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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AZELASTINE AND FLUTICASONE IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, specific, precise, accurate, rapid and reproducible efficient reversed phase HPLC method with PDA detector has been developed and validation for simultaneous estimation of fluticasone (FLP) and azelastine (AZH) in pharmaceutical dosage form. Chromatography was performed on a 150mm X 4.6mm, 5µm particle size, Altima C18 column with a 62: 33:5 v/v/v mixture of buffer pH4.0: acetonitrile: methanol as a mobile phase. The detection of the combined dosage form was carried out at 235nm and flow rate employed was 1.0ml/min. The retention times were 2.1±0.3 and 3.1±0.3 min for fluticasone and azelastine respectively. Linear was established in the concentration range of 10.0 to 75.0µg/ml for FLP and 27.4 to 205.5µg/ml for AZH with a correlation coefficient of both drugs for found to be 0.999. The recoveries obtained were 99.80 -100.12% for FLP and 99.68 -100.26% for AZH. Similarly the %RSD value for precision was also found to be within the acceptable limit. 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. The results of the analysis were validated statistically and recovery studies confirmed the accuracy and precision of the proposed method. Developed method was rapid and convenient which could be successfully applied for the routine control of both the component.

KEYWORDS

Azelastine, Fluticasone and RP-HPLC.

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INTRODUCTION

Fluticasone (FLP) and Azelastine (AZH) is an antihistamine corticosteroid combination available as a nasal spray formulation. Azelastine, which is a second generation H1 receptor antagonist with potent topical activity. Chemically it is known as 4-[(4-chlorophenyl) methyl] -2-(hexahydro-1-methyl-1H-azepin-4yl) -1(2H) -phthalazinone. It occurs as a white almost odorless, crystalline powder with a

bitter taste. It has a molecular weight of 418.37[1-3]. Figure No.1. Fluticasone (FLP) a synthetic corticosteroid with anti-inflammatory properties. Chemically it is known as (6α, 11β-16α, 17β) -6,9 difluoro-11-hydroxy-16-methyl-3-oxo-17- (1-oxo-propoxy) androsta-1,4-diene-17-carbothioic acid-S-(fluoromethyl) ester. FLP is a white to off-white powder with a molecular weight of 500.58[1-3]. Figure No.2. There are very few methods appearing in the literature for the FLP and AZH individually with other combination drugs and in human plasma[4-18]. Thus, an attempt was made to develop a simple, precise, accurate and cost effective RP-HPLC method for the simultaneous estimation of Fluticasone and Azelastine in pharmaceutical dosage form (nasal spray preparations).

MATERIAL AND METHODS

Reagents and Chemicals Used

All the solvent and reagent used were HPLC and spectroscopic grade. HPLC grade , methanol, acetonitrile and Millipore water obtained from (Milli Q) was used in all experiments, potassium dihydrogen phosphate and ortho phosphoric acid was AR grade are used supplied by M/s SD Fine chemicals (Mumbai, India). Azelastine and Fluticasone are used as workings of reference standard were purchased from Dr. Reddy's Laboratory (Hyderabad, India). The Pharmaceuticals AZL and FLP nasal spray (DYMISTA) were purchased from local pharmacy (Meda Pharmaceuticals).

Instrumentation and Conditions

The chromatographic separation performed using Waters 2695 HPLC system with PDA detector. Software was used Empower version 2 to monitor and integrate the output single. Waters auto injector, thermostatted column compartment and Photo Diode Array detector was used. Waters column (Altima C-18 150mm X 4.6 mm X 5µ particle size) was used for the analysis. Before analysis the mobile phase was filtered through a 0.2µm filter and degassed using sonicator at the flow rate of 1.0ml per minutes. Sample solutions were also filtered through a 0.2µm filter and a liquids of 10µL were injected into the chromatographic system. The HPLC system was used in air-conditioned laboratory atmosphere temperature (25±2°C).

Preparation of buffer pH 4.0

Accurately weighed1.36gm of potassium dihydrogen ortho phosphate in a 1000ml volumetric flask and add 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water and pH adjusted to 4.0 with ortho phosphoric acid.

Preparation of Mobile Phase:

Mix a mixture of above buffer 620ml (62%), 330ml of acetonitrile (33%) and 50ml of methanol (5%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filteration.

Diluent

Water Acetonitrile (50:50v/v)

Preparation of Standard solution

Accurately weighed and transferred 5mg of fluticasone and 13.7mg of azilastine working standards into 10ml clean dry volumetric flask add 7.0ml of diluents, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from above the stock solutions was taken into a 10ml volumetric flask and made up to 10ml and pour into the vials to inject the solution.

Sample Preparation

Accurately 13.7 or 5ml from the formulation (nasal spray) was transferred to 25ml volumetric flask and made up to the mark with diluents. From the above solution 2.5ml was diluted to 10ml and pour into the vials to inject the solution.

Optimization of Chromatographic Conditions

The initial literature search indicated that many HPLC methods are available for individual drugs and their combination with different drugs. Based on literature search, attempts were made to develop a simple method which has less retention time and high selectivity, top priority was given for complete separation of azelastine and fluticasone. Several mobile phase were tested until good resolution obtained between two drugs.

In preliminary experiments all the two azelastine and fluticasone were subjected to separation by reverse phase HPLC equipped with the Altima C-18

(150mm X 4.6 mm X 5µm) column and with flow rate 1mL/min and detection wavelength of 235nm. Column temperature was maintained at ambient. Injection volume is 10µL and runtime is for 10min.
The mobile phase consists of buffer pH4, acetonitrile and methanol (75:10:15%v/v/v). These drugs were able to be separated on the chromatogram but failed in peak purity and peak shape was not good. The effect of mobile phase composition was checked. It improved peak purity. Finally a method developed with buffer pH 4: acetonitrile: water (62:33:5%v/v/v). 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 times of azelastine and fluticasone peaks are about 3.1±0.3 and 2.1±0.3 minutes respectively. The chromatograms were shown in the Figure No.3,4.

Validation

The method was successfully validated as per ICH guideline kQ2 (R1): validation of analytical procedures: text and methodology, international conference on harmonization, Food and Drug Administration, USA, November 2005. The method was validated and parameters were linearity, range, accuracy, precision, LOQ, LOD and robustness.

Specificity

The method is found specific and there is no blank or placebo interference.

Precision

To check the system precision (repeatability) for peak response obtained with five replicates of standard at specified concentration. The %RSD found to be within 2.0%. To check repeatability (method precision) of the method six individual sample preparations form same batch were prepared and injected the % RSD with six samples found to be within 2.0%. The results obtained were presented in Table No.2.

Accuracy

The accuracy of an analytical method is established across its range. Accuracy is performed in three different levels for azelastine and fluticasone. The known quantity of azelastine and fluticasone at 50%, 100% and 150% level is analysed for each level. The % recovery values for these drugs were found to be in between 99.68% to 100.26% and %RSD values were found to be less than 2.0%. The accuracy results were tabulated in the Table No.3,4.

Linearity and range

The Linearity of detector response to different concentration of these drugs was studied with a series of working standard solutions prepared by diluting the stock solution with diluents. The Standard plots were constructed between concentrations vs. peak area a linear response of peak area was observed over the concentration range of 10 to 75µg/mL for FLP and 27.4 to 205.5µg/mL for AZL. Ten micro-liter of each sample was injected under above chromatographic conditions and peak area was measured. The data of linearity curve was summarized in the Table No.1 and Figures No.5,6 and it was found that correlation coefficient (R^2) and regression analysis were within the limits.

LOD and LOQ

These methods were evaluated on the basis of signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit. A typical signal-to-noise ratio required for LOQ is 10:1 According to a formula given by miller, the limit of detection (LOD) and limit of quantification (LOQ) were calculated. The resulted are given in Table No.6.

Robustness

The robustness of an analytical method 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. Robustness was done by changing the column temperature, flow rate and the mobile phase.

Ruggedness

This is to prove the lack of influence of operational and environmental variables of the test results by using the method. The average of the six preparations and % RSD for the six observations was calculated and recorded. The method precision was carried out as described above using different

analyst, different column and different instrument. The % RSD for the six determinations shall be NMT 2.0%. The resulted are given in Table No.5.

System Suitability

According to USP system suitability tests are an integral part of chromatographic method validation. 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. 10µL 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 these results are presented in the Table No.8.

RESULTS AND DISCUSSION

To optimize the mobile phase various proportions of buffers with methanol and acetonitrile were tested. Mobile phase composition was changed and the method development was started by Altima C-18 (150mm X 4.6 mm X 5µm) column and with flow rate 1.0mL/min and detection wavelength of 235nm.

Column temperature was maintained at ambient. Injection volume is 10µL and runtime is for 10min. The mobile phase consists of buffer pH4: acetonitrile: methanol (62:33:5 %v/v/v) was used. The retention times of fluticasone and azelastine peaks are about 2.1±0.3 and 3.1±0.3 minutes respectively.

Quantitative linearity was observed over the concentration range of 10 to 75µg/mL for FLP and 27.4 to 205.5µg/mL for AZL. The regression equations of concentration of fluticasone and azelastine are found to be y= 2278.x + 692.1 and y= 10214 x+1439 respectively, where y is the peak area and x is the concentration of drugs (µg/mL). The correlation coefficient of fluticasone and azelastine was found to be 0.999 and 0.999 respectively.

The numbers of theoretical plates obtained were 2565.48 and 2991.48 for fluticasone and azelastine respectively which indicates the efficiency of the column. The high percentage recovery indicates that the proposed method is highly accurate. There is no interference of filters with standard and sample solutions as the difference in responses is within the limit. The %RSD was found to be less than 2.0%.

Table No.1: Linearity data showing equation of regression line and coefficient of determination

S.No	Drug	Conc. Range (µg/mL)	Equation	R^2
1	Fluticasone	10 - 75	y = 2278.x + 692.1	0.999
2	Azelastine	27.4 – 205.5	y = 10214x +1439	0.999

Table No.2: Precision results were summarized for Azelastine and Fluticasone

S.No	Injections	Azelastine (Area)	Fluticasone (Area)
1	1	1370930	117382
2	2	1385998	116002
3	3	1384522	116109
4	4	1382733	116941
5	5	1391190	116849
6	6	1380626	115688
Mean		1382667	116495
SD		6769.1	656.4
% RSD		0.49	0.56

Table No.3: The Accuracy results of Fluticasone

S.No	% Concentration (at specification level)	Amount added (µg/ml)	Amount Recovered (µg/ml)	% Recovery*	% R.S.D
1	50	25.0	25.01	100.03	0.45
2	100	50.0	50.06	100.12	0.57
3	150	75.0	74.85	99.80	0.50

*Mean of three determinations

Table No.4: The Accuracy results of Azelastine

S.No	% Concentration (at specification level)	Amount added (µg/ml)	Amount Recovered (µg/ml)	% Recovery*	% R.S.D
1	50	68.5	68.68	100.26	0.62
2	100	137.0	137.33	100.24	0.24
3	150	205.5	204.84	99.68	0.94

*Mean of three determinations

Table No.5: Ruggedness results were summarized for Azelastine and Fluticasone

Injections	Azelastine (Area)	Fluticasone (Area)
1	1407795	117898
2	1382880	121896
3	1403662	121147
4	1367925	117162
5	1370555	117061
6	1385234	118625
Average	1386342	118965
SD	16503.6	2072.8
% RSD	1.19	1.74

Table No.6: Results of LOD and LOQ for Fluticasone and Azelastine

S.No	Drugs	LOD (µg/ml)	LOQ (µg/ml)
1	Fluticasone	1.00	3.04
2	Azelastine	0.46	1.41

Table No.7: Typical Robustness results of Fluticasone and Azelastine

S.No	Conditions	Fluticasone			Azelastine		
		USP Plate count	USP Tailing	%RSD	USP Plate count	USP Tailing	%RSD
1	Flow rate minus	1596	1.07	0.6	1807	1.02	0.3
2	Flow rate plus	2641	1.08	1.2	2769	1.00	0.2
3	Organic Composition minus	1569	1.09	1.4	1717	1.07	1.5
4	Organic Composition plus	1681	1.07	0.34	1853	1.06	0.26
5	Temperature minus	2267	1.02	0.22	2283	1.04	0.72
6	Temperature plus	1765	1.00	0.23	1844	1.04	1.09

Table No.8: System Suitability data for Fluticasone and Azelastine

S.No	Parameters	Fluticasone	Azelastine
1	Retention time (min)	2.1±0.3	3.1±0.3
2	Theoretical plate	2565±163.48	2991±163.48
3	Tailing factor	1.04±0.117	1.01±0.117
4	Resolution	5.5	

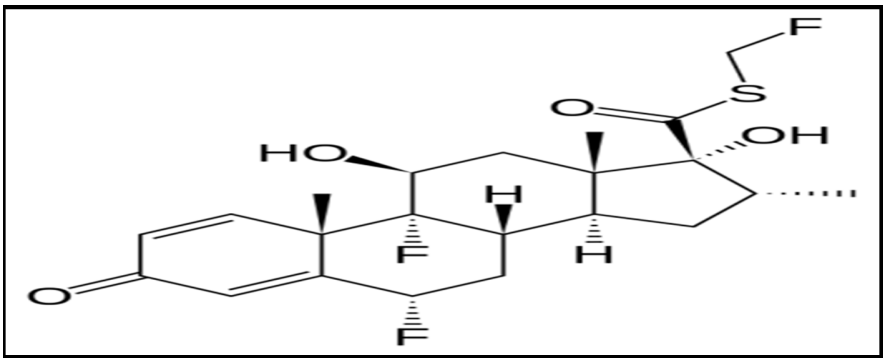

Figure No.1: Structure of Fluticasone

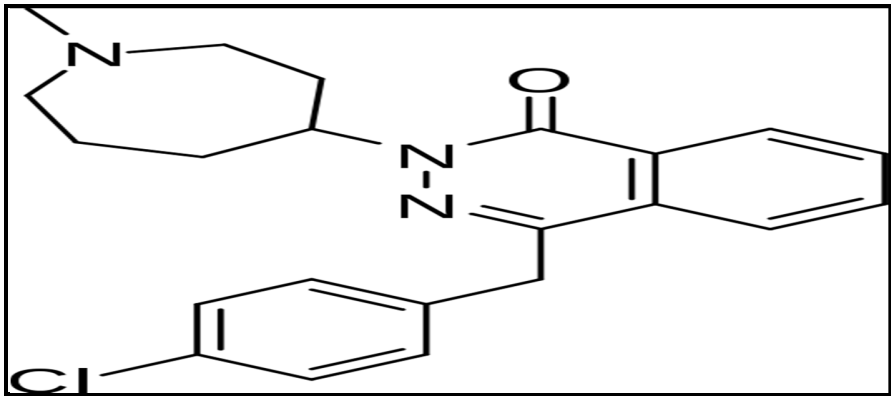

Figure No.2: Structure of Azelastine

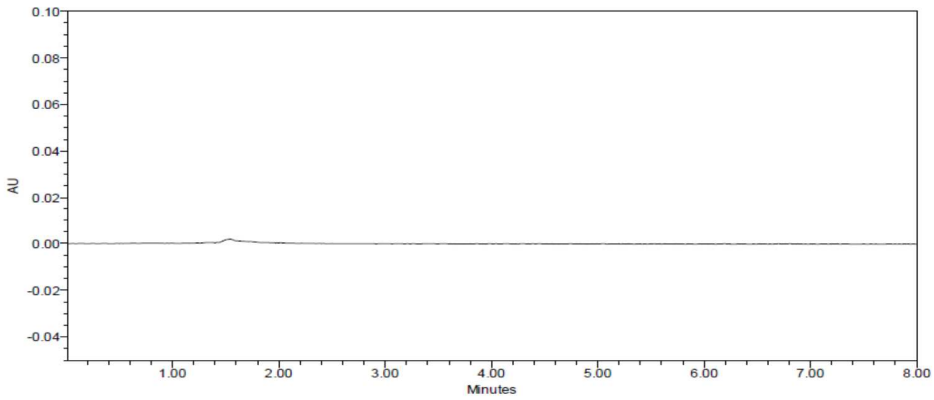

Figure No.3: A Blank chromatogram of fluticasone and azelastine

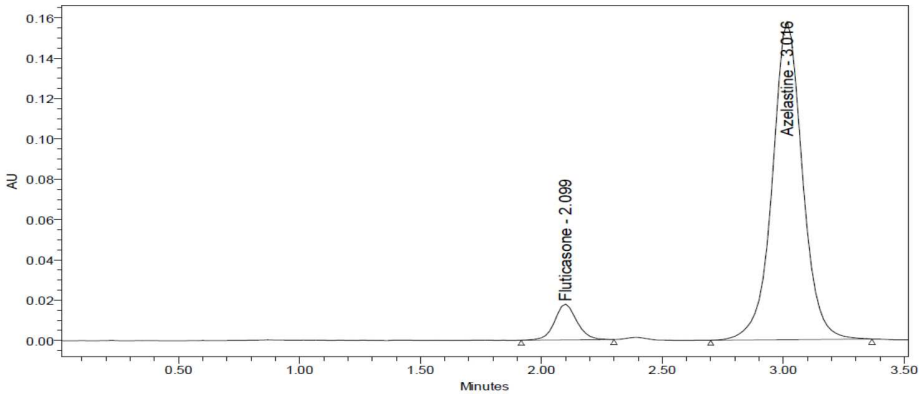

Figure No.4: Standard chromatogram of fluticasone and azelastine

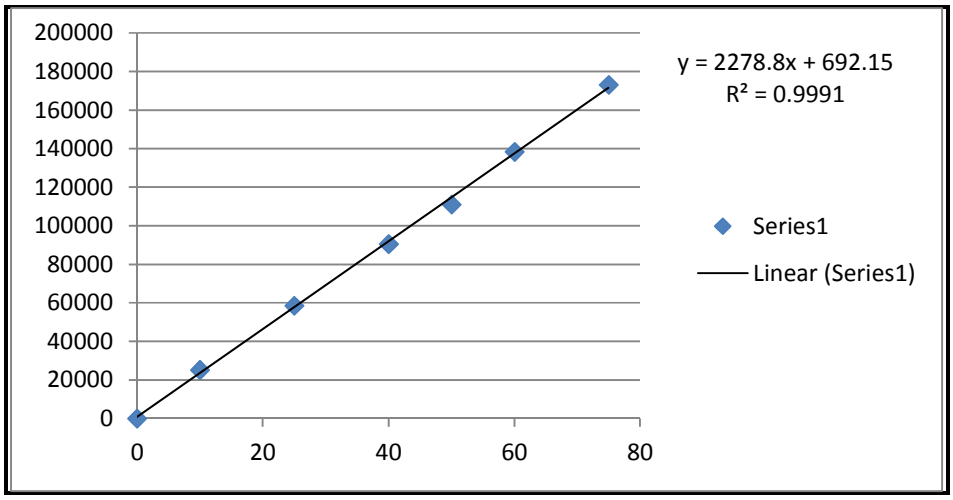

Figure No.5: Linearity graph of Fluticasone

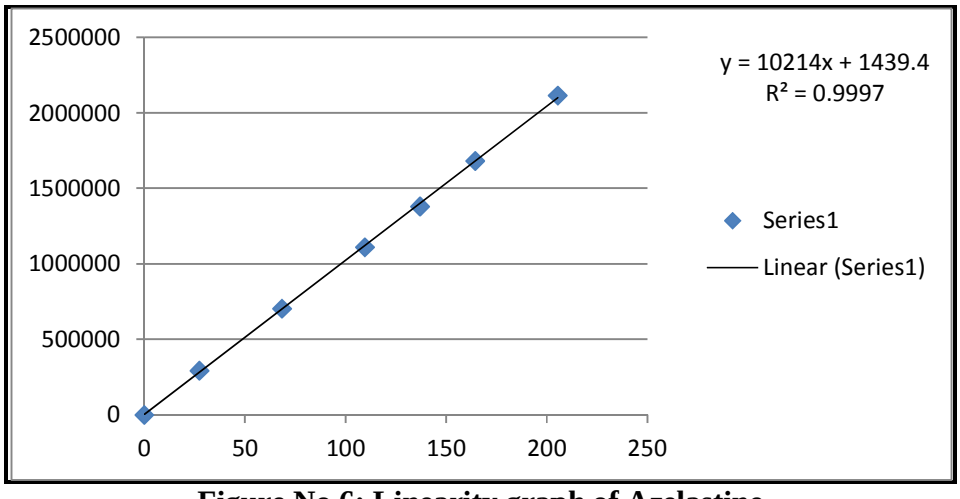

Figure No.6: Linearity graph of Azelastine

CONCLUSION

A simple, specific, accurate, precise, reproducible and efficient reverse phase high performance liquid chromatography method has been developed which can be used accurately for quantitative estimation of fluticasone and azelastine for routine analysis of individual and combination of drugs. Method was validated as per ICH Q2 (R2) so it can be used by analytical department.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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