrate with this buffer (Fig. 3). Acesulphame-K appears as a negative peak, while aspartame and alitame appear as positive peaks. Saccharin appears as a broad negative peak in the electropherogram. Saccharin is present in cordial 5 and in the diet cola and it was of interest to see if saccharin could be determined

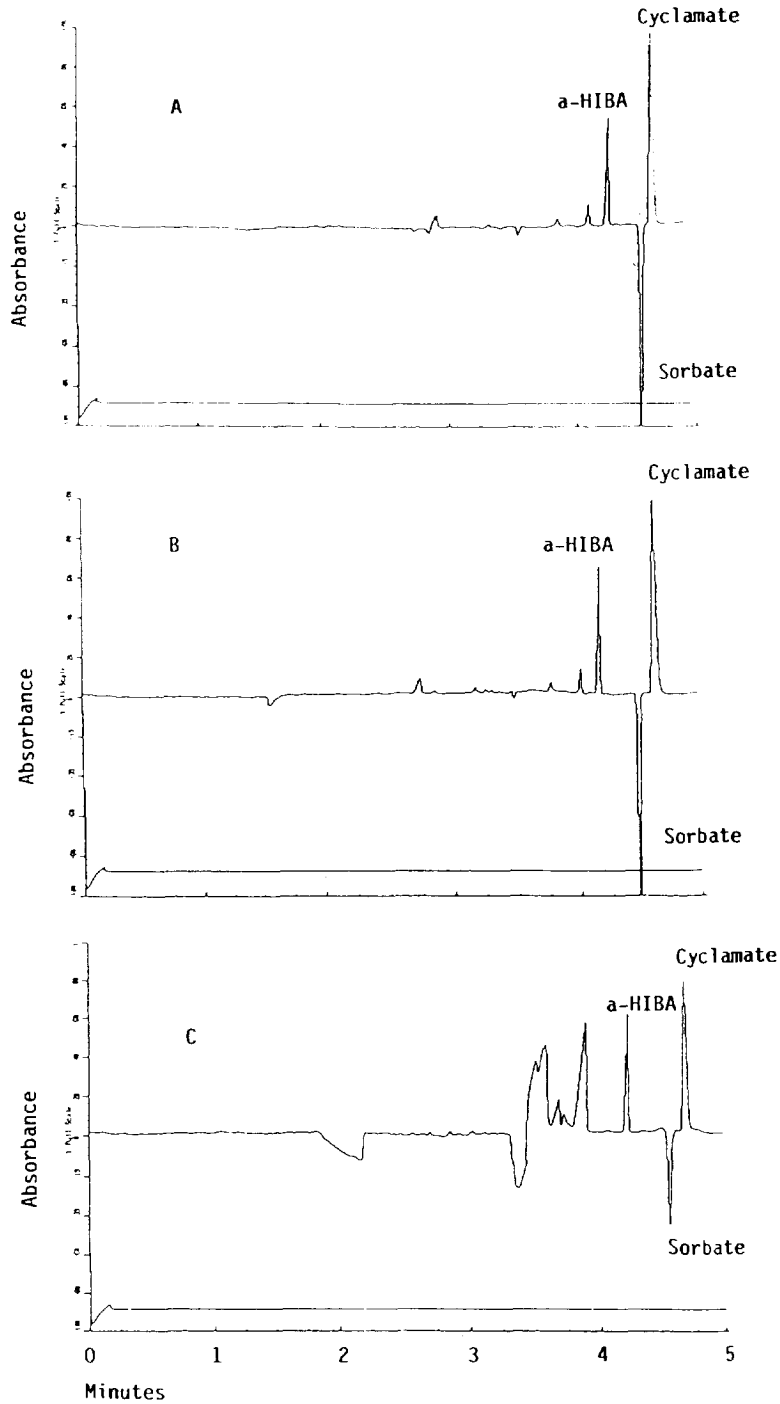

Fig. 2. Electropherograms of (A) standard solution containing cyclamate and sorbate using a 10 m*M* sodium benzoate, 1 m*M* OFM Anion-BT reagent electrolyte, (B) standard solution containing cyclamate and sorbate using a 10 m*M* sodium benzoate, 1 m*M* hexadecyltrimethylammonium hydroxide electrolyte, and (C) cordial 5 containing cyclamate and sorbate using a 10 m*M* sodium benzoate, 1 m*M* hexadecyltrimethylammonium hydroxide electrolyte. Alpha-hydroxyisobutyric acid (a-HIBA) was used as the internal standard. The *x*-axis gives the migration time in minutes.

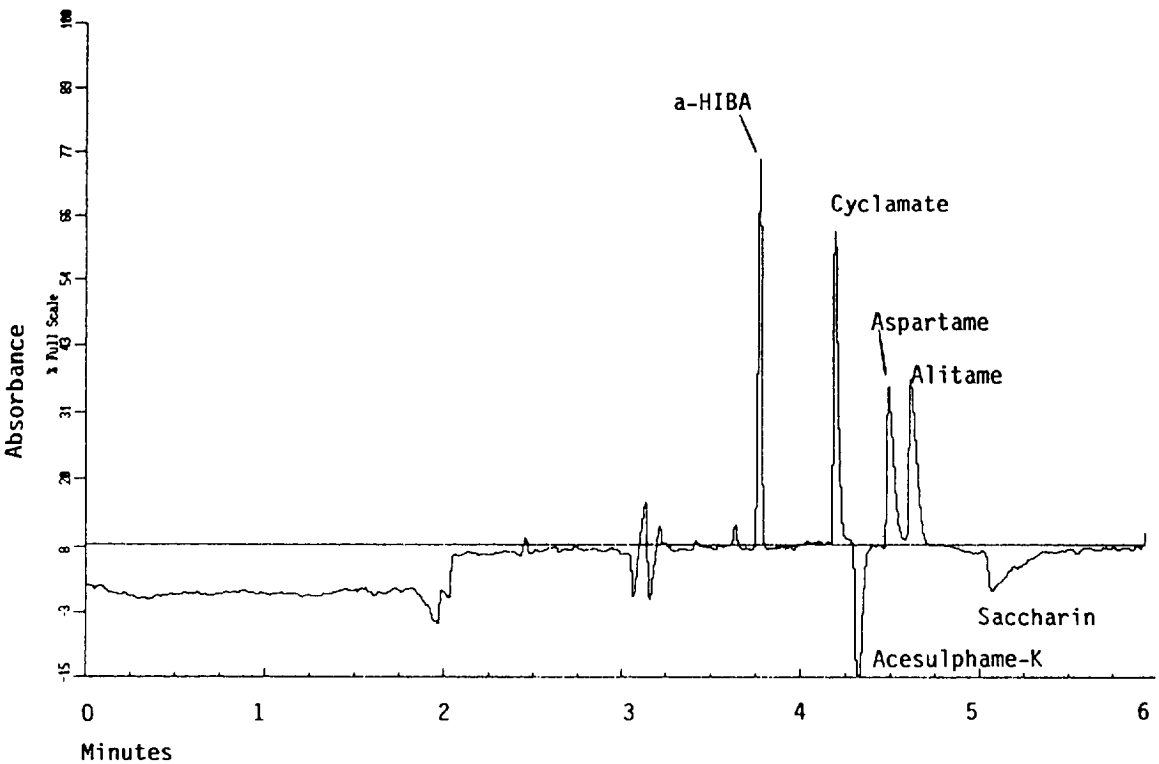

Fig. 3. Electropherogram showing the separation of cyclamate, acesulfame-K, aspartame, alitame, and saccharin using a 10 m*M* sodium benzoate, 1 m*M* hexadecyltrimethylammonium hydroxide electrolyte. Alpha-hydroxyisobutyric acid (a-HIBA) was used as the internal standard. The *x*-axis gives the migration time in minutes.

in the same analysis. However, this could not be done due to the poor detector response for saccharin. The diet cola also contains caffeine and benzoic acid. Caffeine does not migrate with this system and benzoic acid cannot be determined as the electrolyte contains sodium benzoate.

Cyclamate, saccharin and alpha-hydroxyisobutyric acid are well separated with an electrolyte consisting of 10 m*M* potassium hydrogen phthalate and 1 m*M* OFM Anion-BT reagent. In this instance, saccharin appeared as a positive peak in the electropherogram. However, as with the previous system, the detector response for saccharin was too small to be of any use for quantitative purposes.

In an earlier report, saccharin, caffeine, benzoic acid, sorbic acid and a number of other artificial sweeteners were separated and determined in diet drinks and cordials by micellar electrokinetic capillary chromatography (MEKC) [13]. The separation was achieved using a fused-silica capillary with the same dimensions as the one used in the present work and with a buffer consisting of 0.05 *M* sodium deoxycholate, 0.01 *M* sodium borate and 0.01 *M* potassium dihydrogen orthophosphate, pH 8.6. The analyses were performed at 20 kV and at 28°C with the compounds being detected by UV at 220 nm. Cyclamate and saccharin (and the other food additives) can therefore be determined in the same sample using the same column with different buffers and detector settings. After cyclamate has been determined, the column is washed with 0.1 *M* sodium hydroxide, refilled with the MEKC buffer and the detector settings optimised for the other analyses. Fig. 4 shows consecutive electropherograms for the

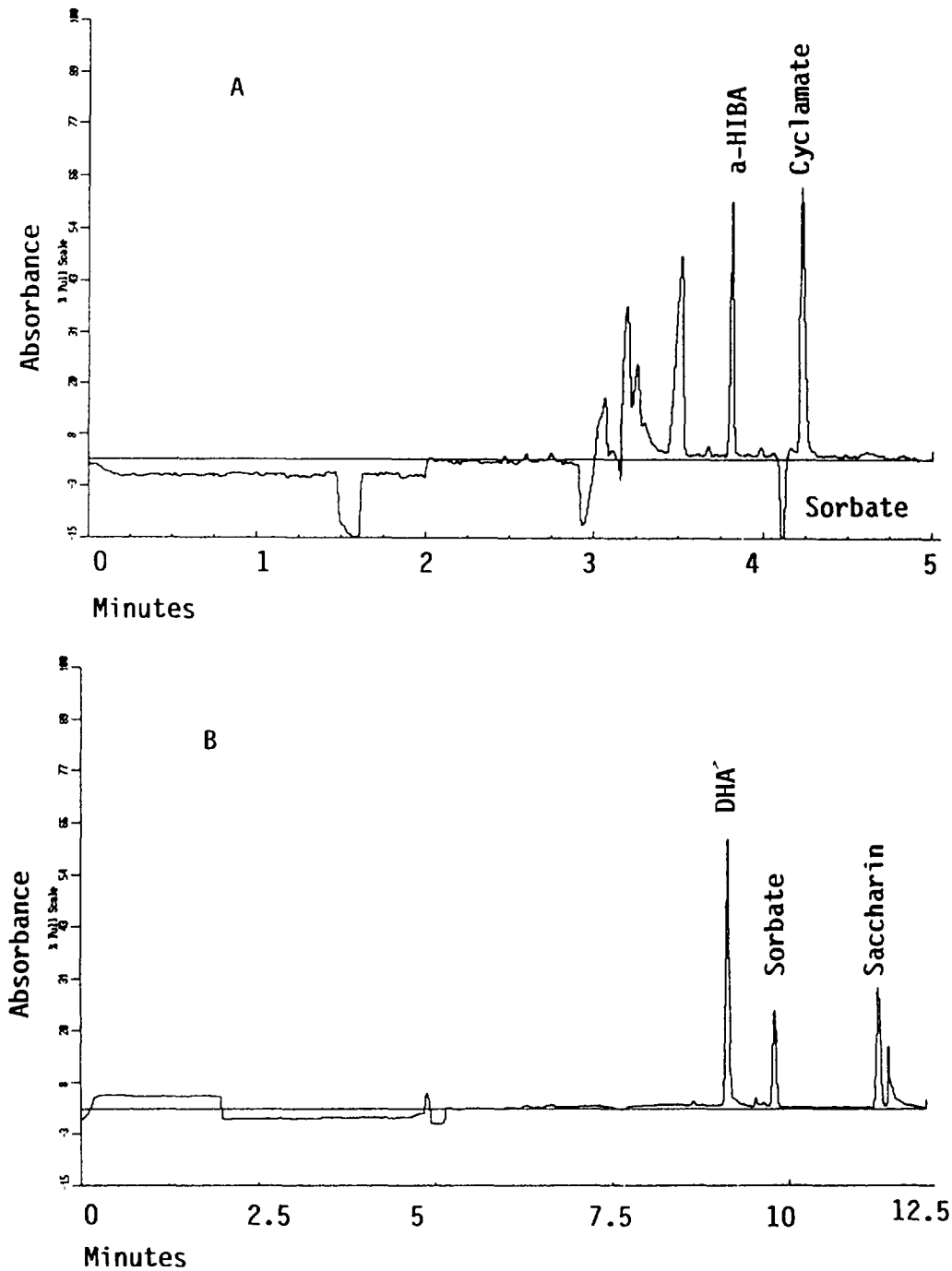

Fig. 4. Electropherograms of cordial 5 containing cyclamate, sorbate and saccharin using (A) a 10 m*M* sodium benzoate, 1 m*M* hexadecyltrimethylammonium hydroxide electrolyte with alpha-hydroxyisobutyric acid (a-HIBA) as the internal standard, and (B) using a 0.05 *M* sodium deoxycholate, 0.01 *M* potassium dihydrogen orthophosphate, 0.01 *M* sodium borate buffer pH 8.6 with dehydroacetic acid (DHA) as the internal standard. The *x*-axis gives the migration time in minutes.

determination of cyclamate, saccharin and sorbate in cordial 5. Although this is not as convenient as determining the analytes in one run, it is a practical alternative and shows the versatility of capillary electrophoresis as an analytical tool. This process can be fully automated with the ISCO Model 3140 electropherograph and other automated instruments.

4. Conclusion

A rapid method for the determination of cyclamate in low joule cordials, diet cola and low joule jam by capillary zone electrophoresis (CZE) is described. The levels of cyclamate were in good agreement with those determined by the standard AOAC gravimetric procedure. Sorbic acid, which is often added to low joule cordials as a preservative, can also be accurately determined with this procedure. Other food additives, e.g. saccharin and benzoic acid, which are often added to low joule cordials containing cyclamate, and caffeine, which is present in low joule colas containing cyclamate, cannot be determined quantitatively with this system. However, the amounts of the additives can be accurately determined in these samples by using a different MEKC system with the same capillary column.

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