Performance Characteristics of an Ion Chromatographic Method for the Quantitation of Citrate and Phosphate in Pharmaceutical Solutions

Dennis Jenke[1,*], Salma Sadain[1], Karen Nunez[1], and **Frances Byrne[2]**

[1]Technology Resources Division and [2]Medication Delivery Division, Baxter Healthcare Corporation, Route 120 and Wilson Road, Round Lake, IL 60073

Abstract

The performance of an ion chromatographic method for measuring citrate and phosphate in pharmaceutical solutions is evaluated. Performance characteristics examined include accuracy, precision, specificity, response linearity, robustness, and the ability to meet system suitability criteria. In general, the method is found to be robust within reasonable deviations from its specified operating conditions. Analytical accuracy is typically 100 ± 3%, and short-term precision is not more than 1.5% relative standard deviation. The instrument response is linear over a range of 50% to 150% of the standard preparation target concentrations (12 mg/L for phosphate and 20 mg/L for citrate), and the results obtained using a single-point standard versus a calibration curve are essentially equivalent. A small analytical bias is observed and ascribed to the relative purity of the differing salts, used as raw materials in tested finished products and as reference standards in the analytical method. The assay is specific in that no phosphate or citrate peaks are observed in a variety of method-related solutions and matrix blanks (with and without autoclaving). The assay with manual preparation of the eluents is sensitive to the composition of the eluent in the sense that the eluent must be effectively degassed and protected from CO_2 ingress during use. In order for the assay to perform effectively, extensive system equilibration and conditioning is required. However, a properly conditioned and equilibrated system can be used to test a number of samples via chromatographic runs that include many (> 50) injections.

Introduction

Citric acid and the inorganic salts of citric and phosphoric acids are common ingredients in pharmaceutical solutions. Chromatographic methods, coupling ionic separation mechanisms with a variety of detection methods, have been proposed for their quantitation in such products (1–4). An ion chromatography method using suppressed conductivity has been developed for pharmaceutical applications (5,6), and it has been adopted by the United States Pharmacopeia (USP) (7). Assays used for the in-process, release, or stability testing of pharmaceutical products must meet stringent performance expectations. The purpose of this study was to establish the performance characteristics of the adopted USP methodology.

*Author to whom correspondence should be addressed: email dennis_jenke@baxter.com.

Experimental

Test method

The test method used was ion chromatography with suppressed conductivity detection. Separation was accomplished with a Dionex (Sunnyvale, CA) IonPac AS-11 anion separator column (250 × 4.6 mm) and an IonPac AG-11 guard column (50 × 4.6 mm). The eluent was 20mM sodium hydroxide at 2 mL/min. An anion trap column (IonPac ATC-3, 24 × 9 mm) was used to scavenge contaminating ions from the mobile phase. Suppression was accomplished with a Dionex ASRS-Ultra suppressor, operated in the autosuppression recycle mode. The sample size was 10 µL, and the separation was performed at ambient temperature. The chromatography was performed with a Dionex DX500 ion chromatograph with an AS50 autosampler, an AS50 thermal column compartment, a GP50 gradient pump, and a Waters (Milford, MA) Model 431 conductivity detector.

Test samples

Method performance was assessed for two general types of pharmaceutical solutions: anticoagulant solutions and multiple electrolyte solutions. Two laboratory-generated samples (TA-1 and TA-2) were prepared using stock solutions of the individual ingredients (Table I), prepared by dissolution of the appropriate raw materials in distilled, deionized water. The laboratory-generated samples were prepared by the admixture and dilution of the stock solutions, and the compositions are summarized in Table II. The composition of these samples was chosen to reflect typical commercial products, and they were prepared so that the level of the potential interferents (e.g., chloride, lactate, and bisulfite) were maximized relative to the analytes of interest (citrate and

phosphate). Specifically, the interferents were present at a level of at least 110% of their levels in typical commercial products. Three samples were prepared for each solution type, and each one contained 80%, 100%, or 120% of their nominal levels of the analytes of interest. Appropriate control and blanks were also prepared.

The proposed USP procedure called for the dilution of test samples to target levels of 20 µg/mL citrate and 12 µg/mL phosphate. The dilutions for the TA-1 solutions for citrate were as follows: dilution 1, 5-mL sample to 100 mL, the diluent was water; dilution 2, 3 mL of dilution 1 to 100 mL with water (10 mL 10mM NaOH added). The dilution factor was 666.7.

The dilution for TA-1 solutions for phosphate were as follows: dilution 1, 5-mL sample to 100 mL, the diluent was water; dilution 2; 6 mL of dilution 1 to 50 mL with water (5 mL 10mM NaOH added). The dilution factor was 166.7.

The dilution for the TA-2 solutions for phosphate were as follows: 4-mL sample to 50 mL with water (5 mL 10mM NaOH added). The dilution factor was 12.5.

Materials

The raw materials used to prepare the test samples, listed in Table I, were USP or reagent grade, as appropriate. The citric acid used to prepare the citrate standard was USP reference standard, Lot FIB092. Additional reagents and chemicals used in the analytical procedures (e.g., the mobile phase) were of analytical grade.

Results and Discussion

Linearity

The method, as described in the USP, called for the use of a

Table I. Stock Solution Preparation

Reagent	Weight (g)	Final volume (mL)	Conc. (mg/mL)	Conc. as citrate or phosphate (mg/mL)
A. Stock solutions for the individual analytes				
Citric acid, anhydrous USP	4.36	100*	43.6	42.9
Sodium citrate, dihydrate USP	13.07	100*	130.7	84.04
Monobasic sodium phosphate, monohydrate USP	2.8	100*	28	19.13
Potassium phosphate, monobasic NF	0.202	100*	2.02	1.41
B. Exipient solution #1 (anticoagulant solution, TA-1).				
Dextrose, anhydrous USP	20.43	500†	40.86	N/A
Adenine	0.33		0.66	N/A
C. Exipient solution #2 (multiple electrolyte solution, TA-2).				
Dextrose, anhydrous USP	100.97	500†	201.94	N/A
Magnesium chloride	0.34		0.68	N/A
Sodium lactate solution	2.84		5.68	N/A
Sodium chloride, USP	0.27		0.54	N/A
Potassium chloride, USP	1.54		3.08	N/A
Sodium bisulfite	0.23		0.46	N/A

* Each material individually prepared.
† All individual materials combined to produce a single common solution.

Table II. Test Solution Preparation

	Volume of stock solution (Table I) used (mL)							Concentration (mg/mL)	
Sample ID	Citric acid	Na citrate	K phosphate	Na phosphate	Excipient # 1	Excipient # 2	Final volume (mL)	Citrate	Phosphate
Control 1*	10.0	10.0	0.0	10.0	0.0	0.0	100	12.65	1.91
Control 2*	0.0	0.0	10.0	0.0	0.0	0.0	100	0.0	0.14
Blank 1*	0.0	0.0	0.0	0.0	50.0	0.0	100	0.0	0.0
Blank 2*	0.0	0.0	0.0	0.0	0.0	50.0	100	0.0	0.0
TA-1-80	8.0	8.0	0.0	8.0	50.0	0.0	100	10.12	1.53
TA-1-100	10.0	10.0	0.0	10.0	50.0	0.0	100	12.65	1.91
TA-1-120	12.0	12.0	0.0	12.0	50.0	0.0	100	15.18	2.30
TA-2-80	0.0	0.0	8.0	0.0	0.0	50.0	100	0.0	0.11
TA-2-100	0.0	0.0	10.0	0.0	0.0	50.0	100	0.0	0.14
TA-2-120	0.0	0.0	12.0	0.0	0.0	50.0	100	0.0	0.17

* The controls contain the analytes of interest, but not the sample matrix. The blanks contain the sample matrix, but not the analytes of interest.

single-point standard containing approximately 20 µg/mL citrate and 12 µg/mL phosphate. The standard linearity was assessed over the range of approximately 100% ± 50% of these target analyte levels. Specifically, standard linearity was determined by triplicate injections of standards containing 10, 15, 20, 25, and 30 µg/mL citrate and 6, 9, 12, 15, and 18 µg/mL phosphate. Calibration data, resulting from a linear regression analysis of the peak response versus the analyte concentration, are contained in Table III. In general, the analyte response (area) was linearly correlated with analyte concentrations in water-based standards.

Test solutions were prepared at 80%, 100%, and 120% of the nominal analyte concentration. Sample linearity was determined via a comparison of the theoretical or preparation concentration of a sameple with the experimental concentration obtained from the analysis of the sample (single-point standardization used per the USP method). The results of such a comparison are shown in Table IV. The near unit slopes and near zero intercepts of the phosphate calibration curves are indicative of an assay with minimal bias. Alternatively, the results obtained for the citrate curve, which indicated significant deviations from the unit slope and zero intercept, reflected a small analytical bias, with the experimentally measured amounts being somewhat lower than the preparation levels. This phenomenon is discussed in greater detail in the Robustness section.

Accuracy and precision

As previously reported (6), the citrate–phosphate method had an accuracy in the range of 95% to 105% recovery, based on the labeling of the products tested. Such performance was not adequate for pharmaceutical applications, including in-process and stability testing. This was true because the product tolerances for such testing were similar to or more stringent than 95% to 105%. Thus, this study focused on the analysis of simulated products containing known amounts of the analytes of interest, as opposed to the analysis of actual products whose composition could only be approximated.

The accuracy and precision data are contained in Tables V and VI. In general, accuracy, based on the recovery (%) versus a control sample, was in the recovery range of 97–103%. Factors influencing accuracy are discussed in the Robustness section. Precision, for either three or six replicate sample dilutions and analyses, was good and was typically less than 1.5% relative standard deviation (RSD). This type of performance is consistent with the requirements of pharmaceutical applications. Typical chromatograms are shown in Figures 1 and 2.

Table III. Standard Linearity

Parameter	Result, citrate*	Result, phosphate†
Slope	62.491	60.560
Response factor‡	59.865	62.416
Intercept	–1.173	0.1838
% Intercept§	–5.8	1.5
Coefficient of determination (r^2)	0.9995	0.9994
Residual sum of the squares	0.1298	0.0585

* Over the concentration range of 10–30 µg/mL.
† Over the concentration range of 6–18 µg/mL.
‡ Response factor = concentration/response for the middle concentration standard at the USP single-point targets.
§ Determined versus the mean experimental response for the 12 µg/mL phosphate or 20 µg/mL citrate standard, as appropriate.

Specificity

To address specificity, matrix blanks were prepared and analyzed. These matrix blanks contained all the formulation components except for those that contained the analytes of interest. Analysis of such samples produced chromatograms with no discernible peaks in the elution region of citrate and phosphate, thus confirming specificity.

Robustness

Mobile phase preparation

The USP assay (7) could be implemented in several manners, differing primarily in the way the mobile phase (eluent) was generated. Specifically, the eluent can be generated in one of four ways: (*i*) preparation of the 20mM NaOH as previously described (single pump operation); (*ii*) preparation of 20mM NaOH, division into two portions, and 1:1 mixing of portions with a binary gradient pump (this was done to increase the amount of the eluent that was available for extended analytical runs); (*iii*) gradient mixing of two solutions, specifically water (0mM NaOH) and 100mM NaOH, in a proportion of 4 parts water to 1 part 100mM NaOH (minimizing the handling of NaOH to reduce CO_2 sorption and increase the amount of available eluent); and (*iv*) the use of commercially available eluent generators. Though the fourth option was recommended by an instrument vendor (5) because of ease of use and more reproducible gradient generation, this option could not be implemented in this study, as an eluent generator was not available. The first, second, and third options were all used at one point in this study, and they produced roughly comparable results. All of the quantitative data reported herein was generated using the second eluent approach.

Degassing the eluent to remove CO_2 was an important success factor because carbonate could have poisoned the column and impaired the chromatographic performance. Degassing could be accomplished either by sparging with an inert gas (such as He)

Table IV. Sample Linearity

	Sample TA-1, both citrate and phosphate		Sample TA-2, phosphate only
Parameter	Result, citrate*	Result, phosphate†	Result, phosphate†
Slope	0.9245	1.096	1.022
Intercept	1.1828	0.0830	0.0675
% intercept‡	6.44	0.64	0.57
Coefficient of determination (r^2)	0.9928	0.9904	0.9993
Residual sum of the squares	0.5104	0.3748	0.0223

* Over the concentration range of 80% to 120% of dilution target, 20 µg/mL.
† Over the concentration range of 80% to 120% of dilution target, 12 µg/mL.
‡ Determined versus the mean experimental concentration for sample TA-x-100.

or by the use of a vacuum coupled with sonication. Both options were experimented with during this study and produced roughly comparable results. All of the quantitative data reported herein was generated using an eluent sparging with He. The eluent was placed under a helium atmosphere cover after sparging.

Salts effect

The calibration standards prescribed by the USP (7) are citric acid for citrate and monobasic sodium phosphate monohydrate for phosphate. The reagents used to prepare the test solutions included sodium citrate dihydrate, citric acid monohydrate, monobasic sodium phosphate monohydrate, and potassium phosphate monobasic. It was possible, therefore, that assay biases could be generated because of slightly different purities of these various reagents. To investigate this possibility, standards using various salts were prepared and used for the analyte quantitation.

In terms of the standards themselves, the difference (%) in the response factor (the ratio of response to analyte concentration) for the citrate standards, with citric acid versus sodium citrate was 1.22%. The difference (%) for the phosphate standards, with potassium phosphate versus sodium phosphate, was 3.03%. These differences reflected the level of bias expected when comparing a sample containing one salt type with a standard containing a different salt type. This expectation was confirmed by determining the difference (%) in recovery obtained for samples using standards prepared from the two different salts. For phosphate, the difference (%) in recovery, standard based on potassium phosphate versus a standard based on sodium phosphate, were as follows: control 1, 3.12%; TA-1-100, 3.05%; control 2, 2.94%; and TA-2-100, 3.09%. These values were all similar to the 3.03% difference in standard response factors. For citrate, the differences (%) in recovery, with a standard based on citric acid versus a standard based on sodium citrate, were as follows: control 1, 1.25%; and TA-1-100, 1.22%. These values were all similar to the 1.22% difference in standard response factors.

This type of bias could be encountered when samples prepared from one salt raw material were tested using a reference standard of the same salt. This was true as it is typically the case that the salts used as the raw material and the reference standards are not from the same lot or batch and, thus, can have different purities. In the case of the USP assay, this situation was exacerbated by the fact that not only are the raw materials and reference materials from different lots of the same salt, but they can also be different salts (e.g., citric acid vs sodium citrate). If the relative purity of the raw material and reference material was not known, then some analytical bias was unavoidable when finished products were tested. It was noted that one means of obtaining such a rel-

Table V. Accuracy and Precision Data For Test Solutions Containing Both Phosphate and Citrate

		Theoretical conc. (mg/mL)		Experimental conc. (mg/mL)		Recovery (%)	
Test sample	Replicate	Citrate	Phosphate	Citrate	Phosphate	Citrate	Phosphate
Control-1	1	18.49*	11.57*	18.11	12.65	N/A	N/A
	2			18.48	12.80		
	3			18.21	12.58		
	Mean			**18.27**	**12.68**		
TA-1-80	1	14.61†	10.14†	14.62	10.08	100.0	99.4
	2			14.65	10.05	100.3	99.1
	3			15.02	10.10	102.7	99.6
	Mean			**14.76**	**10.07**	**101.0**	**99.3**
	% RSD			**1.50**	**0.26**	**N/A**	**N/A**
	95% CI			**13.81–15.71**	**9.96–10.19**		
TA-1-100	1	18.27†	12.68†	18.26	12.92	100.0	101.9
	2			18.60	12.93	101.8	102.0
	3			18.33	12.91	100.3	101.8
	4			18.16	12.82	99.4	101.1
	5			18.21	12.88	99.7	101.6
	6			18.72	13.10	102.5	103.3
	Mean			**18.38**	**12.93**	**100.6**	**102.0**
	% RSD			**1.25**	**0.72**	**N/A**	**N/A**
	95% CI			**17.79–18.97**	**12.69–13.17**		
TA-1-120	1	21.92†	15.21†	21.71	15.24	99.0	100.2
	2			21.47	15.20	97.9	99.9
	3			21.63	15.00	98.7	98.6
	Mean			**21.60**	**15.15**	**98.5**	**99.6**
	% RSD			**0.57**	**0.86**	**N/A**	**N/A**
	95% CI			**21.07–22.13**	**14.59–15.71**		

* Determined based on the weights and volumes used to prepare the solution.
† Determined based on the mean experimental result obtained for the Control-1 Solution adjusted for dilution.

ative potency was to assay the raw material versus the reference standard using the developed ion chromatography (IC) method.

Effect of NaOH concentration in the diluted sample

The effective elution of citrate and phosphate required that these analytes be fully deprotonated. This was accomplished in the analytical separation through the use of a 20mM NaOH eluent. Maintaining the proper analyte speciation during analysis was facilitated if the sample matrix closely matched the mobile phase composition. This was one reason why the USP method called for the dilution of samples in a 1mM NaOH matrix. Because the IC assay may be used to test a number of products, it was possible that the dilution process may not have adequate reproducibility to produce an appropriate sample matrix. Thus, the impact that changing the levels of NaOH in diluted samples has on accuracy was assessed. For example, the "typical" dilution of sample TA-1 for citrate analysis involved the addition of 10 mL of 10M NaOH to a sample before it was diluted to a final volume of 100 mL. Duplicate dilutions of TA-1-100 were performed in this manner, but they were also performed with either 5 or 15 mL of 10mM NaOH. Additionally, it was noted that the citrate dilution was a two-step dilution with no NaOH being added to the first dilution. Thus, two additional replications were performed by adding enough NaOH to the first dilution to make its NaOH level 1mM. This sample was subsequently diluted by adding 10 mL of 10mM NaOH to the second dilution.

A similar strategy was followed for the dilution of TA-1-100 during the phosphate analysis, which was also a two-step dilution. Because the dilution of TA-2-100 for the phosphate analysis was a one-step dilution, nominally requiring the addition of 5 mL of 10mM NaOH to the diluted solution of 50 mL final volume, the effect of the NaOH level was assessed by performing duplicate dilutions with 2, 5, or 10 mL of 10mM NaOH being added. Thus, for a single TA-1-100 sample, four different dilution strategies were used, and each strategy was performed in duplicate (total number of diluted samples = 8). For sample TA-2-100, three different dilution strategies were used and each strategy was performed in duplicate (total number of diluted samples = 6).

If the level of NaOH in the diluted samples effected the analytical performance, then the agreement between the responses obtained for the replicate dilutions performed across all preparation strategies would have been poorer than the agreement between replicate dilutions using a single dilution strategy. For the eight replicates of TA-1-100 made across the four dilution strategies investigated, the % RSD for phosphate was 0.65% and was 0.89% for citrate. For the six replicates of TA-2-100 made across three dilution strategies investigated, the % RSD for phosphate was 0.26%. As these precisions were within the performance expectations for the method and were comparable with the precisions obtained with replicate dilutions using the specified dilution strategy (see, for example, Tables V and VII), it was concluded that the performance of the assay was not materially effected by small changes (± 50%) in the level of NaOH in diluted samples.

Table VI. Accuracy and Precision Data For Test Solutions Containing Phosphate Only

Test solution	Replicate	Theoretical conc. (mg/mL) phosphate	Experimental conc. (mg/mL) phosphate	Recovery (%) phosphate
Control-2	1	11.29*	12.02	N/A
	2		11.91	
	3		11.93	
	Mean		**11.95**	
TA-2-80	1	9.563†	9.501	99.4
	2		9.529	99.6
	3		9.497	99.3
	Mean		**9.509**	**99.4**
	% RSD		**0.18**	**N/A**
	95% CI		**9.435–9.583**	
TA-2-100	1	11.95†	11.92	99.7
	2		11.97	100.2
	3		11.91	99.6
	4		11.91	99.6
	5		11.85	99.2
	6		11.91	99.7
	Mean		**11.91**	**99.7**
	% RSD		**0.32**	**N/A**
	95% CI		**11.82–12.01**	
TA-2-120	1	14.34†	14.16	98.7
	2		14.29	99.6
	3		14.27	99.5
	Mean		**14.24**	**99.3**
	% RSD		**0.50**	**N/A**
	95% CI		**13.93–14.55**	

* Determined based on the weights and volumes used to prepare the solution.
† Determined based on the mean experimental result obtained for the Control-2 Solution adjusted for dilution.

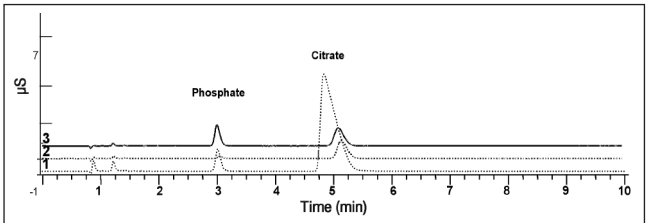

Figure 1. Representative chromatograms of an anticoagulant solution containing both citrate and phosphate (e.g., TA-1). Chromatogram 1, TA-1-100 diluted by 125 for phosphate; chromatogram 2, TA-1-100 diluted by 1000 for citrate; chromatogram 3, standard containing approximately 12 mg/L phosphate and 20 mg/L citrate.

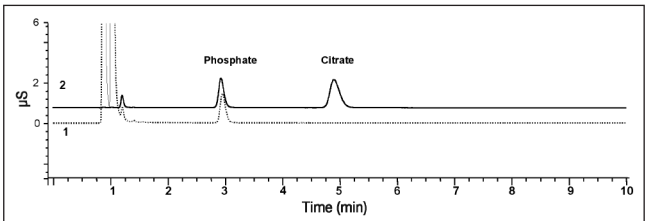

Figure 2. Representative chromatogram for a multiple electrolyte solution containing phosphate only (e.g., TA-2). Chromatogram 1, TA-2-100 diluted by 12.5; and chromatogram 2, standard containing approximately 12 mg/L phosphate and 20 mg/L citrate.

Table VII. Effect of Single Point Standard Versus Calibration Curve on Accuracy

Analyte level (%)	Mean calculated concentration (mg/L)					
	Sample TA-1, with both citrate and phosphate				Sample TA-2, phosphate only	
	Phosphate		Citrate		Phosphate	
	1 point	Curve	1 point	Curve	1 point	Curve
80	10.07	9.89	14.76	14.12	9.51	9.47
100	12.93	12.75	18.38	18.18	11.91	11.91
120	15.15	14.98	21.60	21.78	14.24	14.27
Control	12.68	12.50	18.27	18.05	11.95	11.95

Table VIII. Assessment of Quantitation Using Peak Height versus Peak Area

Analyte level (%)	Mean % Recovery					
	Sample TA-1, with both citrate and phosphate				Sample TA-2, phosphate only	
	Phosphate		Citrate		Phosphate	
	Peak area	Peak height	Peak area	Peak height	Peak area	Peak height
Recovery, 80%	99.3	99.9	101.0	102.0	99.4	100.4
% RSD	0.26	0.30	1.50	1.77	0.18	0.05
Recovery, 100%	102.0	102.2	100.6	100.5	99.7	100.1
% RSD	0.72	0.60	1.25	1.27	0.32	0.43
Recovery, 120%	99.6	99.2	98.5	97.1	99.3	98.9
% RSD	0.86	0.83	0.57	0.55	0.50	0.38

Single-point versus three point standardization

The USP method is based on a single point standard and the dilution of the sample to the same level as the standard. If the actual analyte concentration of the sample is unknown at the time of analysis, dilution to a target of the single-point standard may produce a diluted sample that is actually more or less concentrated than the standard. If the calibration curve was well defined, this minor difference in sample versus standard concentration should have no impact on the analytical accuracy. If the curve is either non-linear or has a significant non-zero intercept, then the difference in concentrations may produce a calibration-derived analytical bias.

To investigate this possibility, several chromatographic runs included not only the single-point standard, but also additional calibration standards at several analyte levels. The results of such an assessment are summarized in Table VII. It is clear from this Table that the two different calibration strategies produced somewhat different concentration results. However, the differences in concentrations, one-point versus the calibration curve, were small and not as statistically significant as the 95% confidence level for the paired results.

Peak height versus peak area

The analyte quantitation, using both peak height and peak area, was examined from the perspective of accuracy and precision. A comparison with the results obtained from a typical analytical run is summarized in Table VIII. In general, both means of quantitation produced essentially equivalent results.

System suitability

System suitability results for all runs are summarized in Table IX. It is noteworthy that all runs met the system suitability requirements, especially considering that these runs were performed using two different analytical columns. A key practical success factor in passing system suitability (and thus producing

Table IX. System Suitability

Parameter	Requirement	Analyte	Performance										
			Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	Run 10	Run 11
T*	T NMT† 2.0	Citrate	1.18	1.32	1.00	1.10	1.25	1.25	1.05	1.18	1.20	1.33	1.23
		Phosphate	1.17	1.15	1.11	1.04	1.19	1.17	1.11	1.08	1.23	1.35	1.05
Precision, short term*,‡	% RSD NMT 1.5%	Citrate	0.43	1.16	0.73	0.17	0.83	0.68	0.31	0.88	0.12	0.35	0.32
		Phosphate	0.38	1.20	0.61	0.27	0.74	0.42	0.29	0.59	0.12	0.22	0.90
Precision, total run§,**	% RSD NMT 2.0%	Citrate	1.00	0.74	1.45	0.82	0.93	1.00	0.56	1.25	0.57	1.98	0.82
		Phosphate	0.67	0.61	1.22	0.73	0.72	0.74	0.44	1.07	0.55	1.96	0.90
Standard prep§,††	Response factor Difference NMT 2.0%	Citrate	2.0	1.7	1.1	1.9	0.2	0.4	0.3	1.5	N/A‡‡	0.7	0.1
		Phosphate	1.9	0.4	1.4	1.9	0.9	0.5	0.7	1.6	N/A‡‡	1.5	1.1

* T = Tailing. System suitability per the USP.
† NMT = not more than
‡ Determined from six sequential injections of the standard.
§ System suitability per internal method requirements.
** Determined from all standard injections made throughout an analytical run (excluding conditioning injections).
†† Agreement between two separate weighings of standard.
‡‡ Not performed in this run.

valid data) was proper system equilibration. Extensive system equilibration, both in terms of extended periods of system equilibration under operating conditions with no injections and system conditioning with repetitive conditioning injections of the standard (10 or more as necessary), was essential to produce a response that was sufficiently stable to meet the precision-based system requirements.

Conclusion

In general, the method was found to be robust within reasonable deviations from its specified operating conditions. Though the method is somewhat labor intensive because of both the multiple dilutions required for some product codes and the extensive system conditioning required, it is generally accurate (accuracy 100 ± 3%) and precise (% RSD not more than 1.5%). The instrument response is linear over a range of 50% to 150% of the standard preparation target concentrations (12 mg/L for phosphate and 20 mg/L for citrate), and the results obtained using a single-point standard versus a calibration curve are essentially equivalent. An observed analytical bias associated with differing salts used as raw materials in tested finished products, and as reference standards in the analytical method, could be overcome by analysis of the raw materials via the test method to establish their potency relative to the reference standard.

The assay is specific in the sense that no phosphate or citrate peaks were observed in a variety of method-related solutions and matrix blanks (with and without autoclaving). The assay with manual preparation of the eluents is sensitive to the composition of the eluent in the sense that the eluent must be effectively degassed and protected from CO_2 ingress during use. In order for the assay to perform effectively, extensive system equilibration and conditioning is required. However, a properly conditioned and equilibrated system can be used to test a number of samples in chromatographic runs that consist of many (> 50) injections.

References

1. A.J. Holden, D. Littlejohn, and G.S. Fell. Determination of citrate in plasma protein solutions by UV-visible spectrophotometry and ion chromatography. *J. Pharm. Biomed. Anal.* **14:** 713–19 (1996).
2. S. Lu, X. Sun, C. Shi, and Y. Zhang. Determination of tricarboxylic acid cycle acids and other related substances in cultured mammalian cells by gradient ion-exchange chromatography with suppressed conductivity detection. *J. Chromatogr. A* **1012:** 161–68 (2003).
3. Q. Chen, S. Mou, K. Liu, Z. Yang, and Z. Ni. Separation and determination of four artificial sweeteners and citric acid by high performance anion exchange chromatography. *J. Chromatogr. A* **771:** 135–43 (1997).
4. D.R. Jenke. Quantitation of oxalate and citrate by ion chromatography with a buffered, strong acid eluent. *J. Chromatogr.* **437:** 231–37 (1988).
5. Dionex Corporation. Application Note 164 Assay for Citrate and Phosphate in Pharmaceutical Formulations using Ion Chromatography. Dionex Corporation, Sunnyvale, CA, 2004.
6. B.M. DeBorba, J.S. Rohrer, and L. Bhattacharyya. Development and validation of an assay for citric acid/citrate and phosphate in pharmaceutical dosage forms using ion chromatography with suppressed conductivity detection. *J. Pharm. Biomed. Anal.* **36:** 517–24 (2004).
7. The United States Pharmacopeia. "Assay for citric acid/citrate and phosphate". In *The United States Pharmacopeia*, USP29. United Sates Pharmacopeial Convention, Rockville, MD, 2006, pp. 2565.

Manuscript received March 17, 2006;
revision received August 16, 2006.