Vitamin Analysis by Capillary Electrophoresis

Paul F. Cancalon, Florida Department of Citrus, Lake Alfred, Florida, USA.

Introduction

Capillary electrophoresis (CE) is a relatively new technique, which has expanded considerably in the last decade. Such rapid development is the result of several factors: sample preparation is reduced to a minimum, separation can usually be achieved quickly and solvent requirements are minimal. With the method we have developed to analyse citrus juices, preparation requires only filtration and dilution. The separation is rapid, up to thirty citrus juice compounds can be separated in 20 min. There is no reconditioning period between each analysis and after a 3 min wash a new sample can be started. These CE characteristics are well suited for continuous analysis with little intervention from the analyst. Finally, the use of the instrument is very cost effective. Seventy centimetres of bare fused-silica capillary cost very little and the same tubing can be used for several years, if maintained properly. Considering that the capillary diameter is 50 µm, samples can be run with very small amounts of buffer; this is also an important point considering the cost of buying and disposing of solvents. Finally, the problem of detection limit, which was a major drawback in early CE, has now been largely resolved and CE detection is now similar to that of high performance liquid chromatography (HPLC).

Numerous CE procedures have been developed to analyse a large variety of organic and inorganic compounds.[1–4] CE has been applied successfully in the areas of pharmaceutical analysis,[5,6] clinical chemistry[7] and food analysis.[8–11] A significant number of these published papers were dedicated to vitamin analysis in pharmaceutical research[13] and in food analysis.[14,15] Vitamins can be divided into two groups: water- and lipid-soluble. A general review of the method of analysis of fat- and water-soluble vitamins has been published by Song et al.[16] Recently, efforts have been made to analyse simultaneously hydrophilic and hydrophobic vitamins. Delgado-Zamarreno et al.[17] analysed water- and fat-soluble vitamins in tablets by electrokinetic capillary chromatography using 25 mM bis(2-ethylhexyl)sodium sulphosuccinate (AOT) as pseudostationary phase, 20% acetonitrile, 2% methanol and 25 mM borate buffer pH 8.4 at 24 kV. A similar separation of fat- and water-soluble vitamins was achieved by Sanchez and Salvado[18] using microemulsion electrokinetic chromatography (MEEKC) with a buffer containing 40 mM borate, 80 mM sodium dodecyl sulphate (SDS), 5% butanol and 15% propanol. Vitamin E, a fat-soluble vitamin, has been measured by micellar electrokinetic chromatography (MEKC)[19] with a buffer of 7 mM borate, 14 mM phosphate, 15 mM SDS, 10 mM sodium cholate, 8% acetonitrile (pH 9.2) at 214 nm, 16 kV and 26 °C.

CE has been applied successfully in the areas of pharmaceutical analysis, clinical chemistry and food analysis.

Many studies were aimed at the separation of water-soluble vitamins. Capillary zone electrophoresis (CZE) with laser-induced fluorescence detection was used by Cataldi et al.[20] to analyse riboflavin and riboflavin derivatives with a 30 mM phosphate buffer pH 9.8 at 15 °C and 30 kV. Total and available niacin were measured by Vidal-Valverde et al.[21] in raw and processed legumes with a buffer of 15 mM borate, 15 mM SDS and 20% isopropyl alcohol pH 9.2 at 30 kV with UV detection at 254 nm. Su et al.[22] determined simultaneously thiamin, riboflavin, pyridoxine, nicotinamide, nicotinic acid and ascorbic acid by MEKC with 4% acetonitrile in 0.02 M borate/phosphate buffer with 0.1 M SDS. Hu et al.[23] analysed nicotinamide, pyridoxine and ascorbic acid by MEKC with amperometric detection and a phosphate/borate buffer pH 8.8 with 0.03 M SDS. Li et al.[24] analysed rutin and ascorbic acid in tablets by CZE with a borate buffer at pH 7.5. Water-soluble vitamins (B1, B2, B6, B12, vitamin C and nicotinamide) were analysed by CZE with a 0.02 M boric acid buffer with UV detection.[25]

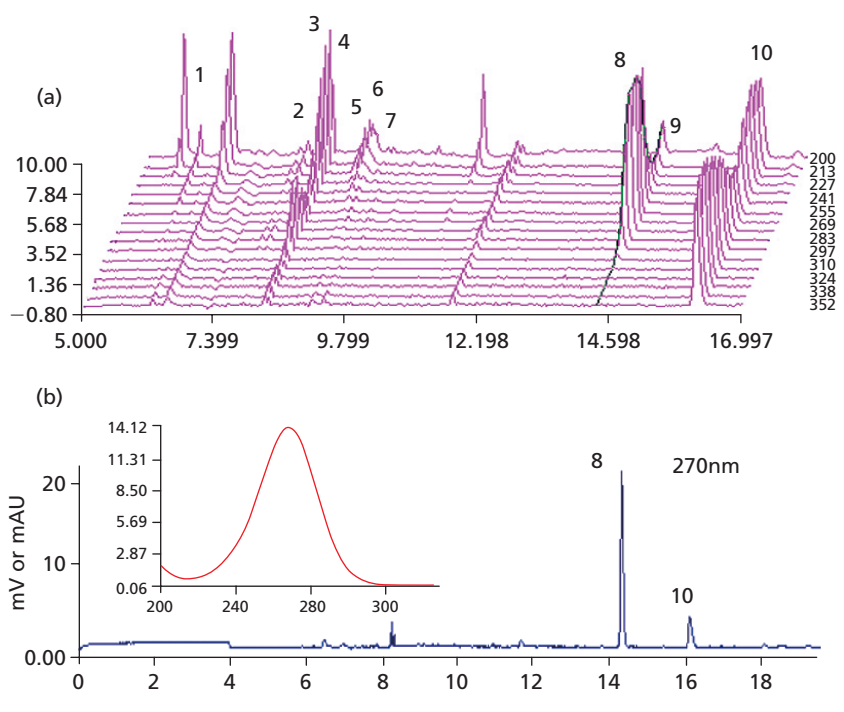
Vitamin C has been most often analysed by CE. Thompson and Trenerry developed an MEKC method with a sodium deoxycholate buffer to assess L-ascorbic acid in fruits and vegetables.[26] The total ascorbic and dehydroascorbic acids were measured after extraction with metaphosphoric acid and reduction of the dehydro form with 0.2% D,L dithiothreitol. Total ascorbic acid was estimated by Herrero-Martinez et al.[27] in various plant extracts after reduction with homocysteine using CZE with a phosphate buffer containing 0.0001% hexadimetrine bromide. A 200 mM borate buffer pH 9 was used by Davey et al.[28] to measure L and D ascorbic acid, at 260 nm, in parsley and mushroom after extraction with metaphosphoric acid, and reported a limit of detection of 84 fmol/injection. When comparing HPLC and CE methods the authors found CE more adequate for the analysis of low vitamin concentration in certain complex matrices, such as

mushroom. L-Cysteine (10 mM) was used by Schiewe et al.[29] to stabilize ascorbic acid in citrus juices without extraction and separation was performed by CZE with a 20 mM phosphate buffer (pH 8). A complex isotachophoresis (ITP)–CZE method was used by Prochazkova et al.[30] to measure trace amounts of ascorbic acid (0.09–0.15 mg/L) in human body fluids. Ascorbic acid and total vitamin C (ascorbic acid and dehydro ascorbic acid (DHAA)) were measured by Liao et al.[31] in various fruits and spinach using 0.2 M borate buffer (pH 9). DHAA was reduced into ascorbic acid by 0.8% homocysteine. We have quantified ascorbic acid and the total vitamin C in citrus juices using a method developed for monitoring many citrus compounds.[32–34] This procedure will be reviewed in greater detail.

Experimental

Analyses were performed with a Spectra-Physics 1000 electrophoresis system (ThermoQuest, Fremont, California, USA) equipped with high-speed scanning detection in the UV range. Separation was achieved as published previously[32,33] with uncoated fused-silica capillary tubings 70 cm × 50 µm (Polymicro Technologies, Phoenix, Arizona, USA) with a 35 mM sodium borate buffer (pH 9.3) containing 5% (v/v) acetonitrile. Electrophoresis analyses were run at 21 kV and 23 °C. Juice samples were diluted fourfold with HPLC-grade water containing 1000 mg/L ethylenediamine tetracetic acid (EDTA) and filtered with a 25 mm GD/X cellulose acetate filter (Whatman, Clifton, New Jersey, USA). A 100 µL solution of ferulic acid (Sigma, St Louis, Missouri, USA), final concentration 31.25 mg/L, was added to the juice as an internal standard (IS). Samples were injected hydrodynamically for 10 s. Quantifications were performed with ThermoQuest PC 1000 software using the internal standard procedure. Standard samples containing 31.25 mg/L of ferulic acid and 0.5–700 mg/L of ascorbic acid were run to establish standard curves.

Figure 1: (a) Three–dimensional electropherogram of orange juice, each wavelength can be extracted and examined separately. (b) To quantify ascorbic acid the electropherogram at 270 nm can be extracted and the spectrum of ascorbic acid examined for identification and purity (inset). (Reproduced from *J. AOAC Int.*, **84**, 987–991 (2001). Copyright 2001 by AOAC International.)



Peaks: 1 = synephrine, 2 = didymin, 3 = hesperidin, 4 = narirutin, 5 = phlorin, 6 = phenyl alanine, 7 = tyrosine, 8 = ascorbic acid, 9 = heat peak (unknown compound generated during storage of citrus juice), 10 = ferulic acid (internal standard).

The total vitamin C was measured by reducing the DHAA acid with a solution of 0.2% D,L dithiothreitol, final concentration.

Results and Discussion

A CE procedure had been developed previously to monitor citrus juice samples.[32,33] As seen in Figure 1(a), many components could be separated simultaneously including ascorbic acid. The ascorbic acid spectrum (inset, Figure 1(b)) gave a maximum at 268 nm and the 270 nm electropherogram was chosen for calculation (Figure 1(b)). The analysis was achieved in 20 min and the required preparation was limited to dilution and filtration. Separation was achieved with an uncoated capillary using a 35 mM sodium borate buffer (pH 9.3) containing 5% (v/v) acetonitrile at 21 kV and 23 °C. Detection was performed by high-speed scanning between 200 and 360 nm. From the multiwave length scan, the electropherogram at 270 nm was extracted and used to quantify ascorbic acid. The ascorbic acid concentration was calculated with an IS method, with ferulic acid as internal standard.[34] However, the level of ascorbic acid decreased rapidly once the juice was diluted; this was a major problem because many samples must be analysed successively. Diluting the juice with water containing 1000 ppm EDTA (750 mg/L final concentration) prevented ascorbic acid oxidation. Figure 2 shows that after 7 h the sample containing EDTA has maintained its ascorbic acid level, while the level in the control juice has dropped by 70%. EDTA prevented oxidation for more than 7 h and allowed samples to be analysed without losses. The values measured with CE were identical to those obtained by HPLC (Table 1).[34] Another problem is the question of ascorbic acid and total vitamin C. Both ascorbic acid and its oxidized form, DHAA, have vitamin C activity as indicated by the Food and Nutrition Board of the Institute of Medicine.[35] However, DHAA does not absorb in the UV range, but its ability to be reduced back into ascorbic acid has been used to determine the total vitamin C level. As already seen, addition of the reducing agent dithiothreitol has been adopted by several groups to determine the total level of ascorbic acid.[6,26] Five commercial orange juices in cartons were analysed before their expiration date, and of the total vitamin C, 15.9 ± 3.1% was DHAA (Figure 3). These values were typical of the level of DHAA routinely measured in juices collected from retail stores.

Conclusion

This juice analysis procedure has been used for several years in our laboratory to analyse more than 1000 citrus juice samples/year. It has allowed us to monitor simultaneously several compounds that have an impact on juice quality, including vitamin C, but also synephrine (a biogenic amine), several flavonoids (didymin, hesperidin, narirutin, neo-hesperidin and naringin), the polyphenol phlorin and three amino acids absorbing in the UV range (tryptophan, phenylalanine and tyrosine). By contrast, monitoring the same compounds by HPLC would require several separate analyses. In our laboratory, the introduction of CE has reduced the number of analysts involved in juice monitoring from three to one. Finally it should be added that, by simply changing the methods, the same capillary can be used to analyse sugars and organic acids. To summarize, we have found CE most effective in the monitoring of citrus juices.

References

1. J.P. Landers, Ed, *A Handbook of Capillary Electrophoresis*, (CRC Press, Boca Raton, USA, 1997).
2. M.G. Khaledi, Ed, *High-Performance Capillary Electrophoresis: Theory, Techniques and Applications*, (Wiley Press, New York, USA, 1998).
3. P. Camilleri, Ed, *Capillary Electrophoresis, Theory and Practice, 2nd edition*, (CRC Press, Boca Raton, USA, 1998).
4. K.D. Altria, *J. Chromatogr. A*, **856**, 443–463 (1999).
5. C.M. Liu and Y.-M. Tzeng, *J. Food and Drug Analysis*, **6**, 391–404 (1998).
6. K.D. Altria, A.B. Chen and L. Clohs, *LC•GC Eur.*, **14**(12), 736–744 (2001).
7. D. Perrett, *Ann. Clin. Biochem.*, **36**, 133–150 (1999).
8. P.F. Cancalon, in *Handbook of Capillary Electrophoresis Applications*, H. Shintani and J. Polonski, Eds, (Blackie Academic and Professional, London, 1996) 583–606.
9. P.F. Cancalon, in *Encyclopedia of Analytical Chemistry*, R.A. Meyers, Ed, (John Wiley & Sons, Chichester, UK, 2000), 3929–3955.
10. F. Kvasnicka, *Electrophoresis*, **21**, 2780–2787 (2000).
11. R.A. Frazier, *Electrophoresis*, **22**, 4197–4206 (2001).
12. M.C. Boyce, *Electrophoresis*, **22**, 1447–1559 (2001).
13. D. Perrett, in *Capillary Electrophoresis. Theory and Practice, 2nd Edition*, P. Camilleri, Ed, (CRC Press, Boca Raton, USA, 1998), 441–485.
14. V.C. Trenerry, *Electrophoresis*, **22**, 1468–1478 (2001).
15. D.L.B. Wetzel, Ed, *Instrumental Methods in Food and Beverage Analysis*, Elsevier, New York (1998).
16. W.O. Song, G.R. Beecher and R.R. Eitenmiller, Eds, *Modern Analytical Methodologies in Fat- and Water-Soluble Vitamins*, (Weimar,Texas, USA, 2000).
17. M.M. Delgado-Zamarreno et al., *J. Chromatogr. A*, **953**, 257–262 (2002).
18. J.M. Sanchez and V. Salvado, *J. Chromatogr. A*, **950**, 241–247 (2002).
19. J.X. Zhao et al., *Electrophoresis*, **22**, 151–154 (2001).
20. T.R.I. Cataldi et al., *J. Chromatogr. A*, **968**, 229–239 (2002).
21. C. Vidal Valverde et al., *Electrophoresis*, **22**, 1479–1483 (2001).
22. S.-C. Su et al., *J. Food Sci.*, **66**, 10–14 (2001).
23. Q. Hu et al., *Anal. Chem. Acta.*, **437**, 123–129 (2001).
24. X.J. Li, H. Xiong and Z.B. Yuan, *Fenzi Shiyanshi*, **20**, 41–43 (2001).
25. J.-Q. Zhang et al., *Zhongguo Yiyao Gongye Zazhi*, **31**, 405–407 (2000).
26. C.O. Thompson and V.C. Trenerry, *Food Chemistry*, **53**, 43–50 (1995).
27. J.M. Herrero-Martinez et al., *Anal. Biochem.*,

Figure 2: Effect of the addition of EDTA at 750 mg/L final solution to prevent ascorbic acid oxidation. (Reproduced from *J. AOAC Int.*, **84**, 987–991 (2001). Copyright 2001 by AOAC International.)

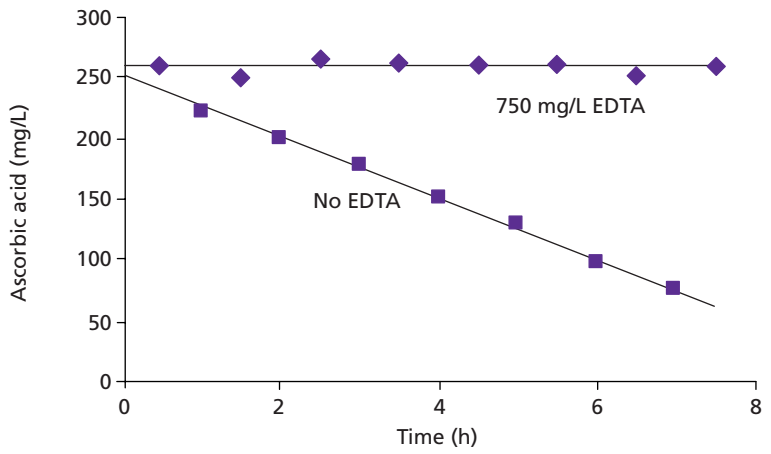


Figure 3: Ascorbic and dehydroascorbic acid (DHAA) in commercial orange juices (OJ) from retail stores.

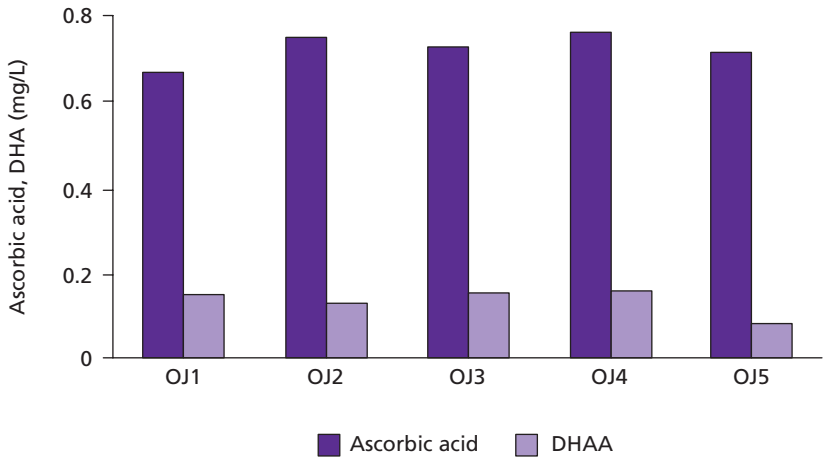


Table 1: Comparision of ascorbic acid quantification by CE and HPLC.

	Ascorbic Acid (mg/L)	
	CE	HPLC
(Samples of tangerine juice quantified by CE and HPLC).		
1	75.57	74.31
2	73.41	74.77
3	81.32	78.37
4	74.53	74.44
5	75.73	73.28
6	71.40	70.05
7	76.35	75.44
8	79.99	77.91

(Reproduced from *J. AOAC Int.*, **84**, 987–991 (2001). Copyright 2001 by AOAC International.)

265, 275–281 (1998).
28. M.W. Davey, G. Bauw and M. Van Montagu, *Anal. Biochem.*, **239**, 8–19 (1996).
29. J. Schiewe, Y. Mrestani and R. Neubert, *J. Chromatogr. A*, **717**, 255–259 (1995).
30. A. Prochazkova, L. Krivankova and P. Bocek, *Electrophoresis*, **19**, 300–304 (1998).
31. T.C. Liao et al., *Electrophoresis*, **22**, 1484–1488 (2001).
32. P.F. Cancalon and C.R. Bryan, *J. Chromatogr.*, **652**, 555–562 (1993).
33. P.F. Cancalon, *J. AOAC Int.*, **82**, 95–106 (1999).
34. P.F. Cancalon, *J. AOAC Int.*, **84**, 987–991 (2001).
35. Food and Nutrition Board, Institute of Medicine. Dietary reference intake: vitamins. Available at http://www.iom.edu/IOM/IOMHome.nsf/Pages/Food+and+Nutrition+Board)

Paul Cancalon gained his PhD in Biochemistry at the State University of New York, Buffalo, USA in 1972 and conducted post-doctoral research at Florida State University where he progressed from Assistant to Associate Professor in the Psychobiology and Neuroscience Programme. He is currently employed as a Research Scientist in the Florida Department of Citrus (FDOC), Lake Alfred, Florida, USA and is responsible for directing the product monitoring laboratory. This involves determining if retail juices bearing the FDOC symbol follow the quality parameters set by the department.

"CE Currents" editor **Kevin D. Altria** is senior principal scientist at GlaxoSmithKline, Harlow, Essex, UK, and is a member of the Editorial Advisory Board of *LC•GC Europe*. Direct correspondence about this column to "CE Currents," K.D. Altria, Building H89 Location 2-030, South Site, Product Line Extensions, GlaxoSmithKline, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK,
tel. +44 1279 643742,
fax +44 1279 643953,
e-mail: KDA8029@gsk.com,
website: http://www.ceandcec.com/