Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 4/5, pp. 353–359, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00

Assay of citrate in pharmaceutical matrices by indirect photometric liquid chromatography*

ARUN CHALGERI‡ and HENRY S.I. TAN†

Division of Pharmaceutics and Drug Delivery Systems, College of Pharmacy, University of Cincinnati Medical Center, Cincinnati, OH 45267, USA

Abstract: The development of a simple HPLC procedure is described for the analysis of citrate in pharmaceutical formulations using the technique of indirect photometric chromatography. A novel mobile phase, using dual eluent species, was developed for rapid elution of citrate. The developed method was found to be linear over the range studied (1–12 μg of citrate injected), showed good per cent recoveries (±RSD) of 97.5 ± 3.05% from a generalized matrix solution, and includes very simple sample preparation steps for analysis of commercial products. This method is quite specific for tricarboxylic acids as most other ions are not retained by the column using the developed mobile phase. The resolution of the method is also good as indicated by a baseline resolution (Rs = 1.5) obtained for citrate and tricarballylate, two structurally similar tricarboxylic acids. It is proposed that the developed method be evaluated as an alternative to the tedious procedures employed by the USP for assay of total citrate in some of its monographs.

Keywords: *Citric acid; indirect photometric chromatography; ion-exchange chromatography; single column ion chromatography; pharmaceutical analysis.*

Introduction

Citric acid is widely used in the pharmaceutical industry. It is used therapeutically as an anticoagulant to preserve blood for transfusion purposes, to dissolve renal calculi, and as one of the ingredients of rectal enemas. Some drugs are used in therapy as their citrate salts. The use of citric acid and its salts as pharmaceutical adjuvant is much more widespread. It is used in preparing effervescent granules and tablets, as a synergist to enhance the effectiveness of antioxidants and in buffering and improving the taste of pharmaceutical formulations.

The analytical methods employed by the United States Pharmacopeia (USP) for the assay of citrate in pharmaceutical formulations are mostly based on acid–base titrimetry. Titrations cannot be used for some of the formulations and, in these cases, USP employs procedures involving gravimetry, colorimetry and ion exclusion chromatography [1]. Not only are these procedures tedious and time consuming, they may also be prone to errors because of strict procedural requirements involving temperature, time, and other variables.

Indirect photometric chromatography (IPC) has been widely used for the chromatographic analysis of ions. This technique uses a photometer to detect ions transparent to UV or visible light. The separation mode in IPC is ion exchange in which the eluent ions in the mobile phase are UV (or visible) light absorbing and, after equilibration with the stationary phase, shows a constant absorbance value at the detector. As the injected sample ions elute at their characteristic retention times, there is a corresponding equivalent decrease in the eluent concentration since the total equivalent concentration of ions must remain constant in the mobile phase, in accordance with the principles of ion exchange. As a result there will be a decrease in absorbance at the detector and a negative peak is recorded [2].

The IPC methodology was selected for this work because it only requires a conventional liquid chromatograph equipped with a photometric detector and this instrumentation is generally available in most analytical laboratories. IPC has been applied to pharmaceutical

*Presented at the sixth annual meeting of the American Association of Pharmaceutical Scientists, Washington D.C., November 1991. This work is also a part of the Ph.D. dissertation research carried out by A.C.

†Author to whom correspondence should be addressed.
‡Present address: Analytical R & D Adria-SP Inc., 4200 Balloon Park Road, Albuquerque, NM 87109, USA.

analysis for the determination of a number of monovalent and divalent anions [3]. However, the per cent recoveries of the ions in this study were mostly low and inconsistent. Analysis of citrate by IPC has been attempted, but not further quantified, because of long retention times associated with citrate due to its high affinity for the ion-exchange stationary phase [4].

This paper describes the development of an analytical method, based on IPC, for the assay of citrate in pharmaceutical formulations. This method is also proposed as an alternative to the tedious procedures prescribed by the USP for the assay of total citrate in some of the monographs. Automation of the developed procedure is also possible with an autosampler connected to the liquid chromatography system.

Experimental

Apparatus

The liquid chromatograph consisted of a Beckman Model 110 B Solvent Delivery Module equipped with a 20 μl injection valve (Beckman Instruments, Fullerton, CA, USA), a Kratos Model 783 programmable absorbance detector (Kratos Analytical, Ramsey, NJ, USA) and a Varian 4270 electronic integrator (Varian Instruments, Walnut Creek, CA, USA).

Chemicals

Potassium hydrogen phthalate (Fisher Scientific, Fairlawn, NJ, USA) trimesic acid (Eastman Kodak, Rochester, NY, USA), citric acid monohydrate (Merck and Co., Rahway, NJ, USA), sodium pyrophosphate decahydrate (Matheson Chemicals, Cincinnati, OH, USA), isocitric acid (sodium salt) and tricarballylic acid (Sigma Chemicals, St Louis, MO, USA) were used as such. All other chemicals used were of analytical grade. All solutions and dilutions were made with distilled and deionized water obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

LC conditions

The chromatographic columns used in this study were: a 25 cm × 4.6 mm i.d. and 4.5 cm × 4.6 mm i.d. Ultrasil AX 10-μm columns (Beckman Instruments). For all quantitative work, the mobile phase consisted of an aqueous solution of 0.15 mM trimesic acid and 0.35 mM sodium pyrophosphate decahydrate, adjusted to pH 6.0 by addition of 1 M phosphoric acid or 1 M sodium hydroxide, and was vacuum degassed before use. The eluent was pumped at a flow rate of 1.0 ml min^{-1}. Two serially connected 4.5 × 4.6 mm i.d. columns were used for all quantitative determinations. The UV absorbance was monitored at 280 nm (0.1 AUFS) against air as reference. The polarity of the integrator was reversed to record the eluting sample bands as positive peaks.

Standard stock solutions

Citric acid stock solution. A proportionate amount of citric acid monohydrate was accurately weighed to give a concentration of about 1 mg ml^{-1} anhydrous citric acid.

Matrix stock solution. This solution was formulated to simulate many USP products containing citrate as an active ingredient. Matrix solution consisted of the following components: dextrose (0.32 mg ml^{-1}), potassium chloride (1.29 mg ml^{-1}), sodium chloride (0.63 mg ml^{-1}), potassium bicarbonate (0.11 mg ml^{-1}), potassium biphosphate (0.05 mg ml^{-1}), and sodium bicarbonate (0.10 mg ml^{-1}).

Standard solution

A standard solution of citric acid was prepared by pipetting 3.0 ml of citric acid stock solution into a 10 ml volumetric flask, and diluting to volume with water.

Sample preparations

For recovery studies. Four different aliquots of citric acid stock solution (0.5, 1.0, 2.0 and 4.0 ml) were pipetted into separate 10 ml volumetric flask, each containing 1.0 ml of the stock matrix solution, and diluted to volume with water.

For commercial products. The liquid products were first diluted with water and an appropriate aliquot of this solution (to yield final citrate concentrations of about 0.3 mg ml^{-1}) was pipetted into a 10 ml volumetric flask and diluted to volume with water. Effervescent tablets were carefully dissolved in water in a 1.0 l, volumetric flask and diluted to volume with water. This solution was filtered and the filtrate was treated in the same manner as the liquid products.

Chromatographic procedure

Using a 50-μl syringe, about 45 μl of the prepared sample or standard solution was introduced into the injection valve to completely rinse and fill the 20-μl sample loop and chromatographed under the conditions described above. Quantitation was based on relating the citrate peak height of the sample to that of the standard.

Linearity determination

From the citric acid stock solution, five different aliquots were pipetted into separate 10 ml volumetric flasks, to give 0.05–0.6 mg ml^{-1} citric acid (anhydrous) after dilution to volume with water. Each of the standard solutions were chromatographed as above and the citrate peak area or height were plotted against the amount of citrate injected.

Results and Discussion

Citrate ion is generally characterized by high affinity for the anion exchangers [5]. The retention time for citrate can therefore be very long, leading to broad peaks, and high concentrations of the eluent in the mobile phase will be required to elute it in a reasonable timeframe. In IPC, there is an inverse relationship between eluent concentration and sensitivity [6]. The absorbance value at the detector increases with increasing eluent concentration and sensitivity decreases with increasing absorbance values in IPC. Therefore, for analysis of citrate by IPC, retention time is reported to be very long [2] and an attempted method was limited in sensitivity [4]. The extent of effect of each of the above three variables on each other should be evaluated to define optimum conditions of analysis by IPC.

One more factor which has a direct bearing on retention time and eluent concentration is the exchange capacity of the ion-exchange columns. With low exchange capacity columns, a more dilute eluent will be capable of eluting a sample ion with a similar retention time as that obtained with a high exchange capacity column. Thus, increased detection sensitivity and reduced retention time of a sample ion can be accomplished by using a column of low exchange capacity. Initially in this project, a 25 cm high exchange capacity (0.28 meq g^{-1}) column was used because separation of closely related ionic species like citrate and isocitrate was desired as this would impart some specificity into the developed method.

Initial attempts to elute citrate with mobile phases consisting of 1.0 mM potassium hydrogen phthalate; 1.0 mM potassium hydrogen phthalate and 40 mM potassium biphosphate; 1.0 mM trimesic acid; and 1.0 mM trimesic acid and 40 mM potassium biphosphate, all at pH 6.0, were unsuccessful. However, it was observed that oxalate eluted only when potassium biphosphate was present in the mobile phase in addition to potassium hydrogen phthalate. As citrate (about 28% trivalent character at pH 6.0) is much more strongly retained, even a combination of trimesic acid (also trivalent at pH 6) and phosphate (divalent) was unsuccessful in eluting it from a 25 cm column within 30 min. It was postulated that perhaps another trivalent anion (at pH 6), in addition to trimesic acid, should result in elution of citrate. Among the common inorganic acids listed [7], only pyrophosphoric acid was trivalent (about 63%) at pH 6 ($pK_{a_3} = 5.77$). Indeed, incorporation of 1.0 mM sodium pyrophosphate along with 0.5 mM trimesic acid resulted in elution of citrate within 15 min at a flow rate of 2.0 ml min^{-1}. Under these conditions, baseline resolution was obtained between citrate and isocitrate (Fig. 1). The sensitivity of this method was rather limited as injecting 4 μg of citrate resulted in a peak height of less than 2 cm.

In order to increase the sensitivity, the column length was reduced by replacing the 25 cm analytical column with two 4.5 cm columns connected together. A corresponding decrease (by about 70%) in the concentration of each of the components of the mobile phase resulted in a retention time of about 10 min for citrate at a flow rate of 1.0 ml min^{-1}. The peak height, on injecting 4 μg of citrate, had now more than tripled to about 6 cm. This was accompanied by a loss in resolution as citrate could not be separated from isocitrate. However, baseline separation was still achieved between citrate and tricarballylate. Tricarballylic acid is similar to citric acid, except for the absence of the hydroxyl group (Fig. 2), and was used in subsequent studies as a resolution marker for system suitability test purposes. All the figures presented here show the tricarballylate peak to indicate that it does not interfere with the elution of citrate.

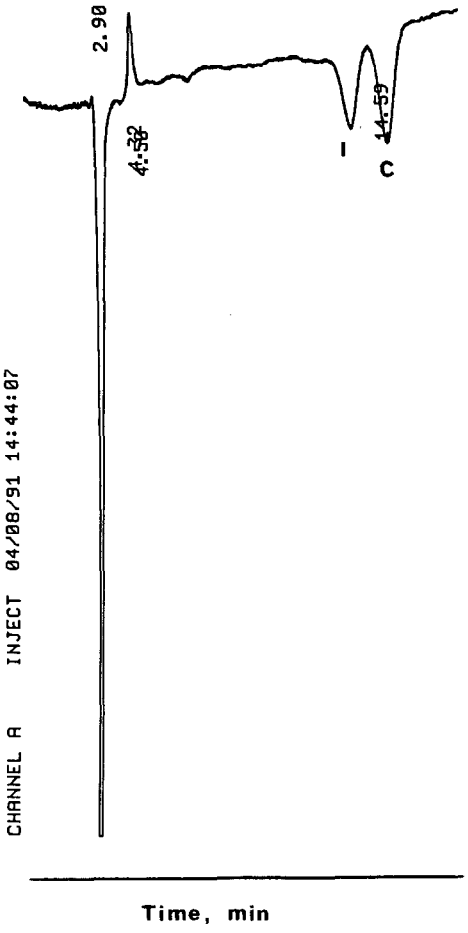

Figure 1
Liquid chromatogram showing baseline resolution between isocitrate (I) and citrate (C). Chromatographic conditions: column: a 45 × 4.6 mm Ultrasil-AX precolumn connected to a 250 × 4.6 mm Ultrasil-AX (10 μm) analytical column. Mobile phase: aqueous solution of 0.5 mM trimesic acid and 1.0 mM sodium pyrophosphate decahydrate, pH 6.0, at 2.0 ml min^{-1} with detection at 280 nm (0.074 AUFS). Integrator polarity was not reversed to record negative peak.

The effect of the mobile phase pH on the retention and resolution is illustrated in Table 1. The resolution between citrate and tricarballylate at pH 6 is quite adequate (Rs = 1.58) and this appears to be the optimum pH to work with. At this pH the tricarballylate peak is symmetrical and the citrate peak shows a tailing factor of 1.2 at 5% peak height. The retention time and resolution decreases as the pH is increased from 5.0 to 7.0. This can be attributed to the increased displacing power of both trimesate and pyrophosphate ions as they acquire more trivalent character with increasing pH. Another consideration in selecting the pH of the mobile phase is the operating range of the silica-based columns, which is from pH 2 to 7. A pH of 6 is within the column operating limits.

Table 1
Effect of mobile phase pH on resolution (Rs) and retention time (t_R)

	Tricarballylate		Citrate		
pH	t_R*	W*	t_R*	W*	RS
5.0	39	10	69	12	2.73
5.5	40	10	65	12	2.27
6.0	34	8	49	11	1.58
6.5	29	8	40	10	1.22
7.0	25	8	32	9.5	0.80

*All peak retention and width (W) measurements in mm.
Chromatographic conditions as described in text.

The effect of mobile phase flow rate on retention, resolution and HETP is shown in Table 2. The results indicate that changing the flow rate from 0.4 to 2.0 ml min^{-1} causes no serious loss in resolution even at a flow rate of 2.0 ml min^{-1}, although the Height Equivalent to a Theoretical Plate (HETP) for citrate almost doubled over this range. A flow rate of 1.0 ml min^{-1} was adopted because the back-pressure was within the desired limits of 1000 psi. The UV absorbance was monitored at 280 nm as it was one of the maxima in the absorbance spectrum of the mobile phase. At this wavelength, the absorbance value of the mobile phase at the detector was about 0.3, well within the optimum range of 0.2–0.8 absorbance units.

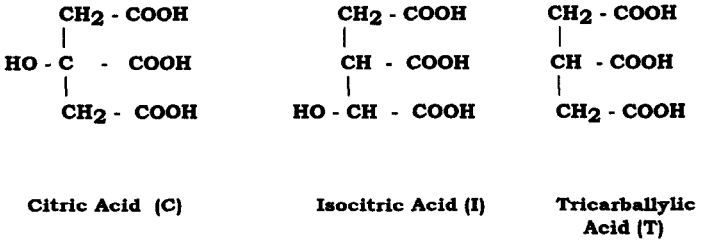

Figure 2
Structures of citric acid (C); isocitric acid (I); and tricarballylic acid (T).

Table 2
Effect of flow rate on retention (t_R), resolution (Rs) and HETP

Flow rate (ml min^{-1})	Tricarballylate		Citrate		Rs	N†	HETP† (mm)
	t_R*	W*	t_R*	W*			
0.4	73.0	19.0	118.5	20.5	2.00	535	0.17
1.0	33.0	8.0	48.5	10.0	1.72	376	0.24
1.3	25.0	6.5	37.0	8.0	1.66	342	0.26
2.0	16.0	4.0	23.0	5.5	1.47	280	0.32

*All peak retention and width (W) measurements in mm.
†N = number of theoretical plates, HETP = height equivalent to a theoretical plate of the column for citrate.
Chromatographic conditions as described in text.

The standard curve, obtained by regressing the peak height or area obtained versus amount of citrate injected (1–12 μg), was found to be linear. For peak height, a typical regression equation of $A = 50.4\ C + 20.2$ ($r = 0.9961$) was obtained, and a typical regression equation using peak area was $A = 9288\ C - 4974$ ($r = 0.9997$), where A = peak area or height and C = amount (μg) of citrate injected. Quantitation by the peak height was chosen because it was found to be less sensitive to the fluctuations and drift observed in the background absorbance. This was reflected in more consistent values obtained on using peak height for quantitation as compared to either peak area alone or citrate–tricarballylate peak area or peak height ratios. The limit of detection, as determined by the standard deviation method [8] and measured in terms of peak height, was found to be 0.085 μg of citrate injected at 0.1 AUFS. The sensitivity of the method can be increased about 4 times if the reference cell of the detector contains the mobile phase rather than air.

No interferences due to the matrix components were observed as indicated by a relatively clean chromatogram on injection of only the matrix solution (Fig. 3). A chromatogram of matrix solution spiked with citric acid and tricarballylic acid is shown in Fig. 4. The per cent recoveries from the spiked matrix solutions are listed in Table 3. The overall weighted per cent recovery (±RSD) was

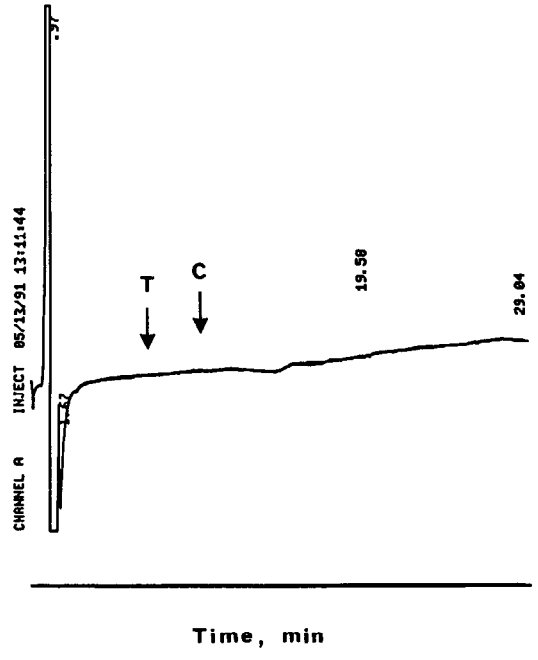

Figure 3
Liquid chromatogram of a blank matrix solution. Chromatographic conditions as described in the text; T and C indicate the locations of tricarballylate and citrate peaks, respectively.

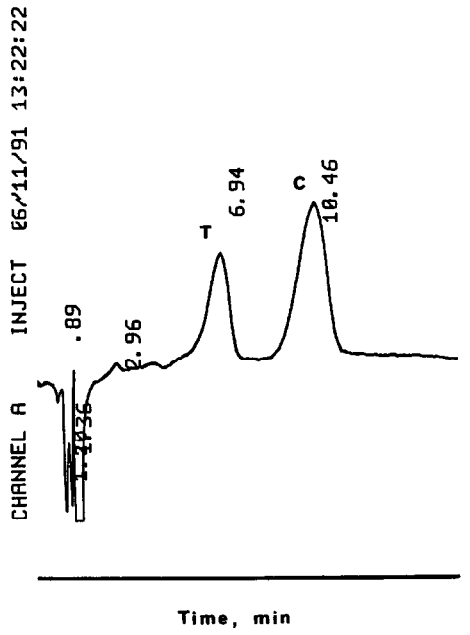

Figure 4
Liquid chromatogram of a matrix solution spiked with tricarballylate (T) and citrate (C). Chromatographic conditions as described in the text.

Table 3
Recoveries from spiked matrix solutions

Conc. level	Amount added (mg ml^{-1})	n	Per cent recoveries obtained
1	0.05	3	95.4
2	0.10	4	97.5
3	0.20	5	95.9
4	0.40	5	100.4
Overall per cent recovery (weighted average)		17	97.5
Pooled standard deviation			2.1

97.5 ± 3.05% (n = 17). The slight negative bias observed could be due to the high ionic content of the matrix solution. The matrix solution was formulated to contain ingredients found in many USP monographs and is therefore more rigorous than any single product.

It was observed that a system peak appears at about 20 min whenever a solution containing citrate was injected. Consequently, the run time for each injection is about 22 min to allow the baseline to revert back to its original level before the next injection can be made. However, the developed method turns out to be quite specific for citrate. Other carboxylic acids, such as tartaric acid, and all other monovalent/divalent anions from the matrix solution were not retained by the column and elute near the void volume.

Assay of some of the commercially available effervescent tablets and liquid oral solutions were carried out by the developed method. Figures 5 and 6 show typical chromatograms

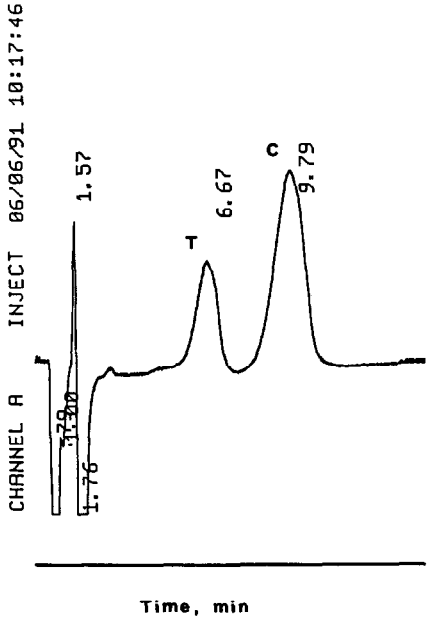

Figure 5
Liquid chromatogram of a sample solution prepared from a commercial liquid oral product containing potassium citrate and citric acid in a syrup base. Chromatographic conditions as described in the text. (T) Tricarballylate, (C) citrate.

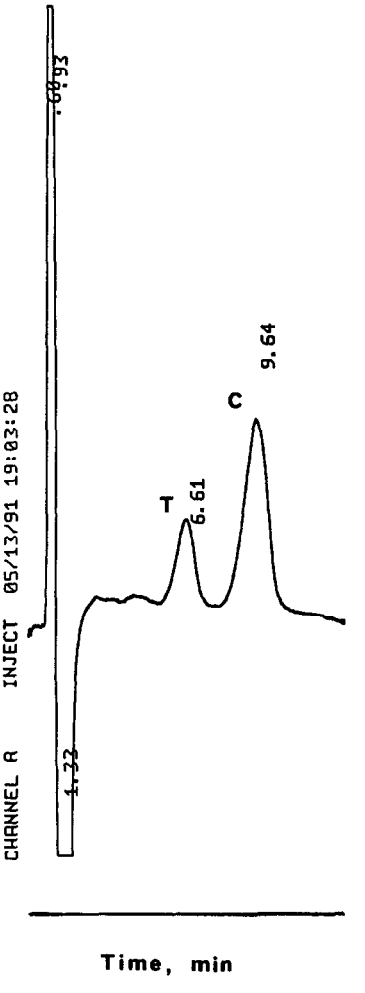

Figure 6
Liquid chromatogram of a sample solution prepared from a commercial effervescent tablet containing potassium citrate, potassium bicarbonate, citric acid, saccharin and other ingredients. (T) Tricarballylate, (C) citrate.

Table 4
Assay results of some commercial products

Product type	Sample	Label claim (citrate)*	% Label found by	
			IPC method	Colorimetric method†
Liquid oral solution	1	657.3 mg/5 ml	92.0	93.1
	2	1046.1 mg/5 ml	97.1	ND‡
	3	1047.3 mg/5 ml	94.1	ND‡
Effervescent tablets	4	2.10 g/tab	103.0	98.6
	5	3.85 g/tab	102.2	97.3
	6	0.55 g/tab	100.5	103.4

*Total citrate as citric acid monohydrate.
†Colorimetric method of anticoagulant citrate dextrose solutions USP.
‡Not determined: because of obvious interferences as evidenced by solutions turning dark on addition of reagents.

obtained with the commercial products. Total citrate in these products were also determined by a colorimetric method, which is used for assaying anticoagulant citrate dextrose solutions in the USP. The assay results are shown in Table 4. A good correlation between the assay values of the two methods ($r = 0.993$, $n = 4$) is observed. It is of interest to note that the developed IPC procedure is more selective since the colorimetric method could not be used for two of the products. This was because of obvious interferences as evidenced by these solutions turning dark on addition of the reagents for colour development. This indicates the ability of the IPC method to analyse a variety of different products without interferences from the matrix components.

The appearance of the system peak late in the chromatogram unnecessarily prolongs the time difference between each injection. It was also observed that the silica-based columns employed provided reproducible results only for about 120 h of use. Efforts are underway in this laboratory to evaluate the use of polymer-based columns for assay of citrate. Tricarballylic acid can be used for system suitability test requirements. At the indicated flow rate of 1.0 ml min^{-1}, the resolution between the citrate and tricarballylate should be no less than 1.7.

References

[1] *The United States Pharmacopeia*, 22nd Rev., pp. 99–103 and pp. 315–316. United States Pharmacopeial Convention, Inc., Rockville, MD (1990).
[2] H. Small and T.E. Miller, *Anal. Chem.* **54**, 463–469 (1982).
[3] D.R. Jenke and N. Raghavan, *J. Chromatogr. Sci.* **23**, 75–80 (1985).
[4] B.P. Downey and D.R. Jenke, *J. Chromatogr. Sci.* **25**, 519–524 (1987).
[5] P.R. Haddad and P.E. Jackson, *Ion Chromatography. Principles and Applications*, p. 23. Elsevier, Amsterdam (1984).
[6] H. Small, *Ion Chromatography*, pp. 196–200. Plenum Press, New York (1989).
[7] D.R. Lide (Ed.), *CRC Handbook of Chemistry and Physics*, 72nd edn, pp. 8–41. CRC Press, Boca Raton, FL (1991).
[8] J.A. Glaser, D.L. Foerst, G.D. McKee, S.A. Quave and W.L. Budde, *Environ. Sci. Technol.* **15**, 1426–1435 (1981).

[Received for review 18 March 1992; revised manuscript received 29 October 1992]