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Development and validation of an assay for citric acid/citrate and phosphate in pharmaceutical dosage forms using ion chromatography with suppressed conductivity detection

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Abstract

The paper describes the development and validation of a simple, rapid, accurate, and sensitive ion chromatographic procedure to assay total citrate (citric acid/citrate) and phosphate in nine dosage forms. The dosage forms chosen represent all dosage forms in USP27-NF22 for which the respective monographs require an assay for either citric acid/citrate or citric acid/citrate and phosphate. Citrate and phosphate were separated in <10 min by a hydroxide-selective column using anion-exchange chromatography with a 20 mM potassium hydroxide eluent and detected by suppressed conductivity. The method showed linear responses over the concentration ranges 0.2–100 μg ml^{-1} (r^2 > 0.9990) for citrate and 0.2–60 μg ml^{-1} (r^2 = 0.9999) for phosphate, with limits of quantitation (signal-to-noise (S/N) = 10) of 0.2 μg ml^{-1} for both analytes. The accuracy of the procedure, determined by spiked recovery measurements, was within 95–105%. The intraday and the interday precision were demonstrated by the relative standard deviations (R.S.D.) of <1 and <2%, respectively, for both analytes. The ruggedness was determined by a full factorial design using analyst, equipment, column lot, and eluent preparation procedure as variables. The results show an overall R.S.D. of <3% and that an electrolytically generated 20 mM KOH eluent produces assay results equivalent to a manually prepared 20 mM NaOH eluent.
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1. Introduction

Citric acid and inorganic citrates are common ingredients in pharmaceutical dosage forms. Citric acid is used in antacids and dentrifices due to the effervescent affect it produces when combined with carbonates or bicarbonates. Citric acid and inorganic citrates can also act as buffering agents and assist in the dispersion of suspensions to help maintain the stability of the active ingredients [1] and improve the effectiveness of antioxidants [2]. Citrate is widely used as a flavoring and stabilizing agent in pharmaceutical preparations. It is also used as an anticoagulant to preserve blood for transfusion and as an ingredient in rectal enemas [2].

Citric acid and citrate have been assayed by ion-exchange chromatography [3,4], ion-exclusion chromatography [5,6], and reversed-phase chromatography [7] in a wide range of sample matrices, including those of pharmaceutical and biological importance. The common detection method is indirect UV [2,8–10]; however, conductivity and refractive index detection have also been used. Because citrate does not absorb UV in the range typically used in chromatography (>200 nm), a mobile phase that contains a compound that has a UV-absorbing chromophore is required for indirect UV detection [10]. In most cases, the mobile phase consisted of an organic acid with a slightly acidic to alkaline pH. Proper

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adjustment of pH is critical because the retention time of citric acid can vary significantly based on the pH of the mobile phase [10]. Furthermore, ion-exclusion separations generally result in long retention times for citric acid unless an organic modifier is used [6].

IC with suppressed conductivity detection has been demonstrated to be the method of choice for the determination of anions, including citrate [11]. Aliphatic tricarboxylic acids, such as citrate, have a high affinity towards the stationary phase of an anion-exchange column. Thus, low ionic strength carbonate/bicarbonate buffer solutions are typically not suitable as eluents. However, when hydroxide eluents are used, citric acid is easily eluted from the column [12]. Previous columns have required concentrated hydroxide eluents to elute the strongly retained citrate from the column. For example, earlier reports using a "hydroxide-selective" IonPac® AS5 column required 75–100 mM NaOH to elute citrate [3,13]. Rapid advances in column technology for anion exchangers specifically designed for use with hydroxide eluents (i.e., "hydroxide-selective") have allowed the separation of strongly retained anions, such as citrate, at lower hydroxide concentrations and shorter retention times. The use of a hydroxide eluent also has the advantages of being readily available, capable of being electrolytically generated at the desired concentration, and having a post-suppression background signal of that of water to yield an exceptionally low background conductance and noise level, thus improving the limits of detection and quantitation. Furthermore, hydroxide eluent is less expensive than organic eluents and is convenient because the waste can be handled easily by neutralizing with a strong acid and disposing in the sink as per OSHA guidelines.

The United States Pharmacopeia—National Formulary (USP27-NF22) has 18 dosage form monographs that require assay for citric acid or inorganic citrate [14]. There are seven different procedures in the monographs including wet chemistry analysis, titration, colorimetry, ion-exchange chromatography, ion-exclusion chromatography, and reversed-phase chromatography, not considering the monograph specific variations (Table 1). Some of the procedures are simple, such as HPLC, but others involve multiple steps. For example, the citric acid assay for magnesium citrate oral solution (a liquid formulation for direct use) requires concentrating the solution followed by fractional precipitation under acidic and then under alkaline conditions, filtration, incineration in a platinum crucible to char the precipitates, and then dissolving in hydrochloric acid followed by back titration. Whereas, the assay for citric acid in magnesium citrate for oral solution (a solid formulation) involves cation-exchange chromatography to convert magnesium citrate to citric acid followed by titration with NaOH [14]. Some of the procedures prescribed in USP27-NF22 are time-consuming, labor-intensive, require extensive analyst training, and may involve significant errors.

This paper reports the development and validation of a simple, accurate, rapid, and robust IC procedure for the assay of citric acid/citrate in dosage forms using a hydroxide-selective anion-exchange column and suppressed conductivity detection. The procedure provides the option of using either an automatically (electrolytically) generated or manually prepared eluent (20 mM KOH or NaOH) that permits isocratic elution to separate citrate, phosphate, and other ions in less than 10 min. The results show that this procedure can replace all of the seven different procedures currently used for the same purpose in USP27-NF22. In addition, the procedure works equally well for the assay of phosphate in anticoagulant solutions (Table 1) permitting simultaneous determination of citrate and phosphate in the anticoagulant solutions. The procedure was evaluated for specificity, linearity, precision, accuracy, ruggedness, and limit of quantitation for phosphate and citrate as defined in the General Chapter ⟨1225⟩ Validation of Compendial Methods in US27-NF22 [15].

2. Experimental

2.1. Standards and reagents

All standards and samples were prepared with a point-of-use deionized water purification system (Labconco, Kansas City, MO) that produces water with a resistivity of at least 18 MΩ cm; the same water was used for eluent preparation and to supply the eluent generator. The official USP Citric Acid Reference Standard (Catalog #1134368, US Pharmacopeia, Rockville, MD) was used as the standard for citrate analysis. Monobasic sodium phosphate monohydrate ($NaH_2PO_4{\cdot}H_2O$) was used as the standard for the analysis of phosphate and was purchased from EM Science (Gibbstown, NJ). Calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$) was obtained from Fisher Scientific (Fairlawn, NJ) and anhydrous sodium acetate (NaOAc) was obtained from Fluka Chemical Company (Milwaukee, WI). Sodium chloride and sodium hydroxide (50%, w/w) were obtained from J.T. Baker (Phillipsburg, NJ). Magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$), sodium citrate dihydrate, and potassium chloride were obtained from Sigma–Aldrich (St. Louis, MO).

2.2. Dosage forms

The dosage forms used in this study are listed in Table 1. The anticoagulant solutions, A1 and A5, were purchased from Sigma–Aldrich and Baxter BioScience (Toronto, Ontario, Canada), respectively. Dosage forms A2, A7, and A9 were purchased from a local pharmacy (Rockville, MD). A6 and A8 were purchased from local grocery stores (Sunnyvale, CA). A mock-formulation of A4 was prepared in the laboratory based on its known composition [16] by dissolving 368 mg of $CaCl_2{\cdot}2H_2O$, 305 mg of $MgCl_2{\cdot}6H_2O$, 4020 mg of NaOAc, 784 mg of sodium citrate dihydrate, 746 mg KCl, and 4851 mg NaCl in water to make a 1-l solution.

Table 1
Identification of pharmaceutical formulations used in this study and current USP monograph procedures for citric acid/citrate and/or phosphate assay

ID	Official title	Active ingredients	Monograph procedure
A1	Anticoagulant citrate, phosphate, dextrose, adenine solution	Citric acid, sodium citrate, monobasic sodium phosphate monohydrate	*Citrate*: Incubation with pyridine and acetic anhydride at 31 °C for 33 min; absorbance measurement at 425 nm. *Phosphate*: Incubation with ammonium molybdate, hydroquinone, and sodium sulfite for 30 min; absorbance measurement at 660 nm
A2	Citric acid, magnesium oxide, sodium carbonate irrigation	Citric acid, sodium bicarbonate, magnesium oxide	Strong cation-exchange HPLC at 40 °C with RI detection
A3	Potassium citrate extended release tablets	Potassium citrate	Dissolution as per the procedure described here in Section 2.5 followed by the procedure for citrate described under A1
A4	Multiple electrolytes injection type 2	Sodium citrate, sodium citrate, potassium chloride, sodium acetate, calcium chloride, magnesium chloride	Strong cation-exchange HPLC at 60 °C with UV detection at 210 nm
A5	Anticoagulant citrate, phosphate, dextrose solution	Citric acid, sodium citrate, monobasic sodium phosphate monohydrate, dextrose	*Citrate*: Same as in CA1/PA1. *Phosphate*: Incubation with ammonium molybdate, sulfuric acid and 1,2,4-aminonaphtholsulfonic acid at 20–25 °C for 10 min, absorbance measurement at 660 nm
A6	Magnesium citrate, oral solution	Magnesium citrate, citric acid, sodium bicarbonate, sodium saccharin	Concentration, fractional precipitation, washing, incineration, dissolution of residues in pre-standardized hydrochloric acid and back titration
A7	Sodium citrate, citric acid oral solution	Citric acid, sodium citrate, sodium bicarbonate	Collect eluate from cation-exchange column, boil for 1 min, cool, titration with NaOH
A8	Sodium bicarbonate, citric acid effervescent tablets	Citric acid, sodium bicarbonate	Dissolution as per the procedure described here in Section 2.5 followed by the procedure described under A7 RP-HPLC with UV detection at 220 nm
A9	Oral rehydration solution	Citric acid, sodium citrate, sodium chloride, potassium chloride, dextrose	

2.3. Ion chromatography

The chromatography was performed using an ICS-2000 Reagent-Free™ Ion Chromatography (RFIC) system (Dionex Corporation, Sunnyvale, CA) equipped with an electrolytic eluent generator (EluGen® EGC-KOH cartridge), a continuously regenerated anion trap column (CR-ATC), a dual piston pump with vacuum degas capability, a six-port injection valve, a heated conductivity cell, and a column oven set at 30 °C. The eluent generator produced a 20 mM KOH eluent at a flow rate of 2 ml min^{-1}. For ruggedness studies, an ICS-2500 modular RFIC system was used. The equipment consisted of a GP50 gradient pump, an EG50 eluent generator, a CR-ATC, an AS50 thermal compartment (set at 30 °C), and an ED50A conductivity detector with a conductivity cell and a heated DS3 stabilizer. A 20 mM NaOH eluent was manually prepared by diluting 1.05 ml of 50% (w/w) NaOH to 1 l with water. Each system was equipped with an AS50 autosampler, an IonPac® AS11 analytical column (4 mm × 250 mm, Dionex Corporation) and an AG11 guard (4 mm × 50 mm) column. These column sets were from different production batches and were installed on each system during the ruggedness study. The sample injection volume was 10 μl throughout. The analytes in the effluents were detected after suppressed conductivity with an ASRS®-ULTRA II (4 mm) operating at a 100 mA current in the recycle mode. Chromeleon® 6.5 Chromatography Management Software was used for system control and data processing.

2.4. Standard preparation

A 250 mg portion of official USP Citric Acid Reference Standard was dried in an oven at 105 °C for 2 h immediately before use [17]. Stock standard solutions were prepared by dissolving 250 mg citric acid and 150 mg monobasic sodium phosphate monohydrate or 250 mg citric acid alone in 500 ml water. To prepare the working citrate/phosphate and citrate standards, the respective stock standards were appropriately diluted with water and 20 mM NaOH was added to each standard to a final concentration of 1 mM NaOH.

2.5. Sample preparation

The liquid formulations, A1, A2, A4–A7, and A9 were diluted with water, and an appropriate volume of 20 mM NaOH was added to each, to obtain solutions of appropriate concentrations of citrate or citrate and phosphate in 1 mM NaOH. The potassium citrate extended release tablets (A3) were prepared by weighing 20 tablets, to obtain the average weight per tablet, and a composite prepared by grinding the tablets into a fine powder. An amount containing about 100 mg citric acid (based on the label amount) was added to 300 ml of hot water (80 °C) and magnetically stirred for approximately 30 min while maintaining the temperature between 70 and 80 °C. The solution was allowed to cool and then quantitatively transferred to a 500 ml volumetric flask and diluted to volume with water. The solution was filtered, with the first 50 ml discarded, to obtain the A3 stock standard, which was diluted further with water and appropriate volumes of 20 mM NaOH were added to obtain solutions containing appropriate citrate concentrations in 1 mM NaOH. The effervescent tablets (A8) were prepared by weighing five tablets, to determine the average weight per tablet, and a composite prepared by grinding them into a fine powder. To prepare the A8 stock solution, an amount containing about 100 mg citric acid was transferred to 300 ml of water in a 500 ml volumetric flask and diluted to the mark. The solution was filtered, with the first 50 ml discarded, to obtain the A8 stock standard. The solution was diluted further as described for A3 to obtain solutions containing appropriate citrate concentrations in 1 mM NaOH. For each dosage form, the solutions were spiked with appropriate standard solutions (containing citrate or citrate and phosphate) for spiked recovery (accuracy) studies.

3. Results

3.1. Method development

To reduce the elution time of the target analytes, the eluent anion should have a high selectivity for the resin. Therefore, the IonPac® AS11, an anion-exchange column with a high selectivity towards hydroxide eluent, in combination with a low anion-exchange capacity of 45 μeq. per column, was chosen.

In preliminary experiments, mixtures of citric acid and monobasic sodium phosphate in the concentration range 5–100 $\mu g\,ml^{-1}$ each in solution containing 1 mM NaOH were analyzed using a 0–100 mM NaOH linear gradient at 1 and 2 $ml\,min^{-1}$. Both phosphate and citrate peaks were eluted from the IonPac® AS11 in 20–25 mM NaOH. Subsequent analysis by isocratic elution using 10, 20, 30, 40, and 60 mM NaOH at 2 $ml\,min^{-1}$ showed that elution by 20 mM NaOH is optimum since the elution is completed within 10 min, which is the typical run time for chromatographic procedures described in USP [14] and baseline resolution of the peaks (peak resolution >2) [19]. In addition, 20 mM NaOH also allow separation of other components present in the dosage forms (Table 1). Furthermore, an isocratic-elution based procedure is simple enough to include in public monographs. Based on peak responses, concentrations of 20 $\mu g\,ml^{-1}$ citric acid/citrate (total) and 12 $\mu g\,ml^{-1}$ monobasic sodium phosphate were chosen as targets.

Fig. 1a and b show typical chromatograms of citrate/phosphate standards and A1, respectively, with approximate retention times of 3.7 and 6.5 min for phosphate and citrate peaks, respectively. Similar results were also obtained with A5. Citrate peaks are also eluted in the similar locus in other samples, as determined by comparing with the citrate peak in the standard. Furthermore, A4 and A9 have additional peaks that are due to other anions present in the formulations (results not shown). The identity and quantitation of these peaks were not investigated. Table 2 summarizes the tailing factor and theoretical plates for the citrate and phosphate peaks. Table 2 also shows the resolution between the citrate and the phosphate peaks in A1 and

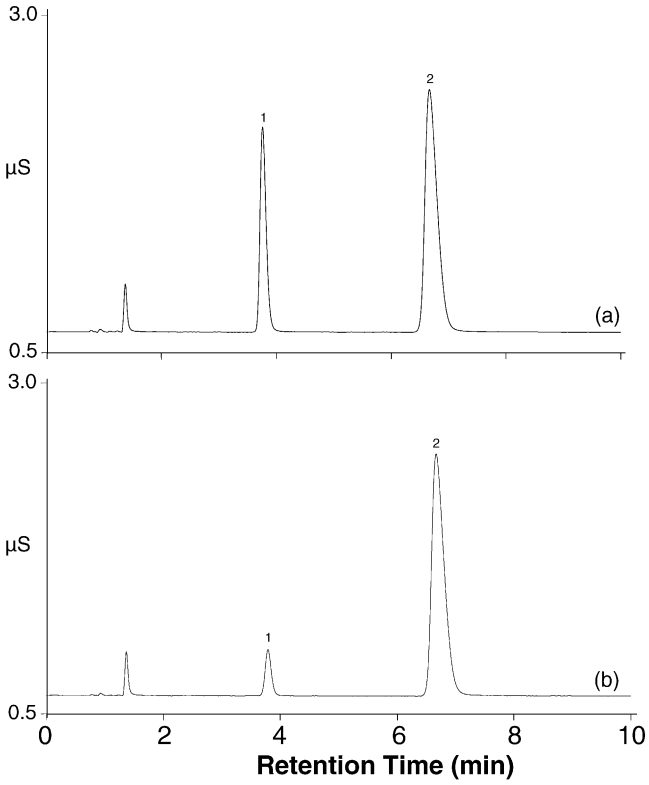

Fig. 1. Chromatograms of: (a) standard solution containing citrate (20.0 $\mu g\,ml^{-1}$ expressed as citric acid anhydrous) and phosphate (12.1 $\mu g\,ml^{-1}$ expressed as monobasic sodium phosphate monohydrate); and (b) the anticoagulant citrate, phosphate, dextrose, adenine solution (A1) diluted to contain 2.6 $\mu g\,ml^{-1}$ monobasic sodium phosphate monohydrate and 21.0 $\mu g\,ml^{-1}$ citric acid anhydrous (based on the label amount). Peaks 1 and 2 represent phosphate and citrate peaks, respectively. See Section 2 for details of run conditions.

Table 2
Peak parameters for citrate and phosphate peaks[a]

Dosage form	Tailing factor	Theoretical plate	*Resolution*
Citrate peak			
A1	1.52 ± 0.05	4558 ± 169	9.75 ± 1.93
A2	1.47 ± 0.05	4714 ± 128	
A3	1.53 ± 0.05	4611 ± 147	
A4	1.59 ± 0.06	4592 ± 171	22.4 ± 0.4
A5	1.55 ± 0.03	4487 ± 75	9.70 ± 0.11
A6	1.91 ± 0.03	3590 ± 58	
A7	1.49 ± 0.03	4676 ± 61	
A8	1.56 ± 0.03	4507 ± 72	
A9	1.69 ± 0.02	4130 ± 74	22.10 ± 0.03
Phosphate peak			
A1	1.39 ± 0.04	5645 ± 121	6.19 ± 0.28
A5	1.35 ± 0.01	5798 ± 80	6.10 ± 0.06

[a] Peak parameters, tailing factor, theoretical plate, and resolution are calculated as described in USP27-NF22 [19].

A5, and that between citrate and its adjacent peak for A4 and A9.

3.2. Method validation

Many of the pharmaceutical dosage forms for which the USP monographs require an assay for citric acid/citrate have similar compositions. For example, several of the formulations contain either citrate alone or citrate and carbonate anions. Thus, based on the composition of the dosage forms, A1–A9 was chosen as representatives of the 18 dosage forms that require citrate assay in USP monographs for the purpose of validation. Furthermore, A1 and A5 have similar composition except A5 does not contain adenine. Similarly, A2, A6, and A7 contain citrate and carbonate (or bicarbonate) anions. Full method validation of the procedure was performed as per General Chapter ⟨1225⟩ of USP27-NF22 [15], which includes specificity, linearity, range, limit of quantitation and detection, accuracy, intermediate (intraday) precision, precision (interday) and ruggedness with the dosage forms A1–A3, whereas limited validation (specificity, accuracy, and precision) was performed with the other dosage forms listed in Table 1.

3.2.1. Specificity

Fig. 1 shows that the retention times of both citrate and phosphate peaks in the standard are essentially the same as those of the respective peaks in the sample A1. Similar results were obtained for the citrate peak in all other samples and the phosphate peak in A5, indicating specificity of the procedure for both citrate and phosphate.

To evaluate the effect of the matrix on peak areas, dilution parallelism between the standards and samples was evaluated [18] for citrate and phosphate peaks in A1 and citrate peak in A2, A3 and A4 (Table 3). The results show similar slopes of the plots of log(response) versus log(dilution) for standards and samples, and low dilution bias, indicating the absence of significant matrix effect.

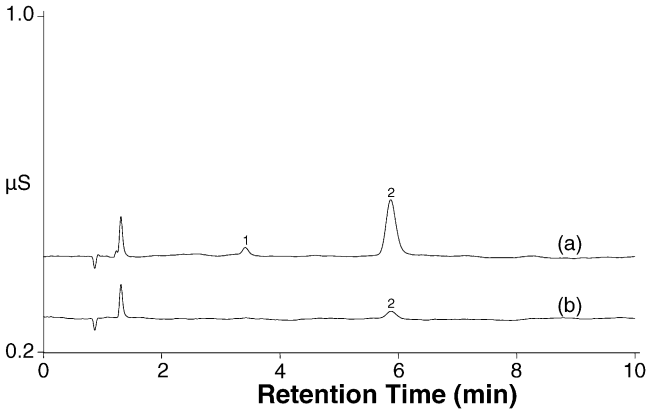

Fig. 2. Chromatograms of anticoagulant citrate, phosphate, dextrose, adenine solution diluted such that the concentrations of phosphate (a) and citrate (b) equal to their respective limits of quantitation (0.2 μg ml^{-1}).

3.2.2. Limit of quantitation, limit of detection, linearity, and range

To determine the limit of quantitation (LOQ), limit of detection (LOD), and linearity of the procedure for citrate and phosphate, standards were injected at nine concentration levels in the range of 0.1–100 μg ml^{-1} for citrate and 0.06–60 μg ml^{-1} for phosphate. The LOQ is defined as the minimum concentration at which the signal-to-noise (S/N) ratio is 10 [15]. The baseline noise was determined by measuring the peak-to-peak noise in a representative 1-min section of the baseline where no peaks are eluting. The baseline noise using the ASRS®-ULTRA II suppressor in the recycle mode was ∼2 nS min^{-1}. For phosphate and citrate, the LOQ was determined to be 0.2 μg ml^{-1}. The limit of detection was estimated to be 0.06 μg ml^{-1} (at S/N = 3) [15] for phosphate and citrate (by extrapolation). However, the determination of LOD is not required for an assay procedure as per General Chapter ⟨1225⟩ [15]. Fig. 2 shows chromatograms of solutions containing citrate and phosphate at their respective limits of quantitation (0.2 μg ml^{-1} each).

The peak areas were plotted against the respective concentrations in the range 0.1–100 μg ml^{-1} for citrate and 0.06–60 μg ml^{-1} for phosphate, and evaluated by a linear regression analysis for the determination of linearity. The regression coefficients (r^2) were 0.9990–0.9994 for citrate and 0.9999 for phosphate, with *y*-intercepts not significantly different from zero at a 95% confidence interval.

The results summarized in Table 3 show similar slopes of the plots of log(response) versus log(dilution) and low dilution bias between the standards and samples for both citrate and phosphate peaks within the range studied (15–22.5 μg ml^{-1} for citrate and 12–18 μg ml^{-1} for phosphate), indicating parallel response.

3.2.3. Accuracy

The accuracy of the procedure was studied by spiking the samples with known amounts of citric acid and monobasic sodium phosphate monohydrate, if present in the unspiked

Table 3
Dilution parallelism: slope of the plot of log of response vs. log of dilution and dilution bias for the dosage forms

	Phosphate peak in A1 in the presence of citrate		Citrate peak in A1 in the presence of phosphate		Citrate peak			
	Standard	PA1	Standard	CA1	Standard	A2	A3	A4
Slope[a]	−1.024	−1.056	−0.963	−0.960	−0.976	−0.975	−0.985	−0.989
Dilution bias (%)	1.64	3.78	2.59	2.80	1.72	1.72	1.03	0.75

[a] Average slope of plots of log(response) vs. log(dilution) from three independent runs.

Table 4
Accuracy (spike recovery) and precision

ID	Analyte	Accuracy (spike recovery, %)						Precision (%, R.S.D., $n = 6$)			
		1 μg ml^{-1} for citrate or 0.6 μg ml^{-1} for phosphate spiked			2.5 μg ml^{-1} for citrate, or 1.5 μg ml^{-1} for phosphate spiked			Intraday			Interday (Overall)
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	
A1	Citrate	104.3	102.5	103.2	99.8	103.7	99.0	0.18	0.37	0.60	0.49
	Phosphate	104.8	98.2	96.5	102.6	97.8	94.8	0.19	0.37	0.18	0.41
A5	Citrate				104.7	97.2	97.6	0.47	0.43	0.67	0.81
	Phosphate				100.7	98.7	95.5	0.32	0.33	0.27	0.51
A2	Citrate	102.0	97.3	102.8	104.5	105.1	101.8	0.16	0.45	0.55	1.54
A3	Citrate	97.4	100.6	97.5	103.9	98.9	96.0	0.73	0.32	0.44	1.28
A4	Citrate	95.3	97.3	98.0	102.0	100.8	103.2				
A6	Citrate				102.9	100.3	99.2	0.82	0.39	0.67	0.77
A7	Citrate				98.6	96.4	101.1	0.64	0.35	0.46	1.31
A8	Citrate				96.6	102.5	98.6	0.41	0.50	0.54	1.20
A9	Citrate				100.5	104.8	98.9	0.91	0.33	0.38	1.20

sample (Table 4). For samples spiked with 1 and 2.5 μg ml^{-1} citric acid, recoveries were in the range 97.4–104.3 and 96.4–105.1%, respectively. For samples containing phosphate, the recoveries were in the range 96.5–104.8 and 94.8–102.6% for 0.6 and 1.5 μg ml^{-1} phosphate spiked, respectively. Response of the target analyte remained linear for all spiked samples as shown by the excellent recoveries, which were all within 95–105%.

3.2.4. Precision

The precision of the procedure was determined by performing replicate injections of the dosage forms prepared at 100% of the target concentrations (20 and 12 μg ml^{-1} for citrate and phosphate, respectively) and determining the relative standard deviations (R.S.D.) of the areas of the citrate and phosphate peaks. Intraday precision was determined for an individual sample by injecting three independent preparations. The above experiments were repeated on three different days to determine the interday precision from the overall R.S.D. of the peak areas. The precision of the replicate injections of the samples within the same day was <1% for citrate and phosphate. The values of interday precision were only slightly greater with a maximum of 1.54% (Table 4).

3.2.5. Ruggedness

The ruggedness was studied using a full factorial design using analyst, instrument (including different batches of the column), and eluent as variables (Table 5). Each of the two analysts analyzed a standard solution containing 20 μg ml^{-1} citric acid and 12 μg ml^{-1} phosphate, and the dosage forms A1–A3 each at 100% target concentration (20 and 12 μg ml^{-1} for citrate and phosphate, respectively) using two instruments, including two different batches of column, and two different methods of eluent preparations (see Section 2 for details). Table 6 shows the overall procedure R.S.D. and the R.S.D. from two different eluent preparation methods for each dosage forms analyzed, indicating ruggedness of the procedure. Evaluation by single-factor ANOVA test shows the results obtained for each sample with electrolytically generated 20 mM KOH eluent and manually prepared 20 mM NaOH eluent are not significantly different at 95% confidence interval.

Table 5
Full factorial design for the ruggedness studies

Assay[a]	Chemist	Eluent[b]	Column[c]
1	A	Manual	E
2	A	Manual	F
3	A	EG	E
4	A	EG	F
5	B	Manual	E
6	B	Manual	F
7	B	EG	E
8	B	EG	F

[a] Samples CA1, PA1, A2, and A3 are used in each assay.
[b] Manual indicates that 20 mM NaOH eluents prepared manually; EG indicates that 20 mM KOH eluents prepared electrolytically by the eluent generator (EG) device.
[c] Column E was also used in all validation studies; column F was only used in the ruggedness study.

Table 6
Results of the ruggedness studies

ID	Overall precision (%, R.S.D.)[a]	Automatic eluent generation[b]		Manual eluent preparation[c]	
		Average ($\mu g\,ml^{-1}$)	R.S.D. (%)	Average ($\mu g\,ml^{-1}$)	R.S.D. (%)
A1 (phosphate)	2.17	17.25	0.51	17.95	0.39
A1 (citrate)	1.51	20.64	1.45	20.71	1.78
A2	2.39	17.90	1.79	17.82	3.17
A3	1.72	19.93	1.50	19.75	2.04

[a] Precision of assay #1–8 indicated in Table 5 for each formulation; all factors combined.
[b] Average and precision of results of assay # 3, 4, 7 and 8 in Table 5 for each formulation.
[c] Average and precision of results of assay #1, 2, 5 and 6 in Table 5 for each formulation.

3.2.6. Stability of samples and standards

Some carboxylic acids are known to be unstable in aqueous solutions [4]. Furthermore, it is critical for any analytical procedure to demonstrate that the standards and analytes are stable over the time required for analysis. In particular, the stabilities of the analytes were of interest in this study because all standards and samples prepared for injection contained 1 mM NaOH. A 20 $\mu g\,ml^{-1}$ citric acid standard and individual samples (A1–A3 and A6–A9) containing citric acid at 100% of the target concentration for analysis were stored at 20–25 °C for up to 5 days (120 h) and evaluated by injecting samples at 0, 6, 12, 24, 40, 100 and 120 h. The relative percent difference between the initial peak area (0 h) and the peak area at any time point, including 120 h, was within ±2% of the initial concentration. This variation is not significantly greater than the intraday precision (Table 4) of the procedure, indicating that the standard and the samples are stable up to 120 h under the conditions of the study.

3.3. System suitability

In addition to the tailing factor, theoretical plates, and resolution between the citrate and adjacent peaks (Table 2), system suitability was also evaluated using a standard solution that contains both citrate (20 $\mu g\,ml^{-1}$) and phosphate (12 $\mu g\,ml^{-1}$) and injecting it at the beginning, middle, and end of the injection sequence for a total of six injections on each of three separate days. For the citrate peak, the R.S.D. of the peak areas was around 0.30% on each day and for phosphate the R.S.D. was 0.28–0.49%.

3.4. Assay results for the pharmaceutical formulations

The dosages forms A1–A9 were assayed for citric acid and A1 and A5 were also assayed for phosphate over three consecutive days using independently prepared standards and samples. The results are summarized in Table 7. For each sample, the R.S.D. of the results were ≤2%. In most cases, the experimental values were very close to the label amounts and within the limits stipulated by their respective USP27-NF22 monographs. However, the results of phosphate assay for A1 and that of the citrate assay for A9 were more than 10% off from the label amounts. The procedures used to determine the label values are based on current USP procedures that are different from the procedure presented in this paper. The difference in the formulation label values and the experimental values may be due to difference in procedure.

Table 7
Comparison of the results obtained by the current method with the label amount

ID	Analyte	Label amounts ($mg\,ml^{-1}$)	Experimental results ($mg\,ml^{-1}$, average[a] ± S.D.)
A1	Citrate	20.17	21.18 ± 0.10
	Phosphate	2.22	2.81 ± 0.01
A5	Citrate	20.17	20.79 ± 0.23
	Phosphate	2.22	2.20 ± 0.02
A2	Citrate	29.6	29.9 ± 0.4
A3	Citrate	10 meq.	10.3 ± 0.2 meq.
A4	Citrate	0.513	0.517 ± 0.003
A6	Citrate	Not less than 75.9	86.9 ± 1.8
A7	Citrate	126.4	128.3 ± 1.6
A8	Citrate	1000 mg/tablet	1044.7 ± 21.5 mg/tablet
A9	Citrate	1.92	2.55 ± 0.05

[a] Average and standard deviation of three independent determinations.

4. Discussion

Chalgeri and Tan [8] described an ion chromatographic procedure for citrate assay of many pharmaceutical dosage forms using indirect UV detection. The procedure used trimesic acid as the UV-absorbing compound in the mobile phase to detect citrate at 280 nm as a negative peak. The lack of sensitivity of photometric detection requires significantly higher load to obtain an adequate citrate peak. Chalgeri and Tan reported an LOQ of 260 ng citrate compared to a 2 ng LOQ reported here. Similarly, the linear range in the current study is 2 ng to 1 μg citrate compared to the linear range of 1–12 μg reported by Chalgeri and Tan. Although the precision and accuracy reported by Chalgeri and Tan are similar to those obtained by the current report, the previous authors determined the parameters with 6 and 12 μg citrate injected whereas the current study injected only 200 ng citrate. The lower injection amount is important to: (1) significantly reduce or eliminate matrix effect; (2) prevent any potential of sample overloading effects that are possible due to the relatively low column capacity; (3) increase peak resolution; and (4) decrease peak tailing, as a whole improving the quality of

the chromatographic profile. In addition, the IC procedure described here can also quantitate phosphate with similar accuracy and precision, and has the potential of quantitating many other anions commonly present in pharmaceutical dosage forms (e.g., chloride, acetate, sulfate).

5. Conclusion

The development of a simple, rapid, accurate, precise, and sensitive procedure for the assay of citric acid and phosphate in pharmaceutical dosage forms using a low capacity hydroxide-selective anion-exchange column with suppressed conductivity detection was reported. The validation of the procedure is in compliance with the current USP requirements [14]. The procedure can replace seven different procedures described in the monographs of the dosage forms in USP27-NF22 to assay citric acid, inorganic citrate, and phosphate.

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