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Indirect photometric detection for determination of citrate in pharmaceutical matrices by ion chromatography[1]

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Abstract

The development of a simple ion chromatographic method for the analysis of citrate in pharmaceutical matrices is described utilizing the technique of indirect photometric detection. A polymeric anion exchange column was used with a mobile phase consisting of an aqueous solution of trimesic acid (pH 10) and indirect detection at 280 nm. The developed method was precise and accurate with percent recoveries ($\pm$%RSD) of 99.3 $\pm$ 0.97% and 98.2 $\pm$ 0.25% at 6 and 12 μg of citrate injected. Linear detector response was found over the range 1–12 μg of citrate injected with a limit of quantitation of 0.26 μg of citrate injected. The method is specific for tricarboxylic acids as most other ions were not retained by the column. Youden's robustness test, involving seven selected operating variables, showed that the method is fairly robust. A system suitability test was also performed. Many commercial USP and non-USP liquid and tablet formulations were assayed for citrate by the developed method and the results were found to be comparable with those obtained by the compendial method.

Keywords: Citrate; Indirect photometric detection; Ion chromatography; Pharmaceutical matrices

1. Introduction

Citric acid and its salts are commonly used ingredients in many pharmaceutical formulations. The USP utilizes a column cation exchange method [1] for assay of a number of citrate formulations based on the method of Tan et al. [2]. Other assay methods employed by the USP for determination of citrate include colorimetry and ion exclusion chromatography carried out at 40°C [3]. The colorimetric method involves color development using pyridine and acetic anhydride with strict control of time and temperature. Most of these methods can therefore be tedious and are not easily automated. The only high performance liquid chromatographic (HPLC) method, involving ion-exclusion chromatography, requires strict

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temperature control of the mobile phase as well as the analytical column.

The technique of indirect photometric chromatography (IPC) was selected in this study because it requires only a conventional liquid chromatograph equipped with a photometric detector and this instrumentation is readily available in most analytical laboratories. IPC uses an absorbance detector to detect ions transparent to UV or visible light. This is accomplished by using UV-absorbing eluent ions in the mobile phase, resulting in a constant absorbance value at the detector, and this baseline absorbance value decreases when the UV-transparent sample ions elute at their characteristic retention time [4]. The absorbance change is proportional to the concentration of the sample ion. Analysis of citrate by IPC has been attempted, but not quantified, because of the long retention times associated with citrate [5].

Earlier, this laboratory reported an IPC method for rapid elution of citrate using novel eluents in the mobile phase [6]. This method used silica-based anion exchange columns and was validated by determining specificity, linearity, accuracy, and the limit of detection. Total citrate in several pharmaceutical products was quantified by this method and the results agreed very well with the USP assay methods. However, the silica-based columns could only be used for about 120 h and the appearance of a system peak late in the chromatogram resulted in unnecessary doubling of the chromatographic run time.

This work describes the development and validation of an improved HPLC method for determination of total citrate in pharmaceutical forumulations by IPC. A polymeric anion exchange column was employed and the method is unique because citrate has been chromatographed at an alkaline mobile phase pH of 10. A typical chromatographic run time is only about 10 min and no system peaks were observed after the elution of citrate. This method is proposed as an alternative to the tedious procedures employed by the USP for the assay of total citrate in some of the monographs.

2. Experimental

2.1. Apparatus

The liquid chromatograph consisted of a Beckmann Model 110B Solvent Delivery Module equipped with a 20 μl injection valve (Beckmann Instruments, Fullerton, CA), a Kratos Model 783 programmable absorbance detector (Kratos Analytical, Ramsey, NJ) and Varian model 4290 and 4270 (used in robustness test) electronic integrators (Varian Instruments, Walnut Creek, CA).

2.2. Chemicals

Trimesic acid (Eastman Kodak, Rochester, NY), citric acid monohydrate (Merck and Co., Rahway, NJ), and tricarballylic acid (Sigma Chemicals, St. Louis, MO) were used as received. 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, Bradford, MA).

2.3. HPLC conditions

The HLPC analytical column consisted of a 150 mm × 4.1 mm PRP-X100 (10 μm) analytical column and was preceded by a 25 mm × 2.3 mm PRP-X100 guard column (Hamilton Co., Reno, NV). The mobile phase was an aqueous solution of 0.875 mM trimesic acid, adjusted to pH 10.0 with 1 M NaOH, and pumped at 1.5 ml min^{-1}. This solution was filtered through a 0.45 μm nylon membrane filter, and vacuum-degassed before use. The eluates were monitored at 280 nm (0.320 AUFS). The eluting analytes were recorded as positive peaks by reversing the input polarity of the integrator.

2.4. Standard stock solutions

2.4.1. Citric acid stock solution

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

2.4.2. Matrix stock solution

This solution was formulated to simulate many USP products containing citrate as an active ingredient. The stock matrix solution consisted of the following components: sucrose (10.26 mg ml^{-1}), dextrose (1.62 mg ml^{-1}), potassium chloride (6.43 mg ml^{-1}), sodium chloride (3.15 mg ml^{-1}), potassium bicarbonate (0.55 mg ml^{-1}), sodium bicarbonate (0.50 mg ml^{-1}), and potassium biphosphate (0.25 mg ml^{-1}).

2.4.3. Standard solution

A standard solution of citric acid was prepared by pipetting 4.0 ml of citric acid stock solution into a 10 ml volumetric flask, and diluting to volume with water.

2.5. Sample preparations

2.5.1. For recovery studies

Four different aliquots of citric acid stock solution of 1.0, 2.0, 3.0, and 6.0 ml were pipetted into four separate 10 ml volumetric flasks, each containing 1.0 ml of the matrix stock solution, and diluted to volume with water to give four spiked sample solutions.

2.5.2. For commercial products

The products assayed by the proposed IPC method are listed in Table 1. Products 1–5 were suitably diluted to give a final concentration of total citrate of about 0.4 mg ml^{-1}. The effervescent granules and tablets (products 6–9) were first carefully dissolved in 1.0 l of water and filtered. An aliquot of the filtrate was then diluted to give a final concentration of about 0.4 mg ml^{-1}.

2.6. Chromatographic procedure

Exactly 20 μl of the sample solution and the standard solution were injected separately by means of the sample loop and chromatographed under the operating conditions described above. Quantitation was based on comparing the citrate peak height of sample to that of the standard.

2.7. Determination of linearity

From the citric acid stock solution, four different aliquots of 1.0, 2.0, 3.0, and 6.0 ml were pipetted into four separate 10 ml volumetric flasks and diluted to volume with water. Each of the solutions was chromatographed as above and the citrate peak heights were plotted against the amount of citrate injected.

2.8. Limit of quantitation (LOQ)

The LOQ was determined following the method of Foley and Dorsey [7]. A blank matrix solution, obtained by diluting 1.0 ml of the stock matrix solution to 10 ml, was injected by means of the 20 μl sample loop to determine the noise level. A standard curve was obtained as described in Section 2.7 with the peak height manually measured in millimeters.

2.9. Test for robustness

The mobile phase factors pH, trimesic acid concentration, flow rate, and water source, along with the detector wavelength, integrator model and column (room) temperature, were selected as the seven variables for Youden and Steiner's [8] robustness test. Each variable was studied at two levels, indicated by upper case and lower case letters in Table 2, to bracket the standard conditions of the variable described under HPLC conditions. Two different batches of spiked placebos were prepared, giving 0.1 and 0.3 mg ml^{-1} of citrate before chromatography, which were assayed following Youden and Steiner's experimental design shown in Table 2. The two levels of each variables studied are shown in Table 3. A total of eight experiments was carried out to evaluate the robustness of this method.

3. Results and Discussion

3.1. Development of the analytical method

One of the primary objectives of this project was to develop a method that is easier to perform

Table 1
List of commercial products analyzed and their ingredients

Product No.	USP product type	Ingredients	Label amount
1	Anticoagulant Citrate Phosphate Dextrose Solution USP	Citric acid	2.99 g l^{-1}
		Sodium citrate	26.3 g l^{-1}
		$NaH_2PO_4-H_2O$	2.22 g l^{-1}
		Dextrose (1 H_2O)	25.5 g l^{-1}
2	Magnesium Citrate Oral Solution	Magnesium citrate	54.5 mg ml^{-1}
3	Tricitrates Oral Solution USP	Potassium citrate	550 mg per 5 ml
		Sodium citrate	550 mg per 5 ml
		Citric acid	334 mg per 5 ml
4	Potassium Citrate & Citric Acid Oral Solution	Potassium citrate	1100 mg per 5 ml
		Citric acid	334 mg per 5 ml
		in a syrupy base	
5	Na-Citrate & Citric Acid Oral Solution	Sodium citrate	500 mg per 5 ml
		Citric acid	300 mg per 5 ml
6	$KHCO_3$ & KCl Effervescent Tablets for Oral Solution	Potassium chloride	1.50 g per tablet
		Potassium bicarbonate	0.50 g per tablet
		L-Lysine · HCl	0.91 g per tabler
		Citric acid	0.55 g per tablet
		Saccharin and others	-
7	$KHCO_3$ Effervescent Tablets for Oral Solution	Potassium bicarbonate	2.5 g per tablet
		Citric acid	2.1 g per tablet
		Saccharin and others	-
8	$KHCO_3$ & K-Citrate Effervescent Tablets for Oral Solution	Potassium citrate	2.7 g per tablet
		Potassium bicarbonate	2.5 g per tablet
		Citric acid	2.1 g per tablet
		Saccharin and others	
9	Sodium Bicarbonate Oral Powder	Sodium bicarbonate	2.340 g per 3.9 g
		Citric acid	1.190 g per 3.9 g
		Sodium citrate	0.254 g per 3.9 g
		Calcium lactatė · 5 H_2O	0.151 g per 3.9 g
		Sodium chloride	0.079 g per 3.9 g
		Sodium biphosphate	0.044 g per 3.9 g
		Magnesium sulfate	0.042 g per 3.9 g

than those currently adopted by the USP in some of its monographs. The developed method would involve simple sample preparation steps, would be easily automated, and could potentially be adopted for assay of total citrate in all the USP monographs. The most common USP method for assaying total citrate is by acid–base titration which may be preceded by cation-exchange when citrate salts are present. However, the USP monographs listed in Table 4 have particularly tedious assay methodologies for determination of total citrate. The developed method is aimed at replac-

Table 2
Experimental design for robustness test

Variable	Experiment #							
	1	2	3	4	5	6	7	8
Eluent concentration	*A*	*A*	*A*	*A*	*a*	*a*	*a*	*a*
pH	*B*	*B*	*b*	*b*	*B*	*B*	*b*	*b*
Wavelength	*C*	*c*	*C*	*c*	*C*	*c*	*C*	*c*
Flow rate	*D*	*D*	*d*	*d*	*d*	*d*	*D*	*D*
Water source	*E*	*e*	*E*	*e*	*e*	*E*	*e*	*E*
Integrator	*F*	*f*	*f*	*F*	*F*	*f*	*f*	*F*
Room temp.(°C)	*G*	*g*	*g*	*G*	*g*	*G*	*G*	*g*
Observed results	*s*	*t*	*u*	*v*	*w*	*x*	*y*	*z*

ing these tedious methodologies in particular. A generalized placebo matrix, incorporating the majority of the ingredients present in the monographs listed in Tables 1 and 4, was formulated to check for possible interferences. The composition of this matrix solution is described in Section 2 and the amount of each ingredient was calculated on the basis of the dilution required to obtain citrate levels of about 0.4 mg ml $^{-1}$ in the final assay solution just prior to injection on the column. This matrix solution did not interfere with the chromatography of citrate as described in this method (Fig. 1).

Earlier work carried out in this laboratory employed two serially-connected, silica-based, strong anion exchange precolumns (45 mm × 4.5 mm) for IPC of citrate using a mobile phase consisting of 0.15 mM trimesic acid and 0.35 mM sodium pyro-phosphate solutions (pH 6.0) [6]. Although the method can be used to assay citrate in pharmaceutical formulations, the silica-based columns did not last beyond 120 h. Since the mobile phase is usually completely aqueous in ion exchange chromatography, it was postulated that a polymer column would perhaps last for a longer period of time. A polymeric column packed with PRP-X100, a strong anion exchange resin of quater-

Table 3
Variables selected for robustness test

Variable type	High level	Low level
Eluent concentration (mM)	0.90 (A)	0.85 (a)
pH	10.5 (B)	9.5 (b)
Wavelength (nm)	285 (C)	275 (c)
Flow rate (ml min $^{-1}$)	1.6 (D)	1.4 (d)
Water source	Milli-Q (E)	Distilled (e)
Integrator type	Model 4290 (F)	Model 4270 (f)
Room temp. (°C)	26 (G)	22 (g)

Table 4
Tedious USP methods for assay of total citrate

USP monographs	Active ingredients[a]	Assay method
Anticoagulant Citrate Dextrose Solutions[b]	Citric acid, sodium citrate, dextrose, Na-phosphate, adenine	Colorimetry
Citric Acid, Magnesium Oxide, and Sodium Carbonate Irrigation	Citric acid, magnesium oxide, sodium carbonate	Ion exclusion chromatography at 40°C
Magnesium Citrate Oral Solution	Magnesium carbonate, citric acid, sucrose, potassium bicarbonate	Precipitation, ignition, acid base titration

[a] As per USP.
[b] These include the following products: Anticoagulant Citrate Dextrose Solution USP, Anticoagulant Citrate Phosphate Dextrose Solution USP, and Anticoagulant Citrate Phosphate Adenine Dextrose Solution USP.

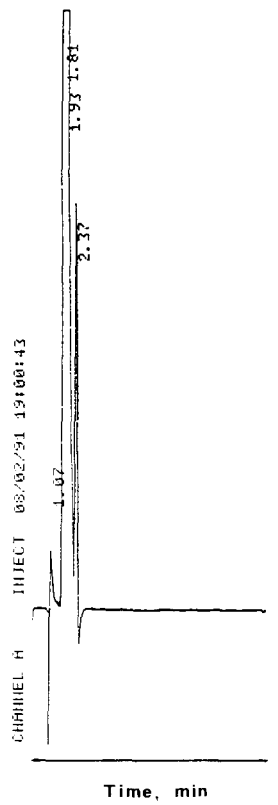

Fig. 1. Liquid chromatogram of a placebo matrix solution chromatographed under the conditions described.

nary ammonium type, was therefore selected for evaluation in this study.

The column manufacturer's (Hamilton Company, Reno, NV) application support group provided a chromatogram that showed the elution of citrate on a 25 cm PRP-X100 column with a mobile phase consisting of 95% 4 mM potassium hydrogen phthalate (pH 4.75) and 5% acetonitrile. However, this could not be duplicated in this laboratory using a 15 cm PRP-X100 column. Because of the strong affinity of citrate for the anion exchanger, a monovalent ion such as potassium hydrogen phthalate is generally not suitable for elution of citrate. Preliminary results indicated that an aqueous solution of 0.5 mM trimesic acid at pH 6.0 eluted citrate on the 15 cm PRP-X100 column. Adjusting the mobile phase pH to 5.0 and 7.0 also gave similar results. However, these initial attempts were also characterized by the appearance of system peaks that prolonged the chromatographic run time and which sometimes co-eluted with the citrate.

The effect of pH on the mobile phase consisting of 0.5 mM trimesic acid was studied in order to determine the pH at which elution characteristics of citrate are highly reproducible with minimal interference from the system peaks. A mobile phase pH of 10.0 was found to provide highly reproducible retention times for citrate with no interference from the system peaks. A more detailed account of the effect of pH and mobile phase variables is documented elsewhere [9]. Based on these studies, a mobile phase composition of 0.875 mM trimesic acid at a pH of 10.0 was found to be a suitable eluent for elution of citrate on a 150 mm × 4.1 mm PRP-X100 column. At a flow rate of 1.5 ml min^{-1}, citrate eluted at about 9 min. In comparison with the method developed earlier on the silica-based column [6], this method has a lower resolving capacity due to the lower exchange capacity of the polymer column (0.158 meq) compared with that of the silica-based columns used earlier (0.346 meq). As such it could not separate citrate and tricarballylate, a tricarboxylic acid similar to citric acid, which was initially selected for use as an internal standard. Consequently, it was decided not to use an internal standard.

3.3. Validation of the method

The validation of the developed method was carried out by determining the accuracy, precision, linearity, specificity, limit of detection, and robustness of the method. The accuracy of the method was determined by spiking the matrix solution with known amounts of citrate and analyzing the spiked samples by the developed method. The accuracy was then calculated as the percent amount recovered from the spiked samples. The overall percent recovery (± %RSD) was 98.9 ± 1.34% ($n = 18$). Table 5 shows the percent recoveries at each of the four citrate concentration levels. Concentration levels of at least 0.3 mg ml^{-1} of citrate (i.e. 6 μg injected) show good repeatabilities of < 1%. Consequently, a concentration level of 0.4 mg ml^{-1} was selected as the concentration of the final assay solution for the

assay of citrate formulations. The repeatability of the assay method, expressed as %RSD, was 0.96% at the 0.3 mg ml^{-1} level and 0.25% at the 0.6 mg ml^{-1} levels. The linearity of the method was determined by regressing the average peak height obtained versus the amount of citrate injected. A linear response was obtained over the concentration range 1–12 μg of citrate injected. A typical regression equation of $A = 57C + 48$ ($r = 0.998$) was obtained (A = peak height, C = amount (μg) of citrate injected). The selectivity of the analytical method is its ability to measure specifically the analyte in the presence of components which may be expected to be present in the sample matrix. This method is selective as indicated by the chromatogram of a placebo matrix solution shown in Fig. 1. No peak is observed at or around the retention time of citrate. The chromatogram of a spiked matrix solution is shown in Fig. 2.

Table 5
Accuracy and precision of the analytical method

Amount added (mg ml^{-1})	Amount found (mg ml^{-1})	%Recovery	Average	%RSD
0.100	0.1019	101.9	99.8	1.40
0.101	0.0989	97.9		
0.101	0.1003	99.3		
0.102	1.1020	100.0		
0.100	0.0997	99.7		
0.202	0.1943	96.2	98.1	2.10
0.201	0.2016	100.3		
0.204	0.1993	97.7		
0.301	0.3031	100.7	99.3	0.97
0.303	0.2988	98.6		
0.302	0.2990	99.0		
0.305	0.3001	98.4		
0.299	0.2987	99.9		
0.601	0.5902	98.2	98.2	0.25
0.607	0.5985	98.6		
0.604	0.5937	98.3		
0.611	0.5988	98.0		
0.599	0.5870	98.0		

Overall percent recovery 98.9
%RSD 1.34

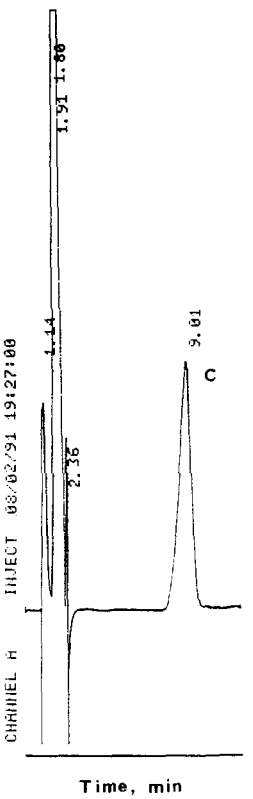

Fig. 2. Liquid chromatogram of a placebo matrix solution spiked with citric acid and chromatographed under the conditions described. C = citric acid.

The determination of the LOQ was based on the method recommended by Foley and Dorsey [7]. This method involves measuring the peak-to-peak noise ($N_{p\ p}$) over 20 base widths of the analytical peak and calculating the standard deviation of the baseline noise (S_b) which is defined as 1/5 $N_{p\ p}$. The LOQ was then determined by the equation LOQ = $(10S_b)/S$, where S is the slope of the standard curve (analytical sensitivity). In this work $N_{p\ p}$, determined with the chromatogram obtained on injecting a placebo matrix solution, was found to be 1.2 mm. The slope of the standard curve, obtained by regressing the citrate peak height obtained at various concentrations of citrate injected, was 9.23 mm μg^{-1}. From these values the LOQ was 0.26 μg of citrate injected. Although the LOQ value is very low, the method in this paper does not intend to quantify citrate at LOQ levels.

Table 6
Robustness test: effect of eluent concentration

Concn. (mM)	Expt. #	Percent recovery at		Std. curve Correlation (r)
		0.1 mg ml^{-1}	0.3 mg ml^{-1}	
0.90	1	95.9	96.6	0.9998
	2	96.0	98.2	1.0000
	3	108.0	95.8	1.0000
	4	106.6	97.4	0.9998
Average (±SD)		101.6 ± 6.6	97.0 ± 1.0	0.9999
0.85	5	97.0	98.0	0.9999
	6	99.1	90.8	0.9998
	7	115.6	96.6	0.9994
	8	111.5	108.1	0.9992
Average (±SD)		105.8 ± 9.1	98.4 ± 7.2	0.9996
Differences		4.20	1.40	0.0003

Robustness, previously known as ruggedness, is a measure of a method's reliability during normal usage. It can be expressed as the lack of influence of operational and environmental variables of the method on the assay results. Youden and Steiner's experimental design [8], based on $(n + 1)$ measurements to test the effects of n variables, was employed to determine if deliberate variations from the prescribed conditions will have any effect on the assay results. Seven variables were chosen and two levels of each variable were studied. The two levels selected were such that, depending on the variable, at least one or both of them was different from the standard conditions. The variables and their levels are listed in Table 3. The experimental design is outlined in Table 2. The observed results to be evaluated are the percent recoveries from matrix solutions spiked at 0.1 and 0.3 mg ml^{-1} concentration levels and the correlation coefficient of the standard curve. These results are designated by lower case "s" to "z" in Table 2. To determine if changing factor "A" to "a" has an effect, the averages of $(s + t + u + v)/4$ and $(w + x + y + z)/4$ are compared. This experimental design gives two groups of four determinations and each group contains the *other* six factors twice at the upper case level and twice at the lower case level. The effects of these factors, if present, cancel out, leaving only the effect of changing "A" to "a". As an example, the effect of the change in the trimesic acid concentration of the mobile phase is summarized in Table 6. The differences obtained between the two levels of each variable are ranked in decreasing order in Table 7.

Table 7
Robustness test: differences obtained between two levels of each variable

Variable	Differences (d) in		
	% Recoveries		Linearity (r)
	0.1 mg ml^{-1}	0.3 mg ml^{-1}	
pH	13.4	3.6	0.0003
Flow rate	2.1	4.4	0.0003
Eluent concn.	4.2	1.4	0.0003
Room temp.	1.2	4.7	0.0001
Integrator type	1.9	4.7	0.0001
Wavelength	0.8	1.9	0.0001
Water source	0.2	0.3	0.0001
Std. dev. (s)[a]	7.7	4.8	–

[a] Calculated from the following formula [8]: $s^2 = 2/7\Sigma d^2$.

The results of the ruggedness test indicate that the mobile phase pH is perhaps the most important variable that needs to be carefully controlled. Increased values were observed when the pH was reduced from 10.5 to 9.5. However, at these two pH levels the suitability parameters height equivalent to a theoretical plate (HETP), tailing, and retention factor are still within the ranges specified below. The repeatability (%RSD) is also within the general USP requirement of 2%. If the pH is carefully controlled and is not considered as a variable, the standard deviation drops from 7.7 to 2.8 for percent recoveries at the 0.1 mg ml^{-1} level. The standard deviation values in Table 7 indicate that such a variation can be expected if inter-laboratory comparisons were to be carried out. This shows that the developed method is quite rugged and able to withstand minor fluctuations in operating variables as fairly low varia-

Table 8
Assay results by the IPC and USP methods

Product #	Label claim[a]	Amount found by		Percent label claim	
		USP	IPC	USP	IPC
1	20.17 mg ml^{-1}	18.3	19.0	90.7	94.2
2	NLT 75.9 mg ml^{-1} [b]	85.9	83.2		
3	191.24 mg ml^{-1}	192.6	183.3	100.7	95.9
4	191.24 mg ml^{-1}	214.4	182.4	112.1	95.4
5	120.16 mg ml^{-1}	124.5	114.1	103.6	95.0
6	0.5027 g per tablet	0.5200	0.5233	103.4	104.1
7	1.9196 g per tablet	1.8925	1.8830	98.6	98.1
8	3.5192 g per tablet	3.4250	3.6110	97.3	102.6
9	1.3894 g per 4 g	1.3220	1.3071	95.1	94.1

[a] Total citrate as anhydrous citric acid.
[b] USP limits; NLT = not less than. Label claims are in terms of magnesium citrate.

tions were observed. The standard curve is essentially linear under all conditions studied and is not affected by deliberate changes introduced in the operating conditions of the developed method.

3.3. System suitability test

To verify that the reproducibility of the chromatographic system is adequate for the assay, a number of factors were determined. Since there is only one peak of interest in the chromatogram, the column efficiency, in terms of the HETP, was determined. The HETP ($\pm$SD, $n = 10$) was 0.20 ± 0.02 mm plate^{-1}. The retention factor k' ($\pm$SD, $n = 10$) for the citrate peak was calculated to be 7.41 ± 0.19. For system suitability testing, a retention factor range of 7.0–7.8 is suggested as all the values observed throughout the entire recovery study fall within this range. Peak symmetry was determined on the basis of the tailing factor at 5% peak height. The citrate peak is fairly symmetrical as indicated by the tailing factor ($\pm$SD, $n = 10$) of 1.08 ± 0.08. A maximum tailing factor of 1.30 is suggested as this will include all the tailing factors observed in the recovery studies. The repeatability, expressed as %RSD, was observed to be about 1%. This is well below the general USP repeatability requirement of 2%.

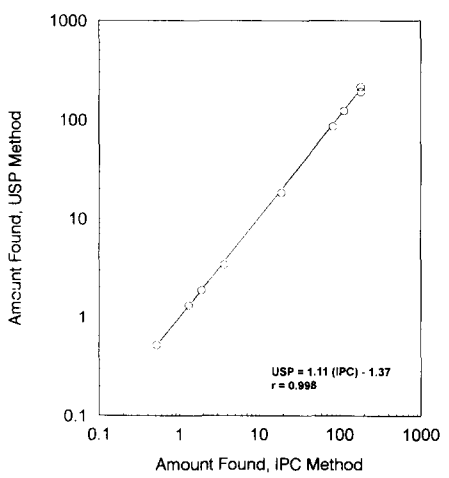

Fig. 3. Relationship between assay values obtained by USP and by the developed IPC method.

3.4. Comparisons with USP methods: assay of commercial products

A number of the commercially available products were assayed for total citrate by the developed method. The liquid chromatograms of these commercial products showed only the citrate peak and were similar to the liquid chromatogram of the spiked matrix solution (Fig. 2). These products were also assayed for total citrate content by their respective USP methods. The colorimetric method used by the USP [3] was followed for determination of total citrate in products 1, 6, 7, 8, and 9, with one exception: a 1 cm cell was used instead of a 2.5 cm cell recommended by the USP.

Products 2–5 were assayed for total citrate by their respective USP methods [1]. The assay results obtained are listed in Table 8. Inspection of the results for products 3–5 shows that the compendial results were higher than those obtained by the IPC method, particularly for product 4. In addition, the USP results for products 3–5 gave a very wide variation of 100.7%–112.1% whereas the IPC results for the same products varied only in the range 95.0%–95.9%. These results show that the IPC method gave better reproducibility. This is not surprising because the USP method for determining total citrate involves a non-specific, classical cation-exchange column whereby the acidic eluate was titrated with 0.02 N sodium hydroxide to a phenolphthalein end point. It is quite possible that commercial product 4 contained ingredients that gave a positive error. Based on the results of the USP method, only products 3 and 5 met the USP potency requirements of 95%–105% of label claim. However, when assayed by the proposed IPC method, products 3, 4, and 5 met USP potency limits requirements. The USP method was further investigated by applying it to three synthetic mixtures containing potassium citrate and citric acid, and three mixtures containing sodium citrate and citric acid, in an aqueous sucrose–sorbitol–glycerol solution. The overall recovery $\pm$ RSD was $100.0 \pm 1.8\%$. This shows that the cation-exchange/titration USP method gave good results in the absence of interferences but may not be suitable for assaying *some* commercial products.

The paired Student's "t" test was used to compare the assay results of the commercial samples obtained by the developed IPC and USP methods as shown in Table 8. In spite of the results for products 3–5, and taking the results as a whole, there was no statistical difference ($p = 0.05$) in the assay values obtained by these two methods. A linear relationship is observed ($r = 0.998$), as shown in Fig. 3, when these two assay values for each of the samples were plotted against each other. This indicates that the developed IPC method can be used for the analysis of total citrate in all USP monographs requiring a citrate assay.

4. Conclusions

The proposed IPC method is simpler than the current compendial procedure. The results indicate that the developed IPC method is reliable for quantitation of total citrate in various pharmaceutical formulations. It is more specific compared with some of the compendial methods.

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