APPLICATION NOTE 73345

Determination of oxalate in cromolyn sodium

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Goal

To develop an ion chromatography (IC) method for the determination of oxalate in cromolyn sodium using a Reagent-Free™ Ion Chromatography (RFIC™) system with suppressed conductivity detection

Introduction

Cromolyn sodium[1] is an important drug for the management of asthma. It acts by inhibiting mediators of inflammation, and cell types that produce inflammation.[2] The United States Pharmacopoeia (USP) monograph for cromolyn sodium describes an assay for oxalate based on absorbance after performing a chemical reaction.[3] The USP monograph prescribes a limit for oxalate of NMT (not more than) 0.35%. This wet-chemical assay involves mixing cromolyn sodium with iron salicylate followed by measuring the absorbance at 480 nm. This method is cumbersome and tedious. The USP has initiated an effort to modernize existing monographs across all compendia.[4] In response to this effort, this application note describes an alternative method for oxalate determination using IC. The assay proposed here is automated, fast, and uses an aqueous mobile phase (eluent). Hence, IC offers a significant improvement to the existing assay described by the USP. Moreover, using an RFIC system with electrolytically generated hydroxide eluent simplifies the method and enhances reproducibility.

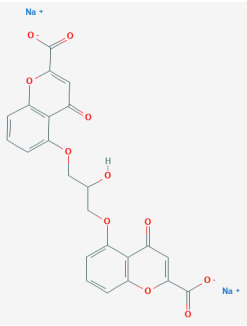

Figure 1. Structure of cromolyn sodium[1]

This application note describes an IC-based method that uses a Thermo Scientific™ Dionex™ IonPac™ AS20 anion-exchange column, an electrolytically generated potassium hydroxide eluent, and suppressed conductivity detection to determine oxalate in cromolyn sodium. The Dionex IonPac AS20 column is a high-capacity anion-exchange column packed with resin functionalized with alkanol quaternary groups and specifically designed for use with hydroxide eluents. The required eluent is generated using a Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator cartridge and purified online using a Thermo Scientific™

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Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap column. The Thermo Scientific™ Dionex™ ADRS™ 600 2 mm Anion Dynamically Regenerated Suppressor produces the regenerant ions necessary for eluent suppression and allows for continuous operation with minimal 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]

Experimental

Equipment

- Thermo Scientific™ Dionex™ Integrion™ HPIC system* including:
  - Eluent Generator
  - Pump
  - Degasser
  - Conductivity Detector
  - Column oven temperature control
  - Detector-suppressor compartment temperature control
- Thermo Scientific™ Dionex™ AS-AP Autosampler with sample syringe, 250 µL (P/N 074306) and buffer line, 1.2 mL (P/N 074989)

*This method can be executed on any Thermo Scientific™ Dionex™ RFIC system

Conditions	
Columns	Dionex IonPac AG20 Guard, 2 × 50 mm (P/N 063066) and Dionex IonPac AS20 Analytical, 2 × 250 mm (P/N 063065)
Eluent source	Dionex EGC 500 KOH Eluent Generator Cartridge (P/N 075778) with Dionex CR-ATC 600 trap column (P/N 088662)
Eluent	30 mM KOH
Flow rate	0.3 mL/min
Column temperature	30 °C
Compartment temperature	25 °C
Injection volume	2.5 µL (Full Loop)
Detection	Suppressed Conductivity, Dionex ADRS 600 Suppressor (2 mm) (P/N 088667), recycle mode, 26 mA current, constant current mode
Backpressure	~1960 psi

Reagent and chemicals

- Sodium oxalate (J.T. Baker P/N 3801-04, Lot no. G04716)
- Sodium phosphate dibasic (MilliporeSigma P/N S3139) [Note: Original part number used was 21998-6, Lot No. 07926EZ]
- Sodium sulfate (MilliporeSigma P/N 239313, Lot no. SLBX9237)
- Cromolyn sodium, USP (USP P/N 1150502, Lot no. R099H0)

Preparation of solutions and reagents

Oxalate stock solution, 1000 mg/L, prepared using sodium oxalate: Accurately weigh 0.152 g of sodium oxalate and dissolve in DI water in a 125 mL polypropylene bottle and adjust the weight to 100 g with DI water. Prepare a 100 mg/L secondary stock solution by 10-fold dilution of the primary stock solution. Dilute the secondary stock solution appropriately to prepare 10 calibration standards: 10, 5, 2, 1, 0.75, 0.5, 0.2, 0.1, 0.05, and 0.03 mg/L. For example, for a 10 mg/L standard, perform a 1:10 dilution of the 100 mg/L standard by adding 10 g of 100 mg/L standard to a 125 mL polypropylene bottle. Add DI water to a final weight of 100 g, mix, cap, and store the bottle at 4 °C until needed.

Robustness study

Following the guidelines of USP Physical Tests, <621> Chromatography, the robustness of this method was evaluated by examining retention time (RT), peak asymmetry, and resolution after imposing small variations (±10%) in procedural parameters (e.g., flow rate, eluent concentration, column temperature).[6] A standard mixture containing 0.5 mg/L sulfate, oxalate, and phosphate was injected in triplicate for each condition. The same procedure was applied to another column set from a different lot.

The variations tested were as follows:

- Flow rate at 0.27 mL/min, 0.3 mL/min, and 0.33 mL/min
- Eluent concentration at 31.5, 35, and 38.5 mM
- Column temperature at 27 °C, 30 °C, and 33 °C [Note: Because the Dionex Integrion system does not have column cooling capacity, for testing lower temperature conditions, the columns were allowed to equilibrate with the room temperatures, which were 25.1 and 24.8 °C for columns 1 and 2, respectively.]

Results and discussion

Separation

Separation of oxalate was achieved using a Dionex IonPac AS20, 2 × 250 mm column under isocratic elution conditions. Figure 2 shows a separation of 5 mg/L oxalate solution prepared using sodium oxalate. The separation of oxalate from sulfate and phosphate was tested to prove the necessary resolution from neighboring peaks. Figure 3 shows the chromatogram for a sample containing 5 mg/L each of sulfate, oxalate, and phosphate analyzed using the proposed method. Both sulfate and phosphate are well resolved from oxalate.

Conditions

Column:	Dionex IonPac AG20 Guard, 2 × 50 mm and Dionex IonPac AS20 Analytical, 2 × 250 mm
Eluent source:	Dionex EGC 500 KOH Eluent
Eluent:	30 mM KOH
Flow rate:	0.3 mL/min
Column temp.:	30 °C
Injection volume:	2.5 µL (Full Loop)
Detection:	Suppressed Conductivity
Peaks:	1. Carbonate 2. Oxalate

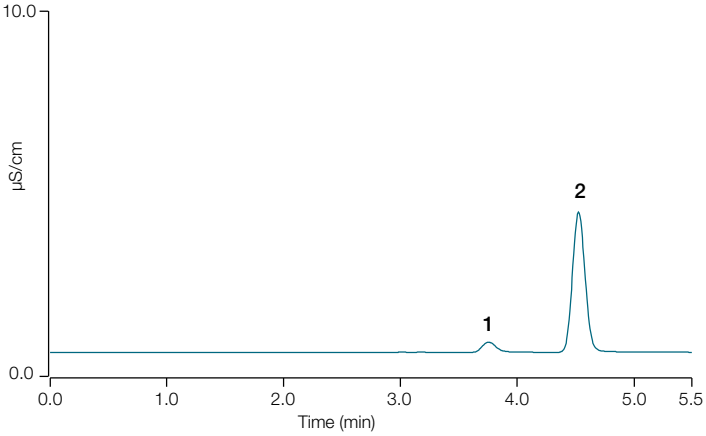

Figure 2. Chromatogram of a solution of oxalate (5 mg/L) analyzed on a Dionex IonPac AS20 column

Conditions

Column:	Dionex IonPac AG20 Guard, 2 × 50 mm and Dionex IonPac AS20 Analytical, 2 × 250 mm
Eluent source:	Dionex EGC 500 KOH Eluent
Eluent:	30 mM KOH
Flow rate:	0.3 mL/min
Column temp.:	30 °C
Injection volume:	2.5 µL (Full Loop)
Detection:	Suppressed Conductivity
Peaks:	1. Carbonate 2. Sulfate 3. Oxalate 4. Phosphate

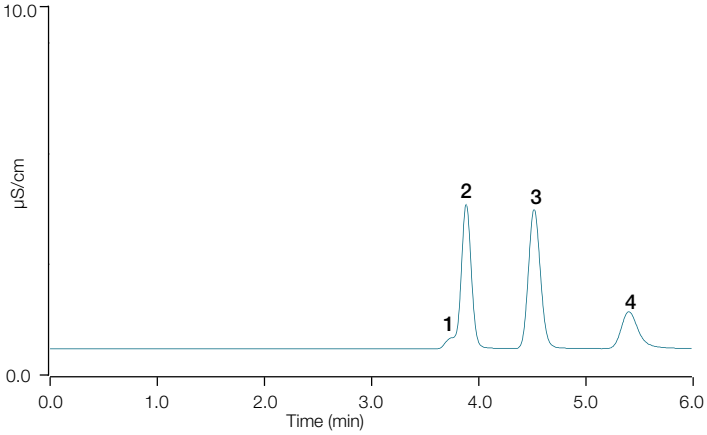

Figure 3. Chromatogram of a solution containing sulfate, oxalate, and phosphate (5 mg/L each) analyzed on a Dionex IonPac AS20 column

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 concentration standards to establish linearity in an assay.[6] 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 the determination of an impurity. In this study, oxalate was calibrated at 10 concentration levels ranging from 0.03 to 10 mg/L. The results yielded a linear relationship of peak area to concentration with a coefficient of determination (r^2) of 0.999 (Table 1). The baseline noise was 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 seven injections. The LOD and LOQ were then set at concentrations that resulted in signal-to-noise ratios of 3 and 10, respectively (Table 1). Figure 4 shows chromatograms obtained using injections of 4 and 10 µg/L of oxalate.

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

Parameter	Value
Linearity (r^2)	0.999
LOD	4 µg/L
LOQ	10 µg/L

Conditions

Column:	Dionex IonPac AG20 Guard, 2 × 50 mm and Dionex IonPac AS20 Analytical, 2 × 250 mm
Eluent source:	Dionex EGC 500 KOH Eluent
Eluent:	30 mM KOH
Flow rate:	0.3 mL/min
Column temp.:	30 °C
Injection volume:	2.5 µL (Full Loop)
Detection:	Suppressed Conductivity
Peaks:	1. Carbonate 2. Oxalate

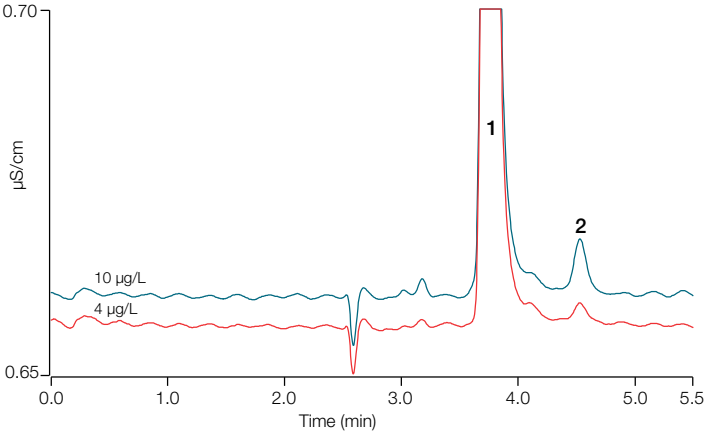

Figure 4. Comparison of 4 and 10 µg/L solutions of oxalate

Table 2. Oxalate recovery studies (n=3)

Base amount (mg/L)	Spiked amount (mg/L)	Spike recovered (mg/L)	Recovery (%)
0.02	0.05	0.049	97.7
	0.3	0.304	101
	1.0	1.05	105

Conditions

Column:	Dionex IonPac AG20 Guard, 2 × 50 mm and Dionex IonPac AS20 Analytical, 2 × 250 mm
Eluent source:	Dionex EGC 500 KOH Eluent
Eluent:	30 mM KOH
Flow rate:	0.3 mL/min
Column temp.:	30 °C
Injection volume:	2.5 µL (Full Loop)
Detection:	Suppressed Conductivity
Peaks:	1. Carbonate 2. Oxalate

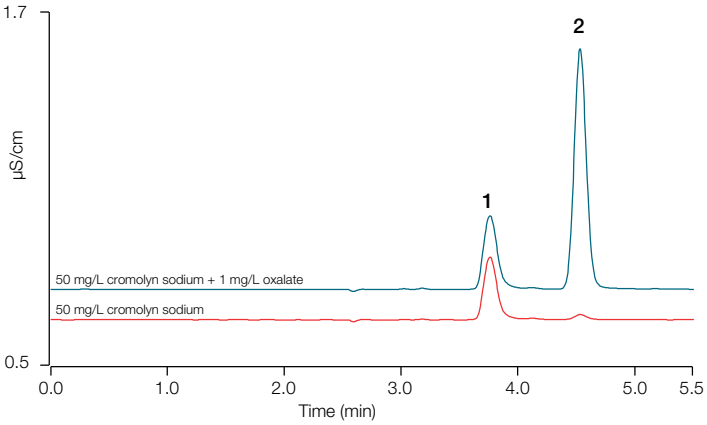

Figure 5. Comparison of 50 mg/L solutions of cromolyn sodium with and without a 1 mg/L oxalate spike

Sample analysis

The USP monograph requires that cromolyn sodium contain NMT 0.35% oxalate.[2] In this study, commercially available sodium oxalate was used to prepare the calibration standards for oxalate. The USP reference standard was used to prepare a 50 mg/L cromolyn sodium solution. The calculated oxalate concentration of the USP reference standard solution was 0.02 mg/L, equivalent to 0.04% oxalate content (Table 2), thus verifying the label claim. This indicates that the method can determine an oxalate concentration below the USP specification.

Accuracy and precision

To test sample accuracy, recovery studies were performed after spiking oxalate samples prepared using the USP Cromolyn Sodium reference standard. Three different spike levels of 0.05, 0.3, and 1 mg/L oxalate were studied, and satisfactory recoveries were obtained for each spike (Table 2). Figure 5 shows representative chromatograms of a 50 mg/L cromolyn sodium sample with and without 1 mg/L oxalate spiked into it.

The assay precision was evaluated by injecting seven replicates at three different concentration levels of 0.1, 1, and 10 mg/L oxalate over four days 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 ≤2.93% (Table 3).

Table 3. Retention time and peak area precisions of oxalate solutions (n=7)

Conc (mg/L)	RSD							
	Day 1		Day 2		Day 4		Day 6	
	RT	Peak area	RT	Peak area	RT	Peak area	RT	Peak area
10	0.03	0.09	0.05	0.21	0.04	1.17	0.03	0.21
1	0.03	1.16	0.05	1.38	0.05	1.21	0	1.42
0.1	0.03	1.38	0.03	2.93	0.05	1.21	0	1.46

Table 4. Robustness of the IC-based assay for oxalate determination performed using a 0.5 mg/L sample containing sulfate, oxalate, and phosphate (n=3)

Method condition	Difference from the standard condition (%)					
	Column 1			Column 2		
	RT	Asymmetry	Resolution (sulfate to oxalate)	RT	Asymmetry	Resolution (sulfate to oxalate)
+10% Flow	8.90	1.83	0.95	8.83	-0.37	1.39
– 10% Flow	-10.8	2.75	-2.17	-11.23	-2.27	-1.60
– 10% Eluent	-11.2	3.52	-14.3	-11.01	-2.34	-11.9
+10% Eluent	7.27	0.92	10.7	7.78	1.10	10.6
+10% Temperature	-3.05	0.21	-3.30	-3.09	-1.10	-5.02
-16.8%** Temperature	5.63	2.18	8.42	5.19	0.22	7.47

**Represents percent difference between 30 °C and average of room temperatures for columns 1 and 2

Robustness

Assay robustness was evaluated by measuring the influence of small variations in procedural parameters (e.g., flow rate, eluent concentration, and column temperature) on the RT, peak asymmetry, and resolution from sulfate peak on two columns from two different lots. The peak asymmetry was measured using the USP formula.[6] A standard injection (0.5 mg/L each of sulfate, oxalate, and phosphate) was made three times (n=3) at each chromatographic condition. Table 4 summarizes the results of the oxalate robustness study. These results indicate that the method is robust and suitable for oxalate determinations.

Conclusion

This study describes an IC-based assay for determination of oxalate in cromolyn sodium. Oxalate was separated on an anion-exchange column and detected by suppressed conductivity in 5.5 min. This method allows the concentration of oxalate to be determined in an automated way rather than performing the cumbersome photometry-based assay. This assay for oxalate 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 oxalate content of cromolyn sodium as per limits set in the USP monograph. Compared to the assay described in the USP Cromolyn Sodium 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 oxalate in the USP monograph and thereby modernize the monograph.

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