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VALIDATED RP-HPLC METHOD FOR ESTIMATION OF SODIUM CROMOGLYCATE IN HUMAN PLASMA

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ABSTRACT

The aim of study is to develop a simple and sensitive RP-HPLC method for the estimation of Sodium Cromoglycate (SCG) in plasma. The method was developed to apply for Bioavailability and Bioequivalence studies. The sample was prepared by extracting the drug from plasma using perchloric acid as a protein precipitating agent and the method was developed using a phenomenex C18 (250x4.6mm i.d., 5µ) with a mobile phase consisting of Acetonitrile and 20mM potassium dihydrogen orthophosphate in the ratio of 25:75and the flow rate1ml/min.The drug was detected at 240nm and the retention time was observed to be 5.1minutes. Study results the method was validated for all the parameters.LOD and LOQ were 3µg/ml and10µg/ml respectively and the linearity was found in the range of 10-35µg/ml with $r^2$0.99. The method was precise(%CV is not more than 10) and accurate (% recovery=92.6%-96.52%). Conclusion is this Bioanalytical method for sodium cromoglycate is useful for Bioavailability and Bioequivalence studies. This RP-HPLC method gives good resolution of compound from plasma. The method was validated and found to be simple, sensitive, precise and accurate.

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INTRODUCTION

Sodium Cromoglycate [Disodium 5, 5'-[(2hydroxytrimethylene) dioxy] bis [4-oxo-4H-1-benzopyran-2-carboxylate] is of synthetic origin and belongs to Chromene. Cromoglicic acid (also referred to as cromolyn, cromoglycate or cromoglicate)shown in figure:1.It is traditionally described as a mast cell stabilizer, and is commonly

marketed as the sodium salt sodium cromoglicate Inhaled sodium cromoglycate is an effective treatment for ACE-inhibitor cough[1].

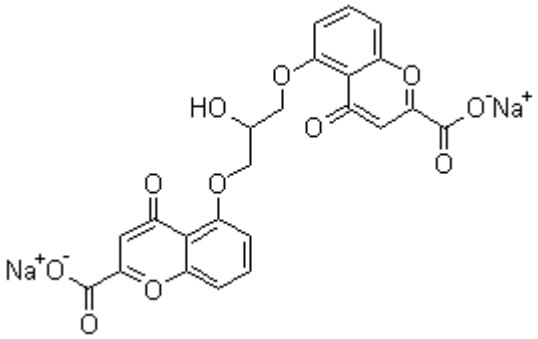

Figure: 1 Structure of sodium cromoglycate

The drug is available for use in dry powder inhalers (DPI), metered dose inhalers (MDI) nebulizers and ophthalmic preparations [2]. The official methods include non aqueous titrations and Spectrophotometry [3, 4]. The drug has been also determined by TLC-densitometric methods [5] and also; electrochemical methods [6]. Several HPLC methods were described for the determination of SCG using UV detection [7], fluorescence detection [8] or tandem mass spectrometric detection [9, 10] and by simple Spectrophotometric methods [11].

In the earlier part of this century colorimetry and Spectrophotometric methods were used for drug analysis due to the reasons of economy and easy availability. These methods, however, are used to a lesser extent today because of their lack of specificity, sensitivity and accuracy. For the estimation of the drugs present in pharmaceutical preparations and in sample drugs, HPLC method is considered to be a most suitable, since this is a powerful and rugged method. It is also extremely specific, linear, precise, accurate, sensitive and rapid.

Several scientists worked on SCG and developed different methods like Liquid high performance chromatography method with solid phase extraction (12), Liquid chromatography mass spectrometric method(13), and simple precise liquid chromatographic method (14).Stability-indicating isocratic HPLC method for the quantitation of cromolyn sodium and its related substances was developed and validated(15). Liquid chromatographic methods for the assay of cromolyn sodium and 3 related compounds(16).A hydrophilic interaction chromatographic (HILIC) procedure for the quantification of Sodium Cromoglicate(SCG) in ophthalmic solution (17).determination of SCG, making use of a sequential injection optosensor with terbium-sensitized luminescence detection(18).

MATERIALS AND METHODS

Chemicals and reagents:

Acetonitrile of HPLC grade was supplied by Merck Limited, Mumbai. Water for HPLC was obtained from Milli-Q RO system. Working Standards of Sodium Cromoglycate was obtained from YARROW CHEM PRODUCTS Pvt. Ltd. Mumbai.

Instruments used:

ELICO –SL159 uv-visible spectrophotometer, Shimadzu gradient HPLC system having the configurations, LC-20AD solvent delivery system, Rheodyne 7725*i* injector with 20 µl loop, SPD 20A dual wavelength detector, LC Solutions data station and Phenomenex Luna C18 (250 x 4.6 mm i.d., 5µ) column.

Optimized chromatographic conditions:

The method was optimized for the good resolution of compound from plasma. The stationary phase is Phenomenex C18 (250 x 4.6 mm i.d., 5µ), Mobile phase is acetonitrile and 20mM potassium dihydrogen orthophosphate of pH 1.5 in the ratio of 25:75 and the flow rate 1ml/min with the sample volume of 20µl, detected at 240nm using SPD-20A wavelength in LC Solution data station.

Preparation of standard and sample solutions

a. Standard stock solution of sodium cromoglycate

10 mg of sodium cromoglycate working standard was accurately weighed and transferred into a 10 ml volumetric flask and dissolved in Acetonitrile and Water (50:50) made up to the volume with the same solvent to produce 1mg/ml of sodium cromoglycate. The stock solution was diluted to suitable concentrations to obtain calibration curve (CC) standards and quality control (QC) samples.

b.Preparation of plasma samples

Standard stock solution was taken and dilutions of 10,15,20,25,30,35µg/ml were prepared,thesewere spiked into plasma andperchloric acid was added which acts as protein precipitating agent and centrifuged at 3000rpm for 5 min andsupernant liquid was collected.

METHOD VALIDATION

HPLC method for the estimation of Sodium cromoglycate in plasma has been developed and validated as per principles of ICH guidelines.

Selectivity/ Specificity

A method is said to be specific when it produces a response only for a single analyte. Method selectivity is the ability of the method to produce a response for the analyte in the Presence of other interferences.

Sensitivity

It is expressed as limit of detection and limit of quantification. It is the lowest amount of analyte in a sample matrix that can be detected and that can be quantifiable.This can be performed by taking different concentrations from lower to higher and check the signal to noise ratio of 3:1 for LOD and 10:1 for LOQ.

Linearity

Linearity is defined as its ability to obtain test results directly proportional to the concentration of the analyte in the samples[19].Linearity and range of the methods were calculated by preparing calibration curves using different concentrations of the drug extracted from plasma i.e.., 10, 15, 20, 25, 30, 35µg/ml. The calibration curve was plotted using peak area Vs concentration of the drug in plasma. Linearity was established over the range of 10-35 µg/ml for sodium cromoglycate.

Precision

Precisionis defined as the closeness of agreement between independent test results obtained under prescribed conditions[20]. It is determined by analyzing six replicates of samples at three concentration levels 10µg/ml as lower quality control (LQC), 20µg/ml as middle quality control (MQC) and 35µg/ml as higherquality control (HQC). It is expressed as the percentage coefficient of variance (%CV)[21],which is calculated as;

% CV = (Standard Deviation /Mean) x100.

Both Intra-day precision and Inter-day precision were performed.

Recovery

The recovery of an analyte in an assay is the detector response obtained from the amount of the analyte added to and extracted from the biological matrix compared to the detector response obtained for the true concentration of the pure authentic standard. Recovery pertains to the extraction efficiency of an analytical method within the limits of variability. Recovery of the analyte need not be 100%, but the extent of recovery of an analyte should be consistent, precise and reproducible. Recovery experiments were performed by spiking three concentrations (LQC, MQC, HQC) of drug in plasma matrix. After centrifugation the supernant liquid was seperated and injected. Analytical results of these extracted samples were compared withunextracted standards that represent 100% recovery.

Stability

To assess the stability of the analyte in the sample matrix under the same conditions of storage as that of the study samples for the time period between the date of first sample

collection and the date of last sample analysis, the following test was performed.

3 samples each of 3 concentrations i.e., 10,20,35µg/ml were prepared and kept at -70^{o} C and the stability of the analyte was evaluated by comparing each of the back calculated concentrations of stability Quality Control sample (QCs) with the mean concentrations of the respective QCs analysed in the first accepted precision and accuracy batch. The time period for short term stability is 1-3hr, Long term stability is 1-4 weeks and for assessing standard stock solution is1-4 weeks, the stored samples were collected and their related concentrations after storage are obtained by injecting into the HPLC. Freeze thaw stability can be performed by freezing the sample then it is thawed and reading is obtained by injecting the thawed sample, it is cycle I. Perform two more cycles i.e.., cycle II, cycle III and take the readings.

Ruggedness

Ruggedness of the method was studied by changing the experimental conditions such as operators, instruments, source of reagents, solvents and columns of similar type.

Robustness

Robustness of the method was studied by injecting the standard solutions with slight variations in the optimized conditions namely, ± 1% in the ratio of Acetonitrile in the mobile phase, varying pH range ±1 and ± 0.1 ml of the flow rate.

RESULTS

Selectivity:

The blank plasma and the plasma spiked with the drug were injected, the retention time for blank plasma and plasma spiked with drug was 2.4 minutes and 5.1 minutes respectively, no interference of the drug peak with the plasma peak was observed as shown in figure 1, 2, and 3.

Sensitivity:

The limit of detection value for SCGwas 3µg/ml and the limit of quantification was 10µg/ml.

Linearity and range:

The method was found to be linear over the range of 10-35µg/ml with the correlation coefficient (r^2) 0.99as shown in the figure: 4.

Precision:

By the precision studies the percentage coefficient of variance (%CV) values were obtained in the range of 0.2-9.1%,for intra day precision the values were2.7-3.2%, for inter day precision the values were 1.9-9.1%.Results were shown in table: 2

Recovery

The mean absolute recovery of Sodium cromoglycatewasfrom 92.6 to 96.5%. Here three levels was performed, the recovery of first level was 96.5%, for second level 93.2% and for third level 92.6%. The results were shown in table1.

Stability:

The stability studies were conducted and thepercentage nominal rangewas 97.7-98.5, 93-98.1, 97.6-99.5, 96.6-98.9 for short term, long term,freez thaw and stock solution stability studies respectively.Results were shown in the table: 3.

DISCUSSION

Solubility of drug was tested in the following solvents like Acetonitrile, methanol and water. Better solubility was achieved in Acetonitrile and water in 1:1 ratio, so this is taken as sample solvent. Wavelength for maximum absorbance (λ_{max}) was determined as 240nm using uv-visible spectrophotometry.Inbioanalytical methods generally plasma peaks appear which may interfere with sample peaks, so to prevent interference resolution between the peaks is necessary, for which different ratios of mobile phase were tested and 25:75 (Acetonitrile:

20mM potassium dihydrogen orthophosphate of 1.5 pH) was found optimal, with which plasma peak and drug peak obtained at 2.4 and 5.1minutes respectively.This method was validated for sensitivity, linearity, precision, recovery and stability. Linearity was performed in the range of 10-35µg/ml concentrations. Sensitivity is determined by LOD and LOQ, which was performed by taking concentrations from lower to higher and comparing the peak areas of signal and noise peaks. Signal to noise ratio for LOD and LOQ was 3:1 and 10:1 respectively. Recovery was performed with 10µg/ml(LQC), 20µg/ml(MQC), 35µg/ml(HQC) concentrationsand is calculated by comparing peak areas of the drug spiked in plasmaand pure drug of same concentrations. Intra day and inter day precision was performed and the percentage coefficient of variance was calculated to know the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogenous volume of biological matrix. The samples are storedfor different periods of time and short term, long term, stock solution stability, freeze thaw stability were performed.

FIGURES

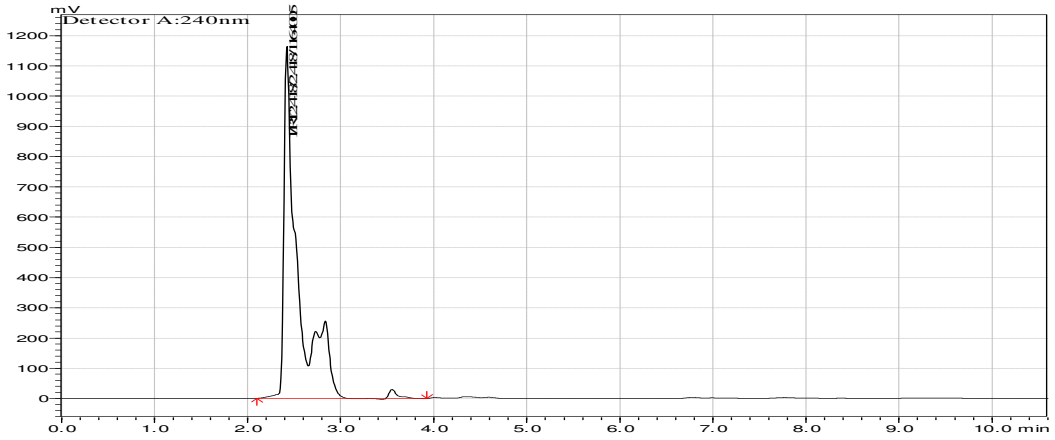

Figure: 1 Chromatogram of Blank plasma

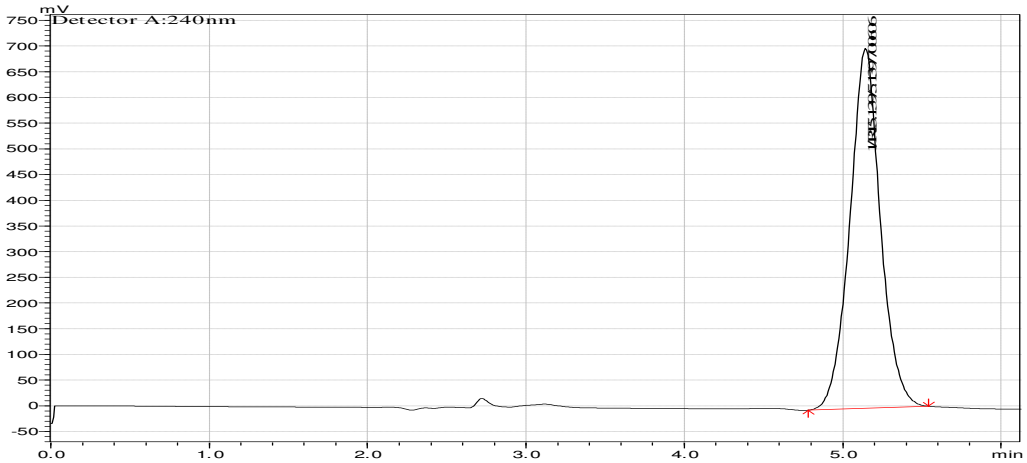

Figure: 2 chromatogram of sodium Cromoglycate

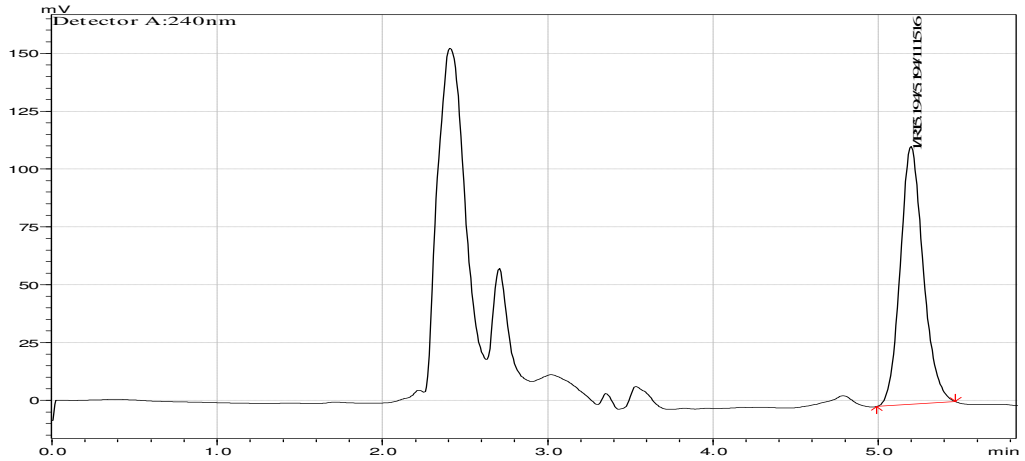

Figure: 3 Chromatogram of sodium cromoglycate and plasma

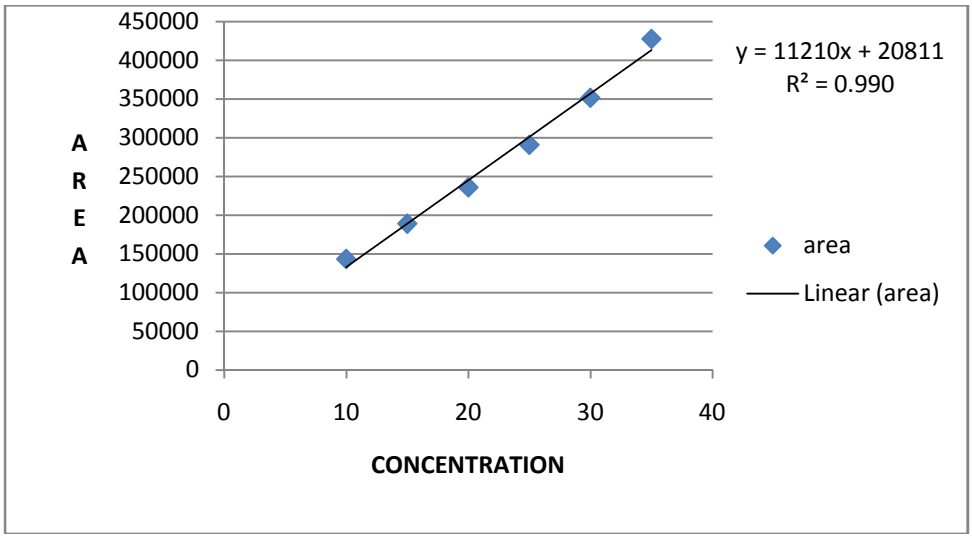

Figure: 4 Linearity Graph

TABLES

Table1: Recovery Table

Levels	Concentration(µg/ml)	Volume of drug(ml)	Volume of plasma(ml)	Concentration recovered	%Recovery
Level I	10	1	9	9.7	96.5%
Level II	20	1	9	18.6	93.2%
Level III	35	1	9	32.4	92.6%

Table: 2 Precision Table

Intra day Precision Table			
	LQC	MQC	HQC
Avg	373086.7	433707.3	754570.2
SD	10141.9	11781.5	24083.6
%CV	2.7	2.7	3.2
N	6	6	6
Inter Day Precision table			

Day -1 (Avg)	373086.7	433707.3	754570.2
Day -2 (Avg)	321843.5	482985.7	770422.8
Day -3 (Avg)	318263	446754.5	783806.3
Avg	337731.1	454482.5	769599.8
SD	30671.2	25531.9	14635.4
%CV	9.1	5.6	1.9

Table 3: stability studies

Short term stability			
TIME	**LQC**	**MQC**	**HQC**
after 1hr	10	19.9	34.9
after2hr	9.9	19.6	34.5
after3hr	9.5	19.1	34.1
Avg	9.8	19.5	34.5
SD	0.2	0.3	0.3
%CV	2.4	1.7	1.1
%Nominal	98.1	97.7	98.5
N	3	3	3
Long term stability			
1st week	9.5	19.1	34.1
2nd week	9.4	18.9	35.1
3rd week	9.1	18.9	33.9
Avg	9.3	18.94	34.35
SD	0.2	0.1	0.5
%CV	1.6	0.3	1.4
%Nominal	93	94.7	98.1
N	3	3	3
Stock solution stability			
1st week	10.1	19.8	35.0
2nd week	9.9	19.6	34.9
3rd week	9.3	19.2	34.5
Avg	9.7	19.5	34.8
SD	0.3	0.3	0.2
%CV	3.1	1.5	0.6
%Nominal	97.6	97.7	99.5
N	3	3	3
Freeze Thaw stability			
Cycle I	9.7	19.8	34.9
Cycle II	9.2	19.6	34.6
Cycle III	10.1	19.0	34.3
Avg	9.6	19.	34.6
SD	0.3	0.3	0.3

%CV	3.5	1.7	0.8
%Nominal	96.6	97.5	98.9
N	3	3	3

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