Analytical Method Development And Validation Of Fluticasone Propionate By Using RP- HPLC

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Reverse Phase High performance liquid chromatography (RP-HPLC), fluticasone propionate, Validation, Mobile phase, Method Development, methanol

Abstract
In this study, a straightforward, reliable, precise, and stable RP-HPLC method for estimating fluticasone propionate. Among the method's many benefits are its straight forward and mobile phase, inexpensive solvents, quick analysis. HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump. rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and Chrom NAVCFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm x 4.6 mm, 5 µm) column using methanol and water as mobile phase at flow rate of 1.0 min. Samples were injected using Rheodyne injector with 20 µL. loop. Detection was carried out at 235nm, with a sharp peak at 4.00 minutes for fluticasone propionate work .the method exhibits good linearity (r2 = 0.9999). The % RSD values for method precision and intermediate precision studies were found to be less than 2%. The % recovery was found to be within an acceptable limit 98%-102%.Thus, the created method was described as robust, accurate, exact, and linear. Because the process eliminates the need for costly reagents and also It takes less time.

1.Introduction

Chronic respiratory diseases which include asthma and chronic obstructive pulmonary diseases comprise a major cause of death and disability for all age groups and regions in the world [1]. In the last estimates of World Health Organization, 235 million people had asthma, 64 million suffered from chronic obstructive pulmonary disease and a few other millions had allergic rhinitis or other often-underdiagnosed respiratory diseases [2].

Traditionally the treatment of these diseases consists in treatment with inhalation drugs (such as corticosteroids and ß-adrenergic agonists) formulated to be used as nasal spray dry powder inhaler, metered-dose inhaler or as nebulizer [1]. Lung delivery systems are advantageous and an alternative to systemic drug delivery because they allow the topical action of drugs , by providing a targeted therapy on the affected airways area with higher drug concentration, but at same time with reduced systemic side effects derived of the lower systemic exposure to the drugs [3].

One of the drugs class used in lung therapy is the corticosteroids, very important in the regulation of inflammatory process and response. Fluticasone propionate (FP), a 2 generation trifluorinated glucocorticosteroid based on the androstane nucleus, is a highly potent drug and one of most used corticosteroids to treat asthma (inhalation) and allergic rhinitis (intranasally)[1]. FP is a very effective drug in the treatment of these diseases, but has a limitation of its water insolubility, that may lead to a reduction in the local absorption of the drug . Fluticasone propionate is a highly selective agonist at the

glucocorticoid receptor and used for prophylaxis and treatment of allergic rhinitis

It mimics the naturally occurring hormone produced by the adrenal glands, cortisol or hydrocortisone used in the management of asthma and chronic obstructive pulmonary disease (COPD). Chemically it [(6S,85,9R 105,115,135,145,16R, 17R)-6,9-difluoro-17-15 (fluoromethylsulfanylcarbonyl)-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydro cyclopenta [a] phenanthren-17-yl) propanoate [4] Its molecular formula is CHFOS and its molecular weight is 5006 g/mol .
High Performance Liquid Chromatography (HPLC) is a method of analysis that is widely used to separate and identify organic and inorganic solutes in any sample, particularly those that are industrial, environmental, food, medicinal, biological, etc. It has becoming more often used to determine stability studies of polar/ionic, thermally unstable, or non-volatile chemicals because has great sensitivity, specificity, and resolution capabilities. Given the previously mentioned data and the literature review, a unique RP-HPLC technique has been created and approved for the estimation of fluticasone propionate.

Chemical structure of fluticasone propionate.

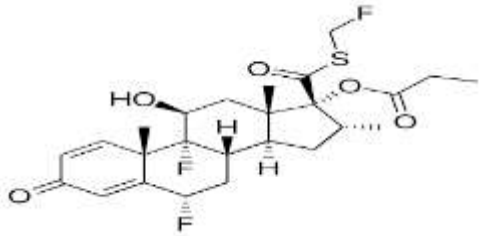

Figure 1: structure of fluticasone propionate

2. MATERIALS AND METHODS

Materials And Reagents:

fluticasone propionate was supplied by aarti pharmaceuticals in the Indian state of Maharashtra. It was purchased from a nearby drugstore. Methanol & water were all HPLC-grade reagents employed in the current study. HPLC analysis was performed using the fluticasone propionate
HPLC-grade water was used in the HPLC study.

Experimental Work:

Instrumentation and Chromatographic Condition

HPLC system used was JASCO system equipped with model PU 4180 RHPLC pump. rheodyne sample injection port (20 µl), JASCO UV-4075 UV-VIS detector and Chrom NAVCFR chromatography software (version 2.0). Separation was carried out on HiQSil C18 (250 mm x 4.6 mm, 5 µm) column using methanol and water as mobile phase at flow rate of 1.0 min. Samples were injected using Rheodyne injector with 20 µL. loop. Detection was carried out at 235nm. And retention time 4.00 minutes for fluticasone propionate All weighing was done on Shimadzu balance (Model AY-120)

Mobile phase

Mobile phase was methanol . removal of gases was carried out in ultrasonic water bath for 15 minutes.

Preparation of standard stock solution

About 10mg of fluticasone propionate was accurately weighed and transferred into 25 mL volumetric flask. 70 mL of diluent was added and then sonicated in ultrasonic water bath for 30 minutes. The solution was cooled and volume was made up to the mark with diluent. Resulting solution was used as test solution.

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Selection of analytical wavelength

It is the characteristic of a compound which helps to provide the electronic structure of the compound or analyte. The structural analysis of fluticasone propionate was carried out under UV ranging from 200-400nm using the standard solution

3. RESULT AND DISCUSSION

Table 1: Final reversed phase High performance liquid Chromatographic Conditions

Sr No	Parameter	Condition Used for Analysis
1	Column	Phenomenex luna
2	Mobile phase	methanol
3	Flow rate	1.0
4	Detection wavelength	235nm
5	Sample injector	20µl
6	Column temperature	30^0 c
7	Retention time	4.1min

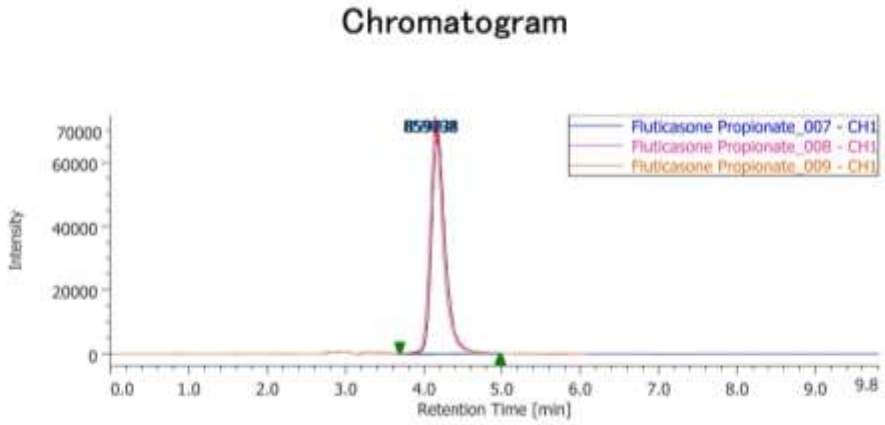

Figure 2 HPLC chromatogram of standard

fluticasone propionate

Observation: fluticasone propionate eluted at 4.00 minutes with acceptable chromatography Conclusion: Method can be used for further analysis and will be subjected for validation.

Method development

The proposed chromatographic method was found to be suitable for the effective preparation with good resolution, peak shape given in the figure. The mobile phase composed of methanol at flow rate 1.0ml/min was selected as it gave well resolved peaks of standard fluticasone propionate**.** The optimum wavelength 235nm selected for detection and quantitation.

Validation of analytical method

Validation of proposed analytical method involves linearity and range, precision, accuracy, limit of detection (LOD), limit of quantitation (LOO) and robustness study. It was validated according to ICH Q2(R1) guideline.

System suitability

System suitability: System suitability is the evaluation of the component of an analytical system to show that the performance of a system meets the standard required by a method. System suitability study was performed before each validation run. Area, Retention time (RT), Tailing factor and Theoretical plates were determined. Tailing factor for the Fluticasone Propionate in standard solution should not be more

than 2.0. Theoretical plates for the Fluticasone Propionate peaks in standard solution should not be less than 2000.

Parameter	Fluticasone Propionate	Acceptance Criteria
Retention time	6 min	±10
Theoretical plate	NLT 2000	>2000
Tailing factor	NMT 2.0	<2.00
% RSD	NMT 2.0	<2.00

Table no 2:System suitability data for Fluticasone Propionate

linearity

The calibration curves were found to be linear for the concentration range of 5-40 ppm. The standard working curve equation for drug was found to be y = 54484x + 5337.3 with correlation coefficient value R^2=0.9982. The results of linearity are given in the table and figure.

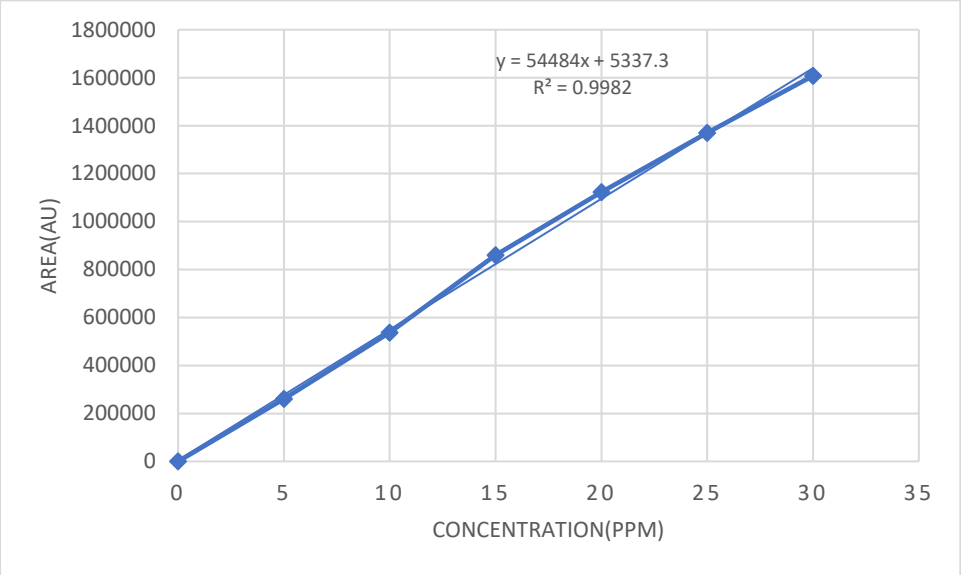

Figure 3: Linearity curve of standard Fluticasone Propionate

Concentration	Area
0	0
5	260297
10	537330
15	860072
20	1123061
25	1369620
30	1607789

Table no 3: linearity of Fluticasone Propionate

Recovery studies

The accuracy was determined from recovery studies. A known but varying amount of sample was spiked into pre-analyzed sample test solution at 50%, 100% and 150% recovery levels of working concentration in triplicate. The spiked test solution was analyzed according to the proposed procedure. The percentage
recoveries were calculated against respective levels and mentioned in Table 04.

Analyte	Recovery level	% Recovery	Average % Recovery
Sample	50%	100.3	100.4
	50%	100.4	
	50%	100.5	
	100%	100.6	100.8
	100%	101.1	
	100%	100.8	
	150%	101.2	100.8
	150%	100.8	
	150%	100.5	

Table no 04: Recovery of Fluticasone Propionate.

Method precision
The six test solutions were prepared separately. Each was analyzed as per proposed procedure. The % assay, average and %RSD was calculated and tabulated in the Table 05

Sample no	% Assay of Sample
1	100.113
2	99.144
3	100.256
4	100.278
5	100.756
Mean	100.109
% RSD	0.591

Table no 05 Method precision

Intermediate precision
The intermediate precision was determined by comparing two independent analysis on 2 different days. The data of the 1" analysis was taken from the analysis of 'Method precision.

Name of analyte	Sr.no	Assay (% w/w analysis-1)	Assay (% w/w analysis-2)
Sample	1	101.478	100.215
	2	101.784	99.635
	3	100.621	101.245
	4	100.154	100.478
	5	100.417	101.596
	6	100.487	100.487
	Average	100.9902	100.6093
	% RSD	0.675126	0.709172
	Overall % RSD	0.69	

Table no 5: Intermediate precision
Secificity
Specificity The specificity of the method for Assay is demonstrated by injecting following solutions into the HPLC system.
Diluent as a blank

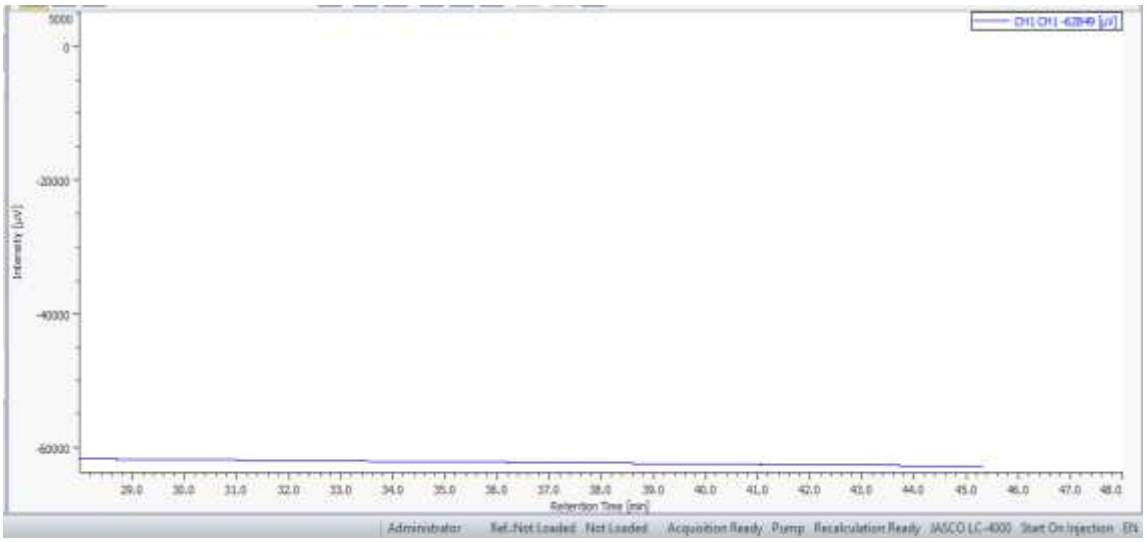

Figure no 04: HPLC Chromatogram of blank solution

Test solution

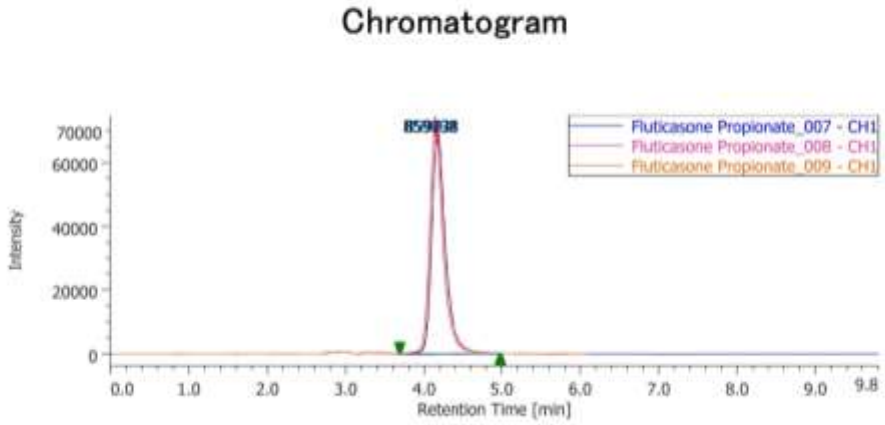

Figure no 05: HPLC Chromatogram of test solution

Robustness

The influences of slightly changed parameters of the chromatographic conditions were tested according to ICH guidelines to demonstrate robustness of the method. The tests are carried out by injecting Blank and standard solution by varying same of the parameters of chromatography mentioned below.

Table no 6: Robustness parameters

Sr no.	**Parameters**	**Working parameter**	**- changes**	**+ changes**
1	Flow	0.9 ml/min	1.0 ml/min	1.1 ml/min
2	Wavelength (±5nm)	237		241

Table no 7: Robustness study with change in wavelength

Changes in wavelength (nm)		
	237	**241**
1	80478	80154

2	79665	81361
3	80457	80457
4	79558	80114
5	80256	82358
Mean	80082.8	80888.8
SD	440.504	962.836
% RSD	0.550062	1.190322

Table no 8: Robustness study with change in flow rate

Change in Flow (min)			
	0.9	**1.0**	**1.1**
1	80568	82565	80478
2	81245	82547	79548
3	80369	81254	80567
4	82415	82325	81245
5	80478	80478	82357
Mean	81244	81833.8	80839
SD	854.165	929.9122	1041.651
% RSD	1.051358	1.136342	1.28855

CONCLUSION

The Specificity of the HPLC test for Assay of was proved by chromatographic comparison and method was found to be specific. The linearity of the proposed method was determined from the correlation coefficient and the method was found to be linear and within the range of 50 to 150% of working concentration The accuracy of the method was calculated by recovery study & the proposed method was found to be accurate as all the parameter of the method complies as per the acceptance criteria.

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