

# IC Assay for Lithium, Sodium, and Calcium in Lithium Carbonate

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## Key Words

Dionex IonPac CS16 Column, Suppressed Conductivity Detection, Pharmaceutical, USP Monograph

## Introduction

Lithium carbonate is used to treat a number of mental health problems associated with chemical imbalance in the brain, particularly bipolar disorder.<sup>1,2</sup> The U.S. Pharmacopeia (USP) monograph describes a lithium carbonate assay by titration with sulfuric acid, followed by sodium hydroxide, to titrate the excess acid using methyl orange as the indicator. Sodium and calcium are possible cationic impurities in lithium carbonate preparations. In the USP monograph, sodium is determined with a flame photometer at 589 nm and corrected for the background transmission at 580 nm. To determine calcium, the USP describes a chelometric titration assay that uses oxalate precipitation and permanganate titration and requires more than 4 h to complete.<sup>3</sup> These assays are time consuming and cumbersome procedures that use hazardous reagents.

The USP has embarked on a global initiative to modernize many of the existing monographs across all compendia.<sup>4</sup> In response to this initiative, an alternative analytical method was developed to determine these analytes in lithium carbonate.

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.<sup>5</sup> In addition, using electrolytically generated methanesulfonic acid (MSA) eluent significantly simplifies the method and enhances method reproducibility between laboratories.

This study describes a method that uses a Thermo Scientific™ Dionex™ IonPac™ CS16 cation-exchange column, an electrolytically generated MSA eluent, and suppressed conductivity detection to determine lithium, sodium, and calcium in lithium carbonate. The Dionex IonPac CS16 column is a high-capacity cation-exchange column packed



with resin functionalized with carboxylic acid groups. This column is specifically designed for disparate concentration ratios of adjacent-eluting cations in diverse sample matrices. Therefore, the Dionex IonPac CS16 column is suitable for the separation of low concentrations of sodium and calcium in a sample with a high lithium concentration.

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 Reagent-Free™ IC (RFIC™) system requires only deionized (DI) water as the carrier, it significantly simplifies system operation and improves analytical reproducibility. This method was validated following the guidelines outlined in USP General Chapter <1225>, Validation of Compendial Procedures.<sup>6</sup>

## Goal

To develop an IC method for the determination of lithium, sodium, and calcium in lithium carbonate using an RFIC system with suppressed conductivity detection

## Equipment

- Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ system, capable of supporting high-pressure IC, including:
  - SP Single Pump
  - EG Eluent Generator
  - DC Detector/Chromatography Compartment
- Thermo Scientific Dionex AS-AP Autosampler with Sample Syringe, 250 µL (P/N 074306) and a Standard 1200 µL Buffer Line Assembly (P/N 074989)
- Dionex EGC III MSA Eluent Generator Cartridge (P/N 074535)
- Dionex CR-CTC II Continuously Regenerated Cation Trap Column (P/N 066262)
- Dionex CERS 500 (2 mm) Cation Electrolytically Regenerated Suppressor (P/N 082543)
- Vial Kit, 10 mL, Polystyrene with Caps and Blue Septa (P/N 074228)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, version 7.2

## Reagents and Standards

- DI water, Type I reagent grade, 18 M $\cdot$ cm resistance or better
- Lithium Carbonate, 300 mg, USP Reference Standard (USP P/N 1369000)
- Sodium Chloride, Certified ACS,  $\geq$ 99% (Fisher Scientific P/N S271)
- Calcium Chloride Dihydrate, Certified ACS, 99 to 105% (Fisher Scientific P/N C79)
- Magnesium Chloride Hexahydrate, Certified ACS, 99 to 102% (Fisher Scientific P/N M33)

## Conditions (Applicable to Figures 1–3)

Columns:	Dionex IonPac CG16 Guard, 3 $\times$ 50 mm (P/N 079931) Dionex IonPac CS16 Analytical, 3 $\times$ 250 mm (P/N 059596)
Eluent:	8 mM MSA from 0 to 15 min, 67 mM MSA from 15 to 20 min, 8 mM MSA from 20 to 25 min
Eluent Source:	Dionex EGC III MSA cartridge with Dionex CR-CTC II trap column
Flow Rate:	0.43 mL/min
Injection Volume:	10 µL
Temperature:	40 °C
Detection:	Suppressed conductivity, Dionex CERS 500 (2 mm) suppressor, recycle mode, 85 mA current
System	
Backpressure:	~2200–2300 psi
Background	
Conductance:	~0.3 µS
Noise:	~0.4 nS/min peak-to-peak
Run Time:	25 min

## Preparation of Solutions and Reagents

*Note: Do not use glassware to prepare the solutions. Polymeric containers made of high-density polyethylene (HDPE) are recommended.*

### Lithium Stock Solution, 1000 mg/L

Accurately weigh 0.5323 g of USP lithium carbonate and dissolve in 100 mL of DI water.

### Sodium Stock Solution, 1000 mg/L

Accurately weigh 0.2542 g of sodium chloride and dissolve in 100 mL of DI water.

### Calcium Stock Solution, 1000 mg/L

Accurately weigh 0.3668 g of calcium chloride dihydrate and dissolve in 100 mL of DI water.

## Working Standard Solutions

To prepare working standard solutions, dilute the stock solutions to the appropriate concentrations with DI water. Prepare the working standard solutions for sodium and calcium on the day of analysis.

## Sample Preparation

Prepare a 20-fold dilution of the 1000 mg/L lithium stock solution made from lithium carbonate to formulate a test solution of 50 mg/L lithium. The acceptance criteria of sodium and calcium in the USP monograph are 0.1% (0.26 mg/L) and 0.15% (0.40 mg/L), respectively. Dilute both the sodium and calcium stock solutions to prepare 100 mg/L each of the sodium and calcium solutions. Then spike 0.26 mL of 100 mg/L of sodium and 0.40 mL of 100 mg/L of calcium to 100 mL of 50 mg/L lithium to prepare the test solution of lithium carbonate fortified with sodium and calcium at the acceptance criterion levels.

## 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 of the three analytes after imposing small variations ( $\pm 10\%$ ) in procedural parameters (e.g., flow rate, eluent gradient concentration, column temperature).<sup>7</sup> Inject a standard mixture containing 50 mg/L lithium, 0.26 mg/L sodium, 0.20 mg/L magnesium (to determine the resolution of calcium), and 0.40 mg/L calcium. Apply the same procedure to two column sets from two different lots. Test the following variations:

- Flow rate at 0.43 mL/min, 0.39 mL/min, 0.47 mL/min
- Column temperature at 40, 36, and 44 °C
- MSA eluent initial/final concentrations at 8 mM/67 mM, 7.2 mM/67 mM, 8.8 mM/67 mM, 8 mM/61 mM, 8 mM/73 mM

## Results and Discussion

### Separation

The separation of lithium, sodium, and calcium was achieved on a Dionex IonPac CS16 column designed for disparate concentration ratios of close-eluting cations. This column enabled accurate determination of sodium in samples with a high concentration of lithium. The eluent program started with 8 mM MSA to separate sodium from lithium, followed by a step change to 67 mM MSA to quickly elute calcium without inferences from other common cations. The MSA concentration was then returned to 8 mM to equilibrate the column for the next injection. Figure 1 shows the separation of six common cations using a Dionex IonPac CS16 column set, indicating no interference from other common cations with lithium, sodium, and calcium. Figure 2 shows a lithium carbonate sample containing 50 mg/L lithium with an enlarged view (Figure 2, Chromatogram B) showing the separation of sodium and calcium.

### Calibration, Limit of Detection (LOD), and Limit of Quantitation (LOQ)

The International Conference on Harmonisation 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.<sup>6</sup> 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 11 concentration levels ranging from 2.5 to 150 mg/L. The results yielded a linear relationship of peak area to concentration with a coefficient of determination ( $r^2$ ) of 1.000. Sodium and calcium were calibrated at seven concentration levels ranging from 0.05 to 5 mg/L with  $r^2$  values of 0.9997 and 1.000, respectively (Table 1).

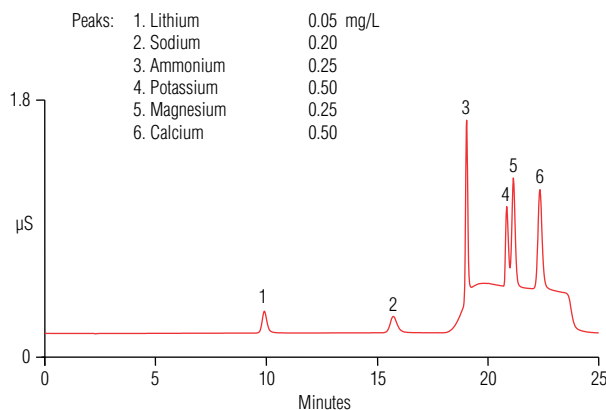


Figure 1. Separation of six common cations.

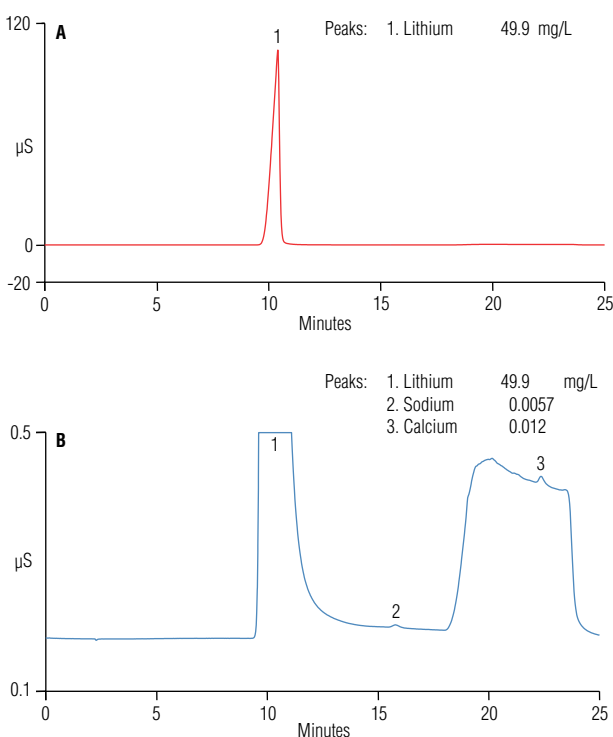


Figure 2. (A) A lithium carbonate sample (50 mg/L lithium) and (B) enlarged view of Chromatogram A showing sodium and calcium peaks.

Table 1. Calibration, LODs, and LOQs of lithium, sodium, and calcium.

Cation	Calibration Range (mg/L)	$r^2$	LOD ( $\mu\text{g/L}$ )	LOQ ( $\mu\text{g/L}$ )
Lithium	2.5–150	1.000	0.27	0.9
Sodium	0.05–5	0.9997	1.1	3.6
Calcium	0.05–5	1.000	7.4	25

Table 2. Recovery data for lithium, sodium, and calcium spiked in lithium carbonate samples containing 50 mg/L lithium.

Cation	Found (mg/L)	RT RSD (n = 3)	Peak Area RSD (n = 3)	Added (mg/L)	Total Found (mg/L)	RT RSD (n = 3)	Peak Area RSD (n = 3)	Recovery (%)
Lithium	49.9	0.01	0.12	25.1	75.5	0.03	0.04	102
				50	100	0.02	0.06	101
				75.5	125	0.01	0.03	100
Sodium	0.0057	0.03	4.39	0.130	0.120	<0.01	0.15	87.8
				0.260	0.240	0.01	0.07	90.0
				0.390	0.358	0.01	0.21	90.2
Calcium	0.012	0.02	4.66	0.200	0.207	<0.01	0.53	97.7
				0.400	0.423	0.01	0.26	103
				0.600	0.610	<0.01	0.33	99.6

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 1 µg/L lithium, 5 µg/L sodium, and 10 µg/L calcium. The LODs and LOQs were then determined by multiplying the signal-to-noise ratio 3× and 10×, respectively (Table 1). The LODs of lithium, sodium, and calcium were 0.27, 1.1, and 7.4 µg/L, respectively. The LOQs of those three analytes were 0.9, 3.6, and 25 µg/L, respectively, which correspond to 0.00034, 0.0014, and 0.0094% of lithium carbonate. The low LODs of sodium and calcium indicate that this IC method can easily assay these two cationic impurities well below the acceptance criteria specified in the USP monograph.

### Sample Analysis

The USP monograph requires that lithium carbonate contain not less than 99.0% lithium carbonate calculated on the dried basis.<sup>3</sup> In this study, the USP lithium carbonate reference standard was used to prepare the test solution of 50 mg/L lithium. The concentration of the test solution was 49.9 mg/L, equivalent to 99.8% lithium carbonate, which is within the USP specification. The concentrations of sodium and calcium in the lithium carbonate sample at the test concentration were 0.0057 and 0.012 mg/L, corresponding to 0.004 and 0.008% of lithium carbonate, respectively.

### Sample Accuracy and Precision

Method accuracy was validated by spiked recoveries of lithium, sodium, and calcium in lithium carbonate samples over three concentration levels (i.e., 50, 100, and 150%), with three replicates of each concentration. For a test solution concentration of 50 mg/L lithium, the recoveries of lithium at the three spike levels were in the range of 100–102%.

The USP monograph limits for sodium and calcium in lithium carbonate are 0.1 and 0.15%, respectively.<sup>3</sup> This correlates to 0.26 mg/L sodium and 0.4 mg/L calcium in 50 mg/L lithium. The lithium solution was spiked with 0.13, 0.26, and 0.39 mg/L of sodium and 0.2, 0.4, and 0.6 mg/L of calcium, respectively. The recoveries of the three spiked levels of sodium and calcium were in the range of 87.8–90.2% and 97.7–103%, respectively. These recoveries are summarized in Table 2. A chromatogram of a spiked lithium carbonate sample is shown in Figure 3.

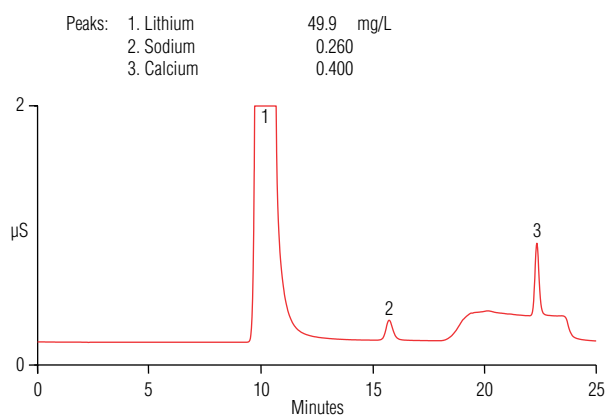


Figure 3. A lithium carbonate sample (50 mg/L lithium) spiked with sodium and calcium at the acceptance criterion levels.

Assay precision was evaluated by injecting seven replicates of the test concentrations (50 mg/L lithium, 0.26 mg/L sodium, and 0.40 mg/L calcium), and expressed as the RSDs of RT and peak area from the series of measurements for the three analytes. The RT RSDs were <0.02% and the peak area RSDs ranged from 0.36 to 1.04% (Table 3).

Table 3. Retention time and peak area precisions of 50 mg/L lithium spiked with 0.26 mg/L sodium and 0.40 mg/L calcium.

Cation	Concn (mg/L)	RT RSD (n = 7)	Peak Area RSD (n = 7)
Lithium	49.9	0.02	0.36
Sodium	0.24	0.01	0.44
Calcium	0.414	0.01	1.04

## Robustness

Assay robustness was evaluated by measuring the influence of small variations ( $\pm 10\%$ ) in procedural parameters (e.g., flow rate, eluent gradient concentration, column temperature) on the RT, peak asymmetry, and resolution of the three analytes on two column sets from two different lots. The peak asymmetry was evaluated following the USP formula. The resolution was determined using a USP formula relative to the previous peak in a chromatogram. A standard mix (50 mg/L lithium, 0.26 mg/L sodium, 0.20 mg/L magnesium, and 0.40 mg/L calcium) was injected three times at each chromatographic condition. The resolution of sodium to lithium ranged from 7.99 to 8.49 on Column 1 and from 7.13 to 7.86 on Column 2. The resolution of calcium to magnesium ranged from 3.56 to 4.18 on Column 1 and from 3.08 to 3.54 on Column 2. Tables 4–6 summarize the results for lithium, sodium, and calcium, respectively. These results indicate the method is robust for all three analytes.

Table 4. Robustness of the IC-based assay for lithium (injected sample: 50 mg/L lithium spiked with 0.26 mg/L sodium, 0.20 mg/L magnesium, and 0.40 mg/L calcium; average of three injections).

Parameter		Column 1				Column 2			
		Lithium RT (min)	Diff.* (%)	Asym.**	Diff.* (%)	Lithium RT (min)	Diff.* (%)	Asym.**	Diff.* (%)
Flow Rate (mL/min)	0.39	11.5	9.6	0.65	0.5	11.3	9.5	0.70	0.9
	0.43	10.4	—	0.66	—	10.2	—	0.70	—
	0.47	9.57	-8.9	0.66	-1.0	9.47	-8.6	0.71	-0.5
Column Temp (°C)	36	10.5	0.5	0.66	0	10.3	0.6	0.70	0
	40	10.4	—	0.66	—	10.2	—	0.70	—
	44	10.4	-0.4	0.66	0	10.2	-0.3	0.70	0
Eluent MSA Initial Concn (mM)	7.2	11.4	8.2	0.65	1.5	11.2	8.2	0.69	1.4
	8.0	10.4	—	0.66	—	10.2	—	0.70	—
	8.8	9.70	-7.5	0.67	-1.5	9.58	-7.4	0.71	-1.4
Eluent MSA Final Concn (mM)	61	10.5	0.7	0.66	0	10.4	0.8	0.70	0
	67	10.4	—	0.66	—	10.2	—	0.70	—
	73	10.4	-0.5	0.66	0	10.2	-0.4	0.70	0

\* Difference

\*\* Asymmetry

Table 5. Robustness of the IC-based assay for sodium (injected sample: 50 mg/L lithium spiked with 0.26 mg/L sodium, 0.20 mg/L magnesium, and 0.40 mg/L calcium; average of three injections).

Parameter		Column 1						Column 2					
		Sodium RT (min)	Diff.* (%)	Asym.**	Diff.* (%)	Resol.***	Diff.* (%)	Sodium RT (min)	Diff.* (%)	Asym.**	Diff.* (%)	Resol.***	Diff.* (%)
Flow Rate (mL/min)	0.39	17.3	9.5	1.19	0.8	8.44	0.8	16.9	9.4	1.18	0.8	7.56	0.9
	0.43	15.7	—	1.20	—	8.40	—	15.3	—	1.17	—	7.49	—
	0.47	14.4	-8.9	1.21	-0.8	8.29	-1.0	14.1	-8.6	1.19	-0.8	7.42	-0.9
Column Temp (°C)	36	16.1	2.6	1.19	0.3	8.78	4.6	15.7	2.5	1.18	0.3	7.86	4.9
	40	15.7	—	1.20	—	8.40	—	15.3	—	1.17	—	7.49	—
	44	15.4	-2.4	1.20	-0.6	7.99	-4.8	15.0	-2.4	1.18	-0.6	7.13	-4.8
Eluent MSA Initial Conc (mM)	7.2	17.2	8.8	1.16	2.2	8.49	1.2	16.8	8.7	1.17	0.6	7.60	1.4
	8.0	15.7	—	1.20	—	8.40	—	15.3	—	1.17	—	7.49	—
	8.8	14.5	-8.1	1.22	-2.8	8.28	-1.3	14.2	-8.0	1.18	-0.3	7.40	-1.3
Eluent MSA Final Conc (mM)	61	15.8	0.6	1.19	0.3	8.40	0.1	15.4	0.5	1.15	0.9	7.50	0.3
	67	15.7	—	1.20	—	8.40	—	15.3	—	1.17	—	7.49	—
	73	15.7	-0.4	1.20	-0.6	8.41	0.0	15.3	-0.4	1.16	-0.9	7.53	-0.2

\* Difference

\*\* Asymmetry

\*\*\* Resolution relative to lithium

Table 6. Robustness of the IC-based assay for calcium (injected sample: 50 mg/L lithium spiked with 0.26 mg/L sodium, 0.20 mg/L magnesium, and 0.40 mg/L calcium; average of three injections).

Parameter		Column 1						Column 2					
		Calcium RT (min)	Diff.* (%)	Asym.**	Diff.* (%)	Resol.***	Diff.* (%)	Calcium RT (min)	Diff.* (%)	Asym.**	Diff.* (%)	Resol.***	Diff.* (%)
Flow Rate (mL/min)	0.39	23.3	4.0	1.13	2.0	4.04	5.1	23.2	3.8	1.19	1.9	3.45	4.2
	0.43	22.3	—	1.16	—	3.82	—	22.2	—	1.22	—	3.28	—
	0.47	21.6	-3.7	1.18	-2.3	3.67	-4.5	21.5	-3.5	1.18	-1.4	3.20	-3.3
Column Temp (°C)	36	22.3	0.1	1.16	1.7	3.78	0.9	22.2	0.1	1.18	1.9	3.28	0.8
	40	22.3	—	1.16	—	3.82	—	22.2	—	1.22	—	3.28	—
	44	22.4	-0.1	1.19	-0.9	3.85	-1.0	22.2	0.0	1.19	-1.4	3.32	-0.4
Eluent MSA Initial Conc (mM)	7.2	22.4	0.2	1.16	1.1	3.80	0.7	22.3	0.2	1.19	1.7	3.27	1.3
	8.0	22.3	—	1.16	—	3.82	—	22.2	—	1.22	—	3.28	—
	8.8	22.3	-0.2	1.18	-0.6	3.85	-0.6	22.2	-0.2	1.19	-0.8	3.34	-0.8
Eluent MSA Final Conc (mM)	61	23.1	3.0	1.11	3.2	4.18	8.5	22.9	2.8	1.16	2.8	3.54	7.3
	67	22.3	—	1.16	—	3.82	—	22.2	—	1.22	—	3.28	—
	73	21.8	-2.7	1.19	-3.8	3.56	-7.6	21.7	-2.6	1.18	-2.2	3.08	-6.7

\* Difference

\*\* Asymmetry

\*\*\* Resolution relative to magnesium

## Conclusion

This study describes an IC-based assay for simultaneous determination of lithium, sodium, and calcium in lithium carbonate. The three analytes were separated on a cation-exchange column and detected by suppressed conductivity within 25 min. The concentrations of lithium, sodium, and calcium in a lithium carbonate sample were determined in a single run. This assay for lithium, sodium, and calcium was validated to meet the analytical performance characteristics outlined in USP General Chapter <1225>, Validation of Compendial Procedures, and demonstrates detection limits well below the limits set in the USP monograph. Compared to the three time-consuming assays in the USP lithium carbonate monograph, this assay offers a simple, accurate, and robust measurement of the three analytes without handling hazardous reagents. Therefore, this method is a candidate to replace the existing assays for lithium carbonate, sodium, and calcium in the USP monograph, and thereby modernize the monograph.

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