Faster and Improved Ease-of-Use Assays of Citrate and Phosphate in Pharmaceutical Formulations Using Ion Chromatography with Suppressed Conductivity

Hua Yang, Terri Christison, Linda Lopez and Jeff Rohrer, Thermo Fisher Scientific, Sunnyvale, CA, USA

Poster Note 72097

ABSTRACT

Purpose: To demonstrate an improved IC method for the assay of citrate and phosphate using a Thermo Scientific™ Dionex™ IonPac™ AS11-HC-4µm microbore column on a Thermo Scientific™ Dionex™ Integrion™ HPIC™ system.

Methods: Citrate and phosphate samples are dissolved in 1 mM NaOH, separated by ion chromatography (IC), and detected by suppressed conductivity detection (CD). Eluent is generated automatically using a Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge.

Results: The improved IC method for the assay of citrate and phosphate was precise (< 2% RSDs over 4 days) and accurate (95–106% recoveries). It increased sample throughput 2x by reducing the run time from 10 to 5 min. It also increased sensitivity with LOQs of 0.03 and 0.06 mg/L for phosphate and citrate, respectively, using 25% less sample than the previous method.

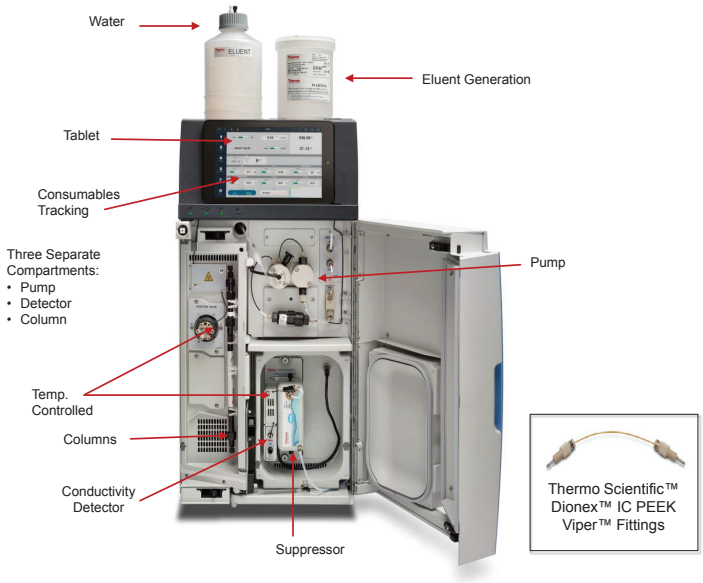
INTRODUCTION

Citric acid/citrate and phosphate are ingredients of many pharmaceutical formulations. As the chromatographic technique of choice for citrate determinations, ion chromatography (IC) with suppressed conductivity detection has been validated[1-2] and featured in United States Pharmacopeia (USP) General Chapter <345>, Assay for Citric acid/Citrate and Phosphate[3]. The method was first published in the official 2006 edition of the United States Pharmacopeia and National Formulary (USP 29–NF 24). In this method, citrate and phosphate are separated on a L61 column (a Dionex IonPac AS11 hydroxyl-selective anion exchange column), using 20 mM NaOH or KOH at a 2 mL/min flow rate. Both phosphate and citrate are eluted from the column and determined within 10 minutes.

A recently introduced compact IC system, the Dionex Integrion HPIC system, includes many advancements in IC instrument technology, such as high-pressure capabilities for Reagent-Free™ IC (RFIC™) (up to 5000 psi), column heater control, and many new features designed to increase customer ease-of-use.

Here, we update AN164 for citrate and phosphate determinations using the high-capacity Dionex IonPac AS11-HC-4µm column, with similar selectivity to L61, on the Dionex Integrion HPIC compact IC system. This method demonstrates reduced run times from 10 to 5 min. Following the guidelines outlined in USP General Chapter, Validation of Compendial Methods[4], the improved IC method is evaluated in terms of linearity, precision, accuracy, robustness, and limit of quantitation (LOQ).

Figure 1. Dionex Integrion HPIC System Configured for Conductivity Detection.



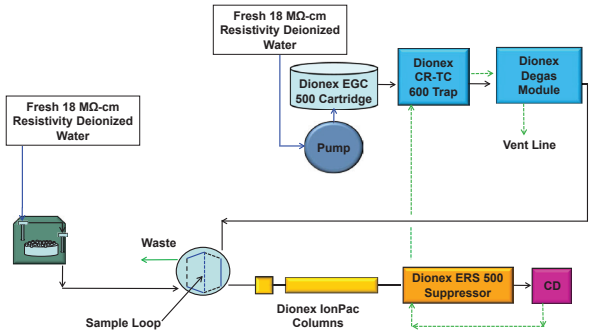
MATERIALS AND METHODS

Chromatography: See chromatograms for conditions.

Instrument: Dionex Integrion HPIC system with RFIC module (Figure 1). The flow diagram is shown in Figure 2.

Data Analysis: Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2 SR4

Figure 2. Flow Diagram for the Dionex Integrion HPIC System.



Sample Preparation

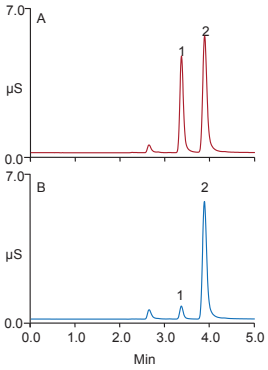
1000 mg/L stock standards were prepared by dissolving citric acid (USP, Catalog #1134368) or sodium dihydrogen phosphate monohydrate (EM Science) in 18 MΩ-cm resistivity or better deionized (DI) water. The 20 mM NaOH solution was prepared daily from sodium hydroxide solution, 50% (w/w), aqueous NaOH (Fisher Scientific). Calibration standards and samples were prepared by diluting stock standards or sample (Anticoagulant Citrate Phosphate Dextrose Solution (CPD) , Novateinbio Cat# NIBB-410) using an appropriate amount of the stock standard solutions, CPD sample, 20 mM NaOH and DI water.

Thermo SCIENTIFIC

RESULTS

The Dionex Integrion HPIC system is capable of running analyses at up to 5000 psi (6000 psi with manual eluent preparation). The Dionex IonPac AS11-HC-4µm columns have smaller (4.0 vs. 13 µm) and higher porosity (pore size 2000 Å vs. <10 Å) resin particles with the same functional groups as the L61 column. As a result, the Dionex IonPac AS11-HC-4µm column has chromatographic selectivity similar to that of L61, but with higher capacity and efficiency than L61 columns. The methods run on the L61, Dionex IonPac AS11-HC column can be easily transferred to the Dionex IonPac AS11-HC-4µm column with the benefit of increased peak resolution and improved separation. The microbore format (2 mm i.d.) provides reduced eluent consumption, which reduces operating costs. Figure 3 shows the separation of phosphate and citrate on the 2 mm Dionex IonPac AS11-HC-4µm column of (A) standard and (B) assay for citrate in an anticoagulant citrate, phosphate, dextrose, and adenine dosage form. Using an electrolytically generated hydroxide at 60 mM, phosphate and citrate were well separated in 5 min, saving 5 min from the method used in AN164. Additionally, the lower flow rate (0.35 mL/min over the previous 2 mL/min) extends the lifetime of the Dionex EGC 500 KOH cartridge.

Figure 3. Chromatograms of Phosphate and Citrate.



Columns: Dionex IonPac AG11-HC-4µm+ Dionex IonPac AS11-HC-4µm, 2 mm
Eluent: 60 mM KOH
Source: Dionex EGC-500 KOH cartridge, with Dionex CR-ATC 600 trap column, Dionex high pressure degasser
Flow Rate: 0.35 mL/min
Inj. Volume: 2.5 µL
Column Temp.: 35 °C
Detection: Suppressed conductivity, Dionex AERS 500, 2 mm, 20 °C, 52 mA, recycle mode

A: Mixed standard
B: Anticoagulant Citrate Phosphate Dextrose solution 1000-fold diluted.

Peaks:	A	B	
1. Phosphate	12	1.6	mg/L
2. Citrate	20	20	

Table 1 summarizes the calibration and LOQs for citrate and phosphate. This method has lower LOQs with 25% the injection volume of the original method (phosphate 0.03 vs. 0.2 mg/L and citrate 0.06 vs. 0.2 mg/L). Over the calibration range of 0.5 to 200 mg/L for phosphate and 0.5 to 50 mg/L for citrate, the calibrations are linear. When concentration is above 50 mg/L, citrate exhibits a quadratic calibration curve.

Table 1. Summary of Calibration and Limit of Quantitation Data for Citrate and Phosphate.

	Calibration Range (mg/L)	Calibration Type	Coefficient of Determination (r^2)	LOQ (mg/L)
Phosphate	0.5–200	Linear	1.0000	0.03
Citrate	0.5–200	Quadratic	0.9998	
Citrate	0.5–50	Linear	0.9997	0.06

The method performance was evaluated by comparing measured value to the label and determining the precision of replicate sample injections and recovery of spiked samples.

As indicated in Tables 2 and 3, this improved IC method is accurate (95–106% recoveries and close to actual value) and precise (< 2% RSDs over 4 days).

Table 2. Comparison of the Citrate and Phosphate Concentrations Obtained to the Label Amounts and USP Monograph.

	Label Amount (mg/mL)	USP Spec. (mg/mL)	Experimental Average ± Standard Deviation (mg/mL) (n=15)
Phosphate	1.75	1.50–1.65	1.65 ± 0.01
Citrate	20.2	19.16–21.18	20.15 ± 0.04

Table 3. Accuracy and Precision for Citrate and Phosphate in the Pharmaceutical Formulation.

	Intraday Precision* Range (%RSD)	Interday* Precision (%RSD)	Range of Recoveries* (%)
Phosphate	0.18–0.34	0.75	95.6–105.5
Citrate	0.05–0.57	1.25	95.2–98.1

* The intraday precision was from independently prepared solutions analyzed on separate days. The interday precision was over a four day period. Recoveries were determined by adding known amounts of analyte to the sample solutions. The precision ranges are for the individual four days, three independently prepared solutions each day. The spike recoveries are from spiking 2.0 mg/L of citrate or phosphate into the samples.

The robustness of this method was evaluated by examining the retention time (RT), peak asymmetry, and resolution of the two analytes in the mixed standard containing 20 mg/L of citrate and 12 mg/L of phosphate after imposing small variations (± 10%) in procedural parameters. The data (not included in this poster) showed that the method was affected by variations in temperature and eluent concentration, but baseline separation of citrate and phosphate was maintained.

CONCLUSION

This application demonstrates an improved IC method for assaying citrate and phosphate using a Dionex IonPac AS11-HC-4µm microbore column on a Dionex Integrion HPIC system. This method:

- Increased sample throughput 2x by reducing the run time to 5 min
- Increased sensitivity with LOQs of 0.03 and 0.06 mg/L for phosphate and citrate, respectively, using 25% less sample injected than the previous method
- Is precise (< 2% RSDs over 4 days) and accurate (95–106% recoveries)
- Is robust because baseline separation of citrate and phosphate was maintained when small variations (± 10%) in procedural parameters were made

www.thermofisher.com

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Canada +1 800 530 8447
China 800 810 5118 (free call domestic) 400 650 5118
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland +358 10 3292 200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591
Japan +81 45 453 9100
Korea +82 2 3420 8600
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Norway +46 8 556 468 00
Russia/CIS +43 1 333 50 34 0
Singapore +65 6289 1190
Spain +34 914 845 965
Sweden +46 8 556 468 00
Switzerland +41 61 716 77 00
UK +44 1442 233555
USA +1 800 532 4752

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific

PN72097-EN 0616S