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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION HPLC METHOD FOR NASAL SPRAY FORMULATION OF AZELASTINE HYDROCHLORIDE AND FLUTICASONE FUROATE WITH ACCELERATED STABILITY STUDIES

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ABSTRACT

The literature survey done reveals that a simple, rapid and economic high-performance liquid chromatography (HPLC) method for simultaneous estimation of Azelastine HCl (AZE) and Fluticasone Furoate (FF) is yet not reported. So it is needed to develop and validate such method. Such stability-indicating HPLC method is developed by using a Inertsil, ODS, C18, 3V, 250×4.6mm, 5µ HPLC column, water: Acetonitrile (30:70) % V/V. The mobile phase A: 20.35mM Phosphate buffer (pH 3.0) and mobile phase B: 100% ACN is used with gradient at a constant flow rate of 1.0mL min^{-1}. The composition was increased from0 to 15 min from 35 to 70% B and remained constant upto 20min. Further, decreased from 20 to 30 min to 35% with total run time of 30min at 236nm and successively validated. The main objective was to develop simultaneous method for estimation of AZE and FF with analysis of the stability profile of formulation under accelerated conditions. The retention time (Rt) of AZE and FF were observed as 8.5min and 18.5min respectively with the linearity range between 25-75µgmL^{-1} and 5-15µgmL^{-1} respectively. Forced degradation studies were performed on samples of AZE and FF Nasal Spray using acidic, basic, oxidative, thermal, photolytic and humid conditions. The resultant method was validated as per ICHQ2(R1) guidelines and successfully applied to the novel nasal spray for assessment of stability under accelerated conditions.

KEYWORDS

Azelastine HCl, Fluticasone Furoate, Stability-indicating HPLC method, Nasal Spray and Forced degradation study.

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INTRODUCTION

Earlier we have developed nasal spray formulation of AZE and FF. In the present research, a stability indicating HPLC method for this formulation was developed and validated as well as applied simultaneously for assessment of the stability of this formulation under accelerated conditions.

AZE is a potent, second-generation, selective, histamine antagonist (histamine-H1-receptor antagonist). Its chemical name is (±)-1-(2H)-phthalazinone, 4-[(4chlorophenyl) methyl]-2-(hexahydro-1-methyl-1Hazepin-4-yl)-, monohydrochloride (Figure No.1). Its molecular formula is $C_{22}H_{24}C_lN_3O$• HCl and molecular weight is 418.362g/mol, with the following chemical structure[1].

It is used to symptomatic treatment of seasonal allergic rhinitis. AZE nasal spray is indicated for the local treatment of the symptoms of seasonal allergic rhinitis and perennial allergic rhinitis, such as rhinorrhea, sneezing and nasal pruritus in adults and children 5 years of age. It is also indicated for the treatment of vasomotor rhinitis in adults and children ≥ 12 years old[2].

The usage of corticosteroids is generally considered as the most effective treatments for the management of inflammatory diseases including asthma and allergic rhinitis[3]. FF is a synthetic corticosteroid derived from fluticasone showing synthetic trifluorinated corticosteroid with potent anti-inflammatory activity[4]. The chemical name of FF is [(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] phena-nthren-17-yl] furan-2-carboxylate (Figure No.2). Its molecular formula is$C_{27}H_{29}F_3O_6S$ and molecular weight is 538.578g/mol with chemical structure as follows[5].

It is marketed by Cipla as Furamist AZ[6], GlaxoSmithKline as Veramyst and Flonase Sensimist, Allermist and Avamys for the treatment of non-allergic and allergic rhinitis administered by a nasal spray. AZE and FF Nasal Spray consists of AZE 0.1% W/W and FF 0.0197% W/W. Former researchers were studied on the development and validation of AZE in nasal spray formulation by RP-HPLC method[7] whereas some of them had developed and validated the HPLC method for estimation of fluticasone[8]. AZE nasal spray and fluticasone nasal spray used in combination may provide a substantial clinical benefit for patients with SAR compared with therapy with either agent alone[9]. Buta simple, rapid and economic method for the simultaneous estimation of the AZE and FF is yet not developed and validated. So it is necessary to develop and validate stability indicating HPLC method for determination and quantitation of the same drug combination simultaneously in nasal spray formulation.

The attention was not on the separation and identification of each and every degradation product. Since the method was to be applied for stability assessment, the separation between drug peak and all other degradation peaks was considered sufficient.

MATERIAL AND METHODS

Materials

Chemicals and reagent

AZE and FF were obtained as a gift sample from Sava Healthcare Ltd Chinchawad., (Maharashtra, India). HPLC grade Acetonitrile (ACN) (Merk, Germany), Analytical grade Triethylamine (Merk, Germany), Potassium Dihydrogen Phosphate and 1-Octane Sulphonic acid, Diluted Orthphosphoric acid (Merk, Germany) were used from the analytical laboratory. High purity water (Miillipore) was obtained from the organization laboratory.

Instrumentation

The HPLC system (Waters Corp Ltd) consisted of a pump with autosampler injection facility. The detector consisted of a Photodiode Array operated at a wavelength range is 200nm- 800nm. The software used was Empower 2.0. The column used was Inertsil ODS C-18 (250mm×4.6mm, 5µm). Absorbance measurements were made on Photodiode Array detector. The balance used was Shimadzu AUW220D Model EQ-621, pH meter used was Equip-Tronics Micro Controller pH Meter having range 0.0-14.00 and Ultrasonicator used was consisted of Model 5.5L-150H provides high efficient solubilization.

Chromatographic Conditions

The analysis of all compounds was carried out at 30ºC using a Inertsil ODS C18 (250mm×4.6mm, 5µm) column. The mobile phase A was prepared of 20.35mM phosphate buffer (pH adjusted to 3) and the mobilephase B was 100% acetonitrile. The

developed gradient program was 0.01 min-35% B, 15.0 min-70% B, 20.0 min-70% B, 25.0 min-35% B, 30.0 min-35% B. The mobile phase was filtered through a 0.22µm membrane filter and delivered at a constant flow rate of 1mL min^{-1}. The diluent used for the preparation of various solutions was water: acetonitrile in the ratio of 30:70% V/V. The injection volume was 20µL and the analytes were detected by UV detector at 236nm.

Methods

Preparation of stressed/ degradation samples

The degradation products were prepared as per degradation conditions specified in ICHQ2(R1) guidelines. An accurately weighed quantity of AZE and FF (50mg and 20mg resp.) was dissolved in 1mL water (50µgmL^{-1} and 10µg/ml). Further, 10mL of 1 N Hydrochloric acid (HCl) was added. The resulting solution was maintained at RT for 48 hrs. The maintained solution subsequently neutralized with 1N NaOH solution (Sodium Hydroxide) for acid hydrolysis/degradation. The volume was adjusted to 100mL using diluent. Likewise, the solution for alkaline hydrolysis of AZE and FF (50mg and 20mg resp.) was prepared by adding 10mL of 1 N NaOH followed by maintaining at RT for 48hrs. And its subsequent neutralization by 1 N HCl. The volume of resulting solution was adjusted to 100mL using diluent. For oxidative degradation, AZE and FF (50mg and 20mg resp.) solution was treated with 10mL of H_2O_2 (30%V/V), further maintained at RT for 48hrs. Then the volume was adjusted to 100mL using diluent. For thermal degradation, AZE and FF (50mg and 20mg resp.) solution was heated at 60ºC for 2 days on a hot plate. Photolytic degradation of AZE and FF (50mg and 20mg resp.) solution was carried out at condition of 1.2 million lux hrs. And 200 watt hrs./m^2.

The stressed solutions of AZE and FF (50mg and 20mg resp.) solution obtained above were filtered through 0.45µ pore size filter (Chrompack). From this resulting solution, 20µL solution was injected into the HPLC system and % degradation in each case was calculated by comparing with a standard peak of pure drug.

Development of stability indicating HPLC method

The separation between the degradation products and the drug there is need of evaluation of various HPLC parameters like type of column, pH of buffers, the ratio of the mobile phase, flow rate, detection wavelength; were optimized which are described under results and discussion section.

Method validation

Preparation of stock solution

The stock solutions of pure AZE (1000µg mL^{-1}) and FF (200µg mL^{-1}) were prepared and used for further analysis.

Linearity and range

A stock solution of AZE was diluted upto 75µg $mL^{-1}$and FF was diluted upto 15µg mL^{-1} using diluent. The prepared samples were injected in HPLC system in triplicate manner (n = 3). Additionally, the linearity curve was obtained by plotting the area of peak against the concentration of the respective solution injected.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were determined by using standard deviation (SD) of the response (area of peak) and slope of linearity curve (Eqs. (1) and (2)).

LOQ= 10 (SD)/Slope (1)

LOD= 3.3 (SD)/Slope(2)

Precision

System precision

The standard solution was prepared in the predetermined manner and injected in six replicates. The precision of the method was calculated by calculating % Relative standard deviation (RSD). % RSD of peak areas for six replicate injections of standard solution should not be more than2.0 for each analyte.

Method Precision

The six samples were prepared as per the proposed method. The % assays of these samples were determined and the precision of the method was evaluated by computing the % RSD of the results.

Intermediate Precision (Ruggedness)

Intermediate precision or ruggedness expresses ability of method to produce reliable result under within laboratories variation like different days,

different analysts, different equipment, etc. The six samples were prepared as per the proposed method. The % assay of these samples was determined and the precision of the method was evaluated by computing the % RSD of the results.

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 value obtained by the method. The standard stock solution was spiked to placebo in triplicate at different levels i.e. 50%, 100% and 150% of assay concentration of both the analytes and analyzed as per method described. All the mixtures were analyzed separately under optimized chromatographic conditions. The analysis was performed in triplicate and % recovery was calculated.

Filter Validation

The sample solutions were prepared as per determined method and then all filtered and centrifuged solutions at 5000rpm for 10min were analyzed in single sequence. The absolute % difference in the area of sample between the centrifuged and filtered solutions should not be more than 2.0 for both analytes.

Robustness

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. Robustness study was performed by making small but deliberate changes related to the flow rate (0.9 and 1.1mL/min), mobile phase B at -5% and +5% and detection wavelength (233 and 239nm), column oven temperature (25ºC and 35ºC), mobile phase buffer pH (2.8 and 3.2) by using optimized HPLC conditions. The peak area, theoretical plates, tailing factor in each replicate injection are recorded and reported the results and calculated the % RSD of area of five replicate injections of the standard solution for both analyte.

Stability of analytical solution

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 2 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 solutions.

Accelerated stability studies of nasal spray formulation

The nasal spray formulation was prepared as per optimized developed composition. The prepared formulation was stored for accelerated stability testing as per ICH Q1A (R2) guideline (at 40ºC ± 2ºC 75% RH ± 5% RH for 6 months and sampling at 0, 1, 3, 6 months). The acceptance criteria for evaluation parameters under accelerated stability study are summarized in Table No.1[10-14].

Quantitative HPLC assay

The AZE standard stock solution was prepared by weighing accurately about 50mg of AZE working standard and transferred in to a 50mL volumetric flask, then about 30mL of diluent was added, sonicated to dissolve, cool and diluted up to the mark with diluent and mixed well. The FF standard stock solution was prepared by weighing accurately about 20mg of FF working standard and transferred in to a 100mL volumetric flask and about 70mL of diluent was added, sonicated to dissolve, cool and diluted up to the mark with diluent and mixed well. Furthermore both the solutions were mixed by transferring 5mL of each AZE and FF standard stock solution in to a 100mL volumetric flask, diluted up to the mark with diluent and mixed well.

The sample solution was prepared from mixed contents of 3 bottles. Transferred quantity of nasal spray equivalent to 5.0mg of AZE and 0.985mg of FF (about 5gm sample) into a 100mL volumetric flask and shook well. Added about 70mL of diluent and sonicated for 20 minutes. Cool and adjusted to volume with diluent, mixed well. Centrifuged the sample solution at 5000 RPM for 10 min and filtered through 0.45µ nylon filter by discarding first few mL of filtrate and used for the estimation of % assay of AZE and FF[11].

RESULTS AND DISCUSSION

Development of stability-indicating HPLC method

The HPLC method developed in the current study exhibited the separation of AZE and FF from its degradation products formed using 0.1 N HCl, 0.1 N NaOH, 30% V/V H_2O_2 and thermal, humidity and photolytic treatment. This method also permits the quantitation of AZE and FF in nasal spray formulations. The retention times (Rt) of the AZE and FF were found to be 8.6 and 18.5 min respectively (Figure No.3B) in the sample solution. However for degradation products it was within the range of 2.5 to 4.5 min. respectively.

Optimized conditions

Mobile phase for HPLC analysis consisted of 2.76gm of Potassium di-hydrogen phosphate, 1.08gm of 1- Octane sulphonic acid sodium salt and 1mL of Triethylamine in 1000mL of water. Adjust the pH to 3.0 with dilute orthophosphoric acid, filter and degas through 0.45 µ filter and use. The injection volume was 20µL prepared using water: CAN (30:70% V/V) as diluent. The flow rate and column temperature were maintained at 1mL/min and 30ºC, respectively. The analysis was carried out at detection wavelength (max) of 236nm. The HPLC chromatogram showed retention time of AZE and FF as 8.6 and 18.5 min resp. (Figure No.3).

Forced degradation study of AZE and FF

HPLC chromatograms obtained by acidic, oxidative and alkaline degradation showed degradation products within the range of 2.1-4.1 min. for AZE and at 13.3min for FF (Figure No.4B).

Method validation

Linearity and range

The linearity was observed within the range of 25-75µg mL^{-1} for AZE which was calculated by linear regression analysis and the correlation coefficient, slope, and intercept in the calibration curve were observed at 0.9997, 38306.46and 19443.05, respectively while the linearity was observed within the range of 5-15µg mL^{-1} for FF which was calculated by linear regression analysis. The correlation coefficient, slope, and intercept in the calibration curve were observed at 0.9999, 61573.63 and 3706.7414, respectively.

LOD and LOQ

LOD for AZE and FF was found to be 0.3µg mL^{-1} and 0.1µg mL^{-1} respectively, moreover LOQ for AZE and FF was found to be 1.1µg mL^{-1} and 0.2µg mL^{-1} respectively. This shows the sensitivity of the developed method.

Precision

The developed HPLC method was found to be precise as indicated by % RSD (less than 2%) observed below than acceptance criteria of ICH Q2(R1) guidelines (Table No.4).

Accuracy

The solutions were analyzed under optimized chromatographic conditions in triplicate manner. The calculated % recovery was detected within prescribed limits as per ICH Q 2(R1) guideline (Table No.5).

Filter validation

The samples were prepared as per developed method. All the solutions were analyzed in single sequence. The results obtained for filtered and centrifuged samples were calculated and reported along with the absolute difference between centrifuged and filtered sample (Table No.6). The absolute % difference in the area of sample between the centrifuged and filtered solutions should not be more than 2.0 for both analytes.

Robustness

The % RSD calculated for the average peak area was observed within acceptable limits (less than 2%) as per ICH Q 2(R1) guideline (Table No.7).

Stability of analytical solution

The cumulative % RSD for the average values of % assay obtained in standard and sample solutions for each analyte at periodic intervals should not be more than 2.0. The solution is considered stable, till the time point where the cumulative % RSD of the stored sample and standard preparation is not more than 2.0 for each analyte (Table No.8).

Accelerated stability studies of Nasal Spray formulation

Quantitative assay of nasal spray formulation

The assay content for formulation was observed (Table No.9) within prescribed limits as per USP monograph (NLT 90.0% and NMT 110.0%)[15].

Accelerated stability study of nasal spray formulation

HPLC assay of the nasal spray (stability samples)

The accelerated stability samples of the nasal spray were analyzed by the established HPLC method. There was no any degradation product as indicated after comparing it with chromatogram of placebo sample which are stored for 1-6 month. The peaks detected in all stability samples were related to formulation while all other extra peaks observed were due to the excipients present as evidenced from the chromatogram of placebo sample (Figure No.5).

All the evaluation parameters under accelerated stability testing of the formulation were observed within the acceptance criteria (Table No.9).

Quantitative assay of the marketed nasal spray formulations (stability samples)

The assay content for marketed formulations was observed (Table No.9) within recommended limits as per USP monograph (NLT 90.0% and NMT 110.0%)[15].

In the present study, the developed method was applied on the in-house formulated nasal spray formulations and Furamist AZ Nasal Spray manufactured by Cipla Limited[6] (Figure No.6) to evaluate % assay of individual formulation (Table No.10).

Table No.1: Acceptance criteria for accelerated stability study

S.No	Test	Standard values	References
1	Appearance	Turbid Solution	10
2	pH	4.5-6.5	10
3	Viscosity	83.2cp	10
4	Assay	90-110% w/v	11
5	%RSD	Less than 2%	12,13,14
6	No. of Theoretical Plates	NLT 5000	12,13,14
7	Tailing factor	NMT 2.0	12,13,14

Table No.2: Optimization of method for HPLC development

Trial No	Optimizing parameters	Column specification	Observation	Inference
1	Isocratic Method with Buffer pH 3.0 : ACN (50:50) % V/V	Sunfire C18	Peaks found but AZE peak is in void volume, FF peak is improper and resolution between two peaks is very less.	Method Rejected
2	Gradient Optimization	Sunfire C18	Peaks found but the base line is improper.	Gradient Optimized
3	Current gradient with same Mobile Phase Buffer pH 3.0: ACN	Sunfire C18	Peaks found but with improper peak shapes.	Column Rejected
4	Current gradient with same Mobile Phase Buffer pH 3.0: ACN	Inertsil, ODS, 3V, 250×4.6mm, 5µm, Old column	Peaks found with improper peak shapes.	Column Rejected

Table No.3: Forced degradation observed during forced degradation study

S.No	Degradation Type	Peaks area of standard		Peak area		% Degradation	
		AZE	FF	AZE	FF	AZE	FF
1	Acid degradation	18,66,665	5,38,149	1961792	544277	1.1	6.1
2	Base degradation			1885530	421515	3.0	27.3
3	Peroxide degradation			1909920	567903	1.7	1.9
4	Thermal degradation			1838196	508424	2.7	1.2
5	Photolytic degradation			1840653	512611	2.4	0.2
6	Humidity degradation			1848904	511710	2.8	1.2

Table No.4: Observations for precision data

S.No	Parameters	AZE	FF
1	Method Precision (%RSD)	0.96	0.43
2	System precision (%RSD)	0.18	0.33
3	Intermediate precision (%RSD)	1.38	0.75

Table No.5: Evaluation of accuracy

S.No	Level (%)	Extra added quantity (%)		Amount recovered (%)		% Recovery	
		AZE (µg/ml)	FF (µg/ml)	AZE (µg/ml)	FF (µg/ml)	AZE (µg/ml)	FF (µg/ml)
1	50	24.510	5.115	24.389	5.068	99.5	99.1
2	100	49.020	10.230	48.479	10.323	99.6	100.2
3	150	73.530	15.345	73.430	15.376	99.9	100.9

Table No.6: Filter validation data

S.No	Sample solution	AZE	FF
		Absolute % Difference in Area	Absolute % Difference in Area
1	Centrifuged	NA	NA
2	0mL discarded	1.74	0.39
3	1mL discarded	1.01	0.42
4	2mL discarded	1.87	1.00

Table No.7: Robustness data obtained for different parameters by HPLC method

S.No	Parameters	Changes in parameters	AZE			FF		
			Mean (Peak area)	SD	% RSD	Mean (Peak area)	SD	% RSD
1	Flow rate ± 0.1mL/min	0.9mL/min	2254514	1545.77	0.07	656292	747.09	0.11
		1.1mL/min	1859442	1258.79	0.07	540471	1167.76	0.22
2	Gradient- B Mobile phase	- 5.0%	2056060	4138.38	0.20	595705	1078.86	0.18
		+ 5.0%	2060917	1505.79	0.07	602514	578.21	0.10
3	Wavelength ± 3nm	233nm	2380958	5960.50	0.25	506263	1598.45	0.32
		239nm	1486073	3824.10	0.26	566105	1647.28	0.29
4	Column oven temp± 5°C	25°C	2046417	1392.93	0.07	588504	1489.32	0.25
		35°C	2048054	1261.71	0.06	594890	1219.58	0.21
5	Buffer pH ± 0.2	2.8	2025894	977.33	0.05	588314	1191.53	0.20
		3.2	2026635	2371.29	0.12	588048	437.42	0.07

Table No.8: Data obtained for analytical solution stability

S.No	Time point	Stability data for Standard solution							
		AZE				FF			
		Cumulative							
		% Assay	Average	SD	% RSD	% Assay	Average	SD	% RSD
1	Day-0	100.0	NA	NA	NA	100.0	NA	NA	NA
2	Day-1	102.6	101.3	1.84	1.82	102.0	101.1	1.56	1.54
3	Day-2	111.6	104.7	6.09	5.82	114.3	105.5	7.70	7.30

S.No	Time point	Stability data for Sample solution							
		AZE				FF			
		Cumulative							
		% Assay	Average	SD	% RSD	% Assay	Average	SD	% RSD
1	Day-0	96.6	NA	NA	NA	97.5	NA	NA	NA
2	Day-1	95.7	96.2	0.64	0.67	96.4	97.0	0.78	0.80
3	Day-2	100.8	97.7	2.72	2.78	105.1	99.7	4.74	4.75

Table No.9: Data for accelerated stability study of formulation

S.No	Condition		% assay for AZE	% assay for FF
1	Initial	NA	101.4	99.5
2	1 month	25ºC/60% RH	98.6	99.0
		30ºC/60% RH	98.2	99.5
		40ºC/60% RH	97.8	99.0
3	3 months	25ºC/60% RH	98.1	99.0
		30ºC/60% RH	98.2	94.9
		40ºC/60% RH	97.7	97.5
4	6 months	25ºC/60% RH	93.2	99.5
		30ºC/60% RH	93.2	97.5
		40ºC/60% RH	94.4	97.0

Table No.10: Data obtained from quantitative evaluation of marketed and in-house formulated samples

S.No	Formulation	Stage	Condition	% Assay for AZE	% Assay for FF
1	In-House (972-13)	Initial	RT	100.0	96.4
2	In-House (972-07)	8M	RT	99.0	96.4
3	Furamist AZ (SA64012)	23M	RT	93.0	101.5

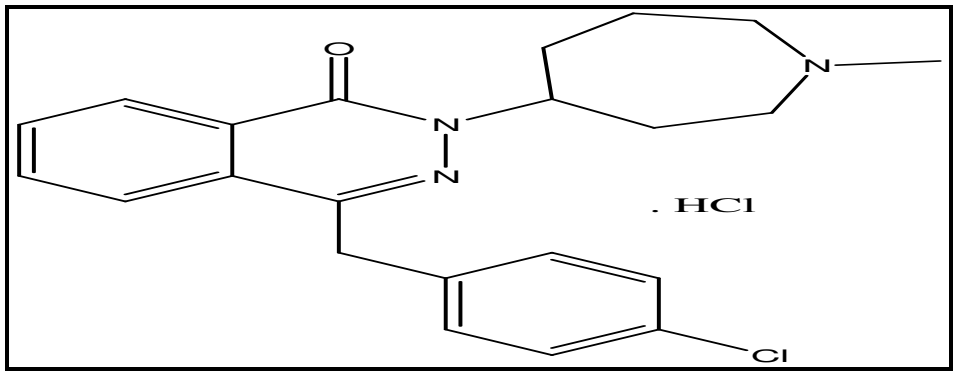


Figure No.1: Chemical structure of AZE

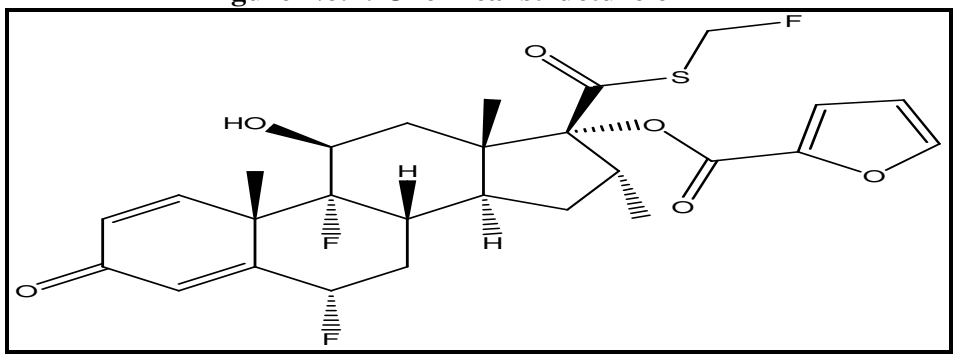
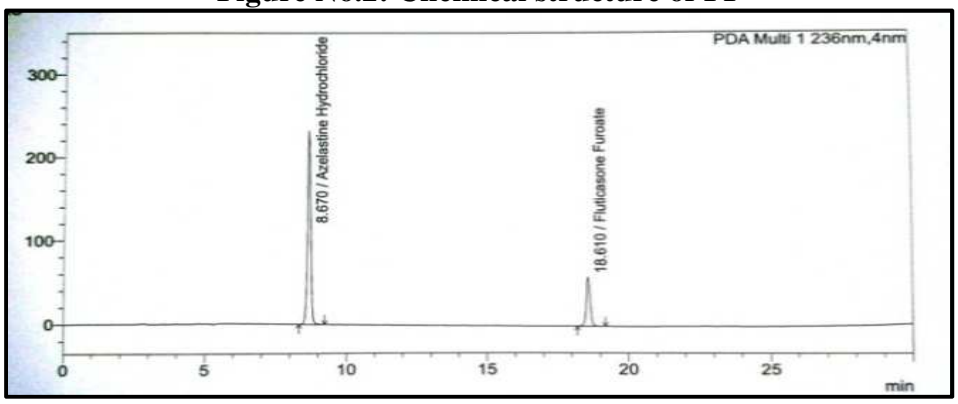
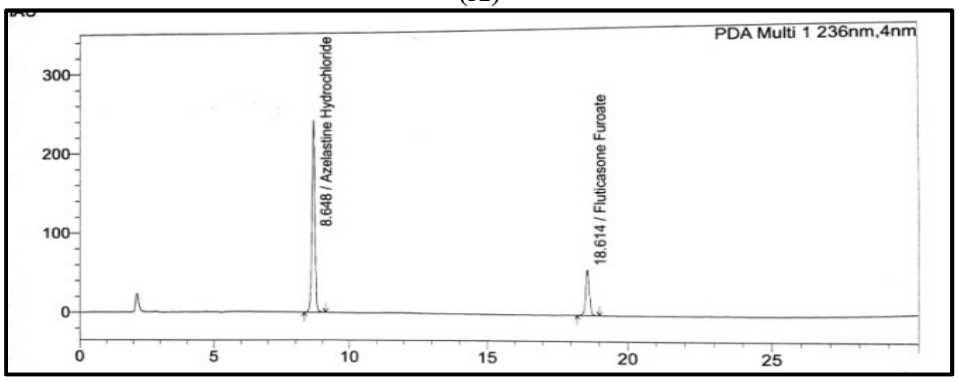


Figure No.2: Chemical structure of FF

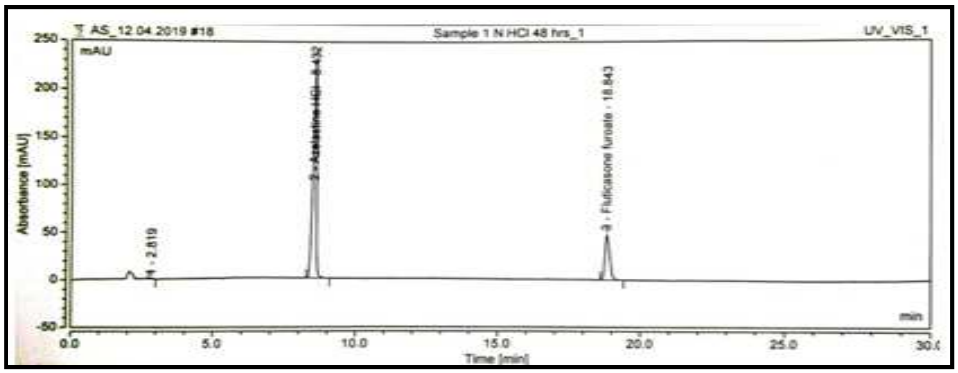


(A)

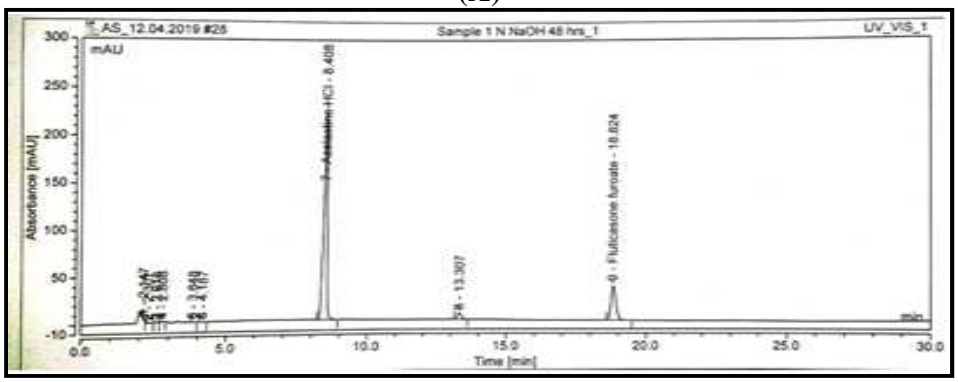


(B)

Figure No.3: HPLC chromatogram for mixed (A) standard solution and (B) sample solution containing AZE and FF



(A)



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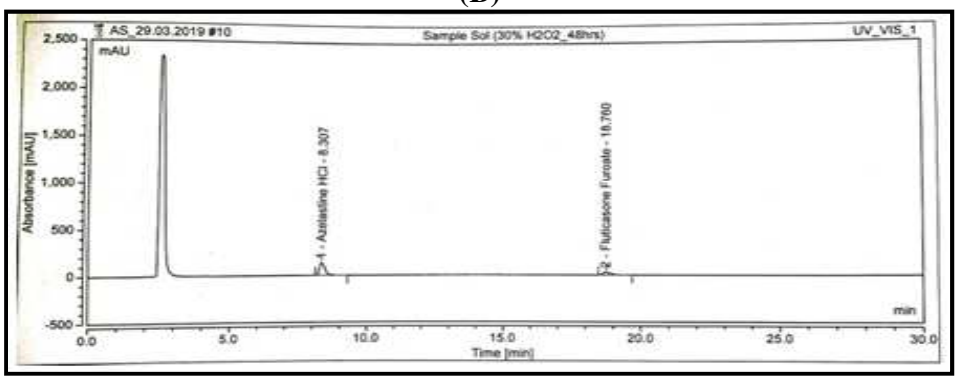
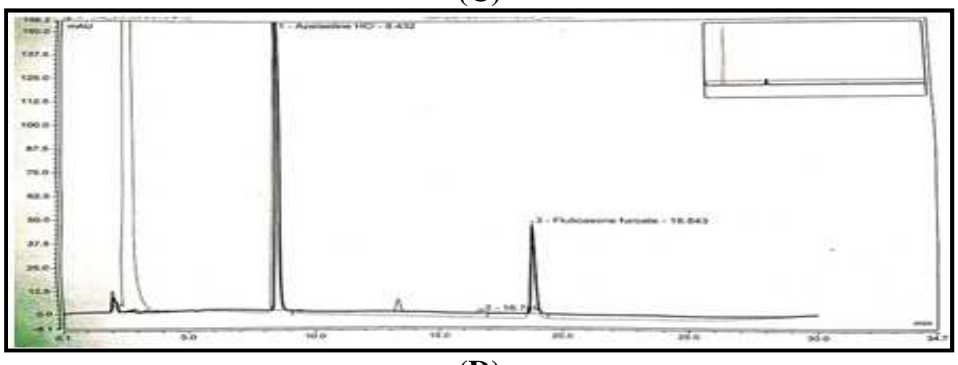
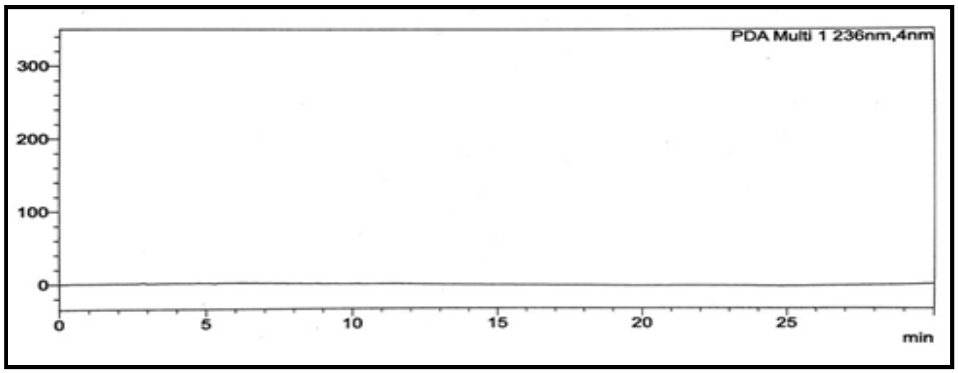


(C)

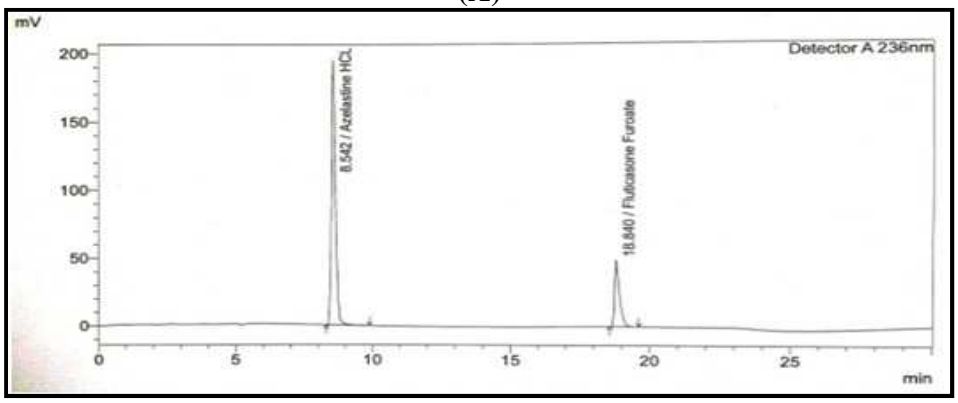


(D)

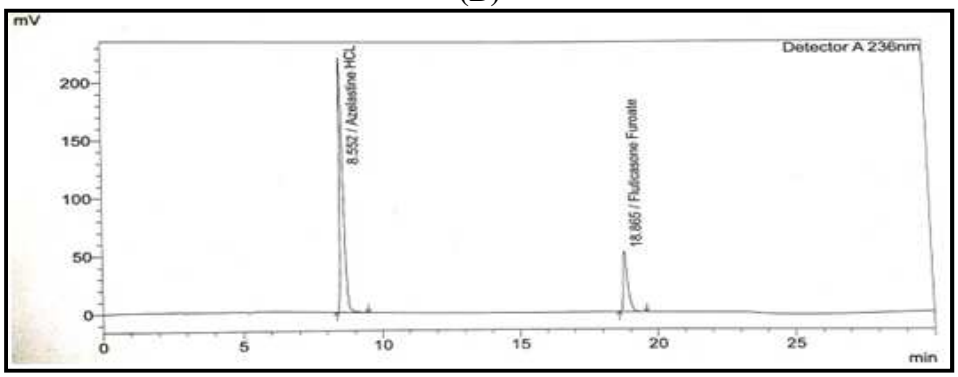
Figure No.4: HPLC chromatogram for (A) Acidic degradation (B) Alkaline degradation (C) Oxidative degradation (D) Overlay of A, B, and C


PDA Multi 1 236nm,4nm
300
200
100
0
0
5
10
15
20
25
min

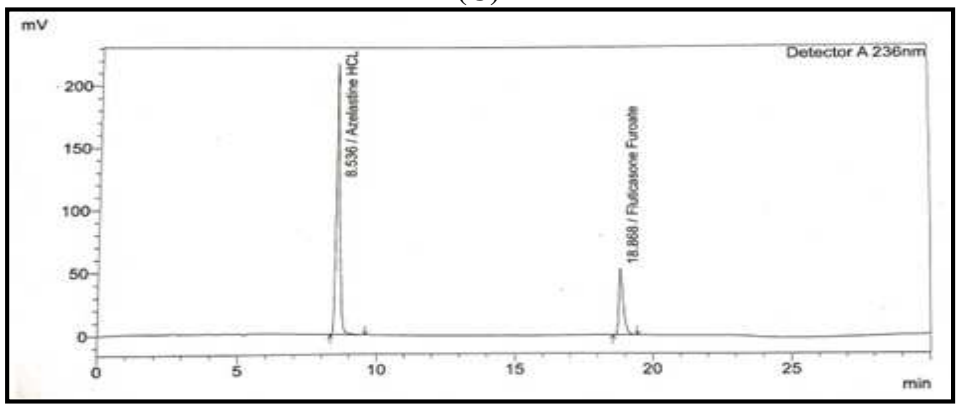
(A)


mV
Detector A 236nm
8.542 / Azelastine HCL
18.840 / Fluticasone Furoate
200
150
100
50
0
0
5
10
15
20
25
min

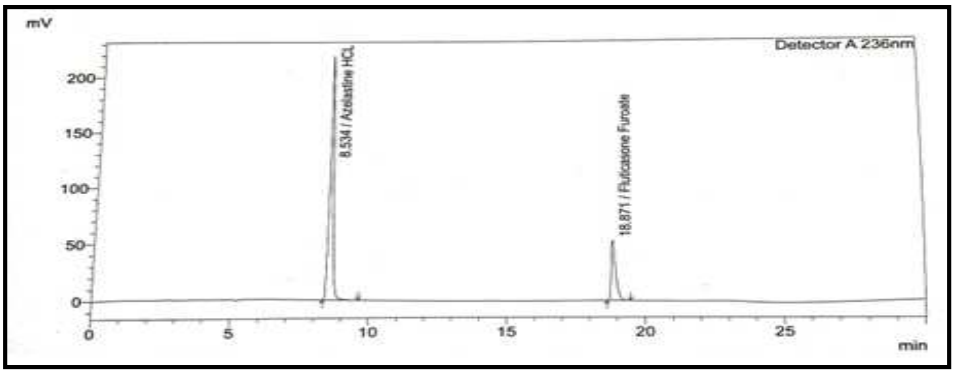
(B)


mV
Detector A 236nm
8.552 / Azelastine HCL
18.865 / Fluticasone Furoate
200
150
100
50
0
0
5
10
15
20
25
min

(C)

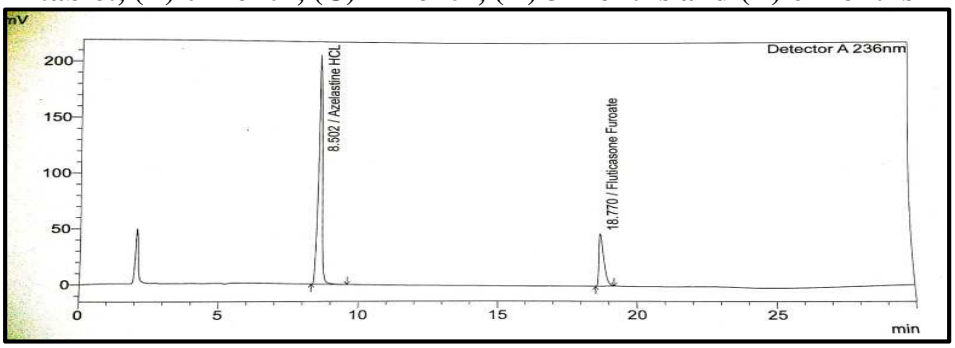

mV
Detector A 236nm
8.536 / Azelastine HCL
18.868 / Fluticasone Furoate
200
150
100
50
0
0
5
10
15
20
25
min

(D)

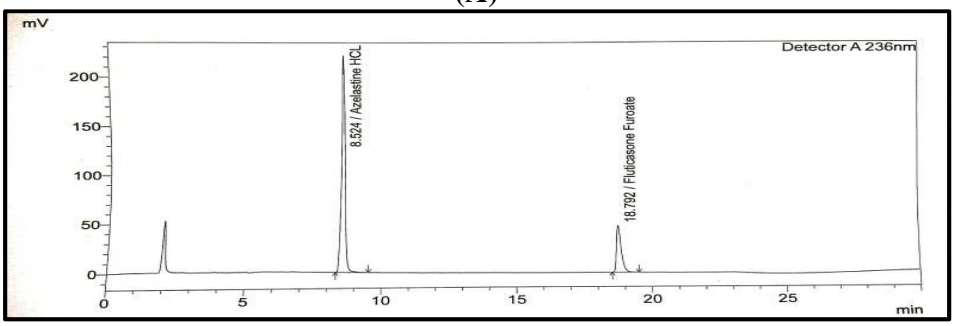


(E)

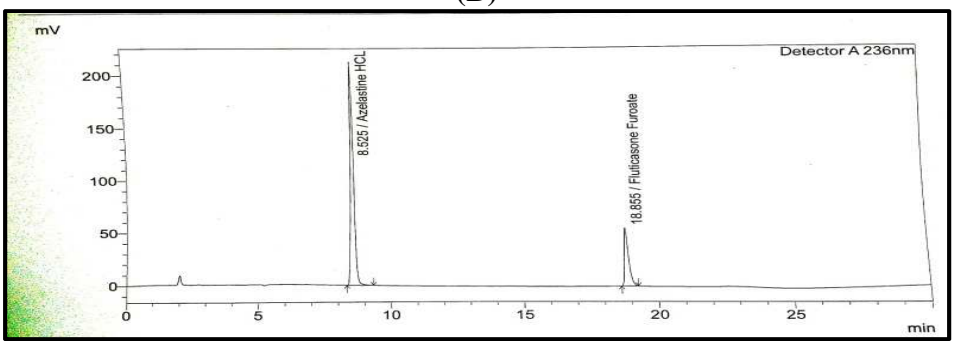
Figure No.5: HPLC chromatograms of accelerated stability study samples of nasal spray (A) Placebo tablet, (B) 0 month, (C) 1 month, (D) 3 months and (E) 6 months



(A)



(B)



(C)

Figure No.6: HPLC chromatograms obtained for the quantitative assay of in-house and marketed samples of nasal spray (A) In-House sample (0 Day), (B) In-House sample (8M) and (C) Furamist AZ (23M)

CONCLUSION

In the current research, the HPLC method was developed and validated as per the ICH guideline, which is efficient to separate AZE and FF using the mobile phase A as 20.35mM buffer pH 3.0 and mobile phase B as 100% ACN. The recovery study performed at 50, 100 and 150% of AZE and FF concentration showed mean % recovery as 99.66% and 100.06% resp. The forced degraded samples were investigated by a developed method, shown degradation of AZE and FF to a reasonable extent in acidic, alkaline, oxidative, thermal, humid and photolytic stress conditions. The HPLC method developed was found to be linear for AZE and FF (r^2 = 0.9997 and 0.9999) within range of 25-75µg mL^{-1} for AZE and 5-15µg mL^{-1} for FF. The method was robust (less than 2% RSD) that remains stable by small changes in process parameters (flow rate, the ratio of mobile phase, detection wavelength, column oven temperature, mobile phase buffer pH). The accelerated stability studies for nasal spray formulation showed the stability of nasal spray up to 6 months as no degradation peaks were observed in the HPLC analysis. This was further confirmed by forced degradation study of the formulation under acidic, alkaline and oxidative conditions where the placebo sample did not show any degradation peaks as like formulation. The developed method was also efficient for the quantitative evaluation of marketed formulation. Therefore, the developed HPLC method could be functional as a routine stability indicating method for the simultaneous estimation of AZE and FF in the nasal spray formulations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

BIBLIOGRAPHY

1. Azelastine Hydrochloride- Drug Bank, *Drug Bank,* 2019. https: //www.drugbank.ca/salts/DBSALT000013.
2. Denise K C Sur, Monica L. Plesa. Treatment of allergic rhinitis, *American Family Physician,* 92(11), 2015, 985-992.
3. Craig La Force. Use of nasal steroids in managing allergic rhinitis, *J Allergy Clin Immunol,* 103(3Pt2), 1999, S388-394.
4. Scadding G K, Keith P K. Fluticasone furoate nasal spray consistently and significantly improves both the nasal and ocular symptoms of seasonal allergic rhinitis: a review of the clinical data, *Expert Opin Pharmacother,* 9(15), 2008, 2707-2715.
5. Fluticasone Furoate-Drug Bank, *Drug Bank,* 2019. https://www.drugbank.ca/drugs/DB08906.
6. Furamist AZ Nasal Spray (Fluticasone furoate + Azelastine hydrochloride) product index, https://ciplamed.com/content/furamist-az-nasal-spray.
7. Gill C, Kulkarni P, Nipte A, Jadhav C, Chate A, Dodake-Supekar A. A study of method development and validation for estimation of Azelastine hydrochloride in nasal spray formulations by RP-HPLC method, *Journal of Drug Delivery and Therapeutics,* 8(6), 2018, 236-240.
8. Sangoi M, Nogueira D, Silva L, Leal D, Dalmora S. Validation of a Stability Indicating Reversed Phase LC Method for the Determination of Fluticasone Propionate in Pharmaceutical Formulations, *Journal of Liquid Chromatography and Related Technologies,* 31(14), 2008, 2113-2127.
9. Paul H Ratner, Frank Hampel, Julius Van Bavel, Amar, Pramila Daftary, William Wheeler, Harry Sacks. Combination therapy with azelastinehydrochloride nasal spray and fluticasone propionate nasal spray in the treatment of patients with seasonal allergic rhinitis, *Ann Allergy Asthma Immunol,* 100(1), 2008, 74-81.
10. Thorat S. Formulation and Product Development of Nasal Spray: An Overview, *Scholars Journal of Applied Medical Sciences (SJAMS),* 4(8D), 2016, 2976-2985.

11. IP Commission, Indian Pharmacopeia, Indian Pharmacopoeia Commission, *Government of India, Ministry of Health and Family Welfare, Ghaziabad,* 2014.
12. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q2(R1), *ICH,* 2005, 1-17.
13. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline Stability Testing of New Drug Substances and Products Q1A(R2), *ICH,* 2003, 1-24.
14. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH Harmonised Tripartite Guideline of Analytical Procedure Development and Revision of Q2(R1), *ICH,* 2018.
15. USPNF, United States pharmacopeia 39-National formulary 34, *United States, Pharmacopoeial Convention, Inc. Rockville, MD,* 2016.

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