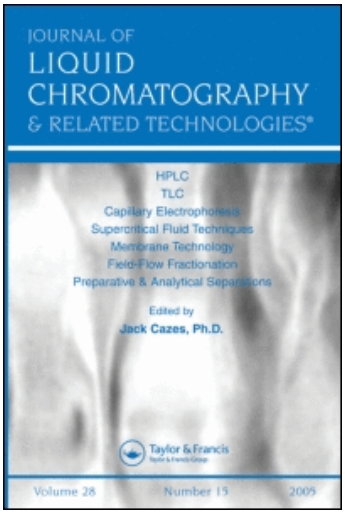

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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING HPLC METHOD FOR THE DETERMINATION OF CROMOLYN SODIUM AND ITS RELATED SUBSTANCES IN CROMOLYN SODIUM DRUG SUBSTANCE AND CROMOLYN SODIUM INHALATION SOLUTION, 1.0%

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ABSTRACT

An HPLC method was developed and subsequently validated for the quantitation of cromolyn sodium and its related substances in cromolyn sodium drug substance and Cromolyn Sodium Inhalation Solution, 1.0%. The current USP monograph for cromolyn sodium assay is a non-selective UV method while related substances are determined by TLC. The TLC method, as written in the USP, does not meet the sensitivity requirements as dictated by current regulations. The HPLC method described in this paper provides more accurate and selective quantitation of cromolyn sodium and its two known potential impurities, cromolyn diethyl ester (Impurity 1) and hydroxy phenoxy 2-propanol (Impurity 2). The method development involved the evaluation of several factors including mobile phase composition, column choice and configuration, wavelength evaluation, and response factors.

Chromatographic parameters include a mobile phase of methanol / buffer (45/55; v/v) and a Nova-Pak C_8, 3.9 x 150 mm column, maintained under ambient conditions. The wavelength of choice to maximize the detection of the related substances was 326 nm. Relative response factor for Impurity 2 was determined to be 0.58. Impurity 1 is not stable in an aqueous environment and eventually hydrolyzes to cromolyn sodium. The structural difference between cromolyn sodium and Impurity 1 is insignificant; therefore, a relative response factor of 1.0 was assigned to Impurity 1.

The described method is linear, reproducible, accurate, and selective over a range of 0.05% - 2.0% of the working analytical concentration (1 mg/mL) for related substances and 46% - 137% of the analytical working concentration (0.2 mg/mL) for assay.

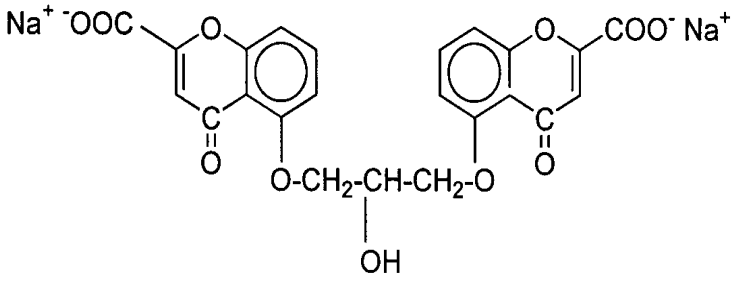
The method precision, relative standard deviation (RSD), among 6 independent samples was not more than 0.4% for the assay and not more than 4.0% for the related substances. Repeatability at the 0.05% (0.0005 mg/mL, n=3) was 2.3%. The intermediate precision was 0.8% (n=18) for assay and 7.8% (n=18) for related substances. The mean absolute recovery for the cromolyn assay between 46 - 137% was 99.9%. The mean absolute recovery for the cromolyn related substance range of 0.05 - 2.0% was 98.5%.

Selectivity was evaluated by subjecting the Drug Substance Sample Preparation for assay (0.2 mg/mL) to thermal, basic, oxidative, and UV stress conditions. Cromolyn precipitates under acidic conditions where the pH < 2.0. No significant interference in the analysis of degradation products and impurities was observed. Cromolyn sodium was very sensitive to base and light stress. Consequently, the validated method for the determination of cromolyn sodium and its related substances in cromolyn sodium drug substance and Cromolyn Sodium Inhalation Solution, 1.0% is regarded as stability-indicating.

INTRODUCTION

Cromolyn sodium is an inhaled anti-inflammatory agent for the preventative management of asthma. The drug acts by inhibiting both the immediate and non-immediate bronchoconstrictive reactions to inhaled antigens. Cromolyn sodium has the following empirical formula ($C_{23}H_{14}Na_2O_{11}$) with a molecular weight of 512.34 grams per mole.

The drug is a water soluble, hydrated crystalline powder that is very hygroscopic. The raw material and solubilized drug is sensitive to light. Cromolyn sodium has the following structure:



Cromolyn Sodium

(4H-1-benzopyran-2-carboxylic acid 5,5'-((2-hydroxy-1-3-propanediyl) bis(oxy)) bis[4-oxo,disodium salt])

Cromolyn Sodium Inhalation Solution, 1.0% is formulated in a sterile, preservative-free solution. The drug product is packaged in single use containers composed of low density polyethylene. Each unit dose container contains 20 mg of cromolyn sodium in 2 mL of purified water.

Current USP methodology is inadequate for assay and related substances.[1] The USP monograph for cromolyn sodium uses a UV method for assay and a TLC method for related substances with a sensitivity of 0.5% (w/w).

Current regulatory requirements dictate that unknown related substances at the 0.1% level to be monitored and identified. The methodologies as described in the current USP do not meet this current regulatory requirement for unknown related substances in drug substances and drug products.

This manuscript describes the development and validation of an isocratic reversed-phase HPLC method. The development required investigating mobile phase composition, column choice and configuration, wavelength, response factors, and sample preparation procedures.

The validated method is sensitive, accurate, reproducible, and stability-indicating for the determination of cromolyn and its related substances in cromolyn sodium drug substance and Cromolyn Sodium Inhalation Solution, 1.0%.

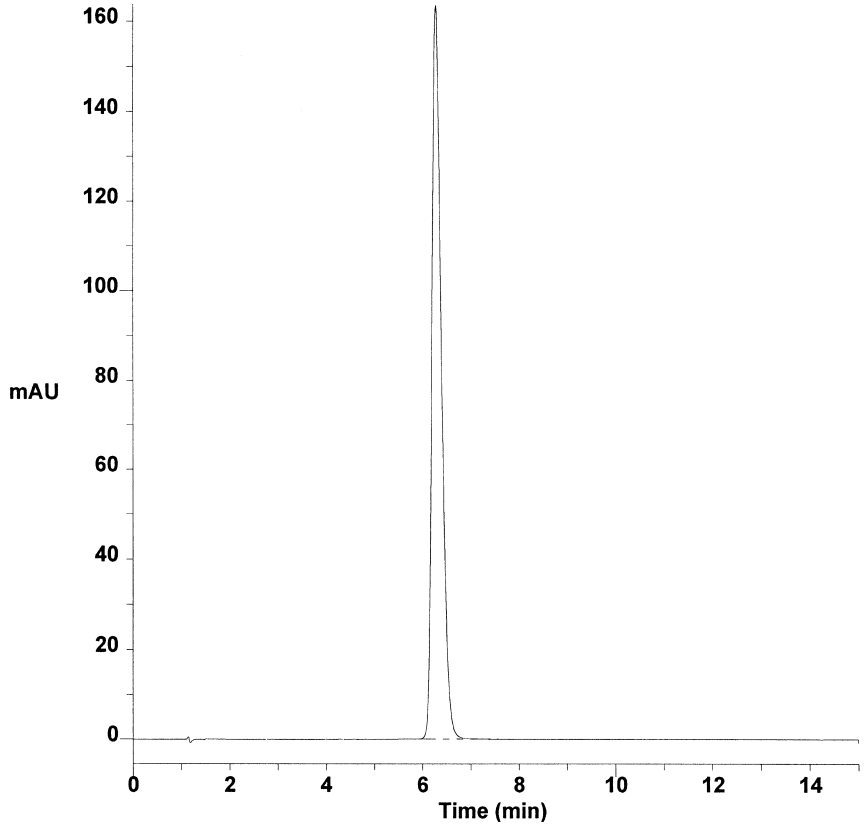


Figure 1. Chromatogram of standard preparation for assay.

EXPERIMENTAL

Chemicals and Reagents

The inhalation solution was formulated at Bausch & Lomb Pharmaceuticals, Inc., Tampa, FL, USA. Cromolyn sodium was a USP reference standard, Lot I. Tetrabutyl - ammonium dihydrogenphosphate (TBA), 1.0M solution in water, was purchased from Aldrich Chemical Company, Milwaukee, WI, USA.

HPLC grade methanol was purchased for Burdick & Jackson, Muskegon, MI, USA. The water was deionized and distilled by the Milli-Q® Water System (Millipore Corporation, Bedford, MA, USA).

Apparatus

The LC chromatographic system consisted of an Hewlett Packard 1100 solvent pumping system, variable wavelength UV-visible detector set as 326 nm, variable volume injector, and Hewlett Packard ChemStation (Version 5.01) (Hewlett Packard, Palo Alto, CA, USA) for integration.

A Waters Nova-Pak C_8 column (3.9 x 150 mm, Waters Associates, Milford, MA, USA) was maintained at ambient temperature.

The flow rate was approximately 1.0 mL/min with a typical operating pressure of 1900 psi.

Preparation of Solutions

Mobile Phase

Add 40 mL of 1.0M TBA to one liter of water (Buffer A). Add 550 mL of Buffer A to 450 mL of methanol, mix well, filter through 0.45 μm filter, and degas.

Dilution Solution

Add 300 mL of methanol to 700 mL of water and mix well.

Standard Preparation

Accurately weigh Cromolyn Sodium, USP Reference Standard and dilute to volume with Dilution Solution to yield a concentration of 0.2 mg/mL (see Figure 1.).

Determine the amount of water in cromolyn sodium reference standard. Calculate the water corrected weight of cromolyn sodium used in the Standard Preparation.

Drug Substance Sample Preparation (DSSP)

Accurately weigh cromolyn sodium drug substance and dilute to volume with Dilution Solution to yield a concentration of 1 mg/mL (see Figure 2). This sample will be used for the analysis of related substances.

For assay, appropriately dilute the 1 mg/mL solution to 0.2 mg/mL with Dilution Solution. Determine the amount of water in cromolyn sodium drug substance. Calculate the water corrected weight of cromolyn sodium used in the Drug Substance Sample Preparation.

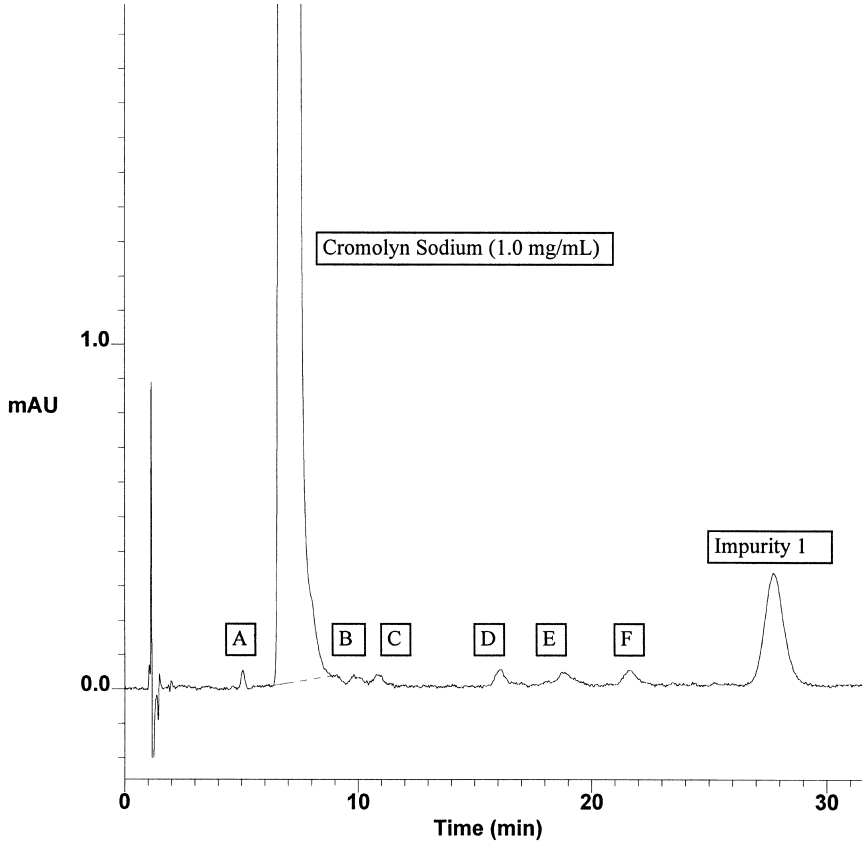


Figure 2. Sample Chromatogram of drug substance preparation for related substances. Peaks A-F are below 0.05%.

Drug Product Sample Preparation (DPSP)

Into a suitable container, dispense the entire contents from 4 unit dose containers. The concentration of cromolyn sodium in the drug product is 10 mg/mL.

Accurately dilute the drug product to 1 mg/mL then to 0.2 mg/mL using water. The 1 mg/mL sample is used for the analysis for related substances and the 0.2 mg/mL solution is used for assay.

Specificity

The DSSP was subjected to stress conditions because it is prepared in 30% methanol in water. The drug product matrix is purified water and the DPSP is prepared in water. Specificity was conducted using the DSSP because stressing the drug in the more complicated matrix would represent worse case situation with regards to degradation and interaction with the matrix.

The specificity of the method was studied through analysis of a Control Solution (unstressed DSSP) and stressed DSSPs. The DSSP was subjected to thermal, basic, oxidative, and ultraviolet light environments for a set period not exceeding two weeks for any stress condition.

A 10 mL aliquot of the Control DSSP was placed into a 20 mL scintillation vial and 1 drop of concentrated NaOH was added to the solution. The pH of the solution was approximately 12. The solution was analyzed after 12 hours and 60 hours. Oxidatively stressed samples were prepared by adding approximately 200 - 300 μL of 30% hydrogen peroxide to the DSSP. The solution was analyzed after 20 hours and 68 hours.

The thermally stressed samples were stored at 60°C for 63 hours. Light stressed samples were stored in an Atlas SunTest Station at 250 watts/meter^2 for one hour and 16 hours. The DSSP was not stressed under acidic conditions because the drug precipitated under acidic conditions (pH < 2) in the 30% methanol/water and in water.

System Suitability

The system suitability results were calculated according to the USP 23 <621> from typical standard chromatograms.[2] The instrument precision as determined by six injections of the standard preparation should provide a relative standard deviation of NMT 1.0%. The tailing factor should not exceed 1.7 at 5% peak height. The retention time specification of cromolyn should be between 4.8 minutes and 8.1. The standard agreement (ratio of response factors x 100%) between duplicate Standard Preparations was set at NMT 1.3%. Table 1 contains the data summarizing the System Suitability results, from which, system suitability specifications were derived.

Data Acquisition

The peak areas of cromolyn sodium and its related substances were measured using Hewlett Packard 3D ChemStation Software (version 5.01). The chromatographic data was automatically processed for peak area.

Table 1

Summary of System Suitability Results

Run	%RSD	Tailing Factor	Standard Agreement	Retention Time
R&D Sys. #18 09/03/97	0.04	1.2	100.14	7.2
R&D Sys. #18 09/05/97	0.10	1.1	100.75	7.3
R&D Sys. #18 09/09/97	0.13	1.1	100.19	7.6
R&D Sys. #19 09/11/97	0.05	1.3	100.36	6.9
R&D Sys. #18 09/12/97	0.06	1.1	100.03	7.3
R&D Sys. #15 09/17/97	0.34	1.2	99.62	7.1
R&D Sys. #19 09/19/97	0.59	1.2	100.26	7.3
R&D Sys. #19 09/29/97	0.85	1.1	100.76	7.6
R&D Sys. #19 09/22/97	0.42	1.4	100.36	5.7
R&D Sys. #19 09/29/97	0.08	1.3	100.38	6.7
R&D Sys. #10 10/06/97	0.11	1.3	100.00	6.5
Specifications:	NMT 1.0	NMT 1.7	NMT 1.3	4.8-8.1 min.

RESULTS AND DISCUSSION

Method Development

To obtain the best overall chromatographic conditions for the analysis of cromolyn sodium and its related substances, the mobile phase was optimized by evaluating various mobile phase compositions, columns of different packing materials (C_{18}, C_8, phenyl), and configurations (5, 10, and 15 cm columns).

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.

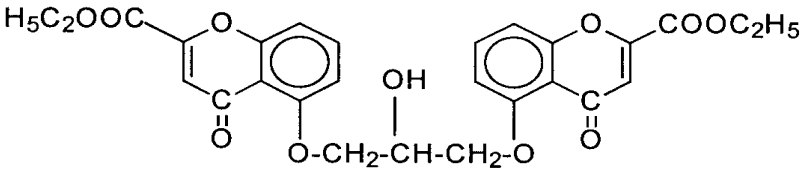
Several methods have been developed for the analysis of cromolyn sodium, some using ion-pairing agents.[3-5] The ion-pairing agents employed for the analysis of cromolyn sodium are tertiary amines that are positively charged at low pH; however, cromolyn will not be charged at low pH. The proper selection of an appropriate ion-pairing agent was essential to the successful development of a reverse phase HPLC method for the analysis of cromolyn and its related substances.

Tetrabutylammonium dihydrogenphosphate (TBA) was selected as the ion-pairing agent because it is a quaternary amine and will remain charged regardless of pH. TBA is inexpensive and provided as a 1.0 M solution in phosphate buffer at pH 6.0. At pH 6.0, cromolyn will be negatively charged (deprotonated) to form an ion-pair with TBA.

TBA was added to the mobile phase to improve retention of cromolyn sodium while having no effect on the retention of the known potential impurities. The TBA concentrations were modified from 5 mM to 35 mM. The chromatography of cromolyn sodium and its related substances was not affected by TBA concentrations above 15 mM. Therefore, 20 mM TBA was selected as the amount of TBA that would be added to the mobile phase.

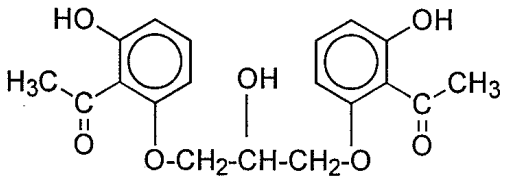
Mobile phase containing acetonitrile precipitated the cromolyn sodium on the column. In addition, the known potential impurities were insoluble in acetonitrile. Methanol was determined to be the most suitable organic modifier for the mobile phase for cromolyn sodium and its related substances. In addition, cromolyn sodium was found to be light sensitive; therefore, analytical samples were placed in amber HPLC vials for analysis.

The two known potential impurities, cromolyn ester (Impurity 1) and the hydroxy phenoxy (Impurity 2), in cromolyn sodium drug substance are shown below:



Impurity 1 (briefly Cromolyn Ester)

(Diethyl-4,4’-dioxo-5-5’-(2-hydoxytrimethylendioxy)di(chromene-2-carboxylate) ester)



Impurity 2 (briefly Hydroxy-Phenoxy)

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

These potential impurities (synthetic precursors) are insoluble in water and are much more lipophilic than cromolyn sodium. These potential impurities are soluble in solvents such as methylene chloride, chloroform, ethyl acetate, and are slightly soluble in acetone.

Several different columns were evaluated with the goal of analyzing cromolyn and its two known potential impurities in one HPLC method. Cromolyn and its known potential impurities could not be analyzed under the same chromatographic conditions using a C_{18} column. Impurity 1 and Impurity 2 did not elute from the column after 90 minutes. Phenyl columns did not resolve all the unknown related substances in cromolyn sodium drug substance. The best resolution of cromolyn sodium and all its detectable related substances was obtained using a Waters C_8 NovaPak 4 µm column. Other C_8 columns did not produce as favorable chromatographic results as the NovaPak column primarily due to the low carbon load of the NovaPak column. C_8 columns with higher carbon loads resulted in excessive retention of the known potential impurities.

A rapid analysis column was evaluated since cromolyn, its unknown related substances, and the known potential impurities were well resolved. Cromolyn sodium USP reference standard contains several unknown low level related substances (~0.05%) and the detection of these peaks were lost with rapid analysis columns. These low level related substances were also present in the drug substance from several manufacturers, but were not detected with rapid analysis columns. Therefore, rapid analysis columns were deemed unsuitable.

Solubility studies were conducted to obtain a methanol/water solvent mixture that would solubilize 1.0% (w/w) Impurity 1 and a 1.0% (w/w) Impurity 2 in the presence of cromolyn sodium. The methanol water solvent mixtures are the most compatible with the HPLC conditions developed for the analysis of cromolyn sodium and its related substances. A solvent system containing 45% methanol will not solubilize cromolyn sodium at a concentration of 1 mg/mL while a solvent system containing 20% methanol will not solubilize the

impurities at the 1.0% (w/w) level. The most appropriate solvent system for cromolyn sodium and its impurities at the 1.0% (w/w) level was methanol /water (30/70; v/v).

Impurity 1 is not stable in methanol/water solutions. Upon dissolution and the subsequent analysis of Impurity 1, three peaks of approximately equal response were obtained. TLC analysis of the Impurity 1 using the USP method produced only one spot. Impurity 1 was dissolved in methanol and analyzed. Initially, three peaks were present on the chromatogram. The relative retention times (RRT) of the peaks in order of decreasing retention time were 0.26, 0.53, and 1.0.

After approximately one month, only one peak (RRT=0.26) was present on the chromatogram and the total peak area of the single peak equaled the sum of peak areas for the three original peaks. In methanol, the ethyl ester impurity of cromolyn reacted with methanol to yield three ester species in solution: the diethyl ester, mono methyl mono ethyl ester, and the dimethyl ester. After one month, only the dimethyl ester was present. The UV spectra of the dimethyl ester degradant was exactly the same as the starting diethyl ester impurity.

A similar reaction takes place in methanol/water solutions where the diethyl ester impurity will react with the solvent to produce the mixed ester and eventually hydrolyze to cromolyn in the presence of water. The monomethyl ester of cromolyn was detected at levels well below 0.1% and it is partially resolved from the unknown related substance that was present in the USP reference standard and drug substances from different manufacturers.

The concentration of cromolyn sodium in the DSSP and DPSP for related substances is approximately 1 mg/mL. The cromolyn peak has a tailing factor approaching 2.0 which is near the recommended maximum tailing factor in the current USP.[2] The 1 mg/mL cromolyn sodium sample preparation was well suited for the analysis of related substances with cromolyn serving as the internal standard.

Only one injection of the DSSP or DPSP for related substances is required. For assay, the 1 mg/mL solution was diluted down to 0.2 mg/mL and the tailing factor was reduced to approximately 1.3 permitting more accurate integration of the cromolyn peak.

Several lots of cromolyn drug substance from three different manufacturers were analyzed using this method to certify and select a vendor that supply high quality material. It should be noted that Impurity 1 and Impurity 2 were not detected in any lot of raw material tested. This method was validated to show quantitation of Impurity 1 and Impurity 2 to clearly demonstrate that they are not present in the drug substance and, if present, they can be accurately quantitated.

Wavelength Determination

Photo diode array detection was investigated from 250 to 350 nm. Cromolyn Sodium, Impurity 1, and its unknown related substance exhibited the greatest response at 326 nm. Impurity 2 exhibited the greatest response at 340 nm. The detection wavelength of 326 nm was chosen for method validation and the RRF was determined for Impurity 2.

Injection Volume Determination

Injection volume was investigated to determine its effect on detection at or below 0.1% w/w (0.1% label claim). Method development was performed with an injection volume of 20 µL. A 10 µL injection of a 0.05% (w/w) Drug Substance Sample Preparation easily met the signal-to-noise requirements for limit of quantitation at approximately 34:1. Cromolyn sodium possesses a very strong chromophor. Hence, a 10 µL injection was selected for validation.

Relative Response Factor

Relative response factors for Impurity 1 and Impurity 2 are 1.00 and 0.58, respectively. Impurity 2 has a different chromophor than cromolyn sodium. The wavelength of maximum absorbance for Impurity 2 was determined to be 340 nm. The relative response factor for Impurity 2 was determined to be 0.58 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. The RRT ranges for these ester impurities were determined during robustness experiments. A RRF of 1.00 was assigned to Impurity 1 and its ester degradation products. If Impurity 1 were present in the drug substance, it would hydrolyzed in water to yield cromolyn. The RRF for Impurity 2 was determined to be 0.58.

Robustness

Late eluting related substances are most affected by small changes in mobile phase composition, namely small variations in methanol content. The methanol content of the mobile phase for the analysis of cromolyn sodium and its related substances is 45%.

The mobile phase was mixed by the instrument to produce a methanol content of 43%, 45%, and 47% and the retention times and RRTs of the cromolyn, Impurity 1 and its degradation products, and Impurity 2 were determined. The results of those experiments are shown below in Table 2.

Table 2

Retention Time Ranges of Cromolyn Sodium and Its Related Substances in a Cromolyn Sodium Standard Preparation Spiked with 1% (w/w) of Impurity 1 and Impurity 2

	Percent Methanol in Mobile Phase					
	43		45		47	
Analyte	RT[a] (min.)	RRT[a]	RT[a] (min.)	RRT[a]	RT* (min.)	RRT[a]
Cromolyn	8.1	1.00	6.2	1.00	4.8	1.00
Unk. Impurity	37.9	4.68	23.6	3.81	14.7	3.06
Impurity 1 Dimethyl Ester[b]	20.7	2.55	15.2	2.45	11.6	2.42
Mixed Ester	39.1	4.83	28.6	4.61	21.8	4.54
Diethyl Ester	74.4	9.19	54.7	8.82	40.4	8.42
Impurity 2	65.7	8.11	47.5	7.66	34.7	7.23

[a] RT = Retention time (minutes), RRT = Relative retention time.
[b] The dimethyl ester was not detected in this sample; however, the retention time of this ester was estimated based upon its known RRT to the diethyl ester (RRT = 0.26).

Table 3

Relative Retention Time Range for Cromolyn Sodium Related Substances

Analyte	Relative Retention Time Range
Dimethyl Ester	2.42 - 2.54
Unknown Related Substance	3.06 - 4.68
Mixed Ester	4.54 - 4.83
Impurity 2	7.23 - 8.11
Impurity 1 (Diethyl Ester)	8.42 - 9.19

From these data, the RRT ranges for each of the known potential impurities were determined allowing peak identification (Table 3). The data in Table 2 also includes the unknown related substance that was detected at approximately 0.1% (by peak area).

The RRT ranges only apply if cromolyn retention is between 4.8 and 8.1 minutes. This requirement was included as part of the system suitability specifications.

Table 4

Assay Precision for Cromolyn Sodium for Assay[a]

Run	Individual Assay Values (% LC)	Average Assay (n=6) (% LC)	RSD (%)
	101.1		
Day 1	100.5		
Chemist 1	100.0	100.58	0.4
HPLC System 1	100.4		
R&D	100.4		
	101.0		
	100.5		
Day 2	99.6		
Chemist 1	100.0	100.0	0.3
HPLC System 1	99.9		
R&D	99.8		
	100.2		
	98.8		
Day 3	98.5		
Chemist 1	99.0	98.9	0.3
HPLC System 2	99.3		
R&D	99.0		
	98.6		
Immediate Precision (n = 18)		99.8	0.8

[a] Acceptance Criteria: RSD NMT 2.0%. Note: Working analytical concentration for assay is 0.2 mg/mL.

VALIDATION

Precision

The precision (repeatability and intermediate precision) of the method was determined from one lot of drug substance (Cromolyn Sodium, USP reference standard, Lot I).

Table 5

Precision of Unknown Cromolyn Sodium Related Substance[a]

Run	Peak Area Percent	Average Peak Area Percent (n = 6)	RSD (%)
Day 1 Chemist 1 HPLC System 2 R&D	0.13 0.14 0.13 0.13 0.13 0.13	0.13	3.1
Day 1 Chemist 1 HPLC System 3 R&D	0.12 0.13 0.12 0.13 0.14 0.13	0.13	5.8
Day 3 Chemist 2 HPLC System 1 R&D	0.15 0.15 0.15 0.15 0.15 0.15	0.15	0.00
Immediate Precision: (n = 18)		0.14	7.8

[a] AT 0.1% (by peak area percent), (related substance level); Acceptance criteria: RSD NMT 25%. Note: working analytical concentration for related substances is 1 mg/mL.

Repeatability

Six DSSPs were analyzed in a single session by Chemist I, with HPLC System 1. The RSD of the six results for the cromolyn assay was 0.4% (Limit not more than (NMT) 2.0%).

Table 6

Accuracy of Cromolyn in Assay Range[a]

% Claim of Sample	Recovery (%)
50 (n=3)	99.4
100 (n=6)	100.5
150 (n=3)	99.2
Overall (n=12)	99.7
% RSD	

[a] Acceptance Criteria: 97.0 - 103.0%. Note: Working analytical concentration for assay is 0.2 mg/mL.

Six drug substance preparations were prepared and the unknown related substance (RRT range 3.06 - 4.68) at approximately 0.1% (by peak area) was analyzed for determining repeatability in the related substance range. The DSSPs were analyzed by Chemist I using HPLC System 2. The RSD of the six results for the cromolyn related substance was 3.07% (Limit NMT 40% for related substance less than 1%). In addition, three DSSPs at the 0.05% level were prepared by Chemist I using HPLC System 2 as part of recovery experiments. These results can also be applied to repeatability where the RSD of the three DSSPs at the 0.05% level was 2.3%.

Intermediate Precision

Intermediate precision was evaluated by Chemist I, with HPLC System 1, to independently prepare and analyze another six DSSPs using the same lot of drug substance on two different days. The intermediate precision was 0.8% (n=18) for the assay.

For the related substances, Chemist I, using HPLC System 3, prepared six DSSPs and analyzed each sample for the unknown related substance that was present in the drug substance.

Chemist II, using HPLC System 1, prepared DSSPs and analyzed each sample for the unknown related substance. The RSD was 7.8% (n=18) for the related substance range. The RSD limit for the assay level is NMT 2.0% and the limit for the related substances in NMT 25%. The results are within established limits. The low scatter in the data supports the high degree of ruggedness for this analytical method (Tables 4 and 5).

Table 7

Accuracy of Cromolyn in Related Substances Range[a]

% Claim of Sample	Recovery (%) n = 3
0.05	97.2
1.0	99.0
2.0	99.3
Overall (n = 9)	98.5
% RSD	1.5

[a] Acceptance criteria: ≤10%, label claim: 75 - 125%. Note: Working analytical concentration for related substances is 1 mg/mL.

Accuracy

The accuracy of the method was shown by analyzing blank solutions (30% methanol/water) spiked with known amounts of cromolyn sodium. The weight of cromolyn sodium was corrected for water. The accuracy met the acceptance criteria in both the assay and related substance ranges (Tables 6 and 7).

Specificity

Cromolyn Sodium DSSP was stressed by thermal, basic, oxidative, and ultraviolet light. The results of the stress studies are presented in Table 8.

Only the base stressed samples produced a degradation product that was not entirely baseline resolved (Rs=0.9) from cromolyn. Cromolyn is very sensitive to extreme basic conditions.

Photodiode array analysis indicated that the cromolyn peak was spectrally pure at the peak apex and peak tail, only the initial peak segment of the cromolyn peak was spectrally impure which is to be expected when a partially resolved degradation product is present.

Since the degradation product produced under extreme basic conditions (pH > 12) is partially resolved and can be quantitated using baseline drop integration techniques, the method is considered stability-indicating.

Table 8

Specificity Results[a]

Stress Condition	Peak Homogeneity Criteria: Peak Purity ≤ 990	Peak Homogenous Yes/No	Percent Degradation
Control 25°C, 9 hrs.	1000	Yes	N/A
Control 25°C, 57 hrs.	1000	Yes	N/A
Thermal 60°C, 63 hrs.	1000	Yes	1.9%
Light 250 watts/m^2, 1 hr	999.997	Yes	6.5%
Light 250 watts/m^2, 16 hrs.	996.042	Yes	70.5%
Base 12 hrs.	937.959	No*	33.7%
Base 60 hrs.	934.155	No*	46.6%
Peroxide 20 hrs.	999.529	Yes	11.1%
Peroxide 68 hrs.	999.943	Yes	16.1%

[a] Resolution between cromolyn and the closest degradation product above 0.05% was 0.9.

The control sample and stress samples were analyzed using a Hewlett Packard 1100 HPLC system equipped with a photo-diode array system. The cromolyn peak was determined to be homogenous for thermal stress, light stress, and peroxide stress samples since the peak purity was greater than the threshold value of 990. (Table 8).

Linearity

A linear response in peak area was observed for cromolyn over the range of 46% to 137% of the analytical working concentration (0.2 mg/mL) for assay and over the range of 0.05% to 2.0% of the analytical working concentration

Table 9

Linearity[a]

% Working Concentration (% Label Claim)	Actual Concentration (μg/mL)	Average Peak Response
Assay Range[b]		
45.7	91.3	1134.86243
54.8	109.6	134020630
91.3	182.6	2237.62354
114.2	228.3	2778.99646
137.0	274.0	3350.09864
		m = 12136.46 r = 1.000 % bias = 0.0
Related Substance Range[c]		
0.05	0.5	6.033
0.1	1.0	12.108
0.5	5.1	60.797
1.0	10.2	122.022
2.0	20.5	244.985
		m = 11947.86 r = 1.000 % bias = 0.0

[a] Coefficient of correlation acceptance criteria: NLT 0.999; Bias acceptance criteria: ± 3.0%.
[b] Nominal analytical concentration is approximately 0.2 mg/mL corrected for water.
[c] Nominal analytical concentration is approximately 1.0 mg/mL corrected for water.

(1 mg/mL) for related substances. The correlation coefficient was 1.000 and the percent bias was 0.0% for both the assay range and related substance range (Table 9). The ratios of the slopes from the assay range and the related substance range was 1.02 which was within the limits of 0.98 to 1.02.

Table 10

Stability of Solutions[a]

Preparation	Time (Days)	Response Factor	Ratio
Sample (0.2 mg/mL)	0	11508.40	
	7	12531.23	1.00
Sample (1 mg/mL)	0	11413.01	
	7	11480.80	0.99

[a] Acceptance criteria: Stable over the interval where ratios of response factor lie between .98 - 1.02.

Table 11

Stability of Related Substance in Cromolyn Drug Substance[a]

Preparation	Time (Days)	Peak Area Percent	Ratio
Sample (1mg/mL)	0	0.151	
	7	0.154	0.98

[a] Acceptance criteria: Stable over the interval where the ratios of response factor lie between 0.98 - 1.02.

Range

The range of the method has been set at 0.05 to 2.0% for cromolyn related substances and 46 - 137% for cromolyn since the method has been shown to be precise, accurate, and linear within these regions.

Stability of Solutions

The stability of analytical solutions was determined for the Drug Substance Preparation at 0.2 mg/mL (assay) and 1 mg/mL (related substance range). In addition, the stability of the unknown related substance (RRT=3.81) was also evaluated. The DSSP also served as the Standard Preparation since a USP Reference Standard was used as the drug substance. The samples were prepared

Table 12

Limit of Quantitation[a]

ID	Peak Areas	Average Area	% RSD	Average S/N
0.05%	5.303 5.467 5.467	5.412	1.6	34:1
0.1%	11.011 10.965 11.030	11.002	0.3	61:1

[a] Acceptance criteria: RSD NMT 10% and S/N NLT 10.

and placed in amber HPLC vials for seven days at ambient temperatures. The samples were then analyzed immediately (time zero) and seven days later. The ratio of the response factors from Day 0 and Day 7 for that sample must lie within 0.98 - 1.02 to be considered stable. Since the ratio was within limits, the Standard and Sample Preparations are considered stable at room temperature for up to 7 days in amber vials (Table 10 and Table 11).

Limit of Quantitation

The limit of quantitation was determined by using a precision (RSD) acceptance criteria of NMT 10% and a signal to noise ratio not less than (NLT) 10. A preparation of 0.05% of the analytical working concentration (1 mg/mL for related substances) of cromolyn exhibited an RSD of 1.6% (n=3) with a signal to noise ratio of 34 (Table 12). In addition, the recovery and repeatability at the 0.05% level was 97.2% and 2.3%, respectively. The data shows that the analytical method can accurately and reproducibly quantitate cromolyn related substances at the 0.05% level (see Figure 3).

CONCLUSION

The described isocratic HPLC method for the determination of cromolyn sodium and its related substances has been evaluated for system suitability, linearity, precision, accuracy, stability of solutions, LOQ, and specificity. The cromolyn sodium peak response has been shown to be precise, accurate, and linear over the range of 0.05 - 2.0% for related substances and 46 - 137% for

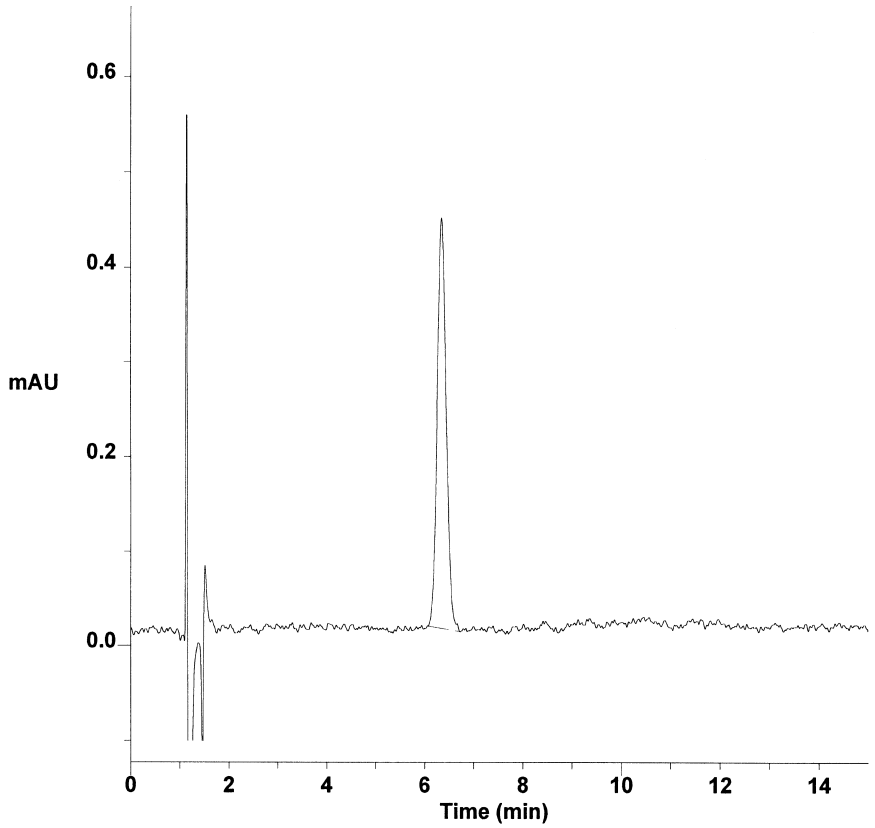


Figure 3. Cromolyn sodium at 0.05% working analytical concentration (1 mg/mL) for the related substance range.

assay. Intermediate precision for assays and related substances between chemists and chromatographic systems was demonstrated to be within 1.0 and 5.0%, respectively. The Standard and Drug Substance/Drug Product Sample Preparations are stable up to 7 days in amber HPLC vials at room temperature. Finally, the method has proven to be specific under a variety of stress conditions, while maintaining peak homogeneity. A partially resolved degradation product was detected in the base stressed samples that degraded over 30%. This partially resolved peak can be easily quantified (by peak area) using baseline drop techniques if it appeared in the drug product, which is very unlikely. Consequently, the validated method for the determination of cromolyn sodium and its related substance is regarded as stability-indicating.

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