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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ASSAY OF FLUTICASONE FUROATE FROM NASAL SPRAY FORMULATION

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Abstract

The literature search reveals that, several HPLC methods for the determination of Fluticasone furoate in combination with other drugs are reported with long run time, high solvent consumption or with less available instrument as compared with HPLC. There is no any reported HPLC method for individual assay of Fluticasone furoate from nasal spray formulation. So the purpose of present experimental work is to develop a rapid, simple, precise, accurate, specific, and sensitive high performance liquid chromatographic method for assay of Fluticasone furoate from nasal spray formulation. The desired chromatographic separation was achieved on the Inertsil ODS-3V 250 x 4.6 mm, 5µ column, using isocratic elution at 240 nm wavelength. The optimized mobile phase constituted of purified water and acetonitrile in the ratio of 20:80 % v/v delivered at the flow rate 1 ml/min with isocratic elution. The retention time of fluticasone furoate was 5 min. The method was validated according to International conference of harmonization guidelines in terms of accuracy, precision, specificity, robustness, linearity and other aspects of analytical validation. Linearity was established in the concentration range of 27.5 to 82.5 ppm (r^2=1.000). The recoveries obtained were 99.4 -100.5 %. Similarly the % RSD value for precision was also found to be within the acceptable limit. Developed method was simple and convenient which could be successfully applied for the routine analysis.

KEYWORD: Fluticasone furoate, Corticosteroid, Asthma, Allergic Rhinitis, RP-HPLC, Validation, ICH guidelines.

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INTRODUCTION

HPLC is an analytical technique used to separate, identify and quantify the component. It finds its use for research, manufacturing, medical, legal purposes. The development of an analytical method for the identification and quantification of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. In the present study attempt is made to develop and validate a simple HPLC method for Fluticasone furoate (FF).

Fluticasone furoate is a synthetic fluorinated corticosteroid having the chemical name [(6S,8S,9R,10S,11S,13S,14S,16R,17R)-6,9-difluoro-17-(fluoromethylsulfanylcarbonyl)-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl] furan-2-carboxylateand the empirical formula is $C_{27}H_{29}F_3O_6S$. Chemical structure of Fluticasone furoate is presented in figure 1.

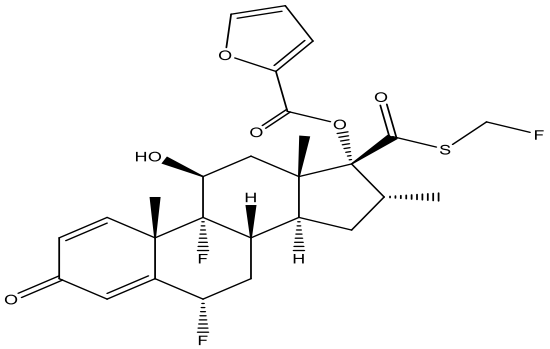

Figure 1: Chemical structure of fluticasone furoate

Fluticasone furoate is a white powder with a molecular weight of 538.58. It is practically insoluble in water. Fluticasone furoate nasal spray is a new topical corticosteroid, with enhanced-affinity and a unique side-actuated delivery device. As it has high topical potency and low potential for systemic effects, it is a good candidate for rhinitis treatment [1]. Fluticasone furoate has demonstrated treatment efficacy for asthma between 100µg and 200µg alone[2-3]. The advantages of the therapeutic profile of Fluticasone furoate have led to increasing use in the clinical practice, which encourages the development of new analytical method to provide driving force in today's pharmaceutical industry [4].

A detailed Literature survey of Fluticasone revealed that Fluticasone furoate and Fluticasone propionate (FP) though have same steroidal backbone but are completely different drugs with Fluticasone furoate showing distinct and superior properties [5]. There are many analytical method are available as spectrophotometry [6], LC [7], LC-MS [8-9], HPLC [10-17], UPLC [18], UPLC-MS [19-20], MEKC [21], RS-RD and MCR [22], others [23-24] for the determination of Fluticasone propionate as single or in combination with another drugs from pharmaceutical formulation and biological fluids. Literature survey also reveals that there are few analytical methods spectrofluorimetry [25], spectrophotometry [26], HPLC [25, 27-28], UPLC [29] methods for determination of fluticasone furoate in combination with another drugs. There is no any reported RP-HPLC method for individual assay of Fluticasone furoate from nasal spray formulation. Therefore there is need to develop a simple assay method for the Fluticasone furoate.

MATERIALS AND METHODS

Materials:

High purity water was generated by using Milli-Q Plus water purification system (Millipore® Elix 100). HPLC grade Acetonitrile and analytical reagent grade Hydrochloric acid were procured from Rankem Chemicals India-Lab chemicals, Mumbai. Analytical grade reagents Sodium Hydroxide, Hydrogen Peroxide were obtained from Merck Chemicals Limited, Mumbai. Reference standard of Fluticasone furoate, drug product, placebo

solution were provided by Sava Healthcare Ltd, MIDC, Chinchwad, Pune.

Chromatographic System:

The HPLC system (model: LC-2010C_{HT}, Make-Shimadzu corporation, Kyoto, Japan.) composed of Lab solutions software certified for QA/QC was used. It consists of vacuum degasser, automatic front panel control, PDA detector, thermostat column compartment with C18 column [4.6mm x 250mm, pore size 5µm], high performance auto sampler, thermostat for high performance auto sampler. Its integrated solvent and sample management capabilities provide the flexibility and ruggedness needed to accommodate an enormous range of HPLC separation challenges.

Chromatographic condition:

The chromatographic condition was optimized using Inertsil ODS-3V 250 x 4.6 mm, 5µ column (G. L. Sciences, Japan). Mixture of HPLC grade milli-Q water and acetonitrile in the ratio of 20:80 % v/v was used as mobile phase. Mobile phase was used as a diluent. The finally selected and optimized conditions were as follows: injection volume 10µL, isocratic elution, at a flow rate of 1 mL/min at 30°C (column oven) temperature, detection wavelength 240 nm. The stress degraded samples and the solution stability samples were analysed using a PDA detector covering the range of 200-400 nm.

Standard solution preparation:

Accurately weighed quantity of about 27.5 mg of Fluticasone Furoate working standard transferred to a 50 mL volumetric flask. Then added 20 ml of diluent and sonicated in an ultrasonic bath for 5 min to dissolve. This solution was then diluted up to the mark with diluent and mixed well. 5ml of this solution is then diluted to 50 ml with diluent to prepare standard stock solution of 55 ppm of Fluticasone furoate.

Sample solution preparation:

An accurately weighed 5 gm of sample (equivalent to 2.75 mg of Fluticasone furoate) solution was taken to into the 50 ml volumetric flask. About 20 ml of diluent was added and sonicated in an ultrasonic bath for 5 min. This solution was then diluted up to the mark with diluent and Sample solution of 55 ppm was prepared. It was then filtered through 0.45 µm nylon syringe filter and the filtrate was collected after discarding first few milliliters.

Placebo (other substance without Fluticasone furoate) solution preparation:

An accurately weighed 5 gm of sample solution was taken to into the 50 ml volumetric flask. About 20 ml of diluent was added and sonicated in an ultrasonic bath for 5 min. This solution was then diluted up to the mark with diluent and mixed well. It was then filtered through 0.45 µm nylon syringe filter and the filtrate was collected after discarding first few milliliters.

Method validation

Validation of the optimized HPLC method was carried out with the following parameters.

System suitability parameters:

System suitability tests are an integral part of chromatographic method validation [30]. The tests were used to verify that the reproducibility of the chromatographic system is adequate for analysis. To ascertain its effectiveness system suitability tests were carried out on freshly prepared standard solution. 10 µL of solution was injected into the optimized chromatographic system. For system suitability six replicates of working standard samples were injected and the parameters like retention time (RT), theoretical plate (N), peak area, tailing

factor and resolution of sample were calculated.

Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample [30]. The linearity of a method demonstrated by preparing solutions over the concentration levels ranging from 50 % to 150 % of working concentration. These solutions injected in triplicate into the system and the peak area of analyte peak recorded. Linearity graph of concentration Vs average peak area of analyte plotted separately. The correlation co-efficient, slope and y intercept evaluated.

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and

the found value [30]. The accuracy of the method was determined by analysing the sample solution at three different concentration levels 50 %, 100 % and 150 % of the usual sample preparation concentration, and injected for the accuracy studies. The area under curve obtained was checked and analysed for the recovery percentage.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [30]. The precision of the method was checked and verified by system precision, method precision and intermediate precision (Ruggedness) variation studies. In system precision studies six injections of standard solution prepared as per the usual analytical method and injected into the system. In method precision six replicates of sample solution of the same batch were prepared and injected into the system. In intermediate precision (ruggedness) six replicates of a single batch samples were prepared and analysed by different analyst, on different day and on different instrument.

Robustness of the method:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [30]. The effect of change in flow rate (±0.1mL/min), column oven temperature (±5°C), wavelength (±3 nm), mobile phase composition (±5%) on the retention time, theoretical plates and tailing factor were studied. During study other chromatographic conditions were kept same as per the experimental section.

Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc [30]. To determine the peak purity selectivity studies was performed by injecting diluents, standard solution and sample solution. Forced degradation study was carried out to prove the specificity of the method.Sample and placebo was exposed under relevant stress conditions: Thermal, photolytic, humidity, acid & base hydrolysis and oxidation (table 1). Peak of Fluticasone furoate was investigated for spectral purity in the chromatogram.

Table 1: Forced Degradation study conditions and % degradation

S.N.	Condition	Degrading agents/ conditions	Exposure period	% Degradation
1	Acid degradation	1N HCl	for 2 Hrs. at R.T.	6.2
2	Base degradation	1N NaOH	for 2 Hrs. at R.T.	0.4
3	Peroxide degradation	30% H_2O_2	for 24 Hrs. at R.T.	0.4
4	Thermal degradation	60°C	for 2 Days	0.6
5	Photolytic degradation	1.2 million lux hours and 200 watt hrs./m^2	-------	2.1
6	Humidity degradation	40°C/75% RH	For 7 days	4.3

Filter validation:

Sample solution was prepared according to the method. The solution was filtered through 0.45 µm filter and vials were filled by discarding 0 mL, 2 mL and 5 mL of solution and these solutions were injected as sample solutionand percentage assay was determined and absolute % difference between centrifuged and filtered sample was calculated.

Solution stability:

The system suitability solution and sample solutions were prepared on day 0 of experiment, stored these solutions at room temperature for every time interval up to 3 days and analyzed these solutions on subsequent days. The standard solution was prepared freshly at the time of analysis and calculated the % assay of analyte in the standard solution and in the sample solution.

RESULTS AND DISCUSSION

Method development and optimization:

The initial literature search indicated that very few HPLC methods are available for the drug as individual or in combination with different drugs which uses the buffer in mobile phase. Based on literature search, attempts were made to develop a simple method which requires less solvent, short runtime and high selectivity. Top priority was given elute the peak with water and acetonitrile only instead of buffer solution or only with organic solvents. In preliminary experiment the water and acetonitrile were used as mobile phase in different ratio with BDS C-18 (150mm X 4.6 mm X 5µm) column with flow rate 1ml/min and detection wavelength 240 nm. The column temperature was maintained ambient. Injection volume is 10µL and runtime is for 20 min. The expected peak with short retention time was obtained with the water and acetonitrile (20:80) ratio but peak shape was broad. The effect of column was checked. It improved peak shape. Finally the method was developed with Inertsil ODS -3V 250 x 4.6 mm, 5µ column. The chromatogram obtained was better than the previous one in all aspects with good peak shape, tailing factor, resolution and theoretical plate as per USP requirement. The retention time of fluticasone peak was about 5.0±1 minute. The Representative chromatogram of standard solution and sample solution is shown in the figure 2 and figure 3 respectively.

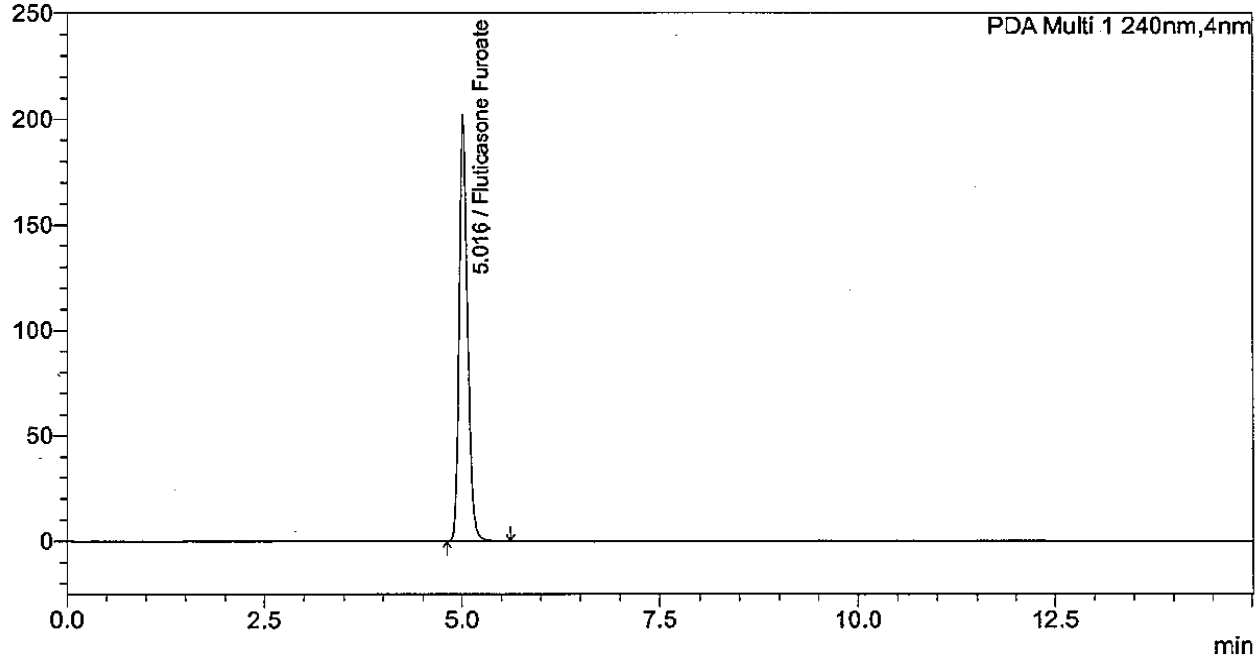

Figure 2: Representative chromatogram of standard solution

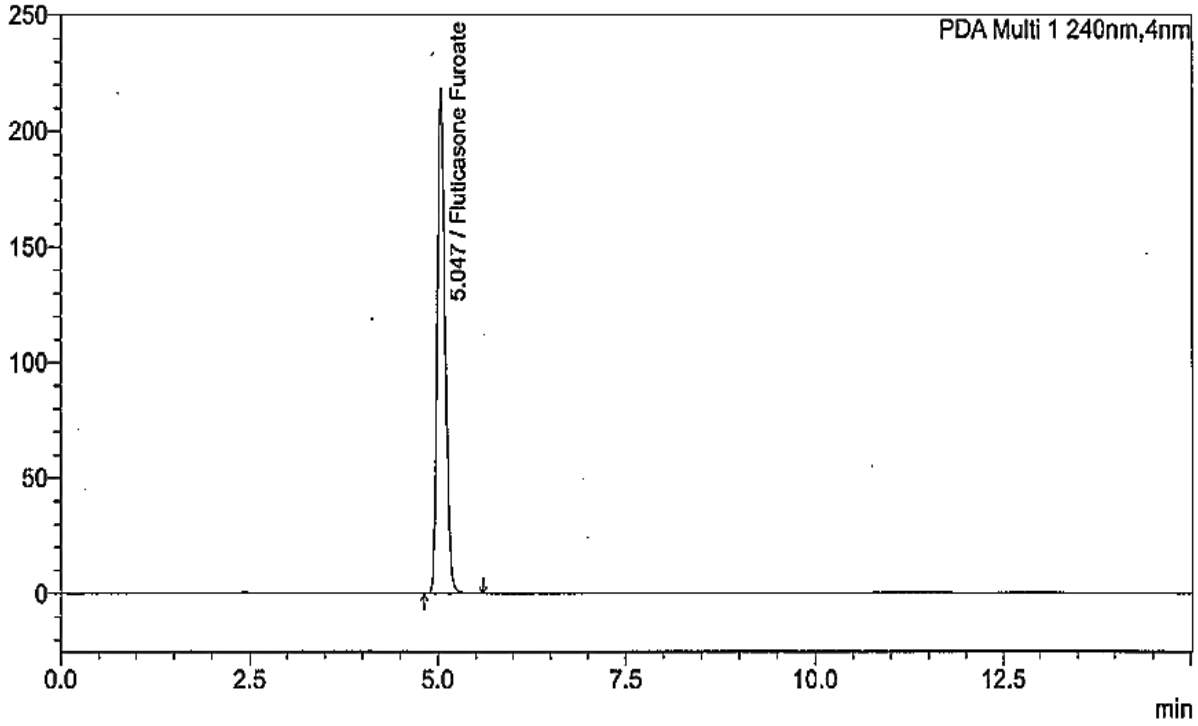

Figure 3: Representative chromatogram of sample solution

Method validation results:

The system suitability testing is used to verify that the reproducibility of the system are adequate for the analysis performed [30]. The system suitability parameters such as runtime, theoretical plates, peak purity, tailing factor were associated with confined values. The plot of peak area response against concentration is linear as shown in figure 4 over the concentration range of 27.5ppm-82.7ppm. The results of linearity are shown in table 2.

Table 2: Linearity results

Linearity Level	Conc. (%)	Conc. (ppm)	Area
1	50	27.58	813243
2	80	44.13	1302729
3	100	55.16	1630071
4	120	66.19	1950490
5	150	82.74	2444963
		Slope	29551.5238
		Intercept	-1762.8544
		Correlation Coefficient [R]	1.000
		R^2	1.000

It was found that correlation coefficient (r^2=1.00) and regression analysis were within the limits (table 4). To ensure the reliability and accuracy of the method recovery studies were carried out at 3 different levels (50 %, 100 %, and 150 %). The results of recovery studies are presented in table 3.

Table 3: Accuracy Results

Level	Amount added(ppm)	Amount recovered(ppm)	%Recovery*	%RSD
50%	27.60	27.72	100.5	0.64
100%	55.20	54.86	99.4	0.26
150%	82.80	82.70	99.9	0.12

* mean of three determinations

From results it is revealed that there is good correlation between amount added and drug found in overall concentration range. The system precision, method precision and intermediate precision (ruggedness) are calculated. The % RSD values (table 4) were below 2.0 % indicating a good precision [30].

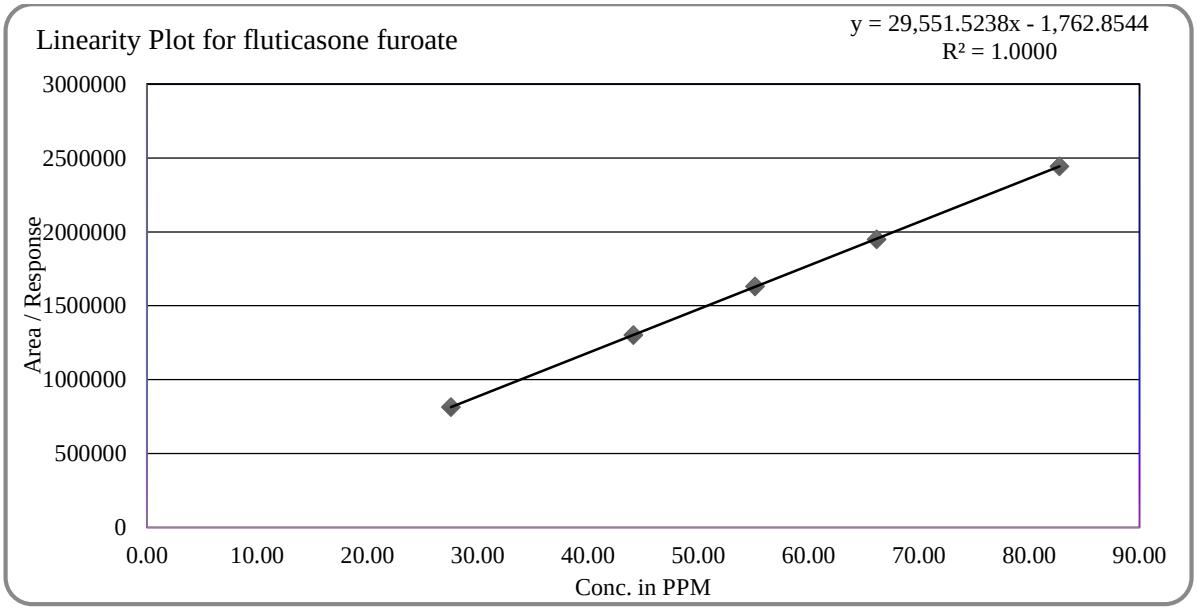

Figure 4: Linearity graph for assay of fluticasone furoate

Robustness of the method was determined by small deliberate changes in method parameters and the content of the drug was not adversely affected by these changes as evident from the low value of % RSD (table 4) that the method was robust. During assay study, there was no change in the content of the drug due to presence of excipient which reveals that the method is specific [30]. Forced degradation studies were performed to demonstrate selectivity and stability indicating capability of the proposed HPLC method. Analyte peaks were investigated for spectral purity in the chromatogram of all exposed sample and found spectrally pure (table 4).

Table 4: Overall results of parameters used in validation

S.N.	Parameter	Results
1	Linearity	27.5 (ppm)- 82.7 (ppm) linearity Equation y=29551.5238x-1762.8544 r2= 1.000
2	Accuracy	Mean % Recovery=99.93 %
3	Precision	
	system precision	% RSD=0.18
	method precision	% RSD=0.90
	intermediate precision	% RSD=0.90
	Overall results of analyst 1&2	% RSD=0.82
3	Specificity	
	Selectivity	No interference of diluent and placebo at the retention time of analyte peak and the analyte peak passes peak purity.

	Forced Degradation	Degradants peaks are well separated from the analyte peak and peak purity passes for analyte peak.	
4	Robustness	low (-)	high(+)
	Change in flow rate	0.9(ml/min) % RSD=0.05	1.1(ml/min) % RSD=0.07
	change in column oven temperature	25°C % RSD=0.03	35°C % RSD=0.13
	change in wavelength	237(nm) % RSD= 0.79	243(nm) % RSD=0.80
	change in mobile phase organic composition	(24:76 %v/v) (16:84 %v/v) % RSD=0.25	% RSD=0.37
5	Filter validation	% difference in result with centrifuged sample	
		0ml discarded=0.27 2ml discarded=0.05 2ml discarded=0.07	
6	Solution stability	stable for 3 days % RSD of standard solution=0.62 % RSD of sample solution=0.75	

The % RSD between results for the analyte obtained for stored standard and sample solution (table 4) is within limit up to the 3 days at room temperature then it can be concluded that solution is stable up to 3 days at room temperature. During filter validation % absolute difference between filtered and centrifuged test solution are less than 2.0 % (table 4) hence nylon membrane filter can be used by discarding first 5 ml.

CONCLUSION

An isocratic RP-HPLC method was successfully developed for the assay of Fluticasone furoate in liquid pharmaceutical formulation. The developed method is selective, precise, accurate, linear, and robust. Forced degradation data proved that the method is specific for the Fluticasone furoate. It can be utilized for assay determination of bulk

and finished product of Fluticasone furoate.

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