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UPLC Method Optimisation and Validation for the Estimation of Sodium Cromoglycate in Pressurized Metered Dosage Form

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Abstract:

UPLC assay method optimised and validated for sodium cromoglycate in metered dose inhaler (MDI) using metronidazoleas an internal standard.The separation of Sodium Cromoglycate was achieved on Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) with gradient mobile phase containing methanol, orthophosphoric acid and acetonitrile in the ratio of 50:15:35 %v/v/v. The flow rate was 0.25 mLmin-1, injection volume 20µl and detection wavelength was set at 326nm, at ambient temperature. The validation of the proposed method was carried outfor linearity, accuracy, precision, robustness, limit of detection and limit of quantification test as per ICH guideline. The retention time of sodium cromoglycate found to be 4.73 min. Calibration graph was found to be linear at range 8-40µg/ml. The regression coefficient (r2) was found tobe 0.9978.The proposed method was rapid with adequate accuracy, precision, ruggedness and robustness and hence be suitable for theroutine analysis of sodium cromoglycate in meter dose inhalation and in bulk.

1. Introduction

Sodium cromoglycate, also referred to as cromolyn, cromoglicic acid chemically it is 5-[3-(2-carboxy-4-oxo-4H-5-chromenyloxy)-2-hydroxypropoxy]-4-oxo-4H-2-chromenecarboxylic acid (M. L. Ozoux et al., 2001 and. Fanta CH et al., 2009)(Figure: 1). It is practically described as a mast cellstabilizer, this drug prevents the release of inflammatory chemicals such as histamine from mast cells. (United State Pharmacopoeia national formulary, 2007)

It is considered a pioneer drug in management of asthma, as the subjects can be confined free from the therapy of steroids mostly, it is mainly effective as a prophylaxis for allergic and exercise induced asthma (JL McGuire, 2000 ad P Kathleen., 2007).

The analytical data are a prerequisite for correct interpretation of any dosage form. The objective of UPLC method development and validation ofsodium cromoglycate in pressurized metered dosage form procedure is to provide information about potency, which can be directly related to the requirement of a known dose. (Nguyen D.T, et al., 2006; Katharina Sterz et al., 2013; and Ashok kumar et al., 2012)

There are many reasons for the need to validate UPLC analytical procedures for sodium cromoglycate in pressurized metered dosage form. A few of them are regulatory requirements, good scientific research and quality control requirements. The Code of Federal Regulations (CFR) 311.165c explicitly states that “the accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented”(BA Moussa, et al., 2011, M. L. Ozoux et al., 2001 and M. Keyvanfard et al., 2013).

As research scholars, we would want to apply good scientific research methodologies to demonstrate that the analytical method used have demonstrated accuracy, sensitivity, specificity, and reproducibility.

The main concept of this research work is intended to examine and identify a genuine and right series of measures which are designed to avoid errors at varied

stages in production of sodium cromoglycate in pressurized metered- dosage form.

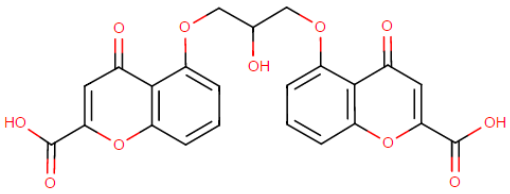

Figure.1: Molecular Structure of Sodium Cromoglycate, 5- [3-(2-carboxy-4- oxo -4H- 5-chromenyloxy)- 2-hydroxypropoxy]- 4-oxo- 4H-2-chromenecarboxylic acid

2. Experimental

2.1 Materials:

Sodium cromoglycate (99.99 % purity) used as analytical standard was procured from Active Pharma Labs (Hyderabad).

HPLC grade methanol, acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai, India. Distilled, 0.45 µm filtered water was used for UPLC quantification and preparation of buffer. Buffers and all other chemicals were of analytical grade.

The pressurized metered dosage (Minirin Nasal Spray 2.5ml) labelled to contain 112 metered dose actuations from the 8.1-gram inhaler and 200 metered dose actuations from the 14.2-gram inhaler of sodium cromoglycate. All chemicals used were of pharmaceutical or special analytical grade.

2.2 Instrumentation:

Acquity, Waters UPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2996 wavelength absorbance detector (PDA) was employed throughout the analysis.
The data was collected using Empower 2 software. The column used was Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm). A Band line sonerexsonicator was used for enhancing dissolution of the compounds. A Band line sonerex sonicator was used for pH adjustment.

2.3 Chromatographic Conditions:

Chromatographic conditions of the validating method are given in table.1

2.4 Preparation of Standard Stock Solution:

2.4.1 Preparation of Diluent:

In order to achieve the separation under the optimized conditions after experimental trials that can be summarized. Stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) column was most suitable one, since it produced symmetrical peaks with high resolution and good sensitivity and with good resolution. The flow rate was maintained 0.25 mL min-1 which shows good resolution. The PDA detector response of sodium cromoglycate was studied and the best wavelength was found to be 326 nm showing highest sensitivity.

The mixture of three solutions methanol, orthophosphoric acid and acetonitrile were taken in the ratio of 50:15:35 %v/v/v. The buffer used is 0.5 M phosphate buffer (pH adjusted to 4.5 with triethylamine) with gradient programming was used as mobile phase at 0.25 mL/min was found to be an appropriate mobile phase for separation of sodium cromoglycate. The column was maintained at ambient temperature.

2.4.2 Preparation of internal standard solution:

Weighed accurately about 10 mg of metronidazole working standard and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter. Metronidazole is taken as internal reference standard as it is most detectable compound for the proposed wavelength and mobile phase.

2.4.3 Preparation of sodium cromoglycate standard solution:

Weigh accurately about 10 mg of sodium cromoglycate and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter. Linearity was determined in the range of 8- 40 µg mL-1

3. Results and discussions

3.1 Validation

The analytical method was validated with respect to parameters such as linearity, precision, specificity, accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in compliance with ICH guidelines.

3.2 Linearity and Range:

The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration of an analyte in the sample. The calibration curve showed good linearity in the range of 8- 40 µg/mL for sodium cromoglycate (API) with correlation coefficient of 0.9978. A typical calibration curve has the regression equation of y = 24738.68x + 397488.9 for sodium cromoglycate. Results are given in Table 2.

3.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD and LOQ of sodium cromoglycate were calculated by mathematical equation. LOD= 3.3×standard deviation ÷ slope and LOQ=10×standard deviation ÷ slope. The LOD of sodium cromoglycate was found to be 0.8390(µg/ml) and the LOQ of sodium cromoglycate was found to be 2.543(µg/ml). Results are given in Table 2.

3.4 Precision:

The Precision of the method was studied in terms of intraday and interday precision of sample injections (24µg/ml). Intraday precision was investigated by injecting six replicate samples of each of the sample on the same day. The % RSD was found to be 0.534. Interday precision was assessed by analysis of the 6 solutions on three consecutive days. The % RSD obtained was found to be 0.925. Low % RSD values indicate that the method is precise. The results are given in table 3.

3.5 Robustness:

Small deliberate changes in chromatographic conditions such as change in temperature (± 2°C), flow rate (± 0.1ml/min) and wavelength of detection (± 2nm) were studied to determine the robustness of the method. The results were in favor of (% RSD < 2%) the developed UPLC method for the analysis of sodium cromoglycate. The results are given in table 5.

3.6 Accuracy:

To study the accuracy of method, recovery studies were carried out by spiking of standard drug solution to pre-analyzed sample at three different levels i.e., at 50, 100, and 150%. The resultant solutions were then reanalyzed by the proposed method. At each level of the amount, six determinations were performed. From the data obtained, the method was found to be accurate. The % recovery and %RSD were calculated and presented in Table 4.

3.7 Analysis of formulation

Assay studies for the analysis of pressurized metered dosage formulation ofsodium cromoglycate. Fixed chromatographic conditions were made use for the analysis of formulation and was found to be 100.507

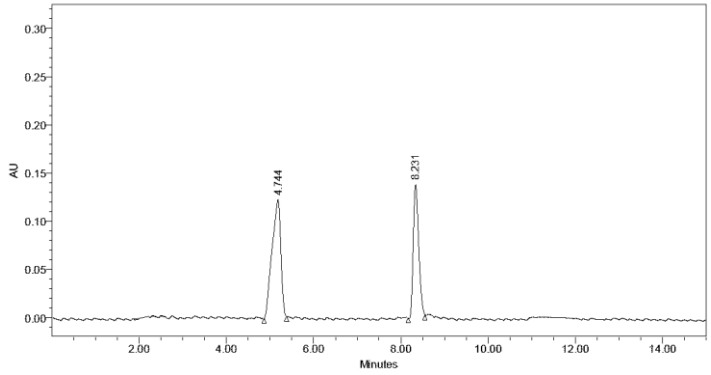

Figure. 2: Optimized chromatogram of Sodium Cromoglycate and internal standard using mobile phase of Methanol, orthophosphoric acid and acetonitrile in the ratio of 50:15:35 %v/v/v

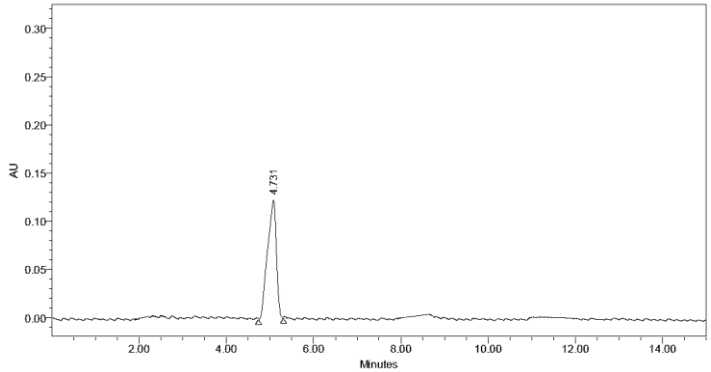

Figure. 3: Standard Chromatogram of Sodium Cromoglycate, using mobile phase of Methanol, orthophosphoric acid and acetonitrile in the ratio of 50:15:35 %v/v/v

Table 1: Chromatographic Conditions of the validating method

Parameter	Value
Column	Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm)
Mobile Phase	Methanol, orthophosphoric acid and acetonitrile in the ratio of 50:15:35 %v/v/v
Flow rate	0.25mL/min
Run time	14 Min.
Column Temperature	Maintained at ambient temperature
Injection volume	20 µL
Detection wavelength	326 nm
Diluent	Mobile Phase

Table 2: Summary of validation parameters for the proposed method

Parameter	Sodium Cromoglycate
Linearity	8- 40µg/ml
Intercept (c)	397488.9
Slope (m)	24738.68
Correlation coefficient	0.9978
LOD	0.8390(µg/ml)
LOQ	2.543(µg/ml)

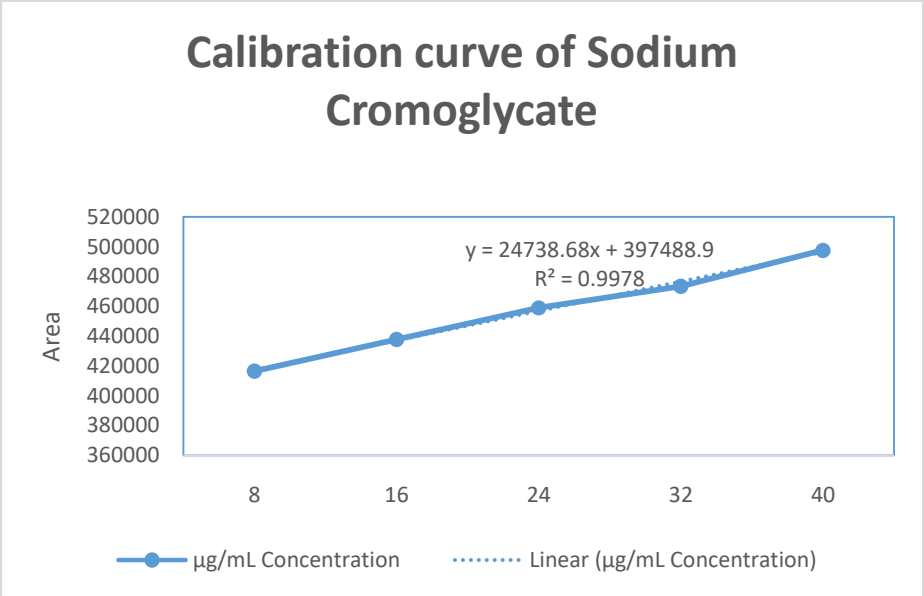

Figure 4: Calibration Curve of Sodium Cromoglycate

Table 3: Results of Precision Studies

Replicate	*Sodium Cromoglycate*			
S.No.	**Concentration Taken (µg/ml)**	**No. of spray taken**	**Area**	**%LC**
1	50.32	10	459055	99.98%
2		10	459076	99.98%
3		10	459154	99.96%
4		10	459176	99.96%
5		10	459206	99.95%
6		10	459264	99.94%
Average				99.96%
Std.Dev				0.016
% RSD				0.02%
Standard weight				50.32mcg
Standard potency				99.98%

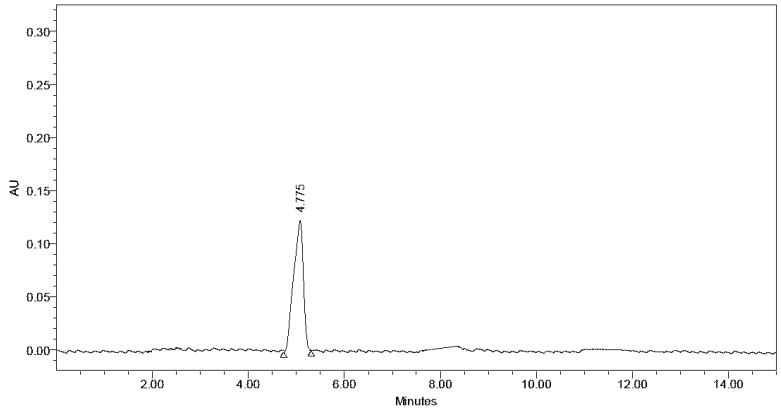

Figure. 5: Chromatogram Showing accuracy results

Table 4: Results of accuracy study

Sodium cromoglycate						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	25.90	25.87	99.98	99.44	0.725	0.73%
100	51.30	51.14	99.74			
150	77.61	76.54	98.62			

Table 5: Results of Robustness studies

Robustness Studies			
Parameter	**Value**	**Peak Area**	**% RSD**
Flow Rate	**Low**	459264	0.11%
	Actual	460022	
	Plus	460212	
Temperature	**Low**	459135	0.19%
	Actual	460498	
	Plus	460754	
Wavelength	**Low**	459113	0.16%
	Actual	460322	
	Plus	460511	

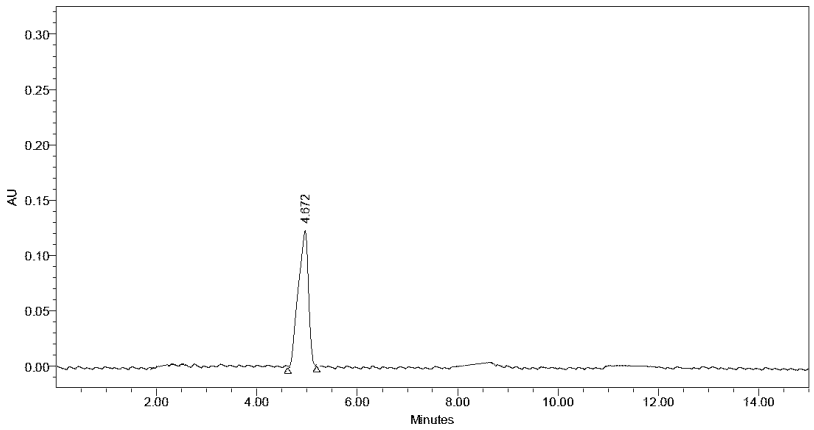

Figure 6: Chromatogram of Assay Studies

4. Conclusion

The proposed UPLC method developed is precise, accurate, linear, robust and specific foranalysis of the drug sodium cromoglycate in pure form and in their respective dosage form. The proposed methodhas the advantage of novelty as there is no reported UPLC method for the determination of pressurized metered dosage formulation of sodium cromoglycate.Satisfactory results were obtained from validation of the method, thus the method can be usedfor routine analysis and quality control of the cited drugs in small laboratories.

Conflict of interest

None declared

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