Journal of Pharmaceutical & Biomedical Analysis
Vol. 10, Nos 10–12, pp. 717–721, 1992
Printed in Great Britain

0731–7085/92 $5.00 + 0.00
Pergamon Press Ltd

Determination of ascorbic acid and isoascorbic acid by capillary zone electrophoresis: application to fruit juices and to a pharmaceutical formulation*

B. LIN LING,†‡ W.R.G. BAEYENS,§ P. VAN ACKER§ and C. DEWAELE‖

‡*Department of Bromatology and Pharmaceutical Analytical Techniques, Faculty of Pharmacy, Complutense University of Madrid, Pza Ramon y Cajal s/n, E-28040 Madrid, Spain*
§*Department of Drug Analysis and of Medicinal Chemistry, Pharmaceutical Institute, State University of Ghent, Harelbekestraat 72, B-9000 Ghent, Belgium*
‖*Bio-Rad RSL, Begoniastraat 5, B-9810 Nazareth, Belgium*

Abstract: Capillary zone electrophoresis was applied to the determination of ascorbic and isoascorbic acid, analysing the various parameters of influence such as the separation voltage, the buffer pH and concentration, the type of separation capillary or the loading conditions. Both analytes could be adequately determined within 5 min. The proposed method uses a 20 cm × 25 μm i.d. coated column, 0.1 M phosphate buffer pH 5.0, 8 kV separation voltage and light absorption detection at 265 nm. Linear calibration curves were obtained in the 0–1 mg ml^{-1} range, with detection limits of 0.5 μg ml^{-1}. This method proved to be very rapid, simple and practical for the qualitative and quantitative determination of ascorbic acid in lemon and orange juices, as well as in a commercially available pharmaceutical formulation.

Keywords: *Ascorbic acid; isoascorbic acid; vitamin C; capillary zone electrophoresis; fruit juice analysis; drug analysis.*

Introduction

Vitamin C is a well-known water-soluble vitamin, widely distributed in the plant and animal kingdom. Its essential rôle in biological systems includes its intake to prevent scurvy, its reaction with certain toxic compounds such as nitrosamines, its induction of the immune response and, as discovered recently, its rôle in inhibiting certain forms of cancer cell growth *in vitro* [1–3]. The main biologically active compound of vitamin C is ascorbic acid (Fig. 1), primarily found in fresh fruits and vegetables. Its epimer, isoascorbic acid (Fig. 1), presents only one-twentieth of the pharmacological activity of ascorbic acid [4] and does not usually appear in fruit juices or in pharmaceutical formulations unless in specific cases of vitamin C degradation. As the quality control of naturally occurring compounds as well as pharmaceutical formulations is of great importance and many compounds are stereospecific in their actions, selective and reproducible purity controls of such mixtures is highly advisable.

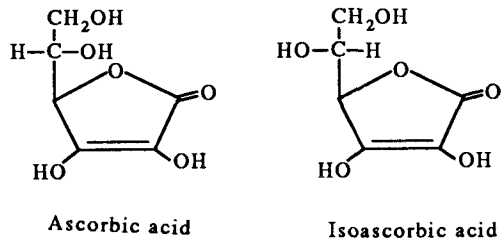

Figure 1
Chemical structures of ascorbic and isoascorbic acid.

There are various methods reported in the literature for the determination of these analytes, mostly based on HPLC with UV, fluorescence or electrochemical detection [5–9]. However, the recently introduced technique of capillary zone electrophoresis (CZE) adds a new dimension to the separation of these type of compounds. Its high efficiency, speed of analysis, wide applicability and versatility, as well as its low consumption of solvent and sample volumes, account for the spectacular development of this method [10]. During the present decade, extensive research work on the various high-performance capillary electrophoretic techniques has been performed, providing improved separation

*Presented at the "Fourth International Symposium on Drug Analysis", May 1992, Liège, Belgium.
†Author to whom correspondence should be addressed.

systems and an increasing number of applications. The present paper describes a CZE method for the analysis of ascorbic acid and the separation from its epimer isoascorbic acid as well as the application of the optimized method to the specific determination of vitamin C in fruit juices and in a pharmaceutical formulation.

Experimental

Chemicals

Ascorbic acid and isoascorbic acid were purchased from Janssen Chimica (Beerse, Belgium). A phosphate buffer solution 0.1 M at different pHs ranging from 4 to 7 adjusted with 0.1 M NaOH and 0.1 M HCl (Bio-Rad, Richmond, USA) was used for the CZE experiments. All compounds were chemically pure and used as such without further purification. Distilled deionized water was used throughout.

Apparatus

An HPE 100 high-performance electrophoresis system (Bio-Rad, Richmond, USA) with variable UV detection was used. Various cartridges containing fused silica columns of two different dimensions: 20 cm × 25 μm and 50 cm × 50 μm coated capillaries were employed. Injection of the samples was performed on the cathodic side by electromigration, using different loading times and voltages as specified. A constant voltage mode was employed for both injection as well as separation of the analytes. The analytes were detected at λ_{ABS} = 265 nm, recording the signals on a Linear recorder, Model 2020-0000 (Reno, USA) and integrating them with a Chromatopac C-R3A integrator system (Shimadzu, Kyoto, Japan).

Results and Discussion

CZE separation of ascorbic acid and isoascorbic acid

A short 20 cm × 25 μm capillary was initially used for the optimization of the CZE experimental conditions. Measurements of the separated analytes were performed at 265 nm, corresponding to a maximum in the absorption spectra of both ascorbic and isoascorbic acids when dissolved in an acidic 0.1 M phosphate buffer. As expected, both compounds presented relatively similar absorption spectra under the same measuring conditions. An aqueous mixture of both compounds each at a concentration of 10 μg ml^{-1} was subject to CZE. Several electrophoretic parameters were analysed and their influence on the overall resolution of the separation studied.

Buffer pH. The influence of the separation buffer pH, at a concentration of 0.1 M, on the migration times of the ascorbic and isoascorbic acids (pK_a values = 4.17) is shown in Fig. 2(A). At low pH values (<4.0) no peaks could be observed in the electropherogram; the analytes do not seem to migrate as no ionized function is developed. With increasing pH values, the negatively ionized alcoholic function of both acids produce a faster migration of the analytes. A pH of 5 proved to be the optimum as higher pH values resulted in reduced selectivity due to a smaller difference in their migration times.

Buffer concentration. Figure 2(B) shows the influence of increasing buffer concentrations, at a pH of 5, on the analytes' migration times. A 0.1 M buffer concentration was chosen for providing the adequate selectivity in a short analysis time (<5 min).

Separation voltage. As expected, increasing separation voltages resulted in decreasing migration times of the analytes [Figs 3(A) and 3(B)] subsequently inducing a selectivity decrease in the present separation. A standard voltage value of 8 kV was used for the separation as well as for the injection.

Injection voltage and time. A linear relationship between the analytes' peak height and the injection voltage (from 2 to 12 kV) could be observed for both analytes assayed. A proportional relationship between the injection time (from 3 to 10 s) and the analytes' peak heights was also noticed. These results account for the increased sensitivity that could be obtained by varying these injection conditions. An arbitrary injection voltage of 5 kV for 8 s was the chosen condition for further CZE experiments.

Length of the capillary. The use of a 20 cm × 25 μm i.d. capillary proved to be very adequate for the determination of ascorbic acid, obtaining a mean migration time of 3.8 min. However, a longer capillary (50 cm × 50 μm

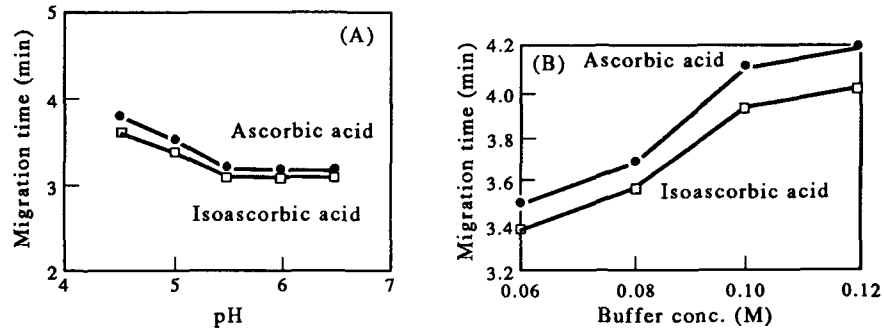

Figure 2
Influence of (A) the buffer pH (at a concentration of 0.1 M) and of (B) the buffer concentration (at a pH of 5.0;) on the migration times of a mixture of 10 μg ml^{-1} ascorbic and isoascorbic acids. Experimental: 20 cm × 25 μm i.d. coated column, 8 kV separation voltage, 8 kV–8 s injection conditions and 265 nm absorption detection.

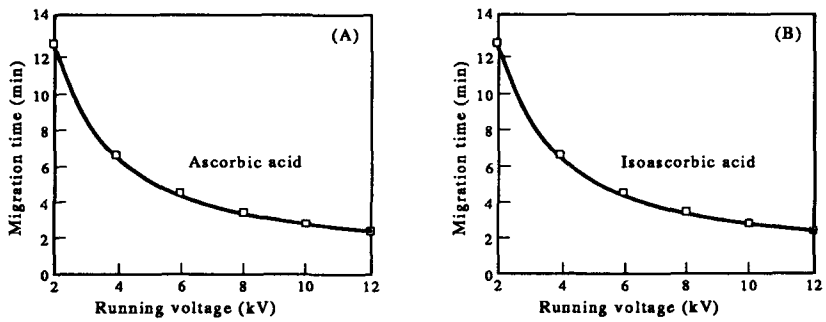

Figure 3
Influence of the separation voltage on the migration times of a mixture of 10 μg ml^{-1} ascorbic (A) and isoascorbic (B) acids. Experimental: 20 cm × 25 μm i.d. coated column, 8 kV separation voltage, 8 kV–8 s injection conditions and 265 nm absorption detection.

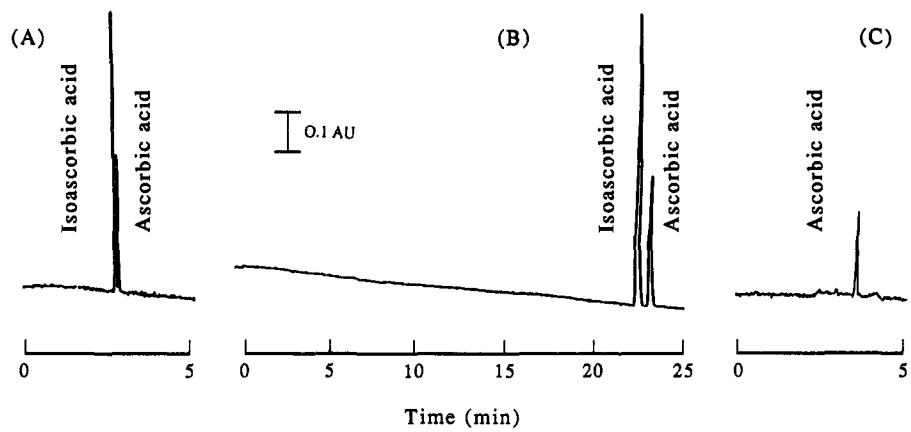

Figure 4
Electropherograms of a mixture of 30:70 (v/v) 10 μg ml^{-1} ascorbic and isoascorbic acid on (A) a 20 cm × 25 μm i.d. coated column and on (B) a 50 cm × 50 μm i.d. coated column and electropherogram of (C) an orange juice sample using the short column, following the proposed CZE experimental method.

i.d.) was required for obtaining the complete separation of both ascorbic and isoascorbic acids. Figures 4(A) and 4(B) show the electropherograms obtained with both capillaries applying the optimized CZE experimental conditions to a mixture of ascorbic and isoascorbic acids. Increased selectivity and sensitivity are the direct consequences of increasing capillary length and diameter values; however, the longer the migration time, the more the band broadening effects of the analyte.

Quantitative determination

Under the optimized CZE experimental conditions (20 cm × 25 μm i.d. coated column, 0.1 M phosphate buffer pH 5.0, 8 kV separation voltage, 8 kV–8 s injection conditions and 265 nm absorption detection) linear calibration curves were obtained for peak height (y) vs concentration (x) plots for both ascorbic and isoascorbic acids in the 0–1 mg ml^{-1} range for 10 different concentration standards, starting from 1 μg ml^{-1}, each analysed 10 times, dissolved in the separation buffer solution (ascorbic acid: $y = 1.032 + 1.061x$, coefficient of correlation >0.99; isoascorbic acid: $y = 1.008 + 1.585x$, coefficient of correlation >0.99). The dilution of the analyte was performed in buffer solution and not in water as the latter solvent would have induced a focusing effect during the injection of the sample resulting in an increased detection limit of the analyte in a nonlinear way. The determined ascorbic acid detection limit values ($S/N > 2$) were therefore different in both cases, being 0.5 μg ml^{-1} when dissolved in buffer solution and 0.25 μg ml^{-1} when dissolved in aqueous solution. These values are similar for isoascorbic acid. However, and as previously mentioned, this detection limit value could be improved by modifying the sample injection conditions. The repeatability of the CZE method hereby proposed gave ascorbic acid relative standard deviations (RSD) ($n = 10$) of 1.46% for the retention times, 1.11% for peak heights and 3.88% for peak areas. These statistical values were similar for isoascorbic acid (2.43, 2.11 and 2.53%, respectively for $n = 10$ samples).

Application to fruit juices

The juice of several lemons, freshly squeezed, were appropriately filtered using injection filters (Sterile Acrodisc, 0.2 μm, Gelman Sciences, Ann Arbor, USA) and subjected to CZE analysis under the optimized experimental conditions previously mentioned. The resulting electropherograms gave one single peak, attributed to ascorbic acid. This was verified by the addition of aliquots of increased concentrations of standard ascorbic acid in phosphate buffer solution to the sample which resulted in an increase of the same peak at the same retention time when analysed under the proposed CZE conditions. Quantitative studies of ascorbic acid were obtained by addition of standard ascorbic acid to the fruit juice samples and calculation of the added analyte by extrapolation. These studies gave a mean concentration of 450 μg ml^{-1} of ascorbic acid in lemon juice which corresponds to literature values [7], for $n = 5$ different determinations (RSD < 5%, mean recovery of 97% with respect to added standard ascorbic acid). Similar applications on fresh orange juices gave a mean concentration of ascorbic acid of 393 μg ml^{-1} which was also in accordance to literature values [9], for $n = 5$ different determinations (RSD < 5%, mean recovery of 101%). Statistical studies regarding retention time and peak area repeatabilities as well as calibration curves gave similar results as previously obtained with analytical solutions.

Application to a pharmaceutical formulation

Tonicalcium (Lab. Bouchara, Belgium) is a drinkable pharmaceutical formulation used as a tonic containing the ascorbate salts of lysine and calcium (0.50 g each salt in a 10-ml ampoule). The contents of one ampoule were dissolved in 0.1 M phosphate buffer pH 5.0 to a final concentration of 2 μg ml^{-1} of each salt, the final solution being analysed by CZE under the optimized experimental conditions. Single ascorbic acid peaks were also obtained. The measured recovery was of 99% with respect to the label claim, with a repeatability of the complete determination procedure of 4.36% RSD ($n = 10$). Other statistical studies ($n = 10$) provided the following data: retention time repeatability = 1.94%, peak area repeatability = 1.39%, detection limit = 0.5 μg ml^{-1} and calibration curves were linear in the 0–1 mg ml^{-1} range.

Conclusions

In conclusion, a relatively simple and fast CZE method for the analysis of vitamin C is proposed. Increased sensitivity and/or selectivity may be obtained by modifying the various injection parameters and/or separation conditions depending on the analyte of interest. Other isomeric compounds as well as biological or pharmacological samples may likewise be assayed and a suitable CZE method accordingly developed.

References

[1] R.E. Wittes, *New Engl. J. Med.* **312**, 178–179 (1985).
[2] B. Borenstein, *Food Technol.* **41**, 98–99 (1987).

[3] A. Bendich, *Food Technol.* **41**, 112–114 (1987).
[4] *The Merck Index*, 11th edn, Merck, Rahway, NJ (1989).
[5] S.J. Ziegler, B. Meier and O. Sticher, *J. Chromatogr.* **391**, 419–426 (1987).
[6] W.A. Behrens and R. Madere, *Anal. Biochem.* **165**, 102–107 (1987).
[7] P. Wimalasiri and R.B.H. Wills, *J. Chromatogr.* **256**, 368–371 (1983).
[8] D. Sybilska, K. Duszczyk and M. Przasnyski, *J. Chromatogr.* **298**, 352–355 (1984).
[9] R.J. Bushway, J.M. King, B. Perkins and J. Krishnan, *J. Liq. Chromatogr.* **11**, 3415–3423 (1988).
[10] R.A. Wallingford and A.G. Ewing, in *Advances in Chromatography Biotechnological Applications and Methods*, Vol. 29 (J.C. Giddings, E. Grushka and P.R. Brown, Eds), p. 2. Marcel Dekker, New York (1989).

[*Received for review 5 May 1992; revised manuscript received 22 June 1992*]