Application Note 1121

Ion Chromatography Assay for Lithium in Lithium Citrate

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Key Words
Dionex IonPac CS12A Column, Suppressed Conductivity Detection, Pharmaceutical, USP Monograph

Goal
To develop an IC method for the determination of lithium in lithium citrate using an RFIC system with suppressed conductivity detection

Introduction
Lithium is considered a primary therapeutic agent for acute and prophylactic treatment of biopolar disorder.[1] Practically, lithium is administered as salts such as lithium citrate. The United States Pharmacopoeia (USP) monograph for lithium citrate describes an assay based on flame photometry.[2] This assay involves mixing lithium citrate with hydrochloric acid followed by measuring the emission at 671 nm. This method is cumbersome, tedious, and it uses a hazardous/corrosive chemical.

The USP has initiated an effort to modernize existing monographs across all compendia.[3] In response to this effort, this application note describes an alternative method for lithium citrate analysis that is automated, fast, and uses an aqueous mobile phase (eluent). Ion chromatography (IC) offers a significant improvement to the existing assays because it can simultaneously determine lithium, sodium, calcium, and other common cations in a single injection.[4] Moreover, using a Reagent-Free™ Ion Chromatography (RFIC™) system with electrolytically generated methanesulfonic acid (MSA) eluent simplifies the method and enhances reproducibility.

This application note describes an IC based method that uses a Thermo Scientific™ Dionex™ IonPac™ CS12A-5µm cation-exchange column, an electrolytically generated MSA eluent, and suppressed conductivity detection to determine lithium in lithium citrate. The Dionex IonPac CS12A-5µm column is a medium-capacity cation-exchange column packed with resin functionalized with carboxylic acid groups. This column is specifically designed for fast analysis of alkali metals, alkaline earth metals, and ammonium. Therefore, the Dionex IonPac CS12A-5µm column is suitable for the separation of lithium from low concentrations of other cationic contaminants. The required eluent is generated using a Thermo Scientific Dionex EGC III MSA Eluent Generator Cartridge and purified on-line using a Thermo Scientific Dionex CR-CTC II Continuously Regenerated Cation Trap Column. The Thermo Scientific™ Dionex™ CERS™ 500 2 mm Cation Electrolytically Regenerated Suppressor produces the regenerant ions necessary for eluent suppression and allows continuous operation with minimum maintenance. Because the RFIC system requires only deionized (DI) water as the carrier, it significantly simplifies system operation and improves analytical reproducibility. The method proposed in this application note was validated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Procedures.[5]

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Equipment

- A Thermo Scientific™ Dionex™ ICS-2100 Reagent-Free™ Ion Chromatography (RFIC™) system was used in this work. The Dionex ICS-2100 system is an integrated ion chromatograph that includes:
  - Pump
  - Column Heater
  - Pump Degas
  - EG Eluent Generator
  - Dionex CR-CTC Continuously Regenerated Cation Trap Column (P/N 066262)
- Thermo Scientific Dionex AS-AP Autosampler with 10 µL injection loop
- Dionex EGC III MSA Cartridge (P/N 074535)
- Thermo Scientific™ Autoselect™ Polyvial™ 10 mL Autosampler Vials with caps and septa (P/N 055058)
- Thermo Scientific Dionex CERS 500 (2 mm) Cation Electrolytically Regenerated Suppressor (P/N 082543)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) 7.2 Workstation

Reagents and Standards

- Deionized (DI) Water, Type I reagent grade, 18 MΩ-cm resistance or better
- Lithium Carbonate, 300 mg, USP Reference Standard (USP P/N 1369000), Lot Number G1J227
- Lithium Citrate (tri basic, tetrahydrate, $Li_3C_6H_5O_7.4H_2O$), Sigma-Aldrich, P/N 62484-100G-F, Lot Number BCBM6951V
- Thermo Scientific Dionex Six Cation-II Standard, Thermo Scientific, P/N 046070, Lot Number 140915

Conditions	
Columns:	Dionex IonPac CS12A-5µm, Analytical, 3 × 150 mm (P/N 057185) Dionex IonPac CG12A-5µm Guard, 3 × 30 mm (P/N 057184)
Eluent:	8 mM MSA from 0–6 min, 67 mM MSA from 6–8 min, 8 mM MSA from 8–20 min
Eluent Source:	Dionex EGC III MSA cartridge with Dionex CR-CTC II Trap column (P/N 074535)
Flow Rate:	0.4 mL/min
Background Conductance:	~0.3 µS
Detection:	Suppressed conductivity, Dionex CERS 500 (2 mm) Suppressor, recycle mode, 79 mA current
Noise:	~1–2 nS/min peak-to-valley
Run Time:	20 min
Injection Volume:	10 µL in Push-Full mode
Column Temperature:	33 °C

Preparation of Solutions and Reagents

Lithium Stock Solution 1000 mg/L, prepared using Lithium Carbonate, USP

Accurately weigh 0.5322 g of USP lithium carbonate and dissolve in DI water in a 125 mL polypropylene bottle and adjust the weight to 100 g with DI water.

Lithium Stock Solution 1000 mg/L, prepared using Lithium Citrate

Accurately weigh 1.354 g of lithium citrate (hydrate) and dissolve in DI water in a 125 mL polypropylene bottle and adjust the weight to 100 g with DI water.

Robustness Study

Following the guidelines of USP Physical Tests, <621> Chromatography, evaluate the robustness of this method by examining the retention time (RT), peak asymmetry and resolution after imposing small variations (±10%) in procedural parameters (e.g., flow rate, eluent gradient concentration, column temperature).[6] Inject a standard mixture containing 5 mg/L lithium. Apply the same procedure to another column set from a different lot. Test the following variations:

- Flow rate at 0.36 mL/min, 0.4 mL/min, 0.44 mL/min
- Column temperature at 30, 33, and 36 °C
- MSA eluent initial concentrations at 7.2 mM, 8 mM, 8.8 mM
- MSA eluent initial concentrations at 60.3 mM, 67 mM, 73.7 mM

Results and Discussion

Separation

Separation of lithium was achieved using a Dionex IonPac CS12A-5µm, 3 × 150 mm column under gradient elution conditions. Figure 1 shows separation of 5 mg/L lithium solution prepared using lithium citrate.

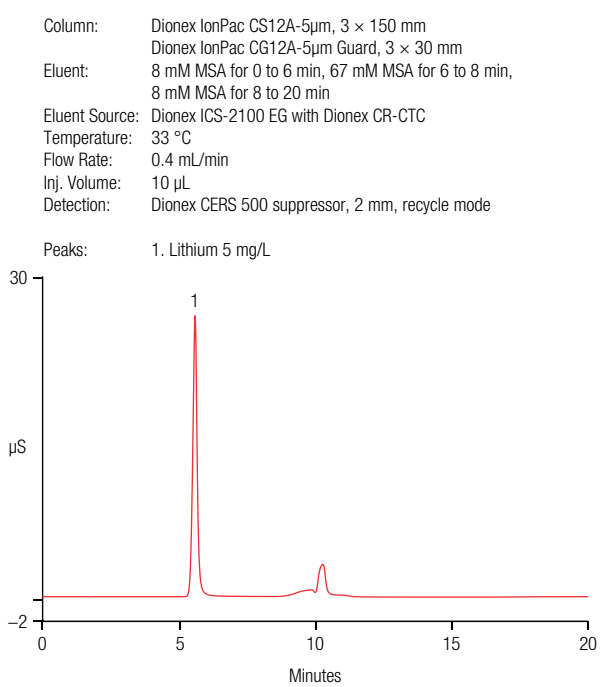

Figure 1. Determination of 5 mg/L lithium in deionized water.

Figure 2, shows separation of a commercially available six cation standard mix using the proposed method. In order to achieve good separation from nearest cation (i.e. sodium), it was required to keep initial eluent concentration at 8 mM which was rapidly increased to 67 mM to elute the remaining cations quickly. The other four common cations elute within 10 min. The remaining 10 min prepare the column for the next sample injection.

Calibration, LOD, and LOQ

The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the USP General Chapter <1225> guidelines recommend a minimum of five concentrations to establish linearity in an assay.[5] For a drug substance or finished product, the minimum specified range is from 80 to 120% of the test concentration. A minimum range from 50 to 120% is required for determination of an impurity. In this study, lithium was calibrated at 14 concentration levels ranging from 0.1 to 15 mg/L. The results yielded a linear relationship of peak area to concentration with a coefficient of determination (r^2) of 0.9999 (Table 1).

To determine the LODs and LOQs, the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1 min segment of the baseline where no peaks elute but close to the peaks of interest. The signal was determined from the average peak height of three injections of 2 µg/L lithium. The LODs and LOQs were then determined by multiplying the signal-to-noise ratio by 3 and 10, respectively (Table 1). The LOD and LOQ of lithium were 1.2 µg/L and 4 µg/L respectively. Figure 3 shows a chromatogram obtained using a 2 µg/L injection of lithium.

Table 1. Method calibration, LOD, and LOQ data for lithium.

Parameter	Value
Linearity (r^2)	0.9999
LOD (µg/L)	1.2
LOQ (µg/L)	4

Column: Dionex IonPac CS12A-5µm, 3 × 150 mm
Dionex IonPac CG12A-5µm Guard, 3 × 30 mm
Eluent: 8 mM MSA for 0 to 6 min, 67 mM MSA for 6 to 8 min, 8 mM MSA for 8 to 20 min
Eluent Source: Dionex ICS-2100 EG with Dionex CR-CTC
Temperature: 33 °C
Flow Rate: 0.4 mL/min
Inj. Volume: 10 µL
Detection: Dionex CERS 500 suppressor, 2 mm, recyle mode

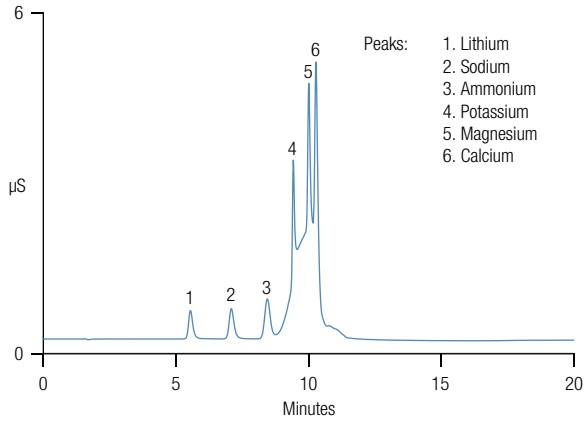

Figure 2. Chromatography of six common cations with the conditions for lithium assay.

Column: Dionex IonPac CS12A-5µm, 3 × 150 mm
Dionex IonPac CG12A-5µm Guard, 3 × 30 mm
Eluent: 8 mM MSA for 0 to 6 min, 67 mM for MSA 6 to 8 min, 8 mM MSA for 8 to 20 min
Eluent Source: Dionex ICS-2100 EG with Dionex CR-CTC
Temperature: 33 °C
Flow Rate: 0.4 mL/min
Inj. Volume: 10 µL
Detection: Dionex CERS 500 suppressor, 2 mm, recyle mode
Peaks: 1. Lithium 2 µg/L

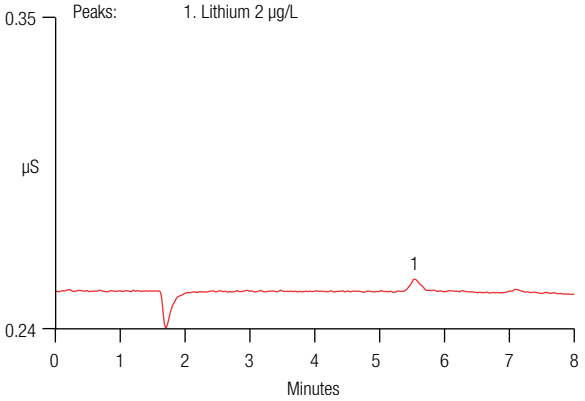

Figure 3. Determination of 2 µg/L lithium in deionized water.

Table 2. Recovery data for lithium spiked in lithium citrate solutions containing 1, 5, and 10 mg/L lithium.

Lithium Concentration (mg/L)	Spike (mg/L)	Total Recovered (mg/L)	Spike Recovery (%)	RT RSD (n=3)	Peak Area RSD (n=3)
5	0	5.02	—	0.04	0.34
	1	6.01	101.5	0.03	0.37
	5	9.99	99.8	0.04	0.36
	10	14.99	99.9	0.04	0.23

Sample Analysis

The USP monograph requires that lithium citrate contain not less than 98.0% and not more than 102% lithium citrate calculated on the dried basis.[2] In this study, commercially available lithium citrate (≥ 99.5%) was used to prepare the test solution of 5 mg/L lithium. The calculated concentration of the test solution was 5.02 mg/L, equivalent to 100.4% lithium citrate content (Table 2), thus verifying the label claim. This indicates that the method is capable of determining lithium citrate concentration within the USP specification. The USP requires that the lithium citrate be dried prior to assay for 3 h at 150 °C; this should result in weight loss of between 24.0 to 28.0% of its weight. But in our hands the drying process resulted in weight loss of only 22.6% indicating residual water content remaining in lithium citrate. Hence this drying process was not performed and water content as provided by the manufacturer was used for making all lithium citrate solutions.

Sample Accuracy and Precision

In order to test sample accuracy, recovery studies were performed after spiking lithium samples prepared using lithium citrate with lithium from lithium carbonate. Three different spike levels of 1, 5, and 10 mg/L lithium were studied and satisfactory recoveries were obtained for the spike. The results of lithium spike recovery experiment are contained in Table 2. Figure 4 shows a representative chromatogram of a 5 mg/L lithium sample spiked with 1 mg/L lithium from lithium carbonate.

Assay precision was evaluated by injecting seven replicates at three different concentration levels of 1, 5, and 10 mg/L lithium and expressed as the RSDs of RT and peak area from the series of measurements. The RT RSDs were ≤0.05% and the peak area RSDs were ≤0.35% (Table 3).

Column: Dionex IonPac CS12A-5µm, 3 × 150 mm
Dionex IonPac CG12A-5µm Guard, 3 × 30 mm
Eluent: 8 mM MSA for 0 to 6 min, 67 mM MSA 6 to 8 min, 8 mM MSA for 8 to 20 min
Eluent Source: Dionex ICS-2100 EG with Dionex CR-CTC
Temperature: 33 °C
Flow Rate: 0.4 mL/min
Inj. Volume: 10 µL
Detection: Dionex CERS 500 suppressor, 2 mm, recycle mode

Peaks: 1. Lithium 5 mg/L + 1 mg/L Lithium Spike

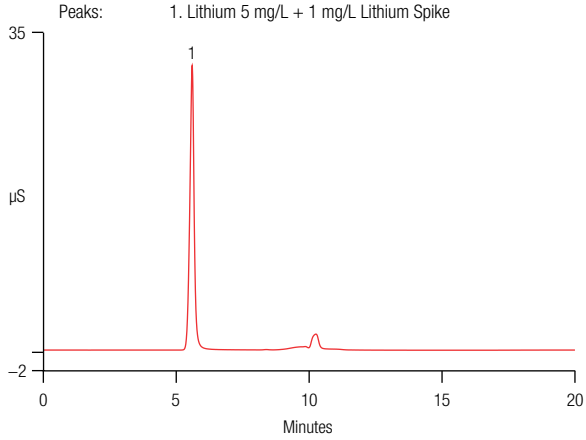

Figure 4. A 5 mg/L lithium sample spiked with 1 mg/L lithium from lithium carbonate.

Table 3. Retention time and peak area precisions of lithium solutions.

Target Lithium Concentration (mg/L)	Found (mg/L)	RT RSD (n=7)	Peak Area RSD (n=7)
1	0.99	0.01	0.35
5	5.02	0.04	0.34
10	10.07	0.05	0.32

Table 4. Robustness of the IC-based assay for lithium determination performed using a 5 mg/L sample.

Parameter	Value	Column 1						Column 2					
		Lithium RT (min)	Lithium RT Difference (%)	Peak Asymmetry	Peak Asymmetry Difference (%)	Resolution (From Na)	Resolution (From Na) Difference (%)	Lithium RT (min)	Lithium RT Difference (%)	Peak Asymmetry	Peak Asymmetry Difference (%)	Resolution (From Na)	Resolution (From Na) Difference (%)
Flow Rate (mL/min)	0.36	6.19	10.7	1.03	–1.28	4.99	2.32	6.33	10.73	0.95	–1.39	5.32	2.64
	0.40	5.59	—	1.04	—	4.88	—	5.72	—	0.96	—	5.18	—
	0.44	5.10	–8.75	1.05	0.64	4.79	–1.91	5.23	–8.63	0.96	0.35	5.11	–1.41
Column Temp (°C)	30	5.61	0.26	1.06	1.28	5.04	3.35	5.74	0.31	0.96	0	5.41	4.44
	33	5.59	—	1.04	—	4.88	—	5.72	—	0.96	—	5.18	—
	36	5.56	–0.54	1.04	–0.64	4.73	–3.07	5.69	–0.52	0.95	–0.69	4.98	–3.99
MSA Eluent Initial Concentration (mM)	7.2	6.04	8.06	1.02	–2.56	5.06	3.76	6.18	8.04	0.94	–2.43	5.38	3.79
	8.0	5.59	—	1.04	—	4.88	—	5.72	—	0.96	—	5.18	—
	8.8	5.22	–6.58	1.07	2.88	4.72	–3.28	5.35	–6.52	0.98	2.43	5.05	–2.51
MSA Eluent Final Concentration (mM)	60.3	5.59	0	1.03	–0.96	4.87	–0.27	5.72	0	0.96	–0.35	5.20	0.32
	67.0	5.59	—	1.04	—	4.88	—	5.72	—	0.96	—	5.18	—
	73.7	5.58	–0.25	1.05	0.32	4.88	0	5.71	–0.21	0.95	–0.69	5.19	0.19

Robustness

Assay robustness was evaluated by measuring the influence of small variations in procedural parameters (e.g., flow rate, eluent concentration during gradient, and column temperature) on the RT, peak asymmetry, and resolution from sodium on two columns from two different lots. The peak asymmetry was measured using the USP formula.[6] A standard injection (5 mg/L lithium spiked with 0.1 mg/L sodium) was injected seven times (n=7) at each chromatographic condition. Table 4 summarizes the results of the lithium robustness study. These results indicate that the method is robust and suitable for lithium analysis.

Conclusion

This study describes an IC-based assay for determination of lithium in lithium citrate. Lithium was separated on a cation-exchange column and detected by suppressed conductivity in 20 min. This method allows the concentration of lithium to be determined in an automated way circumventing the need to perform the cumbersome flame photometry based assay. This assay for lithium was validated to meet the analytical performance characteristics outlined in USP General Chapter <1225>, Validation of Compendial Procedures, and was shown to measure accurately the lithium content of lithium citrate as per limits set in the USP monograph. Compared to assay described in the USP lithium citrate monograph, this assay offers a simple, accurate, and robust measurement without handling hazardous reagents. Therefore, this method is a candidate to replace the existing assay for lithium citrate in the USP monograph, and thereby modernize the monograph.

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