Journal of Chromatography, 299 (1984) 288–292
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,936

Note

Determination of organic acids in foods by high-performance liquid chromatography

Citric acid

SAMY H. ASHOOR* and M. J. KNOX
Division of Agriculture, Arizona State University, Tempe, AZ 85287 (U.S.A.)
(First received March 15th, 1984; revised manuscript received May 21st, 1984)

Citric acid is one of the earliest and most popular food acidulants. It is used extensively in the manufacturing of carbonated beverages, fruit and vegetable drinks, cheeses and other dairy products. Citric acid is also a natural component of many fruits and vegetables. The citric acid content of these foods determines their acceptability and keeping quality. The accurate quantification of citric acid in such foods is therefore essential for quality control requirements and meeting legal regulations.

Several analytical methods for the determination of citric acid have been reported in the literature. They include the AOAC method[1], gas chromatographic (GC) methods[2,3], and liquid chromatographic (LC) methods[4–7]. These methods, however, may be lacking specificity, may be time-consuming or may require derivatization of the acid before analysis. The present LC methods were developed for the separation of standard citric acid and other organic acids or were used to quantify citric acid in a few food products.

In this study, a simple and specific high-performance liquid chromatographic (HPLC) method for the determination of citric acid in a wide variety of food products is presented.

MATERIALS AND METHODS

Apparatus

A Waters Assoc. (Milford, MA, U.S.A.) liquid chromatograph equipped with Model 6000 A pump, Model U6K injector and a data module was used for HPLC analysis. A Gilson (Middleton, WI, U.S.A.) Model 222 variable-wavelength detector set at 210 nm with a sensitivity of 0.1 a.u.f.s. was also used. The HPLC column was 300 × 7.8 mm I.D. Aminex HPX-87H with a Micro-Guard ion-exclusion cartridge (Bio-Rad Labs., Richmond, CA, U.S.A.). A Sorval Model RC-5 refrigerated centrifuge with rotor No. SS-34 (DuPont Company, Newton, CT, U.S.A.) was used for centrifugation.

Reagents

HPLC mobile phase. The mobile phase was 0.009 *N* sulfuric acid solution

0021-9673/84/$03.00 © 1984 Elsevier Science Publishers B.V.

prepared from reagent-grade sulfuric acid and HPLC-grade water (double distilled, passed through 0.45-μm filter membrane). The mobile phase was used at a flow-rate of 0.5 ml/min.

Disodium ethylenediaminetetracetate (Na_2EDTA) stock solution. A 1% Na_2EDTA solution was prepared from reagent-grade Na_2EDTA and HPLC-grade water.

Citric acid standard solutions. Five standard solutions with citric acid concentrations of 0.242, 0.482, 0.720, 0.960 and 1.20 mg/ml were prepared from analytical-grade anhydrous citric acid (Sigma, St. Louis, MO, U.S.A.) and HPLC-grade water. Proper volumes of Na_2EDTA stock solution were added to citric acid standard solutions to give a final Na_2EDTA concentration of 0.01%.

Sample preparation

Carbonated beverages and other liquids. The carbonated beverages were first degassed for 5 min in an ultrasonic bath, 50 ml of the degassed beverage were then transferred to a 100-ml volumetric flask. A 1-ml volume of Na_2-EDTA solution was added and the volume was made to mark with HPLC-grade water. Proper volumes of other liquids (*e.g.* 1 ml of lemon juice) were treated in a similar manner without degassing. Sample preparations were then refrigerated until HPLC analysis.

Semi-solids and solids. Proper weights (3 g frozen orange concentrate, 2–4 g powdered drinks, 10 g frozen strawberries, 25 g fresh tomatoes, 5 g process cheese, 50 g cottage cheese) were blended with 50 ml of distilled water for 3 min. The blends were either filtered or centrifuged at 11,950 *g* for 10 min. The supernatant was separated and the sediment was washed with 10–20 ml distilled water, recentrifuged and the washing was combined with the supernatant. The filtrate or the supernatant was then transferred to a 100-ml volumetric flask, 1 ml of Na_2EDTA solution was added and the volume was made to mark with HPLC-grade water. All sample preparations were refrigerated until HPLC analysis.

Calibration curve

Of each citric acid standard solution, 10 μl were injected and the corresponding data module area units (average of 3 runs) were plotted against the amounts of citric acid injected.

Quantification of citric acid in samples

A known volume (10 μl) of a citric acid standard solution was injected and the data module was calibrated accordingly (external standard method). A volume of 10–20 μl of the sample preparation was then injected and the amount of citric acid was obtained directly from the data module. The data module calibration was checked regularly with citric acid standard solutions.

Recovery

Selected samples were spiked with known amounts of standard citric acid to approximately double their citric acid content. The spiked samples were prepared for HPLC analysis as described in the sample preparation section.

RESULTS AND DISCUSSION

The manufacturer of the HPLC column used in this study recommends to avoid free metal ions in the system since they decrease the column efficiency[8]. Na_2EDTA is a good chelating agent and was therefore added to sample preparation to mask any metal ions present. Although citric acid is also a chelating agent, the presence of 0.01% Na_2EDTA in the sample preparation was required for maintaining the column efficiency and obtaining reproducible results. In a previous study, ascorbic acid was quantified using a similar column and 0.05% Na_2EDTA in the sample preparation[9]. However, in this study an Na_2EDTA concentration of 0.01% was

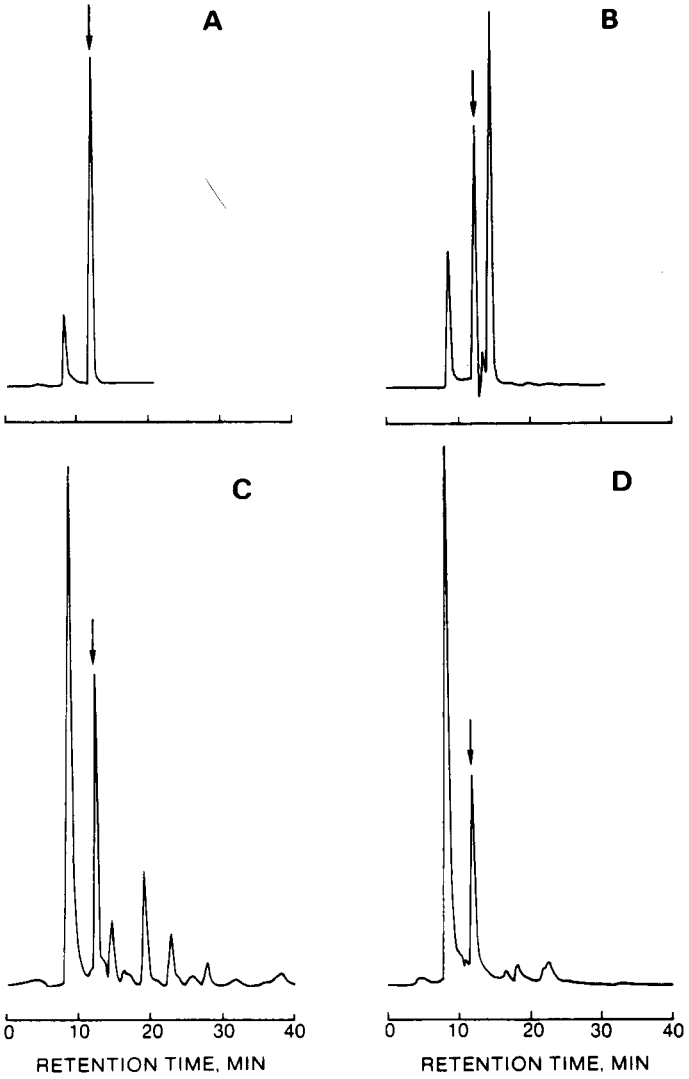

Fig. 1. Chromatograms of standard citric acid in 0.01% Na_2EDTA (A); lemon lime soda (B); tomatoes (C); and cottage cheese (D). The citric acid peak is marked with an arrow.

TABLE I

CITRIC ACID CONTENT OF VARIOUS FOOD PRODUCTS

Sample	*Citric acid content**	*C.V. (%)*
Lemon lime soda brand A	123.0 ± 1.9	1.5
Lemon lime soda brand B	132.9 ± 1.5	1.1
Lemon lime soda brand C	178.7 ± 2.7	1.5
Orange soda	141.5 ± 1.2	0.8
Grape soda	132.0 ± 5.3	2.8
Diet soda brand A	164.5 ± 3.4	2.1
Diet soda brand B	165.0 ± 2.6	1.6
Ginger ale	144.0 ± 2.8	1.9
Root beer	63.0 ± 1.3	2.2
Lemon juice	96.1 ± 0.2	0.2
Frozen orange concentrate	30.5 ± 1.3	4.4
Frozen strawberries	5.06 ± 0.12	2.4
Tomatoes	3.28 ± 0.11	3.5
Powdered drink brand A	24.4 ± 0.53	2.2
Powdered drink brand B	42.3 ± 0.84	2.0
Cottage cheese	1.47 ± 0.04	2.7
Process cheese spread	11.4 ± 0.3	2.9

* Average of six determinations. Carbonated beverages mg/100 ml, lemon juice mg/ml, other samples mg/g.

sufficient for chelation. The Na_2EDTA has a shorter retention time (8.3 min) than the citric acid (11.9 min) and does not interfere with the quantitative determination of citric acid (Fig. 1A). The Na_2EDTA–sulfuric acid system may have adverse effects on the chromatograph stainless steel. However, the Na_2EDTA–sulfuric acid system has been used in HPLC analysis of organic acids in our laboratory for the last two years without any apparent harmful effects.

The calibration curve constructed for citric acid indicated a linear response over a range of 2–12 μg in the presence of 0.01% Na_2EDTA. The curve indicated also that as low as 2 μg of citric acid can be quantified reliably by the HPLC method.

Citric acid was resolved as a single peak in all samples analyzed with no interference from other compounds. These results indicated that the method is specific for citric acid. Selected chromatograms obtained by the method are shown in Fig.

TABLE II

RECOVERY OF CITRIC ACID FROM VARIOUS FOOD PRODUCTS

Sample	*Recovery (%)**	*C.V. (%)*
Lemon lime soda brand A	99.0 ± 1.6	1.6
Lemon lime soda brand B	101.9 ± 3.9	3.8
Ginger ale	101.6 ± 2.7	2.7
Frozen orange concentrate	98.0 ± 1.7	1.7
Tomatoes	98.4 ± 3.1	3.2
Powdered drink brand B	101.4 ± 1.8	1.8
Process cheese spread	98.1 ± 1.6	1.6

* Average of six determinations.

1B, C and D. The identity of the citric acid peak was confirmed by determining its relative retention time (relative to the Na_2EDTA peak) and by spiking with standard citric acid. The relative retention time of the citric acid peak in all samples analyzed was 1.43 ± 0.01. In spiked samples, the citric acid peak area was always increased proportionally to the amount of standard citric acid added to the samples.

The citric acid content of the various food products analyzed by the HPLC method is presented in Table I. These results indicated that the method is versatile and convenient.

The coefficients of variation (C.V.) obtained by the method ranged from 0.2 to 4.4% (Table I) indicating that the method is precise with a high degree of reproducibility. The recoveries of citric acid from various samples were also high, as shown in Table II. They ranged from 98.0 ± 1.7 to 101.9 ± 3.9% reflecting the reliability and accuracy of the method.

In summary, the developed method possesses all the features of a successful analytical method: simplicity, specificity, versatility, reproducibility and accuracy.

REFERENCES

1 *Official Methods of Analysis*, Association of Official Analytical Chemists Arlington, VA, 13th ed., 1980.
2 C. E. Dalgleish, E. C. Horning, M. G. Horning, K. L. Knox and K. Yarger, *Biochem. J.*, 101 (1966) 792.
3 M. A. Harmon and H. W. Doelle, *J. Chromatogr.*, 42 (1969) 157.
4 J. K. Palmer and D. M. List, *J. Agr. Food Chem.*, 21 (1973) 903.
5 V. T. Turkelson and M. Richards, *Anal. Chem.*, 50 (1978) 1420.
6 R. T. Marsili, H. Ostapenko, R. W. Simmons and D. E. Green, *J. Food Sci.*, 46 (1981) 52.
7 E. Rajakylä, *J. Chromatogr.*, 218 (1981) 695.
8 *Bio-Rad HPLC Columns Bulletin 2069*, Bio-Rad Labs., Richmond, CA, 1980.
9 S. H. Ashoor, W. C. Monte and J. Welty, *J. Ass Offic. Anal. Chem.*, 67 (1984) 78.