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Short Communication

A liquid chromatographic assay for citric acid in over-the-counter carbamide peroxide products using indirect UV detection

THOMAS A. WALKER

Pharmaceutical and Analytical Sciences, Marion Merrell Dow Inc., P.O. Box 9627, Kansas City, MO 64134, USA

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Introduction

Citric acid, a commonly used excipient in pharmaceutical formulations, has been used to improve the effectiveness of antioxidants, as a buffering agent, in the preparation of effervescent formulations and for improving the taste of pharmaceutical formulations [1].

Several different analytical methodologies have been used for determining the amount of citric acid present in diverse samples. The analytical methods that have been used include: potentiometric titrations [2], gravimetry [3], colorimetry [3], ion exclusion chromatography [3], gas chromatography [4, 5], enzymatic flow injection [6] and liquid chromatography [3, 7, 8]. These procedures have been shown to work for the analysis of citric acid, however, problems are inherent to these assay methods. These methods are time consuming, tedious and difficult to use.

The separation and quantitation of citric acid using reversed-phase liquid chromatography has several problems associated with it. If a reversed-phase column is used, the mobile phase pH must be low enough to insure that citric acid is not ionized in order for the analyte to be retained. If ion exchange chromatography is used, citric acid will be highly retained (if it is ionized) unless a high ionic strength mobile phase is used or a strong counterion is added. Since citric acid does not have a strong chromophore, these mobile phases can easily swamp the detector and prevent citric acid from being detected. Ion exchange columns are not as efficient as reversed-phase columns, hence resolution and peak shape may not be acceptable for the separation.

Indirect photometric chromatography [9] is a separation scheme which has been employed for the indirect detection of transparent ions where a UV-absorbing counterion is added to the mobile phase. The UV-absorbing counterion has the dual purpose of being involved in the indirect detection of a transparent analyte ion and in the retention of the analyte ion on the stationary phase. Indirect photometric chromatography has been used for the separation and indirect detection of various analyte ions, including excipients commonly used in pharmaceutical formulations. Both strong ion exchangers [1, 10–12] and low-capacity ion exchangers have been used for these separations [12–16]. An advantage of indirect photometric chromatography is that no extravagant instrumentation is required since only instruments that are common to an LC system are used.

This paper discusses the separation and indirect UV detection of citric acid in carbamide peroxide formulations. The separation scheme uses a low-capacity polymer-based anion exchange column and indirect UV detection at 251 nm. The formulations were diluted with water and injected into the LC system. The citric acid assay was found to be

linear over a range of 80–304 μg g^{-1} with a detection limit of 20 μg g^{-1} and a quantitation limit of 50 μg g^{-1}. The system precision (RSD) was 0.3% while the method precision was less than 4.0%.

Experimental

Chemicals

LC-grade water and acetonitrile were obtained from Burdick and Jackson (Muskegon, MI, USA). 1,3,5-Benzenetricarboxylic acid was obtained from The Aldrich Chemical Company (Milwaukee, WI, USA). Citric acid was obtained from Mallinckrodt Specialty Chemical Company (Paris, KY, USA). Sodium hydroxide was obtained from Fisher Scientific (Pittsburgh, PA, USA). All chemicals were of reagent grade. The carbamide peroxide samples were purchased at local pharmacies.

Apparatus

The instrumentation used in this study consisted of a Hewlett–Packard HP1090 chromatography system. The PRP-X100, a 4.1 × 100 mm low-capacity anion exchange column (Hamilton Company, Reno, NV, USA), was used for the separation. The column is a poly(styrene-divinylbenzene)trimethylammonium strong base anion exchanger. Aqueous analyte samples of about 100 μg g^{-1} and sample aliquots of 100 μl were used. Inlet pressures of 300–600 psi were observed. A wavelength of 251 nm was used for indirect UV detection.

Mobile-phase preparation

The mobile phase was prepared by weighing out an appropriate amount of 1,3,5-benzenetricarboxylic acid, transferring it to a 600-ml beaker and adjusting the pH with sodium hydroxide. The solution was transferred to a 1-l volumetric flask, an appropriate amount of acetonitrile was added, the volumetric flask was diluted to volume with LC-grade water, and the solution was mixed. The mobile phase was vacuum filtered through a 0.45-μm PTFE membrane and purged with helium for approximately 10 min.

Standard preparation

Standards were prepared by weighing out approximately 25, 50 and 100 mg of citric acid into separate 100-ml volumetric flasks, diluting to volume with LC-grade water and mixed. The working standards were prepared by transferring an aliquot of standard to an appropriate volumetric flask, diluting to volume with LC-grade water and mixing.

Sample preparation

Either 100 mg or 1 g of the carbamide peroxide sample was weighed into a 25-ml screw-top vial, 5 ml of LC-grade water was added and the sample was mixed. A portion of the sample solution was transferred to an autosampler vial and injected into the LC system.

Results and Discussion

The amount of citric acid that is present in carbamide peroxide formulations is important for calculating the stability of the product and in determining if the buffer concentration is high enough to prevent changes in the product pH. Currently, there are few analytical methods in place for determining the amount of citric acid contained in carbamide peroxide formulations. Methods that have been tried include a potentiometric titration using phenolphthalein as an endpoint indicator, and a colorimetric reaction between citric acid and pyridine. These methods are not specific for citric acid and may give erroneous results. Therefore, a liquid chromatographic separation using indirect UV detection was developed for citric acid and was found to be suitable for determining the amount present in carbamide peroxide formulations.

The stationary phase that was used in this study was a Hamilton PRP-X100. The column is a poly(styrene-divinylbenzene) trimethylammonium strong base low-capacity anion exchanger. This column has been shown to contain both fixed anion exchange sites and adsorption sites [12–14]. Unique separations have been shown to take place on these columns, especially for analytes that contain both a fixed charge site and a hydrophobic centre.

The mobile phase variables that may have an effect on the retention of citric acid were studied and the assay was optimized according to those results. The LC assay that was developed easily separates citric acid from excipients that are present in the different carbamide peroxide formulations.

Effect of 1,3,5-benzenetricarboxylic acid concentration

The first mobile phase variable that was studied was the mobile phase concentration of 1,3,5-benzenetricarboxylic acid and what affect it would have on the retention of citric acid. Typically, analyte retention will decrease as the concentration of UV-active counterion added to the mobile phase is increased. The sensitivity of the system will also be affected by the concentration of UV-active counterion. Small *et al.* [15] have shown that the sensitivity of the system increases when the mobile phase concentration of the UV-active counterion is decreased, however, analyte retention times increase. Therefore, a compromise between sensitivity and analyte retention will need to take place in order to optimize the separation.

Another way of increasing or decreasing analyte retention is to change to a UV-active counterion of a different charge. Typically, as the charge of the counterion increases, analyte retention decreases due to the stronger affinity that the counterion has toward the stationary phase. The retention of citric acid was found to decrease as the concentration of 1,3,5-benzenetricarboxylic acid was increased (Fig. 1). At low concentrations of counterion, sensitivity was significantly greater. Concentrations greater than 2.0 mM of 1,3,5-benzenetricarboxylic acid were tried, however, the background noise was so great that peaks could not be detected. A concentration of 0.5 mM of 1,3,5-benzenetricarboxylic acid was determined to provide the best compromise between sensitivity and citric acid retention.

Effect of ionic strength

In ion exchange chromatography, ionic strength plays a role in the retention of analyte ions by competing for the ion exchange sites present on the stationary phase. As the concentration of a competing ion in the mobile phase is increased competition increases for the fixed ion exchange sites and the analyte ion elutes more rapidly from the column [16].

In this study, sodium chloride was added to the mobile phase in order to determine the effect that increasing ionic strength would have on citric acid retention. Figure 2 shows that the retention of citric acid decreased as the concentration of sodium chloride was increased. Higher concentrations of sodium chloride were tried, however, the background noise was too high for peaks to be detected.

Effect of acetonitrile concentration

The effect that different concentrations of acetonitrile had on citric acid retention was also studied. Organic modifiers have been shown to have an effect on analyte ion retention. Analyte retention is generally reduced as the amount of organic modifier that is added to the mobile phase is increased [17]. However, several cases have been reported where the retention of analyte ions increased with in-

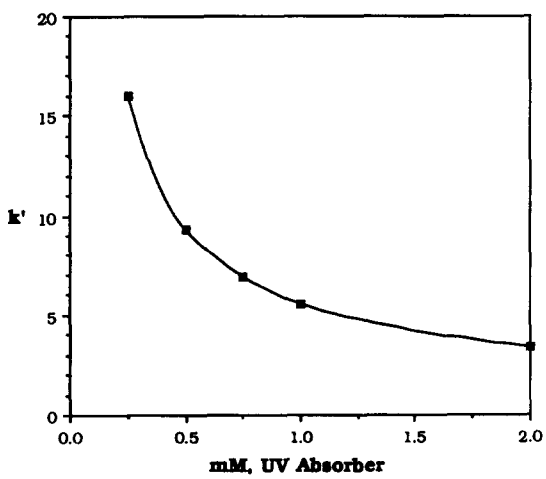

Figure 1
Effect of the mobile phase concentration of 1,3,5-benzenetricarboxylic acid on citric acid retention. Mobile phase: 1,3,5-benzenetricarboxylic acid, pH adjusted to 4.5 with NaOH, acetonitrile–water (25:75%); flowrate: 1.0 ml min^{-1}.

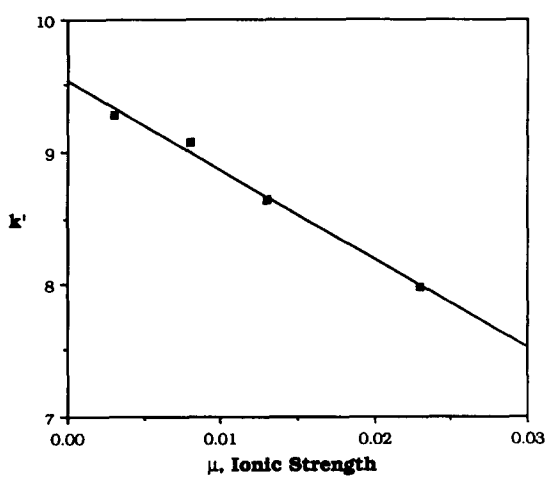

Figure 2
Effect of mobile phase ionic strength on citric acid retention. Mobile phase: 0.5 mM 1,3,5-benzenetricarboxylic acid, pH adjusted to 4.5 with NaOH, NaCl, acetronitrile–water (25:75%); flowrate: 1.0 ml min^{-1}.

creasing organic modifier concentration [13–15].

When the mobile phase concentration of acetonitrile was increased, a corresponding increase in the retention of citric acid was observed. The effect that acetonitrile had on the retention of citric acid is shown in Fig. 3. The increase in retention may be due to changes in the polarity of the stationary phase with respect to the mobile phase. Citric acid is ionic and will migrate toward the phase that is more polar. Therefore, as the mobile phase becomes less polar with higher concentrations of acetonitrile, the citric acid will be attracted more strongly to the stationary phase with a corresponding increase in retention [15].

Effect of mobile phase pH

The mobile phase pH plays a critical role in determining what charge, if any, the UV-active counterion and citric acid will have. The ionization of the analyte, UV-active counterion, and, to some extent, the stationary phase are dependent on the mobile phase pH. As the pH of the mobile phase is increased, the charge on the UV-active counterion (1,3,5-benzenetricarboxylic acid, a weak acid) increases until it is completely dissociated. In the same way, the sample ion (citric acid, a weak acid) will increase in charge until it is completely ionized. When both the UV-active counterion and the analyte are polyprotic weak acids, as in this case, a simple change in retention of the analyte ion with changing mobile phase pH is more difficult to predict.

The effect that mobile phase pH had on citric acid retention is shown in Fig. 4. The retention of citric acid was found to decrease over the mobile phase range of pH 3.2–4.5. The retention of citric acid was found to increase for a mobile phase between pH of 4.5 and 6.0. Retention did not change appreciably once a mobile phase pH of 6.0 was reached. A mobile phase pH of 4.5 was chosen as the mobile phase of choice due to the acceptable retention of citric acid and also since no interference from the system peak or from the sample matrix were observed.

Typical separations

Figures 5–7 show typical separations for a 101 μg g^{-1} citric acid standard and two different carbamide peroxide formulations, respectively. The excipients from the carbamide peroxide sample as well as the system peak did not interfere with the citric acid peak. The citric acid peak shape was found to be acceptable with a runtime of less than 25 min.

Calibration data

The linearity of the system was determined by analysing citric acid standards over a concentration range of 80–304 μg g^{-1}. The system was found to be linear over this range with a correlation coefficient of 0.9999, a *y*-intercept of −287.72 and a slope of 14.684. A quanti-

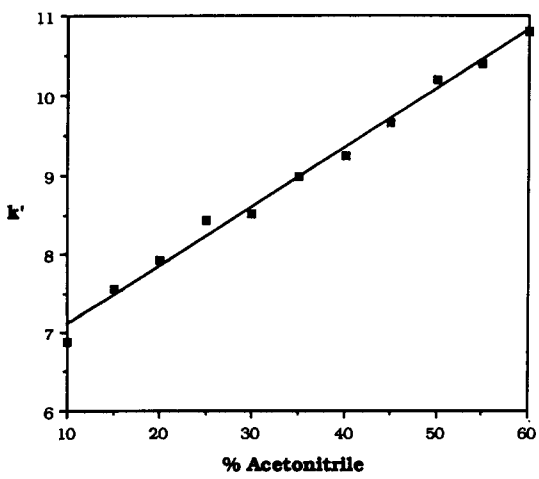

Figure 3
Effect of acetonitrile concentration on citric acid retention. Mobile phase: 0.5 mM 1,3,5-benzenetricarboxylic acid, pH adjusted to 4.5 with NaOH, acetonitrile–water; flowrate: 1.0 ml min^{-1}.

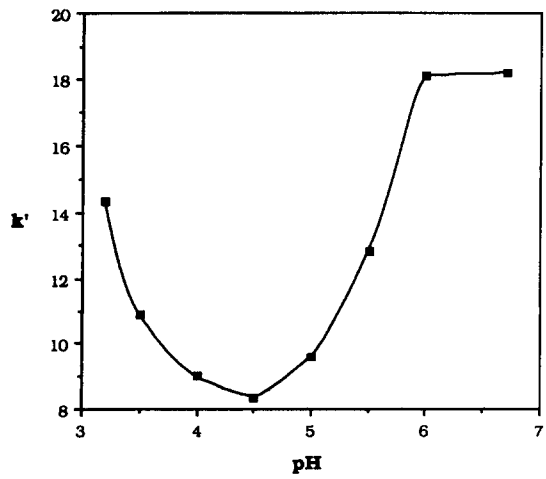

Figure 4
Effect of mobile phase pH on citric acid retention. Mobile phase: 0.5 mM 1,3,5-benzenetricarboxylic acid, pH adjusted with NaOH, acetonitrile–water (25:75%); flowrate: 1.0 ml min^{-1}.

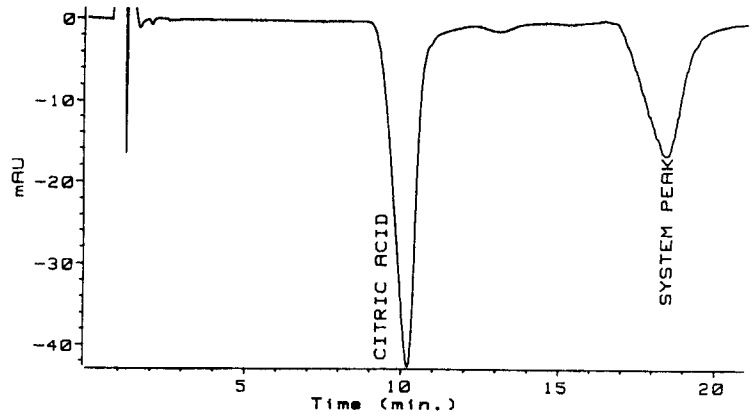

Figure 5
Typical chromatogram of 101 μg g^{-1} citric acid standard. Mobile phase: 0.5 mM 1,3,5-benzenetricarboxylic acid, pH adjusted to 4.5 with NaOH, acetonitrile–water (25:75%); flowrate: 1.0 ml min^{-1}; detection: UV, 251 nm.

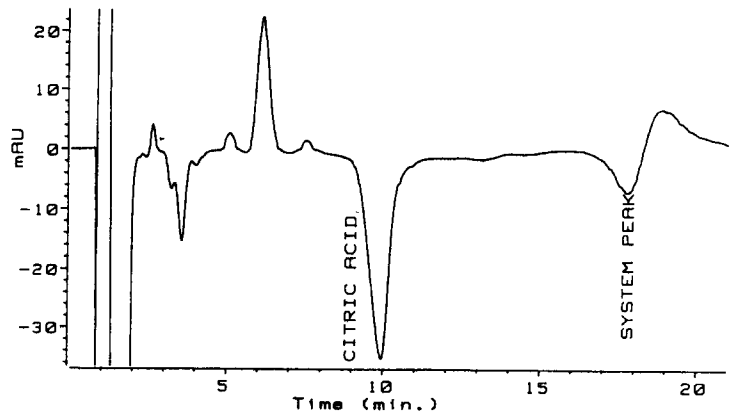

Figure 6
Typical chromatogram of carbamide peroxide sample A. Mobile phase: 0.5 mM 1,3,5-benzenetricarboxylic acid, pH adjusted to 4.5 with NaOH, acetonitrile–water (25:75%); flowrate: 1.0 ml min^{-1}; detection: UV, 251 nm.

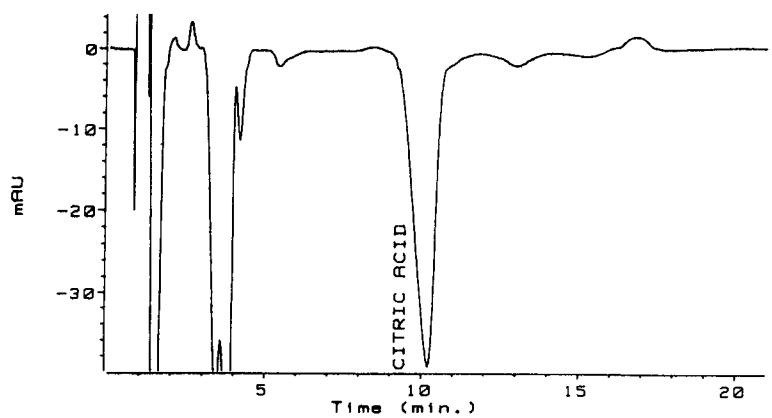

Figure 7
Typical chromatogram of carbamide peroxide sample B. Mobile phase: 0.5 mM 1,3,5-benzenetricarboxylic acid, pH adjusted to 4.5 with NaOH, acetonitrile–water (25:75%); flowrate: 1.0 ml min^{-1}; detection: UV, 251 nm.

tation limit of 50 μg g^{-1} and a detection limit of 20 μg g^{-1} (2:1 signal to noise) were determined from the data.

The system precision was determined by injecting a known citric acid standard (101 μg g^{-1}) five times into the LC system and calculating the reproducibility. The system precision was found to be 0.3%. The method precision was determined by injecting carbamide peroxide samples into the chromatographic system and determining the %RSD (six separately weighed samples). The method precision was calculated to be 4.0%. A recovery of 103.9% (4.4% RSD) of the theoretical value of citric acid contained in the different carbamide peroxide formulation was obtained. The carbamide peroxide samples used in this study contained approximately 0.595% citric acid on a weight to weight basis.

The data obtained in this study indicate that this chromatographic method can be used to determine the amount of citric acid present in carbamide peroxide samples.

References

[1] A. Chalgeri and H.S.I. Tan, *J. Pharm. Biomed. Anal.* **11**, 353–359 (1993).
[2] N.E. de Souza, O.E.S. Godinho and L.M. Aleixo, *Analyst* **110**, 989–991 (1985).
[3] *The United States Pharmacopeia*, 22nd Rev., pp. 99–103 and 315–316. United States Pharmacopeial Convention, Inc., Rockville, MD (1990).
[4] C.E. Dalgleish, E.C. Horning, M.G. Horning, K.L. Knox and K. Yarger, *Biochem. J.* **101**, 792 (1966).
[5] M.A. Harmon and H.W. Doelle, *J. Chromatogr.* **42**, 157 (1973).
[6] M. Planta, F. Lazaro, R. Puchades and A. Maquieira, *Analyst* **118**, 1193–1197 (1993).
[7] S.H. Ashoor and M.J. Knox, *J. Chromatogr.* **299**, 288–292 (1984).
[8] M.A.M. Rodriguez, M.L.V. Oderiz, J.L. Hernandez and J.S. Lozano, *J. Chromatogr. Sci.* **30**, 433–437 (1992).
[9] H. Small and T.E. Miller, *Anal. Chem.* **54**, 463–469 (1982).
[10] D.R. Jenke and N. Raghavan, *J. Chromatogr. Sci.* **23**, 75–80 (1985).
[11] B.P. Downey and D.R. Jenke, *J. Chromatogr. Sci.* **25**, 519–524 (1987).
[12] T.A. Walker, N. Akbari and T.V. Ho, *J. Liq. Chromatogr.* **14**, 619–641 (1991).
[13] T.A. Walker, *J. Liq. Chromatogr.* **11**, 1513–1530 (1988).
[14] T.A. Walker, T.V. Ho. and N. Akbari, *J. Liq. Chromatogr.* **14**, 1351–1366 (1991).
[15] T.A. Walker, T.V. Ho and N. Akbari, *J. Liq. Chromatogr.* **12**, 1213–1230 (1989).
[16] T.A. Walker and T.V. Ho, *J. Chromatogr. Sci.* **28**, 254–257 (1990).
[17] L.R. Snyder and J.J. Kirkland, *Introduction to Modern Liquid Chromatography*, 2nd edition, pp. 410–452. John Wiley and Sons Inc., New York (1979).

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