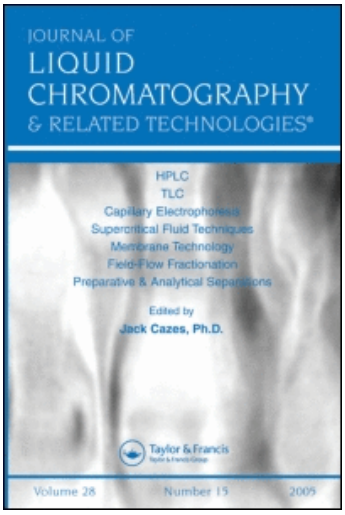

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THE SELECTION OF AN ION PAIRING REAGENT FOR DEVELOPING AND VALIDATING A STABILITY-INDICATING HPLC METHOD FOR CROMOLYN SODIUM AND ITS KNOWN IMPURITIES

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ABSTRACT

A robust, stability-indicating isocratic HPLC method for the quantitation of cromolyn sodium and its related substances was developed and validated. The two known synthetic impurities likely to be present in cromolyn drug substance, designated as impurity 1 and impurity 2, are non-ionic compounds while cromolyn is a weak acid. The described HPLC method exploits the additional selectivity offered by using an ion pairing reagent. Several quaternary amine ion pairing reagents were evaluated for their chromatographic effects on cromolyn and its impurities, i.e., resolution and retention time. The retention time of cromolyn and the two known impurities varies greatly depending on the

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type of ion pairing reagent present in the mobile phase. Myristyltrimethylammonium bromide provided baseline resolution between the impurities and cromolyn within the shortest run time. The chromatographic conditions, such as the organic modifier, pH, temperature, and flow rate, were investigated and adjusted to provide a robust method. Forced degradation studies demonstrated that the method is selective for CROMOLYN, providing a spectrally pure peak that is resolved from all detectable degradation products.

The method is, thereby, stability-indicating. Cromolyn sodium and its related compounds can be quantitated in a single chromatographic run of about 30 min. The limit of quantitation (LOQ) was determined to be 0.05% for cromolyn and the two known impurities. The method was shown to be precise, accurate, and linear in the assay range (i.e., 75–125%; 100% = 0.5 mg/mL) and in the related substances range (i.e., 0.05–1.0%). The validation data is summarized below:

Assay Range: 75–125% (100% = 0.5 mg/mL of Cromolyn Sodium, anhydrous basis)

Validation Parameter	*Result*
Repeatability (n = 6)	RSD = 0.3%
Intermediate Precision (n = 18)	RSD = 0.3%
Accuracy (n = 12)	Recovery = 99.5%; RSD = 0.3%
Linearity	r = 1.0000; bias = 1.4%

Related Substance Range: 0.05–1.0%

Validation Parameter	*Result*
Accuracy – Cromolyn (n = 10)	Recovery = 101.2%; RSD = 9.6%
Accuracy – Impurity 2 (n = 9)	Recovery = 100.2%; RSD = 2.0%
Linearity – Cromolyn	r = 0.998; bias = 1.7%
Linearity – Impurity 2	r = 1.000; bias = 1.4%

INTRODUCTION

Cromolyn sodium is an anti-inflammatory reagent for the preventative management of asthma. The drug acts by inhibiting both the immediate and

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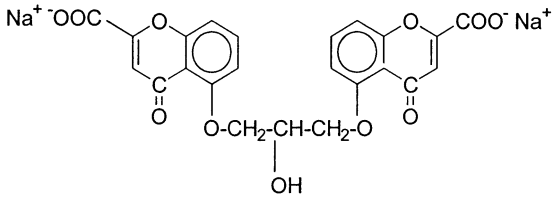
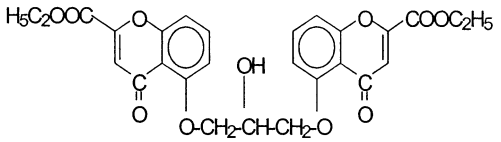
non-immediate bronchoconstrictive reactions to inhaled antigens. This compound is used in many different formulations such as nasal solutions, ophthalmic solutions, etc. Cromolyn sodium has the following empirical formula ($C_{23}H_{14}Na_2O_{11}$) with a molecular weight of 512.34 gm/mole. The compound is a water soluble, hydrated crystalline powder that is hygroscopic.[1] The raw material and solubilized drug are sensitive to light. Cromolyn sodium and its two known potential impurities have the following structures:



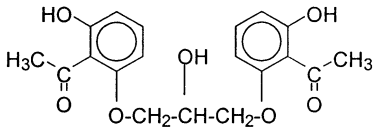
CROMOLYN SODIUM

4H-1-benzopyran-2-carboxylic acid 5,5'-((2-hyroxy-1.3-propanediyl bis(oxy)) bis[4-oxo,disodium salt]



IMPURITY 1 (briefly DEC)

Diethyl-4,4'-dioxo-5,5'-(2-hydroxytrimethylendioxy)di(chromene-2-carboxylate) ester



IMPURITY 2 (briefly 1,3 Bis)

1,3-bis(2-acetyl-3-hydroxyphenoxy)-2-propanol

The current USP methodology does not meet current regulatory requirements for assay and related substances.[2] The USP monograph for cromolyn sodium uses a non-selective UV method for assay and a TLC limits test for related substances. There are several methods published in the literature for determining cromolyn using a variety of conditions and methods of detection from a variety of matrices.[3–6] An HPLC method for determining cromolyn sodium and cromolyn sodium related substances is published in the literature, which uses tetrabutylammonium dihydrogenphosphate as an ion pairing reagent. This method was a stability indicating assay that was also accurate and precise for determining related substances at the 0.05% w/w level. However, there was a large retention time difference between cromolyn and its two known impurities, resulting in a chromatographic run time of about 90 min.[7]

This manuscript describes the development and validation of an isocratic reversed-phase HPLC method for assay for cromolyn sodium and its related compounds with a reduced run time. Several quaternary amine ion pairing reagents were evaluated using a preliminary gradient method. The ion pairing reagent that minimized the retention time differences between cromolyn and its two known impurities was selected. Ion pairing reagents do not significantly affect the cromolyn impurities since these compounds are neutral. cromolyn is ionizable and, thereby, the ion pairing reagent can alter its retention time. Method development investigated mobile phase composition, especially ion pairing reagent concentration; and the validated method was shown to be sensitive, accurate, reproducible, and stability-indicating for the determination of cromolyn sodium, its two known impurities, and degradation products in cromolyn sodium drug substance.

EXPERIMENTAL

Chemicals and Reagents

Cromolyn sodium was available as a USP Reference Standard, Lot J. European Manufacturers Associated, Inc. Whitehouse, N.J, supplied the two known impurities. Ion pairing reagents tetrabutylammonium bromide (TBA), dodecyltrimethylammonium bromide (DTA), myristyltrimethylammonium bromide (MTA), hexadecyltrimethylammonium bromide (HTA), and tetraheptylammonium bromide (THA) were purchased from Aldrich Chemical Company, Milwaukee, WI, USA. HPLC grade acetonitrile, KH_2PO_4 and methanol were purchased from Fisher, Norcross, Georgia. The HPLC grade water was deionized and distilled by a Barnstead E-Pure System.

Apparatus

The HPLC chromatographic system consisted of a Hewlett Packard model 1050 solvent pumping system, variable wavelength UV-visible absorbance detector set at 326 nm, variable volume injector.

A Zorbax Stable Bond^{R} Octyl (SB-C_8) column 5 micron (4.6 × 150 mm) was maintained at 40°C. The flow rate was approximately 2.0 mL/min with a typical operating pressure of 225 bar (approximately 3300 psi). Samples and standards were analyzed by injecting 20 µL of the preparations at approximately 0.5 mg/mL onto the chromatographic system. The run time is 30 min.

Data Acquisition

The peak areas of cromolyn sodium and its related substances were measured using Hewlett Packard 3D ChemStation Software (version A.06 and A.07). The chromatographic data was automatically processed for peak area.

Preparation of Solutions

Mobile Phase

For 2 L, add approximately 20 g of MTA and 5 g of KH_2PO_4 to 900 mL of water and pH the solution to 6.5 ± 0.1 with sodium hydroxide (NaOH) or hydrochloric acid (HCl). Add 1100 mL of methanol, mix well, filter through 0.45 µm filter, and degas. The mobile phase contains 55% methanol, 30 mM MTA, and 20 mM KH_2PO_4.

Diluent

Mix acetonitrile and water in a ratio of 30 : 70 and mix well. Acetonitrile is used in diluent to prevent the formation of mixed esters of impurity[1] that would form if methanol were used in the diluent.[7]

Standard Preparation

Determine the amount of water in cromolyn sodium reference standard using suitable means. Accurately weigh cromolyn sodium, USP reference standard and dilute to volume with diluent to yield a final anhydrous concentration of about 0.5 mg/mL.

Sample Preparation

Determine the amount of water in cromolyn sodium drug substance by suitable means. Accurately weigh cromolyn sodium drug substance and dilute to volume with diluent to yield a concentration of about 0.5 mg/mL. This sample will be used to determine assay and related substances.

Specificity

The sample preparation was subjected to various stress conditions as a solution in diluent. The specificity of the method was established through the analysis of a control solution (unstressed sample preparation) and stressed sample preparations. The sample preparation was subjected to thermal, photolytic (i.e., light), basic, and oxidative stress conditions. Acid stress was not conducted since cromolyn precipitates from solution under acidic conditions (pH < 4).

Basic stress samples were prepared by adding an 8 mL aliquot of the control sample preparation into a 10 mL volumetric flask and 1 mL of 0.1 N NaOH was added to the solution. After approximately 15 hours at about 50°C, the solution was neutralized with 1 mL of 0.1 N HCl and analyzed. Oxidatively stressed samples were prepared by adding 1 mL of 30% hydrogen peroxide to a 10 mL volumetric flask and diluting to volume with control sample preparation. The solution was analyzed after approximately 15 hours at about 50°C. The thermally stressed samples were stored at 50°C for about 209 hours (~ 9 days). Photolytic stress was conducted in an Atlas Electric Devices Co. SunTest CPS + . The source was a xenon arc lamp filtered (quartz filter, glass filter, and ID65 filter) so the output is similar to the ID65 emission standard ICH Option 1 for forced degradation studies.[8–10] The sample preparation was placed in teflon capped quartz cells and exposed to the source at an irradiance setting of 765 watts/m^2 for twenty minutes (Total exposure was 23 watt hours/m^2 of UV radiation and 56.6 Klux hours of Visible radiation). A sample preparation in a teflon capped quartz cell wrapped in aluminum foil served as the dark control.

System Suitability

The system suitability results were calculated according to the USP 24 ⟨621⟩ from typical standard chromatograms. The instrument precision, as determined by six injections of the standard preparation, should provide a relative standard deviation (RSD) of NMT 1.0%. The tailing factor should not exceed 2.0 at 5% peak height. The capacity factor specification for cromolyn should be between 10 and 17, which is also supported by robustness experiments. The standard

Table 1. Summary of System Suitability Results

Run	Column No.	%RSD ($n = 6$)	Tailing Factor	Cromolyn Capacity Factor (k′)
080500A.s	C222	0.1	1.2	15.7
CR080500.s	C220	0.6	1.1	15.6
CR08260A.s	C220	0.1	1.1	16.2
CR09230A.s	C222	0.1	1.2	14.9

agreement (ratio of response factors $\times 100\%$) between duplicate standard preparations was set at 98.0–102.0% based on precision results. Table 1 contains representative data summarizing some system suitability results, from which, system suitability specifications listed above were derived.

RESULTS AND DISCUSSION

Method Development

Wavelength Determination

Photo diode array detection was investigated from 190 to 500 nm. Cromolyn sodium and impurity 1 exhibited a λ_{max} absorbance at 326 nm. Impurity 2 exhibited a λ_{max} absorbance at 340 nm.[7] The detection wavelength of 326 nm was chosen for method validation and the relative response factor (RRF) was determined for impurity 2.

Relative Response Factor

The relative response factors for impurity 1 and impurity 2 are 1.00 and 0.61, respectively. Impurity 2 has a different chromophor than cromolyn sodium. The relative response factor for impurity 2 was determined to be 0.61 relative to cromolyn sodium at 326 nm. Impurity 1 is quite similar in structure to cromolyn sodium differing in molecular weight by only 2% and the chromophor is unchanged. Thus, a relative response factor of 1.0 was assigned to this impurity. In addition, the determination of the RRF for impurity 1 would be complicated since impurity 1 readily hydrolyzes to cromolyn.

Injection Volume Determination

Injection volume was evaluated to determine the effect on chromatographic detection at or below 0.05% (100% = 0.5 mg/mL). Method development was performed with an injection volume of 20 µL. A 20 µL injection of a 0.0025mg/mL sample preparation resulted in a signal-to-noise of 18 : 1, exceeding the ICH requirements for LOQ.[11] Cromolyn sodium possesses a very strong chromophor. Hence, a 20 µL injection was selected for validation.

Cromolyn and its known potential impurities are vastly different with regards to lipophilicity and solubility. Cromolyn sodium is ionic and hydrophilic, while its known potential impurities are nonionic and hydrophobic. Zorbax SB-C8 columns were used in all method development and validation experiments presented in this paper.

To obtain the optimum chromatographic conditions for the analysis of cromolyn sodium and its related substances, various ion pairing reagents were evaluated using a preliminary gradient HPLC method (Fig. 1). The ion pairing reagents are quaternary amines that are positively charged regardless of solution pH. A separate sample of impurity 1 was also evaluated using the various ion-pairing reagents and the gradient HPLC method to determine which ion-pairing reagents minimized the retention time difference between cromolyn and impurity 1 (Table 2).

Based on the gradient HPLC method, DTA and MTA appeared to be the best candidates for minimizing the retention time differences between cromolyn and impurity 1. Using DTA, cromolyn elutes prior to impurity 1 while impurity 1 elutes prior to cromolyn using MTA. A preliminary isocratic method was subsequently developed to better evaluate DTA and MTA. The mobile phase contained 35% acetonitrile and 20 mM KH_2PO_4 at pH 6.5 with a flow rate of 1.5 mL/min. The concentration of DTA and MTA was 30 mM and the column temperature was maintained at 40°C. Cromolyn co-eluted with impurity 2 using DTA (Fig. 2) while cromolyn and the two known impurities were baseline resolved using MTA (Fig. 3). The chromatograms show that MTA provided the best separation between cromolyn and its impurities without requiring a long chromatographic run time; therefore, MTA was selected as the ion pairing reagent.

Robustness

Using the preliminary isocratic HPLC method and a cromolyn sodium sample preparation, the concentration of MTA in the mobile phase was varied to determine a suitable concentration of ion pairing reagent for the method. The MTA concentrations that were investigated were 5, 10, 20, 30, and 35 mM. The concentration of MTA

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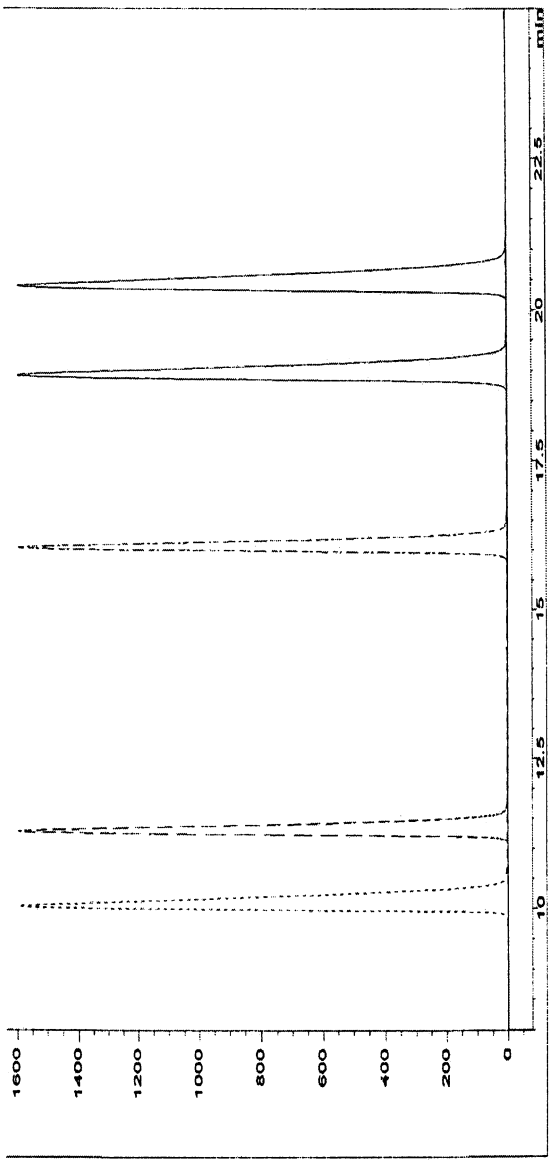

Figure 1. Chromatographic overlays of cromolyn showing the retention time increase with increasing molecular weight of ion pairing reagent added to the mobile phase. Ion pairing reagent in increasing retention time: (1) TBA; (2) DTA; (3) MTA; (4) HTA; and (5) THA. Other conditions: Flow rate = 1.0 mL/min; Column temp. = 45°C; Gradient = 0–70% ACN in 30 min.

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Table 2. The Effect of the Ion Pairing Reagents on the Retention Times of Cromolyn and Impurity 1

Ion Pairing Reagent Concentration = 30 mM	Retention Time (Minutes)	
	Cromolyn	Impurity 1
TBA	10.1	13.0
DTA	11.5	12.3
MTA	16.1	14.8
HTA	19.0	16.0
THA	20.7	14.6

mostly affected the cromolyn tailing factor and retention time. A new column was used in these experiments and the data is summarized below in Table 3.

The data in Table 3 shows that there is no significant change on the cromolyn chromatography using MTA concentrations between 20 and 35 mM. Since there was some difference between 10 and 20 mM MTA concentrations, 30 mM MTA was selected as the most appropriate concentration of ion pairing reagent in the mobile phase.

The effects of column temperature, mobile phase pH, and percent and type organic modifier were evaluated using a cromolyn sodium sample preparation spiked with 1% impurity 1 and 1% impurity 2. In all robustness experiments, the capacity factor, cromolyn tailing, resolution between impurity 1 and impurity 2 and the resolution between cromolyn and impurity 2 were determined. The retention time and resolution between cromolyn and its impurities were significantly affected by small changes in the acetonitrile concentration. The results of those experiments are shown below in Table 4. A slight excess of acetonitrile in the mobile phase causes cromolyn to co-elute with impurity 2. Methanol was subsequently evaluated as the organic modifier. The flow rate was increased from 1.5 to 2.0 mL/min using 55% (v/v) methanol (Fig. 4). The methanol content was varied at 52%, 55%, and 58%. The results of those experiments are shown in Table 5. Cromolyn remains well resolved from its impurities with small changes in the methanol content in the mobile phase. Methanol provided better separation, ultimately making the method more robust.

Once methanol was selected as the organic modifier, the effects of temperature and mobile phase pH were evaluated using the same spiked sample preparation. Temperature was adjusted from 35°C to 45°C. Other conditions for these experiments were as follows: mobile phase pH 6.5, 55% methanol, and flow rate of 2.0 mL/min. The results of those experiments are shown in Table 6. Mobile phase pH was then adjusted to 6.3, 6.5, and 6.8. Other conditions for

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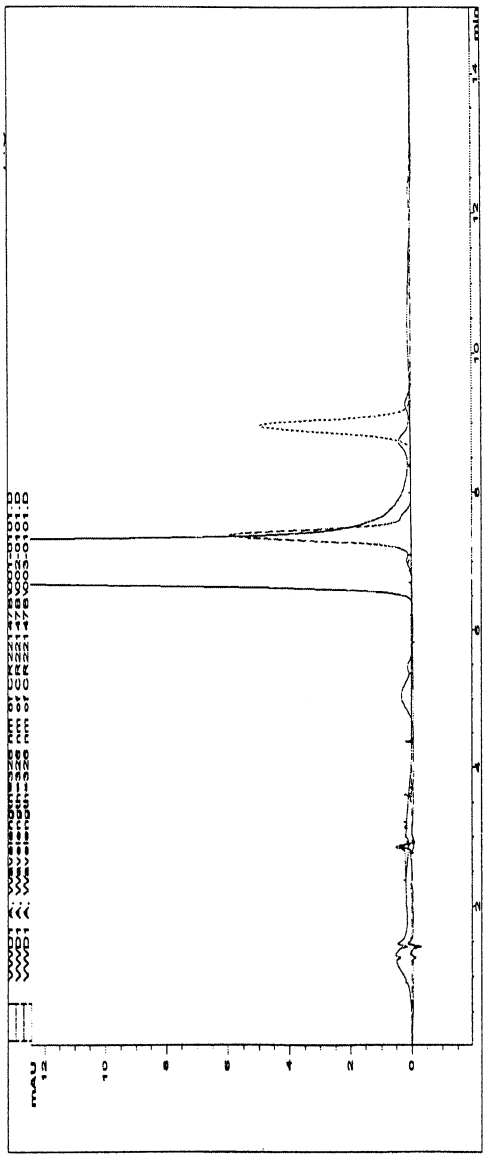

Figure 2. Chromatographic overlay of cromolyn, impurity 1, and impurity 2, using DTA as the ion pairing reagent and 35% ACN. Cromolyn co-elutes with impurity 2 and is resolved from impurity 1.

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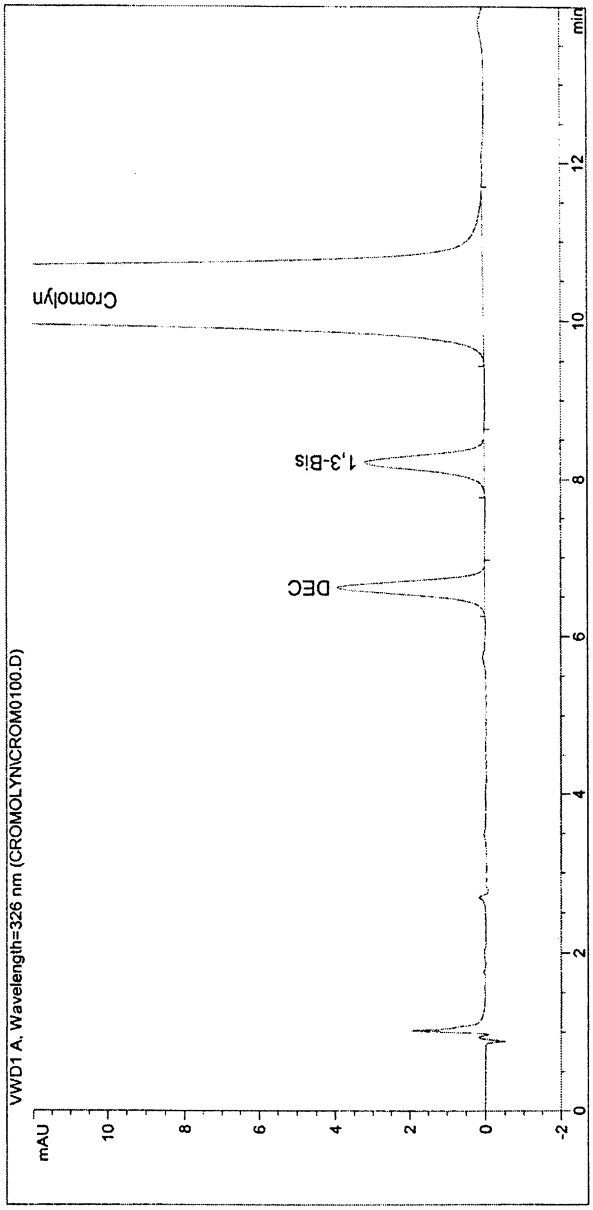

Figure 3. A chromatogram of cromolyn, impurity 1 (DEC) and impurity 2 (1,3-Bis) using MTA as the ion pairing reagent and 35% ACN.

Table 3. The Effect of MTA Concentration on Cromolyn Chromatography

MTA Concentration (mM)	Cromolyn Retention Time (Minutes)	Cromolyn Tailing Factor
5	4.3	0.83
10	6.5	0.95
20	8.5	1.21
30	8.7	1.23
35	8.5	1.17

these experiments were as follows: column temperature 40°C, 55% methanol, and flow rate of 2.0 mL/min. The results of those experiments are shown in Table 7. The data demonstrates that temperature and mobile phase pH have little effect on the quality of the chromatography.

Specificity

A cromolyn sodium sample preparation was stressed by thermal, basic, oxidative, and photolytic stress. Cromolyn degraded under stress conditions of base (Fig. 5), photolysis (Fig. 6), and peroxide (Fig. 7). Cromolyn was stable under thermal stress conditions. Photodiode array analysis indicated that the cromolyn peak was spectrally pure. The results of the stress studies are presented in Table 8. The base and peroxide stress samples were stored at 50°C to facilitate degradation. These samples experienced about 50% degradation in less than 1 day, while the thermally stressed sample (i.e., control sample placed at 50°C) experienced less than one percent degradation after 9 days. Therefore, the

Table 4. Robustness Summary Using ACN as the Organic Modifier

			Resolution R	
% ACN (v/v)	Cromolyn k′	Cromolyn Tailing Factor	Imp. 1 and Imp. 2	Crom. and Imp. 2
30%	27.0	1.5	5.8	10.8
33%	17.9	1.4	5.4	8.2
35%	11.1	1.3	4.9	4.8
40%	5.9	0.7	NA	Co-elution

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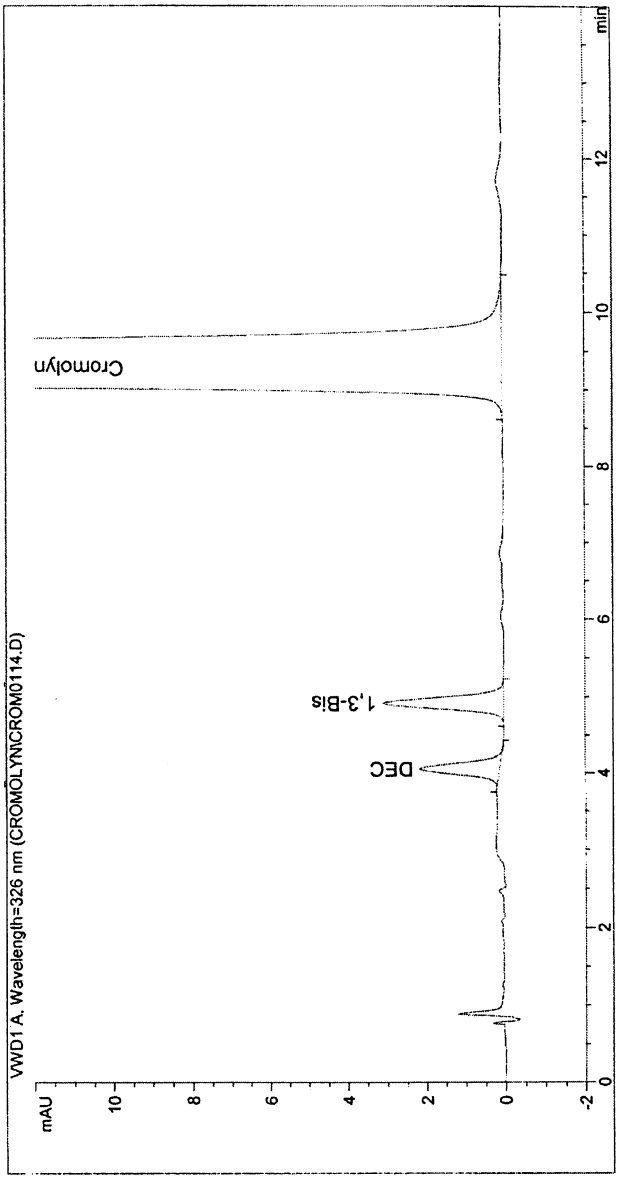

Figure 4. A chromatogram of cromolyn, impurity 1 (DEC) and impurity 2 (1,3-Bis) using MTA as the ion pairing reagent and 55% methanol.

Table 5. Robustness Summary Using Methanol as the Organic Modifier

			Resolution R	
% MeOH (v/v)	Cromolyn k′	Cromolyn Tailing Factor	Imp. 1 and Imp. 2	Crom. and Imp. 2
52%	15.7	1.3	4.2	10.7
55%	13.7	1.2	3.1	11.3
58%	10.6	1.2	2.7	11.2

degradation observed in the base and peroxide stress samples was due to the stress reagent added. Based on these results, the method is stability-indicating.

Validation

Repeatability/Intermediate Precision

The precision (repeatability and intermediate precision) of the method was determined from one lot of drug substance (cromolyn sodium, USP reference standard, Lot J). Six sample preparations were prepared and analyzed by chemist I, with HPLC System 1. The RSD of the six results for the cromolyn assay was 0.3%. Intermediate precision was evaluated by chemist I, with HPLC System 1, on a second day and by chemist II, with HPLC System 2 on day 1. Each chemist independently prepared and analyzed another six sample preparations using the same lot of drug substance. The intermediate precision was 0.3% ($n = 18$) for the assay. The low scatter in the data supports the high degree of ruggedness for this analytical method (Table 9).

Table 6. Effects of Column Temperature on Cromolyn Chromatography

			Resolution R	
Column Temp. (°C)	Cromolyn k′	Cromolyn Tailing Factor	Imp 1. and Imp. 2	Crom. and Imp. 2
35	14.8	1.1	4.3	10.8
40	13.7	1.2	3.1	11.3
45	11.9	1.1	1.7	12.0

Table 7. Effect of Mobile Phase pH on Cromolyn Chromatography

			Resolution R	
Mobile Phase pH	Cromolyn k′	Cromolyn Tailing Factor	Imp. 1 and Imp. 2	Crom. and Imp. 2
6.3	13.7	1.2	3.2	11.3
6.5	13.7	1.2	3.1	11.3
6.8	13.5	1.3	3.2	11.0

Accuracy

Three independent sample preparations were prepared at 75% and 125% of the target analytical concentration (i.e., 0.5 mg/mL) to demonstrate precision and accuracy at these levels (Table 10). Accuracy at 100% was demonstrated by the precision experiments. Accuracy in the related substance range for cromolyn and impurity 2 was established by preparing at least three sample preparations at 0.05, 0.5, and 1% (Table 11). In addition, impurity 2 was spiked into a sample to establish the recovery of the impurity in the presence of cromolyn at 100% (Table 12). Accuracy determinations were not attempted using impurity 1 since it is hydrolyzed to cromolyn, which would complicate the accuracy determination of the method using this impurity.

Linearity

A linear response in peak area was observed for cromolyn over the range of 75% to 125% of the analytical working concentration for assay and over the range of 0.05% to 1.0% of the analytical working concentration for cromolyn and impurity 2. The correlation coefficient, *r*, for each experiment was 1.0000, 0.998, and 1.000, respectively. The percent bias was less than 2.0% for both the assay range and related substance range (Table 13). The ratios of the slopes from the assay range and the related substance range for cromolyn was 0.99.

Range

The range of the method is 0.05 to 1.0% for cromolyn related substances and 75 to 125% for cromolyn since the method has been shown to be specific, precise, accurate, and linear within these ranges.

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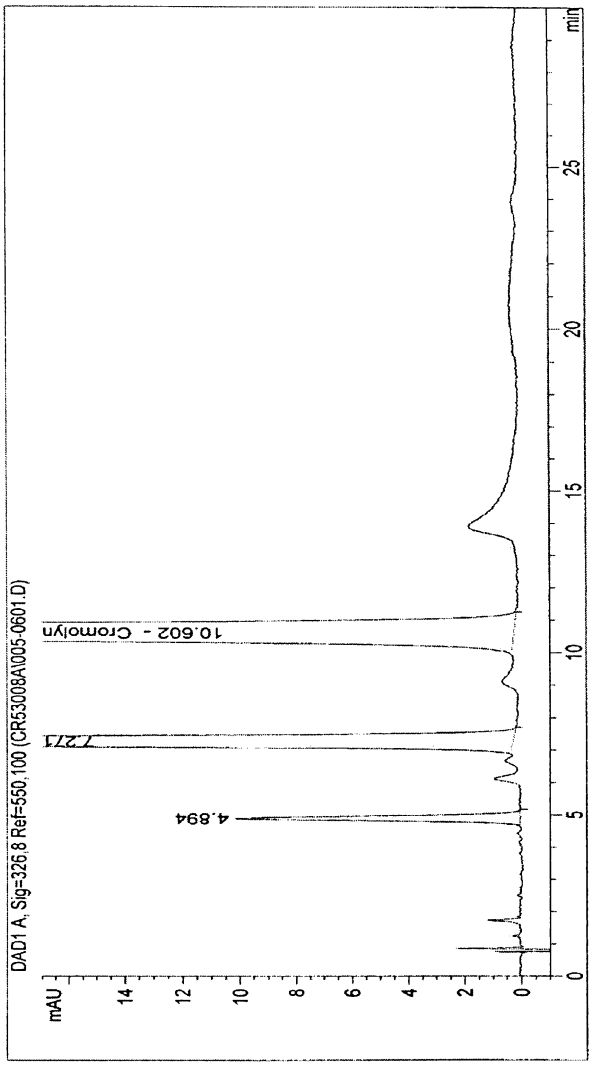

Figure 5. A chromatogram of a base stressed sample preparation.

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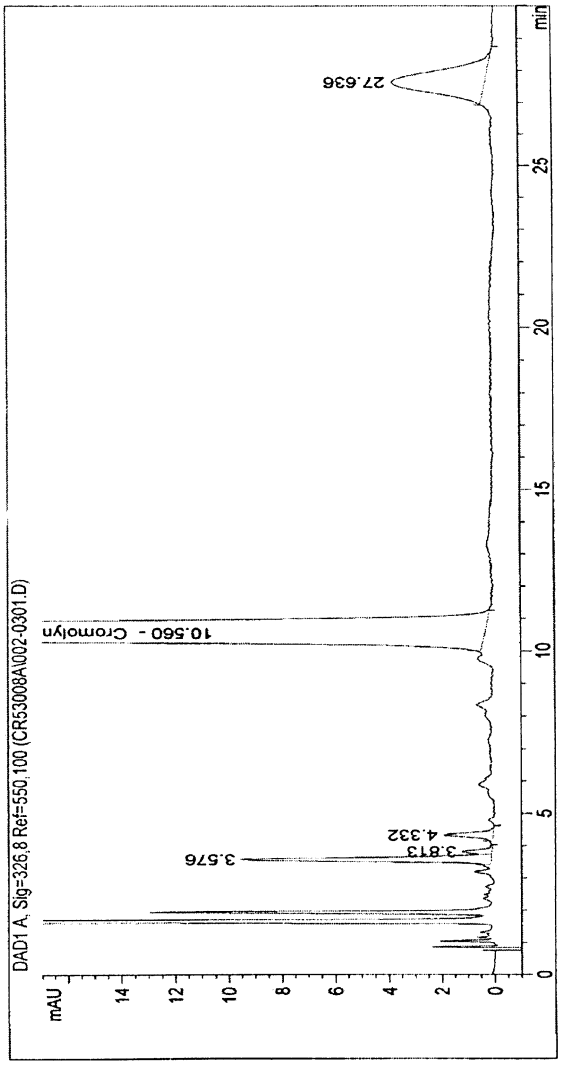

Figure 6. A chromatogram of a photolytic stressed sample preparation.

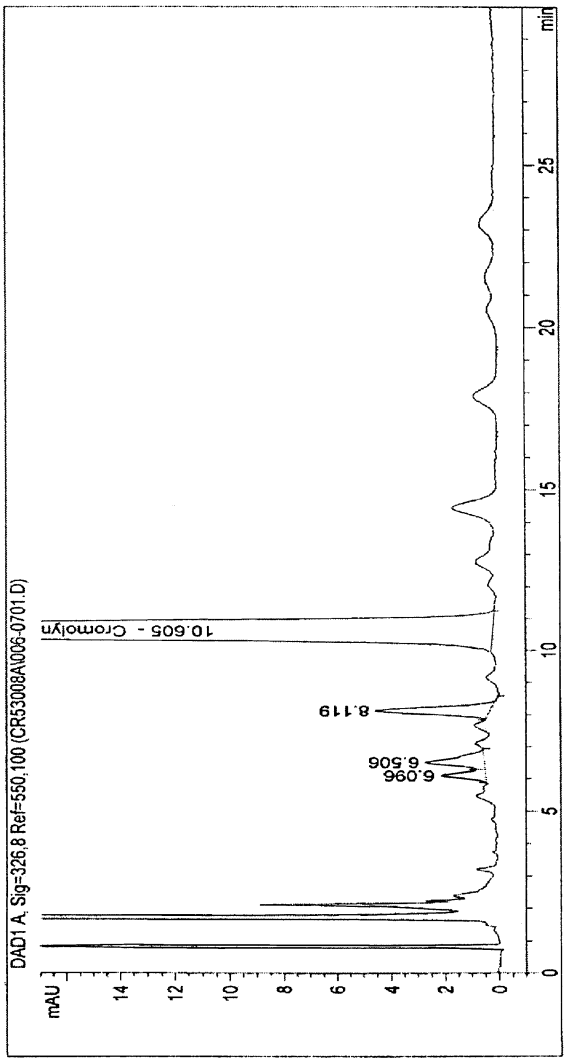

Figure 7. A chromatogram of a peroxide stressed sample preparation. Note: The peak at about 9.1 min is less than 0.1%.

Table 8. Specificity Results

Stress Conditions	Peak Purity Factor*	R to Closest Degradation Product ≥ 0.1%	Percent Degradation
Control	999.487	N/A	0
Photolytic: 23 watt-hrs/m^2 UV (300–400 nm) 56 Klux-hrs of Visible (400–800 nm)	999.657	15.3	21
Dark control (same conditions as above)	999.672	N/A	0.8
Thermal 50°C, ~209 Hours	999.463	N/A	0.5
Base (0.01N NaOH) 50°C, ~15 hours	999.549	2.3	53.2
Peroxide (3% v/v) 50°C, ~15 hours	999.587	5.1	52.0

*Peak purity factor > 990 considered spectrally pure from λ 220–400.

Table 9. Precision Results for the Cromolyn Assay

Analysis	Assay Results (% w/w)	
Repeatability		
Day 1	98.94	99.75
Chemist 1	99.15	99.81
HPLC System 1	99.84	99.51
Column #220	% RSD = 0.3	
Intermediate Precision		
Day 2	99.53	98.29
Chemist 1	99.66	99.45
HPLC System 1	99.35	99.75
Column #222	% RSD = 0.5	
Intermediate Precision		
Day 1	99.74	99.89
Chemist 2	99.96	100.17
HPLC System 2	99.94	99.93
Column #220	% RSD = 0.1	
Intermediate Precision (n = 18)	% RSD = 0.3	

Table 10. Accuracy Results for Cromolyn in the Assay Range

Sample Concentration (%)*	Average Recovery (%)
75 ($n = 3$)	99.2 (RSD = 0.5%)
100 ($n = 6$)**	99.5 (RSD = 0.3%)
125 ($n = 3$)	99.9 (RSD = 0.1%)
Overall ($n = 12$)	99.5 (RSD = 0.3%)

*Working analytical concentration for assay is 0.5 mg/mL.
**Repeatability results, Table 9.

Table 11. Accuracy Results for Cromolyn and Impurity 2 in the Related Substance Range

	Average Recovery (%)	
Sample Concentration (%)*	Cromolyn	Impurity 2
0.05 ($n = 3$)	93.2	100.2
0.5 ($n = 3$)	111.0	100.6
1.0 ($n = 4$)	99.9	100.0
Overall ($n = 10$)	101.2 (RSD = 9.6%)	100.2 (RSD = 2.0%)

*Relative to 0.5 mg/mL cromolyn sample (100% = 0.5 mg/mL).

Stability of Solutions

The stability of analytical solutions was determined for the drug substance preparation at 0.5 mg/mL (assay). The sample preparation also served as the standard preparation since a USP reference standard was used as the drug

Table 12. Recovery of Impurity 2 at 0.05% (LOQ) in the Presence of Cromolyn at 100%

Sample Preparation Number	Recovery (%)
1	102.2
2	104.7
3	107.6
Average and RSD (n = 3)	104.8 (RSD = 2.6%)

Table 13. Linearity Results for Assay and Related Substances

Concentration (%)	Peak Area Response	Linearity (Peak Area vs. Conc. %)
Assay Range*		
124.26	6108.58008	$m = 49.80$
111.83	5507.02393	$r = 1.0000$
99.41	4893.05908	y-intercept $= -70.54$
86.98	4250.45947	bias = 1.4% (at 100%)
74.56	3642.55786	
Related Substance Range* For Cromolyn		
0.0458	2.08419	$m = 49.36$
0.0917	4.62139	$r = 0.998$
0.458	24.74179	y-intercept $= 0.42$
0.917	44.87897	bias = 1.7% (at 0.5%)**
Related Substance Range For Impurity 2		
0.05	1.64579	$m = 32.93$
0.10	4.18019	$r = 1.000$
0.49	17.04254	y-intercept $= 0.46$
0.97	33.31979	bias = 1.4% (at 0.5%)**

*Nominal analytical concentration is approximately 0.5 mg/mL, corrected for water.
**Current specification for impurities in the USP monograph.

Table 14. Solution Stability Results

Sample	Time (Days)	Assay (% w/w)	Difference (Day 0–Day 3)
Precision Sample #6	0	99.51	−0.76
	3	100.24	

Table 15. LOQ Results

Sample	S/N Ratio
Impurity 2	26
Cromolyn	18

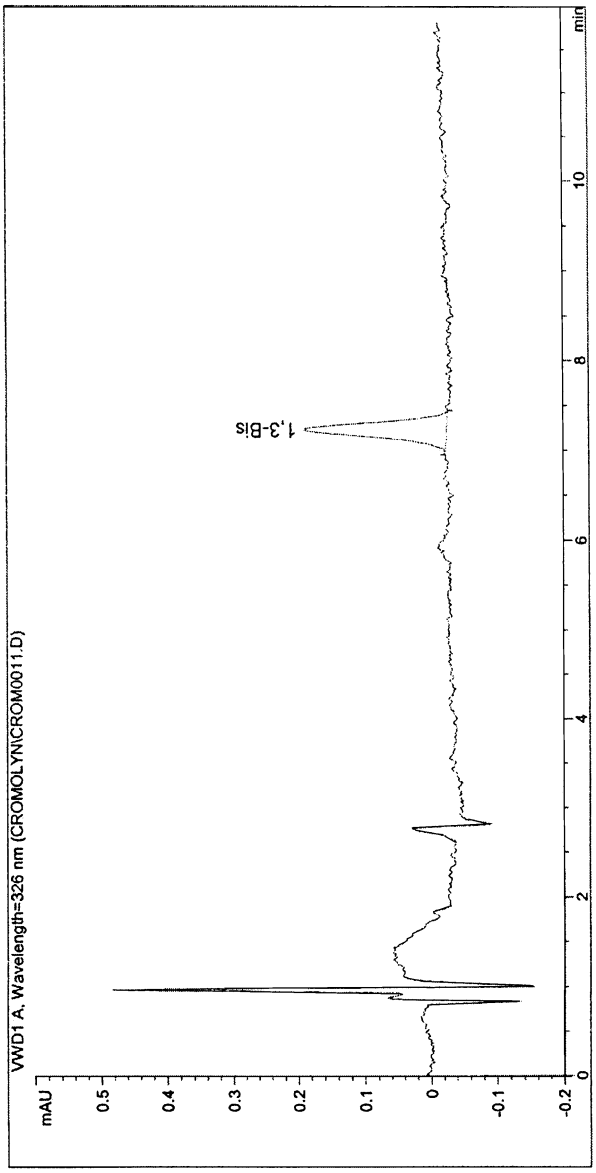

Figure 8. A chromatogram showing impurity 2 (1,3-Bis) at LOQ (0.05%).

substance. The repeatability sample number six was used to evaluate solution stability. After three days, this solution was assayed against a fresh standard preparation. The assay results did not change by more than 2.0% over a three-day period of time. The standard and sample preparations are considered stable at room temperature for up to 3 days in clear glassware (Table 14). No new degradation products greater than 0.05% were observed in the three-day-old sample. Thus, the standard preparation and sample preparation for assay and related substances are stable for three days in clear glassware under ambient laboratory conditions of light and temperature.

Limit of Quantitation

The LOQ was determined by using a signal to noise ratio (S/N) not less than NLT.[11] A preparation of 0.05% of the analytical working concentration (0.5 mg/mL = 100%) of cromolyn exhibited an S/N of 18. The S/N for 0.05% for impurity 2 was 26 (Table 15 and Fig. 8). The LOQ for impurity 1 was not determined since the compound is not stable in diluent; however, since impurity 1 is structurally similar to cromolyn, the LOQ for impurity 1 is assumed the same and assigned 0.05%.

CONCLUSION

An isocratic reverse phase HPLC method was developed based on the evaluation and selection of an ion pairing reagent that would separate cromolyn from its two known impurities within a 30 min chromatographic run time. Robustness experiments were conducted to establish the best mobile phase composition to provide a robust method. The resulting optimized parameters were 20 mM KH_2PO_4, 30 mM MTA, 55% methanol, pH of 6.5, and a column temperature of 40°C using a Zorbax C-8, 5 μm, 4.6 × 150 mm HPLC column. The method was shown to be robust in regards to small changes in methanol concentrations, pH, and column temperature. Forced degradation studies demonstrated that the method was stability-indicating and the LOQ of the method was determined to be 0.05% for cromolyn and its related substances. The validation data shows that cromolyn sodium and its related substances (impurities and degradation products) can be accurately quantitated in a single chromatographic run of 30 min.

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