Journal of Chromatography, 196 (1980) 163–165
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,846

Note

Application of high-performance liquid chromatography to the separation of ascorbic acid from isoascorbic acid

MAI HUONG BUI-NGUYÊN

Swiss Federal Dairy Research Institute, Lausanne Laboratory (Director: Prof. Dr. B. Blanc), Swiss Institute of Vitamins, 1011 Lausanne (Switzerland)*

(First received January 28th, 1980; revised manuscript received March 12th, 1980)

The problem of separating ascorbic acid (antiscorbutic acid or vitamin C) from isoascorbic acid (an epimer of ascorbic acid, Fig. 1) is of importance in control analyses of so-called "vitamin C" enriched food products. Isoascorbic acid is cheaper than ascorbic acid, and some manufacturers use the biological product of lower activity instead of vitamin C. Isoascorbic acid is not found in natural products. Its biological activity is 20 times less than that of ascorbic acid. Conventional methods of vitamin C analysis (titration[1], spectrophotometry[2], polarography[3]), which are based on its oxidation–reduction potential, do not allow differentiation between the two compounds because they have the same oxidation–reduction potential.

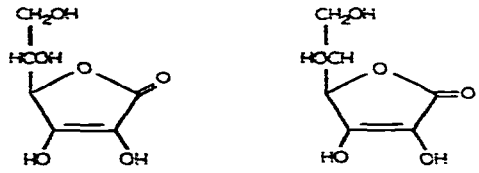

Fig. 1. Structures of ascorbic acid isomers.

High-performance liquid chromatography (HPLC) has been used for the measurement of vitamin C, with anion-exchange[4–6], reversed-phase C_{18} (ref. 7), ion-pair reversed-phase C_{18} (refs. 8, 9) and bonded-phase NH_2 (ref. 9) techniques and electrochemical[5–7] or UV (254 nm)[4,9] detection.

We have carried out this HPLC separation on a column containing a weak anion exchanger (reversed-phase NH_2) with UV detection at 268 nm, the wavelength of maximum absorption of vitamin C.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 1084 B liquid chromatograph equipped with a Hewlett-Packard UV detector with variable wavelength was used.

0021-9673/80/0000–0000/$02.25

The column (stainless steel, 250 × 4.6 mm I.D.), packed with LiChrosorb NH_2 (particle size 10 μm) (Merck, Darmstadt, G.F.R.), was obtained from Brownlee Labs. (Santa Clara, CA, U.S.A.). LiChrosorb NH_2 is a primary amino phase chemically bonded on to SI-100 silica gel.

Reagents

Acetonitrile (HPLC grade S) was purchased from Ratburn Chemicals (Walkerburn, Great Britain). Potassium dihydrogen phosphate, isoascorbic acid and ascorbic acid (analytical grade) were obtained from Fluka (Buchs, Switzerland). Doubly distilled, deionized water was used to prepare all solutions.

Chromatographic conditions

The mobile phase was 75% acetonitrile in a 0.005 *M* solution of potassium dihydrogen phosphate, (pH 4.4–4.7). The solvents were filtered before use through a 0.22-μm Millipore filter (Millipore, Bedford, MA, U.S.A.). Before use the phase was degassed by applying a vacuum to the solvent reservoir for approximately 5 min. The flow-rate was 3 ml/min. The oven temperature was set at 60°C. The absorbance at 268 nm was monitored at a chart speed of 1.5 cm/min.

RESULTS

The results obtained by HPLC on a C_{18} reversed phase with and without ion pairing were not good. HPLC on reversed-phase LiChrosorb NH_2 was satisfactory. Fig. 2 illustrates the separation of the isoascorbic and ascorbic acid reference compounds with detection at 268 nm. The reproducibility was satisfactory; we obtained standard deviations of 1% and 3% for isoascorbic and ascorbic acid, respectively. Ascorbic acid appears to be more sensitive than isoascorbic acid to pH changes (Fig. 2).

Chromatograms of two fruit juices from two different manufacturers are presented in Fig. 3. Identification of the peaks of interest was based on retention

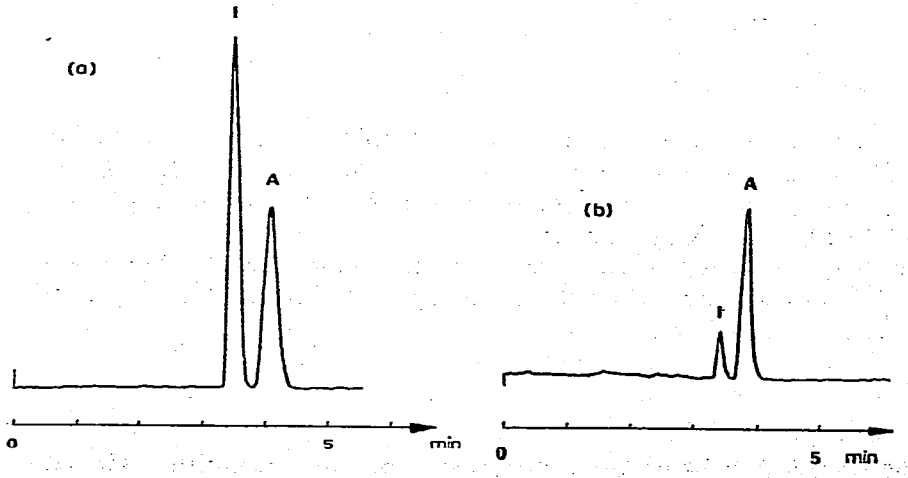

Fig. 2. Chromatograms of isoascorbic (I) and ascorbic (A) acid at (a) pH 4.4 and (b) pH 4.7.

behaviour and co-chromatography with the reference compounds. Fig. 3a shows no constituent that interferes with vitamin C and no isoascorbic acid. Fig. 3b shows two peaks which were confirmed as isoascorbic and ascorbic acids by their retention times and by adding a known amount of isoascorbic acid to the fruit juice.

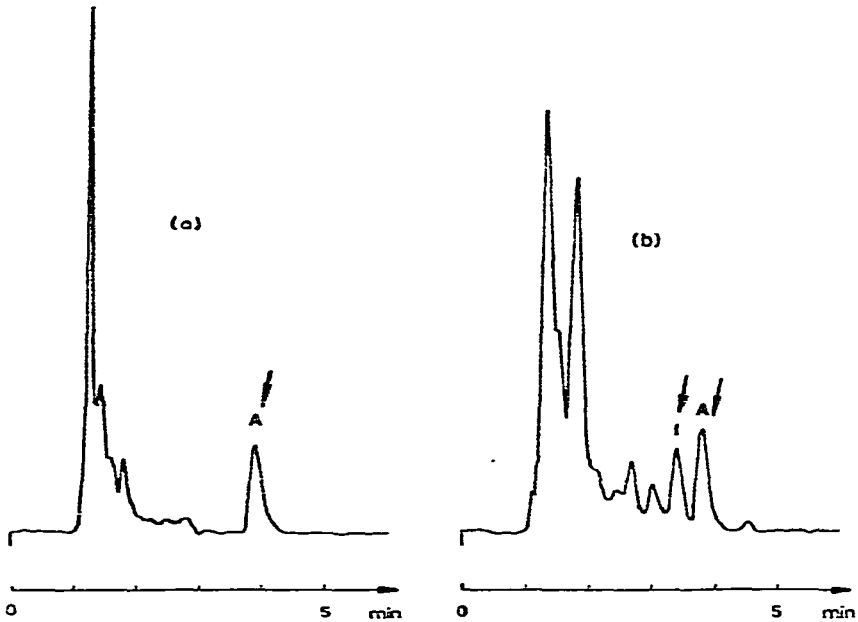

Fig. 3. Chromatograms of (a) an extract of fruit juice found to contain ascorbic acid; (b) an extract of fruit juice found to contain both ascorbic and isoascorbic acids.

For analyses of food, a daily clean-up of the column with water, followed by methanol or acetonitrile, is recommended. Reconditioning can be carried out, *e.g.*, by pumping water for 15 min, then methanol overnight, changing over to the HPLC mobile phase in the morning.

REFERENCES

1 J. Tillmann, *Z. Lebensm.-Unters.-Forsch.*, 54 (1927) 33.
2 D. J. Cameron, *Proc. Nutr. Soc.*, 34 (1) (1975) 7A.
3 J. Lindquist and S. M. Farroha, *Analyst (London)*, 100 (1975) 377.
4 R. C. Williams, D. R. Baker and J. A. Schmit, *J. Chromatogr. Sci.*, 11 (1973) 618.
5 K. V. Thrivikraman, C. Refshavge and R. N. Adams, *Life Sci.*, 15 (1974) 1335.
6 L. A. Pachla and P. T. Kissinger, *Anal. Chem.*, 48 (1976) 364.
7 R. Stillman and T. S. Ma, *Mikrochim. Acta*, (1974) 641.
8 S. P. Sood, L. E. Sartori, D. P. Wittmer and W. G. Haney, *Anal. Chem.*, 48 (1976) 796.
9 R. B. H. Wills, C. G. Shaw and W. R. Day, *J. Chromatogr. Sci.*, 15 (1977) 262.
10 E. S. Wagner, B. Lindley and R. D. Coffin, *J. Chromatogr.*, 163 (1979) 225.