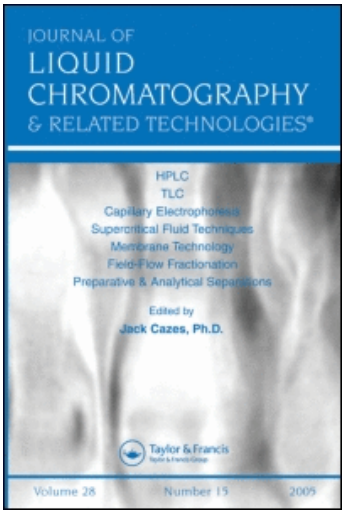

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

CAPILLARY ELECTROCHROMATOGRAPHY: ANALYSIS OF SUCRALOSE AND RELATED CARBOHYDRATE COMPOUNDS

Runhua R. Zhao[a]; Bruce P. Johnson[a]
[a] Technical Center, Athens, GA, U.S.A.

Online publication date: 13 July 2000

J. LIQ. CHROM. & REL. TECHNOL., 23(12), 1851–1857 (2000)

CAPILLARY ELECTROCHROMATOGRAPHY: ANALYSIS OF SUCRALOSE AND RELATED CARBOHYDRATE COMPOUNDS

Runhua R. Zhao, Bruce P. Johnson*

McNeil Specialty Products Company
Technical Center
1440 Olympic Drive
Athens, GA 30601-1645, USA

ABSTRACT

Sucralose is a high intensity, non-nutritive sweetener, which is approximately 600 times sweeter than sucrose. The analysis of this sweetener and similar carbohydrates is challenging, due to both the difficulty of detection as well as the need to separate numerous very similar structures. Capillary Electrochromatography (CEC) was used to analyze sucralose and related carbohydrate compounds. Separation of sucralose and several very similar compounds was achieved by CEC with a C18-silica packing, and low organic content mobile phases. Effects of mobile phase composition on the separation of these compounds are discussed.

INTRODUCTION

Sucralose is a high-intensity, non-nutritive sweetener, which is made from sucrose. It is approximately 600 times sweeter than sucrose, and possesses sensory characteristics that are very similar to sucrose. Sucralose is a chlorodeoxysugar (4,1',6'-trichlorogalactosucrose), whose structure is shown in Figure 1. As part of this work, two other compounds were synthesized, which are very similar except for the addition of an acetate ester (compound A in Figure 1), or

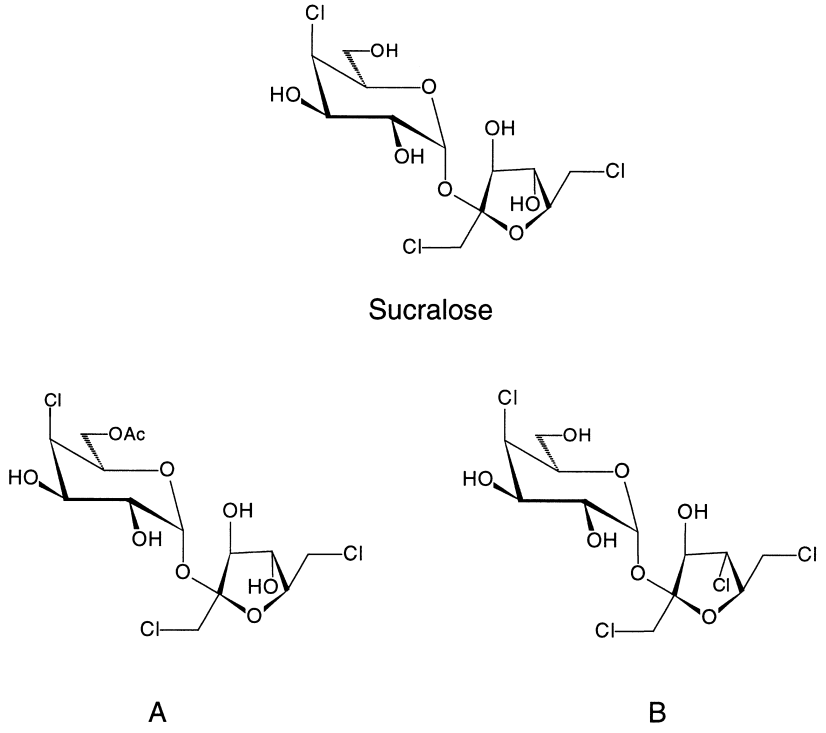

Figure 1. Structures of sucralose and related carbohydrate compounds A and B.

an additional chlorine atom (compound B in Figure 1). While the analysis of sucralose and related compounds is obviously important for the manufacture of sucralose, it presents a number of challenges for analytical chemists, due to both the difficulty of detection, as well as, the need to separate very similar structures. To date, reversed-phase HPLC with evaporative light-scattering, refractive index, and optical rotation detection have been the major technologies for the analysis of these compounds, each with distinct advantages and disadvantages.[1-3]

Capillary electrochromatography has grown rapidly in the last several years, although its first paper was published over twenty years ago.[4-6] As a combination of capillary electrophoresis and HPLC, CEC offers both the high efficiency and resolution of CE and the versatility of HPLC. Both charged and neutral species can be separated at the same time in CEC.[7-8] The use of CEC in the analysis of sucralose and related compounds will not require charged ana-

lytes. Most published CEC papers involved analyses of relatively hydrophobic compounds such as aromatic hydrocarbons with high organic content mobile phases.[6, 8] Successful use of a low organic content mobile phase in CEC is very meaningful for expanding CEC applications to more hydrophilic compounds, such as carbohydrates, drugs, and drug metabolites. To our knowledge, there is only one previous report of the use of CEC in the analysis of carbohydrates.[9]

In this paper, we report CEC analysis of sucralose and related compounds. Effects of mobile phase composition on the separation of these compounds are discussed.

EXPERIMENTAL

Sucralose and related carbohydrate compounds were synthesized in-house.[10,11] Aqueous sodium borate (50 mM) was purchased from Hewlett Packard (Palo Alto, California).

A Hewlett-Packard[3D] Capillary Electrophoresis system with a photo-diode array detector was used for all analyses. A low wavelength (195 nm) was used to detect these compounds. The CEC column used in this work was purchased from Unimicro Technologies, Inc. (Pleasanton, California), and was packed with a 25 cm bed of 3-μm ODS particles. The column had a total length of 33 cm and a 100 μm inner diameter. All electrochromatograms were collected with Hewlett-Packard Chem Station software.

Columns were conditioned with mobile phase. A15 bar external pressure from a helium tank was applied on the inlet of the column, along with a 5 kV voltage, until the current remained constant. Mobile phases of different compositions were made by mixing acetonitrile with 4 mM borate buffer. Samples and CEC mobile phases were filtered with 0.2 μm Nylon filters (Hewlett-Packard) prior to use. To prevent bubble formation, a 6 bar external pressure was applied to both home vials during each run. Sample solutions were made by dissolving the pure compounds in the mobile phase. The concentrations of all carbohydrate compounds were about 4 mg/mL in the prepared sample solutions. All injections were made by a 10 kV voltage for 2 seconds. Cartridge temperature was controlled at 20°C.

RESULTS AND DISCUSSION

Various mobile phases and voltages were used to optimize the separation of sucralose and its related compounds. An initial acetonitrile-4 mM borate buffer (80:20) mobile phase and a 10 KV voltage were used to separated a mixture of sucralose and compound A. No separation was observed as in Figure 2(a). This is due to the strong hydrophilic nature of these compounds. Organic

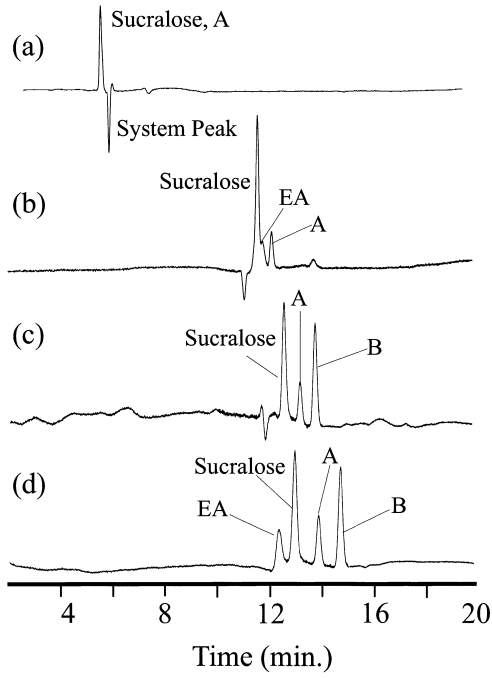

Figure 2. Electrochromatograms of sucralose and related compounds with different mobile phases at 10 kV run: (a) 80/20 acetonitrile/4 mM borate, (b) 55/45 acetonitrile/4 mM borate, (c) 50/50 acetonitrile/ 4 mM borate, and (d) 45/55 acetonitrile/4 mM borate.

content of mobile phase was reduced stepwise. No significant separation was achieved until the organic content was decreased to fifty-five percent, as shown in Figure 2(b), where sucralose and Compound A were fully resolved. Individual components were injected to identify elution order in this study. The shoulder on the sucralose is due to the presence of traces of ethyl acetate (EA) in Compound A. The elution order of EA with respect to the carbohydrates was found to change with mobile phase composition. Change of elution order with the change of mobile phase organic content in CEC was first reported and discussed by M. M. Dittmann in 1997.[12]

Sucralose, and Compounds A and B, were baseline resolved in a mobile phase of fifty percent acetonitrile as illustrated in Figure 2(c). Such elution order was predicted. Sucralose is the least hydrophobic among the three compounds. Both Compounds A and B are more hydrophobic than Sucralose, with addition of an acetate ester (A) and a chlorine atom (B), respectively (Figure 1). Although, it is hard to judge which of Compound A and B should have stronger affinity with C18 column, the elution order is consistent with HPLC results.

In Figure 2(c), EA did not appear, and may coelute with sucralose in this mobile phase. Sucralose, Compounds A, B, and EA were well separated in forty-five percent acetonitrile, shown in Figure 2(d). It should be noted that EA eluted before sucralose in this mobile phase. In Figure 3(a), organic content was further decreased to thirty-five percent, resulting in further separation of sucralose and related compounds. Voltage was adjusted from 10 kV to 15 kV in this and following chromatograms, in order to obtain approximately the same retention time. The first peak at 8.33 min, is the system peak which can be positive or negative, present or absent, depending on the solvent used to prepare the samples.

It was observed that, if a mobile phase is used to prepare the sample, a minimum or no solvent peak is more likely to be obtained. Sucralose and related compounds were further separated in Figure 3(b) and 3(c) with mobile phases composed of thirty and twenty-five percent of acetonitrile, respectively. These conditions are sufficient to resolve other sucralose related compounds.

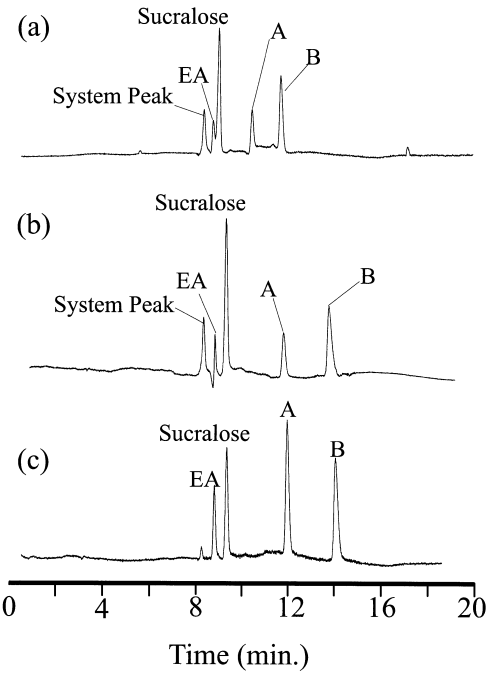

Figure 3. Electrochromatograms of sucralose and related compounds in different mobile phases at 15 kV run: (a) 35/65 acetonitrile/4 mM borate, (b) 30/70 acetonitrile/4 mM borate, and (c) 25/75 acetonitrile/ 4 mM borate.

Separation under these conditions is comparable to the separation with gradient HPLC, yet within a shorter analysis time. Higher concentration samples were used to overcome the low UV detection sensitivity. This may be solved by the use of indirect UV detection or other detection techniques in the future.

CONCLUSIONS

CEC with a C18 packing, and a acetonitrile / borate buffer mobiles phase was investigated for sucralose and its related compounds. Mobile phases, composed of 25 to 80% acetonitrile, were compared for the separation of these compounds. Sucralose and several similar compounds were well separated with a low (25-30%) acetonitrile mobile phase at 15 KV voltage. The separation under these conditions is comparable to that of HPLC, but within a shorter time.

ACKNOWLEDGMENT

The authors of this paper wish to thank Dr. Chan Yan of Unimicro Technologies, Inc., for his technical support and helpful discussions.

REFERENCES

1. B. P. Johnson, C. A. Lyons, **Proceedings of the 46th Pittsburgh Conference and Exposition on Analytical Chemistry**, Presentation 428, New Orleans, LA, Mar 6 - Mar 10, 1995.

2. H. Ichiki, M. Semma, Y. Sekiguchi, M. Nakamura, Y. Ito, Jpn. J. Food Chem., **2**, 119-121 (1995).

3. M. E. Quinlan, M. R. Jenner, J. Food Sci., **55**, 244-226 (1990).

4. V. Pretorius, H. B. J. Hopkins, J. D. Schieke, J. Chromatogr. A, **99**, 23-30 (1974).

5. J. W. Jorgenson, K. D. Lukacs, J. Chromatogr, **218**, 209-216 (1981).

6. R. Dadoo, C. Yan, R. Zare, D. Anex, D. Rakestraw, G. Hux, LC/GC **15**, 630-635 (1997).

7. L. Colon, Y. Guo, A. Fermier, Anal. Chem., **69**, 461A-467A (1997).

8. M. M. Dittmann, K. Wienand, F. Bek, G. P. Rozing, LC/GC, **13**, 802-814 (1995).

9. Z. El Rassi, C. Yang**, The Eighth Annual Conference on Capillary Electrophoresis**, Frederick, Maryland, October 20-22, 1997.

10. T. O'Conner, "Chlorination of Carbohydrates and Other Alcohols," US Patent 4,783,526, 1988.

11. J. L. Navia, R. E. Walkup, N. M. Vernon, R. E. Wingard, Jr., "Sucralose Pentaester Production," US Patent 5,298,611, 1994.

12. M. M. Dittmann, G. P. Rozing, Proceedings of the 19th International Symposium on Capillary Chromatography and Electrophoresis, Wintergreen, Virginia, 1997, p. 84-85.

Received August 27, 1999 Author's Revisions March 5, 2000
Accepted September 14, 1999 Manuscript 5157