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Assay Method Development and Validation for Simultaneous Estimation of Mometasone Furoate and Azelastine HCL for Nasal Spray by RP-HPLC

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Abstract

A simple, precise, accurate, method was developed and validated for analysis of Mometasone Furoate and Azelastine Hydrochloride in nasal spray formulations. For development, different chromatographic condition and stress conditions like acid, base, peroxide, thermal and humidity as per ICH guidelines were used. Method was developed on reversed-phase C_{18} column using a mobile phase consisting of potassium dihydrogen, phosphate buffer and acetonitrile. Other HPLC parameters were flow rare 1 ml/min, detection wavelength 239 nm, injection volume 20 µl and column temperature 30°C. The developed method was further validated with respect to linearity, precision, accuracy, specificity and robustness. The results obtained were within the acceptance criteria as per ICH guidelines.

Keyword: *Azelastine hydrochloride, acetonitrile, buffer, mometasone furoate, HPLC*

INTRODUCTION

INTRODUCTION TO ANALYTICAL CHEMISTRY

Analytical chemistry involves separating, identifying and determining the relative amounts of the compounds making up a sample of matter [1-5]. Analytical chemistry is concerned with chemical characterization of matter, both qualitative and quantitative.

Important factors, which must be taken into account while selecting an appropriate method of analysis, include:

- The nature of information, which is sought.
- Size of the sample available and the proportion of the constituent to be determined.
- The purpose for which analytical data is required.

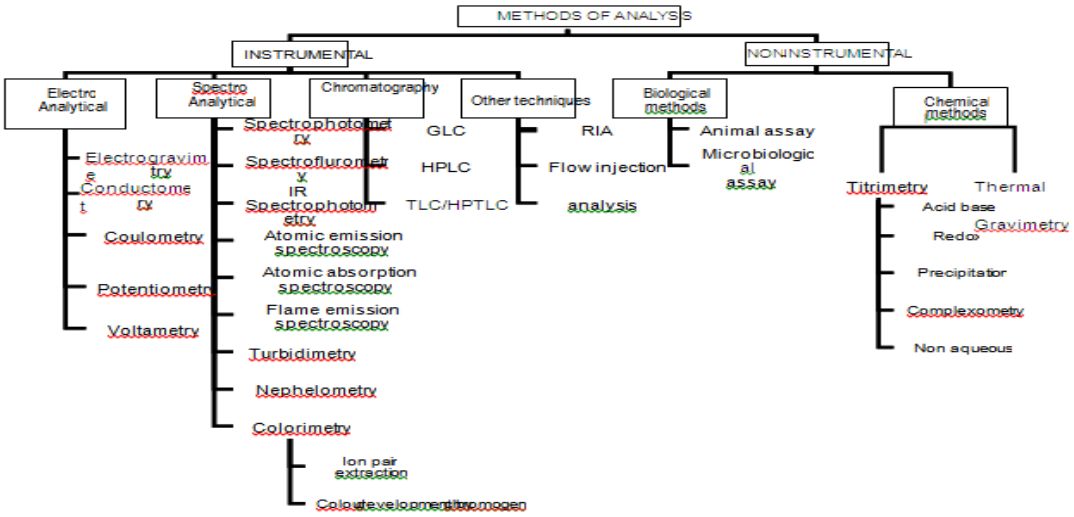


Figure 1: *Analytical Chemisty*

High Pressure Liquid Chromatography (HPLC)

Principle of Separation in HPLC

The principle of separation in normal phase mode and reverse phase mode is adsorption. When mixtures of components are introduced in to a HPLC column, they travel according to their relative affinities towards the stationary phase (Fig. 1). The component which has more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated. Today, HPLC is the most widely used analytical separation method [6]. The method is popular because it is non-destructive and may be applied to thermally labile compounds (unlike GC); it is also very sensitive technique since it incorporates a wide choice of detection methods. The wide applicability of HPLC as separation methods makes it a valuable separation tool in scientific fields [7-10].

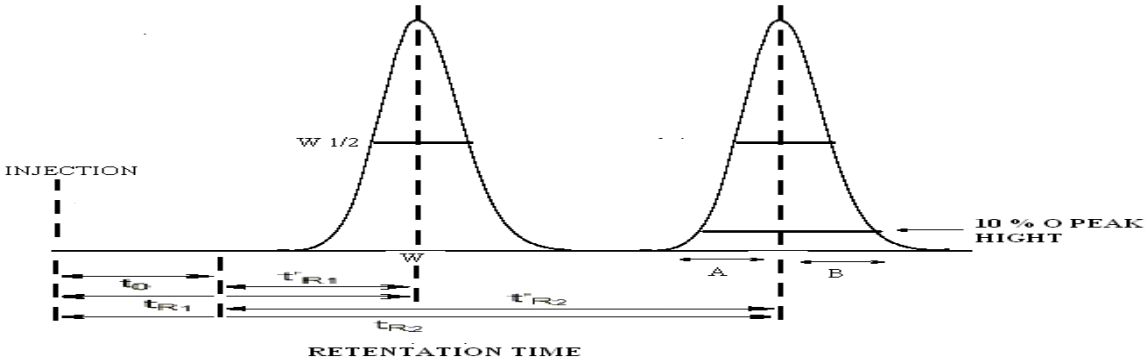


Figure 2: *Fundamental parameters of chromatography.*

Where,

$w_{1/2}$ = peak width at half height

w = band width of the peak (intersection point of the inflection tangents with the Zero line)

A = peak front at 10% of peak height to peak maximum

B = peak maximum to peak end at 10% of retention times

t_0 = dead time of a column = retention time of untreated substance

t_{R1}, t_{R2} …= retention time of components 1, 2…

t'_{R1}, t'_{R2}…= net retention time of components 1, 2…

EXPERIMENTAL

Material Used

Mometasone Furoate: Active Pharmaceutical Ingredient (API) was supplied by Glenmark Pharmaceutical Ltd.

Azelastine Hydrochloride: Active Pharmaceutical Ingredient (API) was supplied by Glenmark Pharmaceutical Ltd.

Reagents and Chemicals Used

All chemicals used throughout the work were of analytical grade and the solvents were of HPLC grade purchased from Merck, Mumbai [11-15].

Table 1: *Reagents and chemicals.*

Sr. No.	Name	Specification
1	Acetonitrile	HPLC grade
2	Potassium Dihydrogen Phosphate	AR grade
3	Sodium Salt of Octanic Acid	AR
4	Ortho Phosphoric Acid	AR
5	Water	Milli Q

Instruments Used

Table 2: *Instruments.*

Sr. No	Name	Model	Manufacturer/Supplier
1	Weighing balance with LC P45 printer	Sartorious	Sartorious
2	Digital pH meter	LABINDIA	LABINDIA
3	Degasser	X15522025	MILLIPORE
4	Sonicator		Meltronics
5	HPLC	- LC-2010c - 2996 alliance Photodiode array Detector	- Shimadzu - Waters

Development and Optimization of RP-HPLC Method for MOMETASONE Furoate and Azelastine HCl

Selection of Column

On the basis of reversed phase and number of carbon atom, C_{18} column having configuration Zorbax SB CN, 150*4.6 mm, 5µ is used for further study [16].

Selection and Optimization of Mobile Phase

For selection and optimization of mobile phase, the various mobile phase compositions containing water and ACN, water and methanol, buffer and ACN were tried, but the resolution, peak shape, theroritical plates were not found to be satisfactory. Finally, mobile phase containing Potassium Dihydrogen Phosphate Buffer: ACN (55:45 v/v) was found to give best resolution for both the drugs. The observation observed with different compositions of mobile phase has shown in the table of trial taken [17-20].

Selection and Optimization of Detection Wavelength

The sensitivity of HPLC method is depends upon proper selection of detected wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected. In the present study, as per BP wavelength of Azelastine HCl is 210nm and wavelength of Mometasone Furoate is 248 nm. We scaned the sample of Azelastine HCl and Mometasone Furoate separately using Water: Acetonitrile (60:40) as a solvent over 400-200nm, and we got absorption maxima at 210nm and 248 nm respectively for both drugs. We have taken overlay of both spetra and got isoabsorptive point ta 239nm. On HPLC, we found area response good at 239 nm as compared to 210nm and 248nm. So we carried out detection at 239nm.

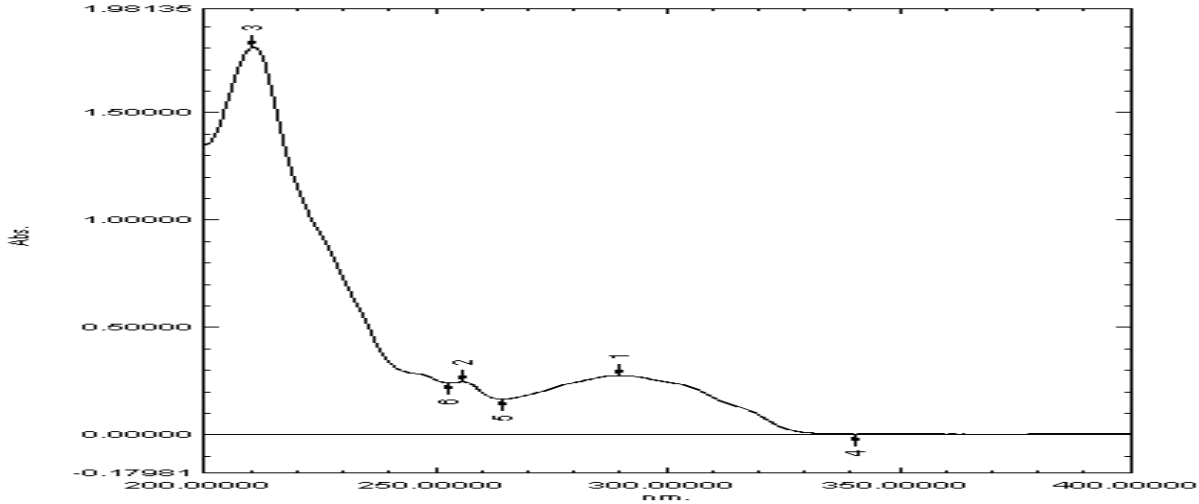


Figure 3: *Spectra of Azelastine HCl.*

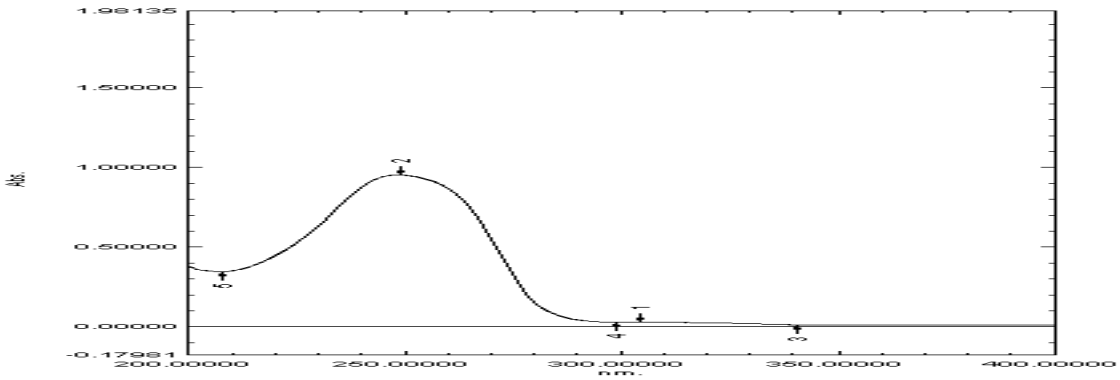


Figure 4: *Spectra of Mometasone Furoate.*

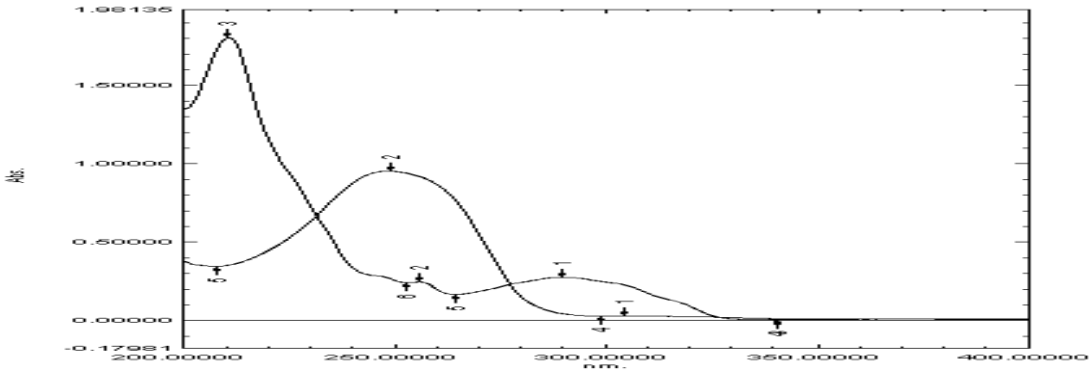


Figure 5: *Overlay Spectra of Azelastine HCl and Mometasone Furoate.*

Optimization of Flow Rate

Various trials were carried out using different flow rates like 0.8 ml/min, 1.0 ml/min, 1.5 ml/min with an objective to get good resolution and sharp peaks of Mometasone Furoate and Azelastine HCl. The flow rate which provides satisfactory resolution of Mometasone Furoate and Azelastine HCl peaks was seleted. The observation obtained at different flow rate is shown in table of trials taken [21].

Table 3: *Trials Taken.*

Sr. No.	Chromatographic Condition				Observation
	Mobile phase	Column	λ max	Flow rate	
1	Water : ACN (50:50)	Zorbax SB CN, 150*4.6 mm, 5µ..	210 nm	1.0ml/min	Only one peak observed
2	Water : ACN (70:30)	Zorbax SB CN, 150*4.6 mm, 5µ..	248nm	1.5ml/min	Not good resolution
3	Water : ACN (60:40)	Zorbax SB CN, 150*4.6 mm, 5µ..	248 nm	0.8ml/min	Theoretical plate was not in limit
4	Methanol:ACN (50:50)	Zorbax SB CN, 150*4.6 mm, 5µ.	239 nm	1.0ml/min	Peak shape not good
5	Methanol:ACN (70:30)	Zorbax SB CN, 150*4.6 mm, 5µ.	248 nm	1.0ml/min	Only one peak observed
6	Methanol:ACN (60:40)	Zorbax SB CN, 150*4.6 mm, 5µ.	239 nm	1.0ml/min	Peak shape was not good
7	Buffer : ACN (50:50)	Zorbax SB CN, 150*4.6 mm, 5µ.	239 nm	0.8ml/min	Resolution was not good
8	Buffer : ACN (70:30)	Zorbax SB CN, 150*4.6 mm, 5µ.	239 nm	1.5ml/min	Peak shape was not good
9	Buffer : ACN (60:40)	Zorbax SB CN, 150*4.6 mm, 5µ.	239 nm	1.0ml/min	Therotical plate was not in limit
10	Buffer : ACN (55:45)	Zorbax SB CN, 150*4.6 mm, 5µ.	239 nm	1.0ml/min	Good peak shape and resolution

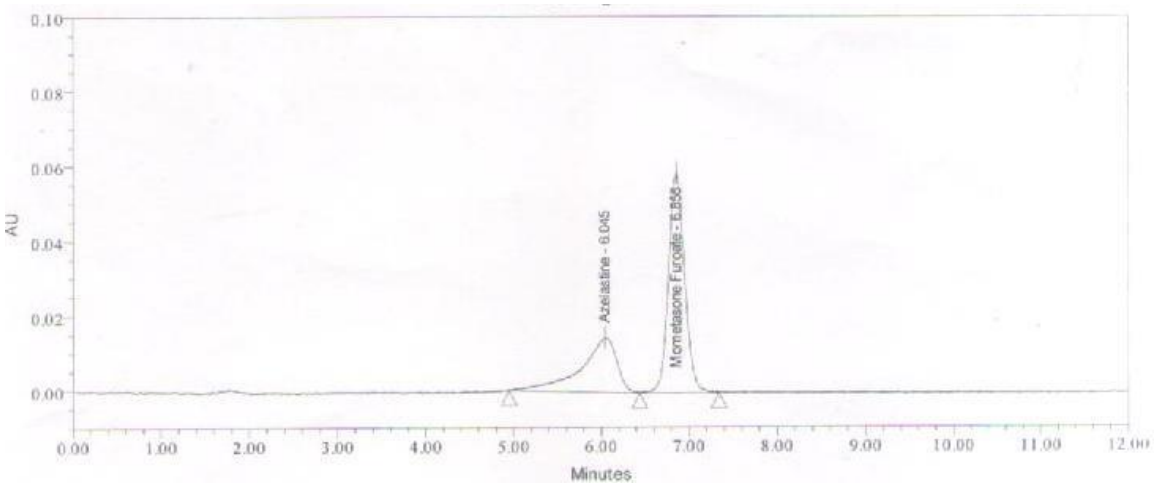


Figure 6: *Chromatographic Cond.: MP: Water:ACN (70:30), 248 nm, 1.5 ml/min.*

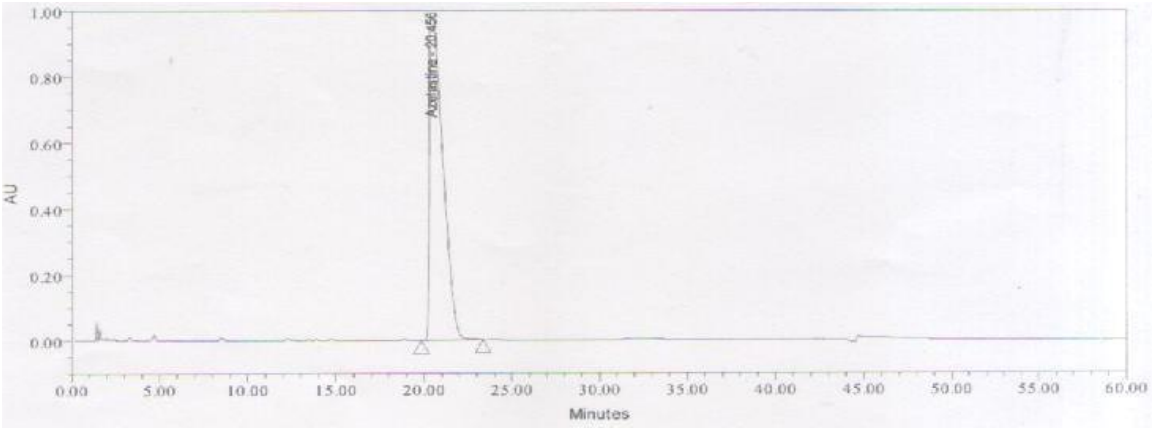


Figure 7: *Chromatographic Cond.: MP: Water:ACN (70:30), 248 nm, 1.5 ml/min.*

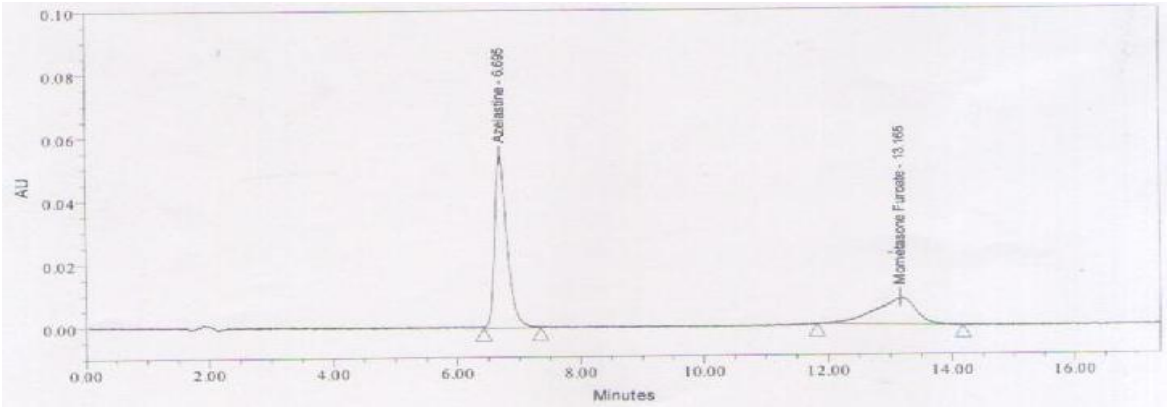


Figure 8: *Chromatographic Cond.: MP: Water:ACN (70:30), 248 nm, 1.5 ml/min.*

Table 4: *Optimized Chromatographic Conditions.*

Parameter/ Conditions	Description/Values
Column name	Zorbax SB CN, 150*4.6 mm, 5µ.
Detector	239 nm
Flow rate	1.0 ml/min
Injection volume	20 µl
Temperature	30^0C
Mobile phase	ACN : Buffer (55:45)

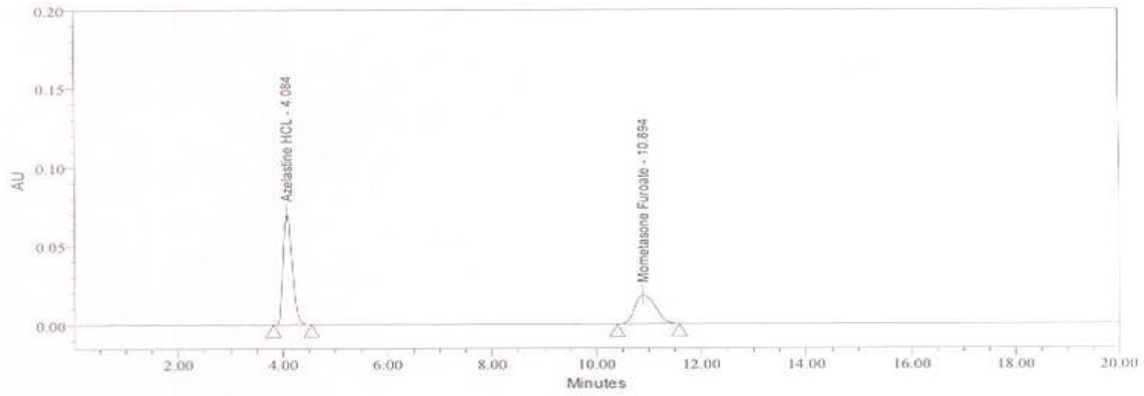


Figure 9: *Chromatographic Cond.: MP: Water:ACN (70:30), 248 nm, 1.5 ml/min.*

Table 5: *System Suitability.*

COMPOUND	SYSTEM SUITABILITY	
	PARAMETER	VALUE
Azelastine HCl	Area, % RSD	846013, 0.474
	Theoretical plates	2860
	Retention time	4.1
	Peak Tailing	1.22
Mometasone Furoate	Area, % RSD	493622, 0.293
	Theoretical plates	4025
	Retention time	10.9
	Peak Tailing	1.13
	Resolution	6.8

Assay of Formulation

Preparation of Buffer

Dissolve 1.24 gm of Potassium Dihydrogen Phosphate and 3.95 gm of Sodium salt of Octanic Acid in 1000 ml of milli Q water, Adjust pH 3.0 to 3.1 with diluete Orthophosphoric acid. Filter through 0.45µ filter.

Preparation of Mobile Phase

Mixed Buffer and Acetonitrile in ratio (55:45 %v/v) and sonicate to degas.

Preparation of Diluent

Mixed Water and Acetonitrile in ratio (40:60 %v/v).

Preparation of Standard Solution A (Mometasone Furoate)

25 mg of Mometasone Furoate working standard/ reference standard was weigh accurately, transfered in to 100 ml volumetric flask, add 70 ml of diluent and sonicate to dissolve. Make up to the mark with diluent and mix. (Conc. Of Mometasone Furoate is 250 mcg/ml) [22-25].

Preparation of Standard Solution B (Azelastine HCl)

28 mg of Azelastine HCl working standard/ reference standard was weigh accurately, transfered in to 100 ml volumetric flask, add 70 ml of diluent and sonicate to dissolve. Make up to the mark with diluent and mix. (Conc. Of Azelastine HCl is 280 mcg/ml).

Preparation of Mix Standard Solution (A and B)

Dilute 4 ml of the standard solution A and 10 ml of the standard solution B in 100 ml volumetric flask and make up to the mark with diluents. (Conc. Of Mometasone Furoate is 10 mcg/ml and Conc. Of Azelastine HCl is 28 mcg/ml) [26-28].

Preparation of Sample Solution

After priming the first six sprays to waste, tare 50 ml dry volumetric flask and actuate sprays 10 times into 50 ml volumetric flask and weigh accurately the sample

quantity for calculation, (equivalent to 500 mcg Mometasone Furoate and 1400 mcg Azelastine HCl), add 30 ml diluents and sonicate for 5-10minutes. Cool to room temperature; make up volume with diluents and mix. Filter the solution through 0.45 µ. Teflon filter. (Conc. Of Mometasone Furoate is 10 mcg/ml and Conc. Of Azelastine HCl is 28 mcg/ml) [29].

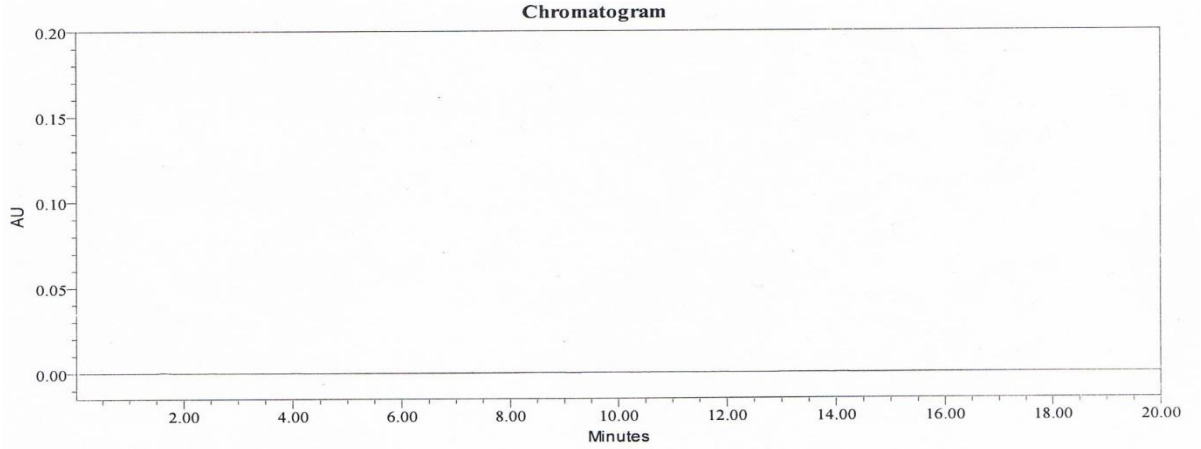
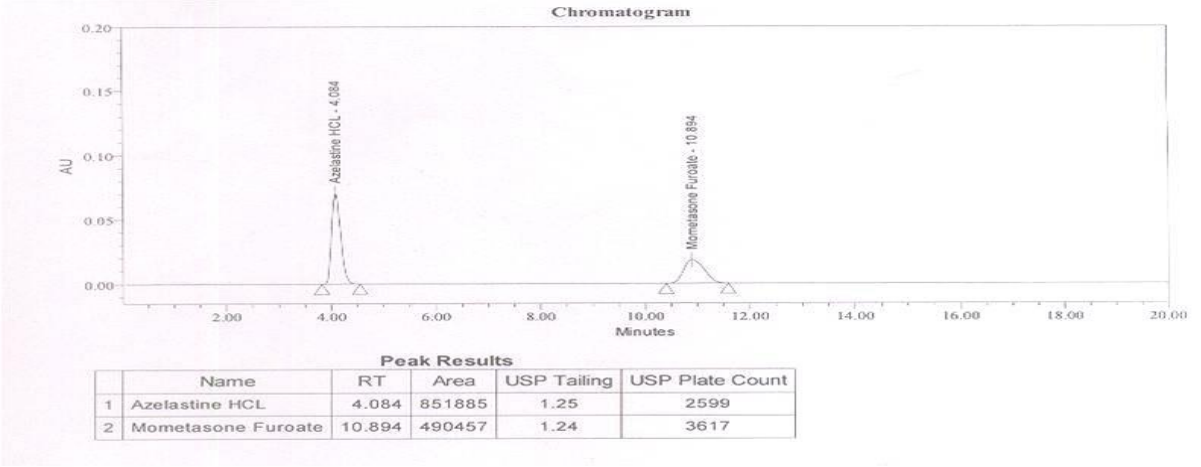


Figure 10: *HPLC Graph for Diluent.*



	Name	RT	Area	USP Tailing	USP Plate Count
1	Azelastine HCL	4.084	851885	1.25	2599
2	Mometasone Furoate	10.894	490457	1.24	3617

Figure 11: *HPLC Graph for Assay of Formulation.*

Table 6: *Assay of Formulation.*

Sr.No.	Drugs	Label Claim (mcg/spray)	Amount Found (mcg/spray)	% Amount Found
1.	Mometasone Furoate	50	50.75	101.5
2.	Azelastine HCl	140	140.39	100.3

Validation of the Developed RP-HPLC Method

Precision

Preparation of Standard Solution A (Mometasone Furoate)

25 mg of Mometasone Furoate working standard/ reference standard was weigh accurately, transfered into 100 ml volumetric flask, add 70 ml of diluent and sonicate to dissolve. Make up to the mark with diluent and mix. (Conc. Of Mometasone Furoate is 250 mcg/ml) [30].

Preparation of Standard Solution B (Azelastine HCl)

28 mg of Azelastine HCl working standard/ reference standard was weigh accurately, transfered in to 100ml volumetric flask, add 70 ml of diluent and sonicate to dissolve. Make up to the mark with diluent and mix. (Conc. Of Azelastine HCl is 280 mcg/ml) [31].

Preparation of Mix Standard Solution (A and B)

Dilute 4 ml of the standard solution A and 10 ml of the standard solution B in 100 ml volumetric flask and make up to the mark with diluents. (Conc. Of Mometasone Furoate is 10 mcg/ml and Conc. Of Azelastine HCl is 28 mcg/ml).

Preparation of Sample Solution

After priming the first six sprays to waste, tare 50 ml dry volumetric flask and actuate sprays 10 times into 50 ml volumetric flask and weigh accurately the sample quantity for calculation, (equivalent to 500 mcg Mometasone Furoate and 1400 mcg Azelastine HCl), add 30 ml diluents and sonicate for 5-10 minutes. Cool to room temperature, make up volume with diluents and mix. Filter the solution through 0.45 µ. Teflon filter. (Conc. Of Mometasone Furoate is 10 mcg/ml and Conc. Of Azelastine HCl is 28 mcg/ml).

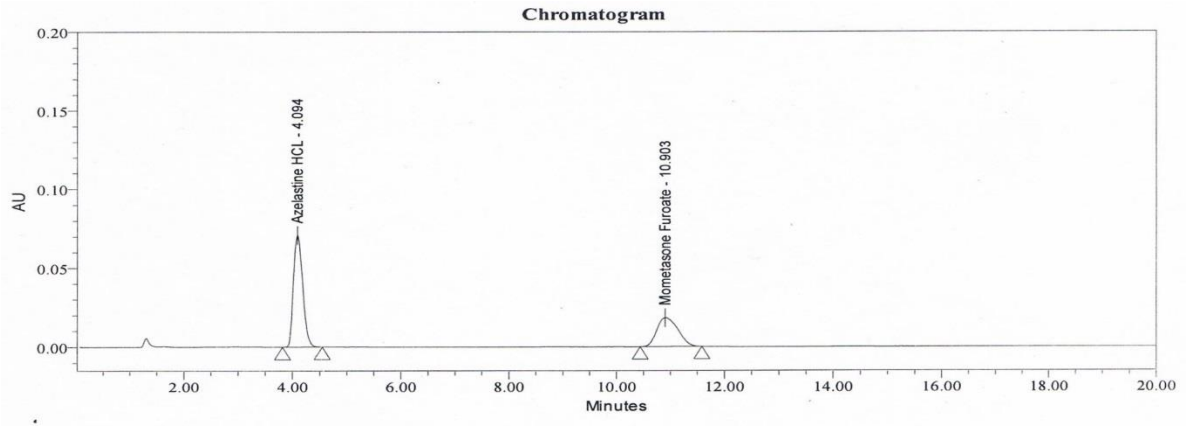


Figure 12: *HPLC graph for Sample for Mometasone Furoate and Azelastine HCl.*

Table 7: *Data for Repeatability of Mometasone Furoate and Azelastine HCl.*

Sr. No	Concentration (mcg/ml)		Average Area		Amount	(%
	Mome. Furoate	Aze. HCl	Mome. Furoate	Aze. HCl	Mome. Furoate	Aze. HCl
1	50	140	497169	838432	101.5	100.3
2	50	140	508188	829697	102.0	99.2
3	50	140	495872	869165	101.2	104.0
4	50	140	509068	867665	103.9	103.8
5	50	140	500330	828821	102.1	99.1
6	50	140	498178	840776	101.7	100.6
			Average		101.4	101.2
			Std dev.		1.236	1.199
			% RSD		1.207	1.173

Linearity

Solutions of the concentration level 50, 80, 90, 100, 110, 120, 150, 200 % of the Mometasone Furoate and Azelastine HCl were prepared.

Preparation of Linearity Stock Solution A (Mometasone Furoate)

10.14 mg of Mometasone Furoate working standard/ reference standard was weigh accurately, transfered in to 100ml volumetric flask, add 70 ml of diluent and

sonicate to dissolve. Make up to the mark with diluent and mix.

Preparation of Linearity Stock Solution B (Azelastine HCl)

28.48 mg of Azelastine HCl working standard/ reference standard was weigh accurately, transfered in to 100 ml volumetric flask, add 70 ml of diluent and sonicate to dissolve. Make up to the mark with diluent and mix.

Table 8: *Preparation of Linearity.*

Sr.No.	Linearity Level	ml of Stock solution A	ml of Stock solution B	Volume upto made with diluents
1	50 %	5 ml	5 ml	100 ml
2	80 %	4 ml	4 ml	50 ml
3	90 %	9 ml	9 ml	100 ml
4	100 %	5 ml	5 ml	50 ml
5	110 %	11 ml	11 ml	100 ml
6	120 %	3 ml	3 ml	25 ml
7	150 %	3 ml	3 ml	20 ml
8	200 %	5 ml	5 ml	25 ml

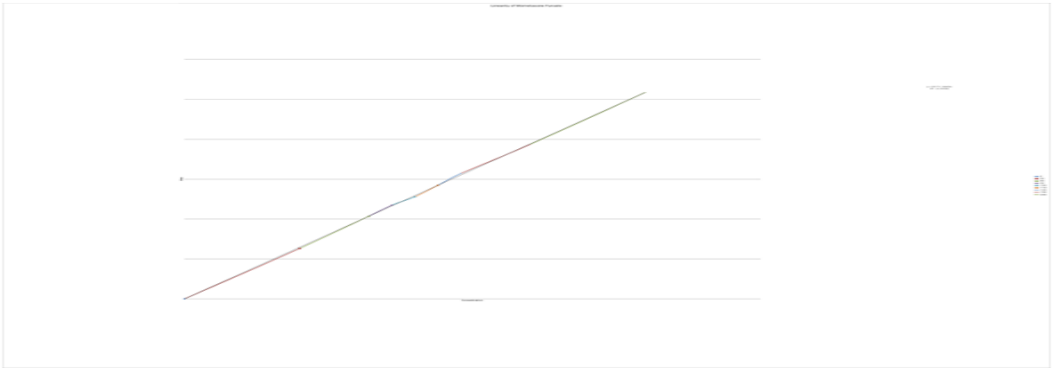


Figure 13: *Linearity of Mometasone Furoate.*

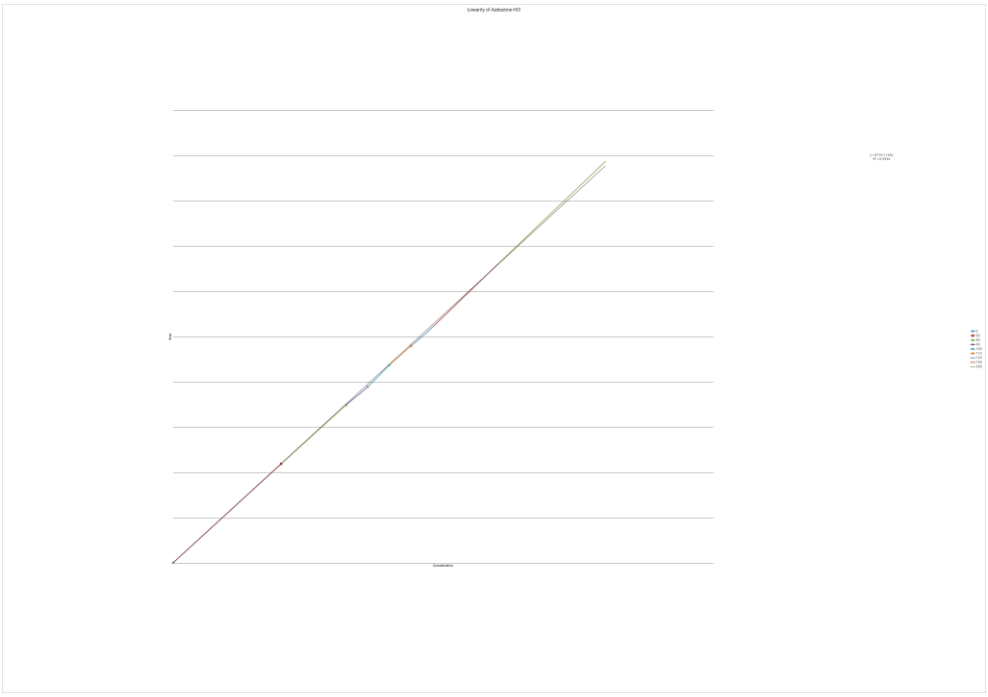


Figure 14: *Linearity of Azelastine HCl.*

Table 9: *Data for Linearity Level of Mometasone Furoate.*

Sr. No.		Average Area		
	Linearity Level %	**Mometasone Furoate**	**SD**	**% RSD**
1	50 %	252315	127.99	0.051
2	80 %	414156	1600.18	0.386
3	90 %	469476	833.68	0.178
4	100 %	511060	1161.78	0.227
5	110 %	568568	1843.43	0.324
6	120 %	628154	120.92	0.019
7	150 %	773249	1100.97	0.142
8	200 %	1034210	687.31	0.066
	Slope	51215		
	Intercept	2832		
	Correlation	0.9999		

Table 10: *Data for Linearity Level of Azelastine HCl.*

Sr. No.		Average Area		
	Linearity Level %	**Azelastine HCl**	**SD**	**% RSD**
1	50 %	437368	324.56	0.074
2	80 %	697709	1533.01	0.220
3	90 %	778037	1138.44	0.146
4	100 %	872984	889.54	0.102
5	110 %	958141	992.78	0.104
6	120 %	1041981	477.30	0.046
7	150 %	1318020	931.97	0.071
8	200 %	1774431	2161.63	0.122
	Slope	31555		
	Intercept	19400		
	Correlation	0.9999		

Accuracy

It was done by recovery study. Sample solutions were prepared by spiking at about 40 %, 80 %, 100 %, 120 %, and 180 % of specification limit to Placebo and analyzed by the proposed HPLC method.

Preparation of Recovery Stock Solution A (Mometasone Furoate)

13.73 mg of Mometasone Furoate working standard/ reference standard was weigh accurately, transfered in to 50 ml volumetric flask, add 30 ml of diluent and sonicate to dissolve. Make up the volume with diluents and mix.

Preparation of Recovery Stock Solution B (Azelastine HCl)

35.20 mg of Azelastine HCl working standard was weighted accurately and transferred into a 250ml volumetric flask, add 30ml of diluent and sonicated to dissolve. Make up the volume with diluents and mix.

Table 11: *Data of Accuracy for Mometasone Furoate.*

Conc. Level %	Actual Amount added in mg	Average Area	Amount Recovered in mg	% Recovery	Mean
40	219.68	218382	222.27	101.2	101.3
40	219.68	218658	222.55	101.3	
40	219.68	218924	222.82	101.4	
80	439.36	439525	447.35	101.8	100.8
80	439.36	435395	443.15	100.9	
80	439.36	430244	437.91	99.7	

100	549.20	530743	540.20	98.4	98.3
100	549.20	530536	539.99	98.3	
100	549.20	529482	538.91	98.1	
120	659.04	656865	668.56	101.4	101.6
120	659.04	659413	671.16	101.8	
120	659.04	658177	669.90	101.6	
180	991.31	990293	1007.93	101.7	101.2
180	991.31	983363	1000.88	101.0	
180	991.31	981783	999.27	100.8	
				Average	100.6
				SD	1.34
				% RSD	1.33

Table 12: *Data of Accuracy for Azelastine HCl.*

Conc. level %	Actual Amount added in mg	Average Area	Amount Recovered in mg	% Recovery	Mean
40	559.43	338965	554.58	99.1	99.9
40	559.43	345723	565.63	101.1	
40	559.43	339943	556.18	99.4	
80	1090.88	654441	1070.72	98.2	100.5
80	1090.88	656279	1106.45	101.4	
80	1090.88	678891	1110.73	101.8	
100	1398.57	870411	1424.07	101.8	100.8
100	1398.57	859123	1405.60	100.5	
100	1398.57	856031	1400.54	100.1	
120	1678.28	1013136	1657.58	98.8	100.7
120	1678.28	1041467	1703.93	101.5	
120	1678.28	1042738	1706.01	101.7	
180	2510.43	1505862	2463.73	98.1	99.9
180	2510.43	1540126	2519.79	100.4	
180	2510.43	1551776	2538.85	101.1	
				Average	100.4
				SD	0.43
				% RSD	0.43

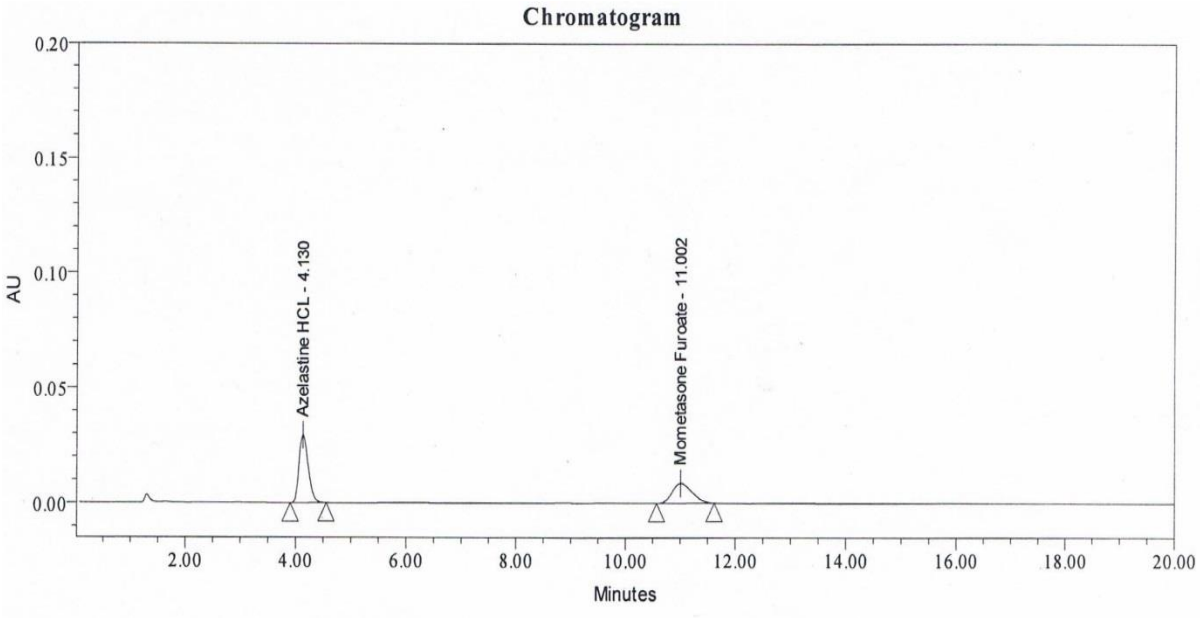


Figure 15: *HPLC Chromatogram of Accuracy 40%.*

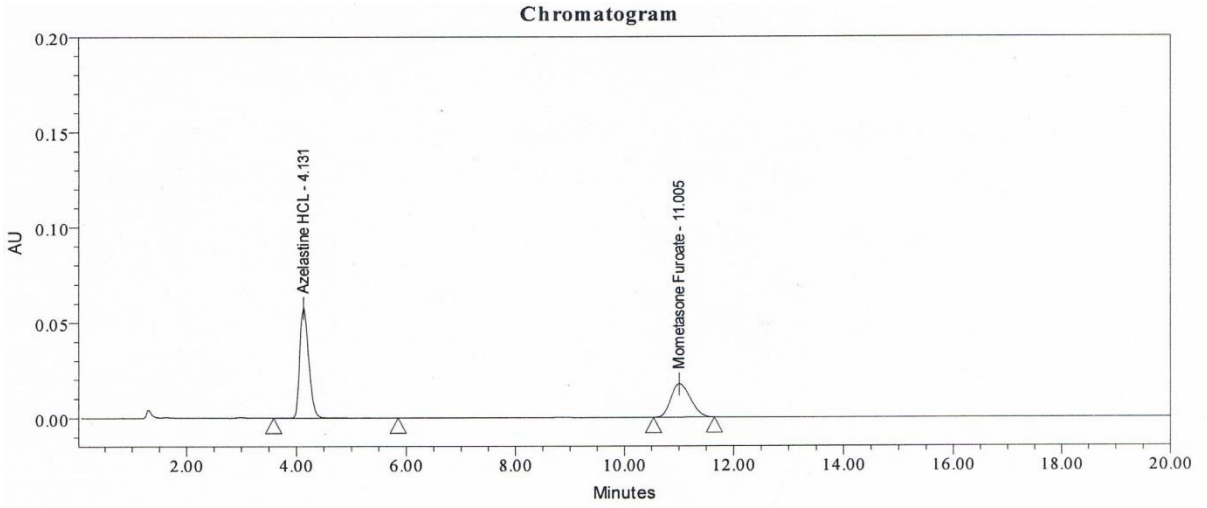


Figure 16: *HPLC Chromatogram of Accuracy 80%.*

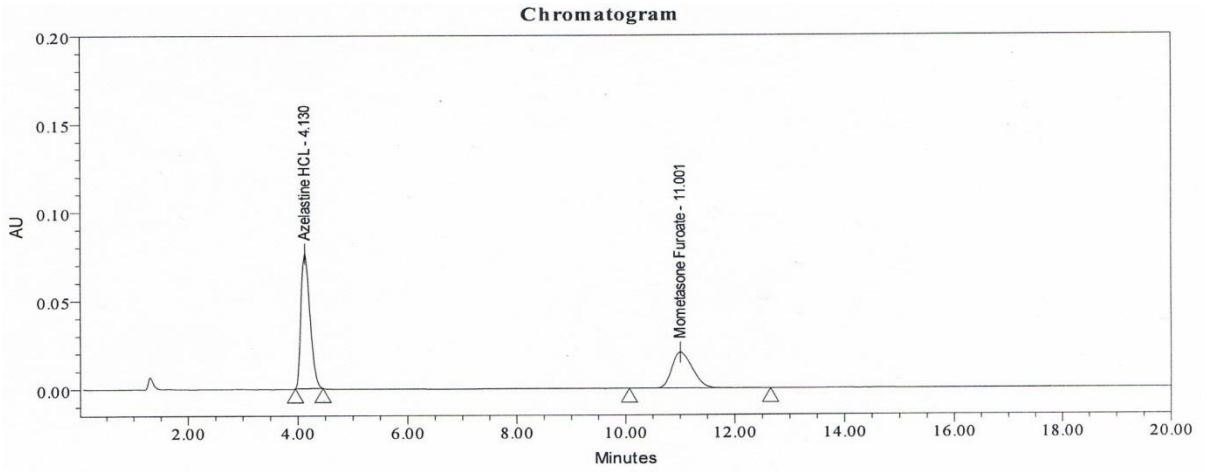


Figure 17: *HPLC Chromatogram of Accuracy 100%.*

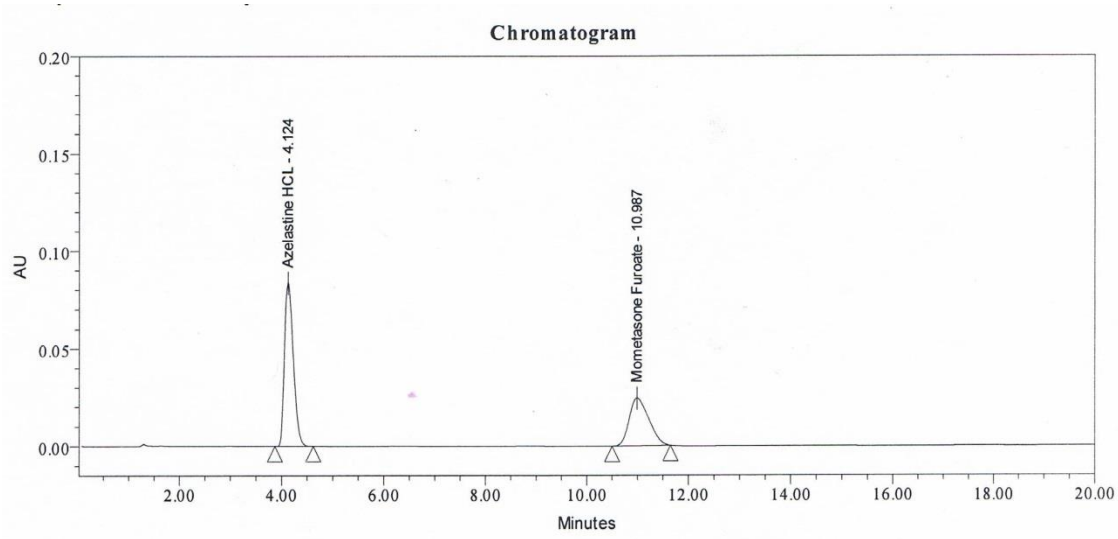


Figure 18: *HPLC Chromatogram of Accuracy 120%.*

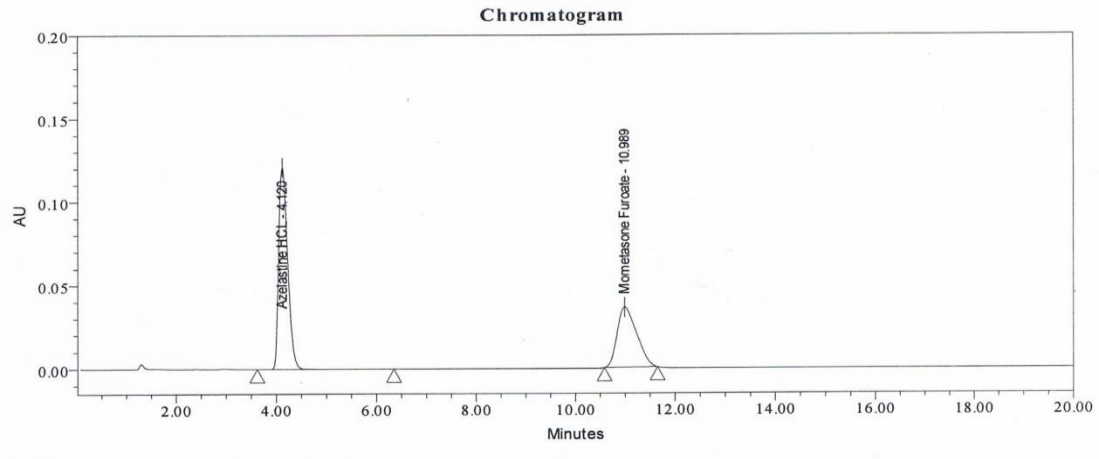


Figure 19: *HPLC Chromatogram of Accuracy 180%.*

Specificity

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is the procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. The method is quite selective. There was no other interfering peak around the retention time of Mometasone Furoate and Azelastine HCl, also the baseline did not show any significant noise.

Preparation of Sample

Actuate 50 sprays in 100 ml volumetric flask. Add 70 ml diluents and sonicate for 10 minutes. Make up upto the mark with diluents.

Table 13: *Preparation of Degradation Sample.*

Sr. No	Sample Identity	Sample Preparation
1	Acid degradation sample	20 ml sample + 5 ml diluents, 1 ml, 1 M HCl (Water). Heat at 70ºc for 15 min and dilute to 50 ml with diluents.
2	Base degradation sample	20 ml sample + 5 ml diluents, 1 ml, 0.05 M NaOH (Water). Heat at 70ºc for 1 min and dilute to 50 ml with diluents.
3	Peroxide degradation sample	20 ml sample + 5 ml diluents, 2 ml 3% H_2O_2. Heat at 70ºc for 15 min and dilute to 50 ml with diluents.
4	Thermal degradation sample	105ºc / 24 hrs
5	Humidity degradation sample	92% RH/24 hrs

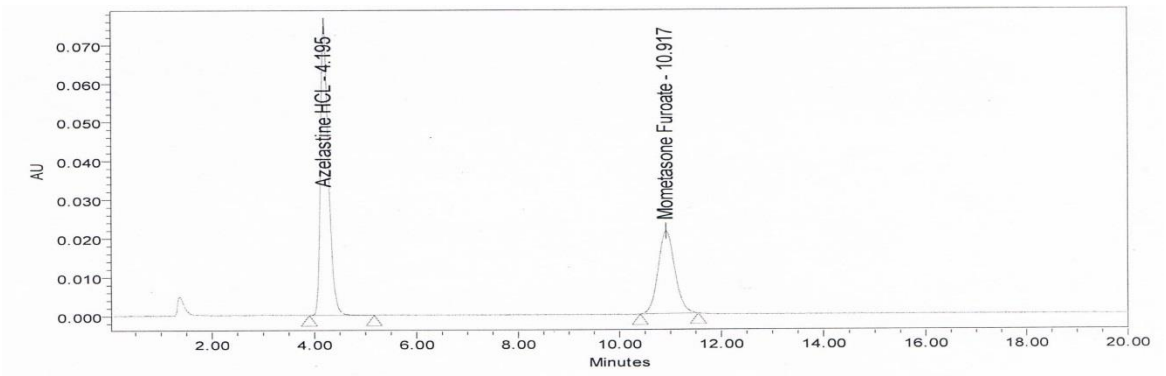


Figure 20: *HPLC Chromatogram of Specificity.*

Stability indicating HPLC method

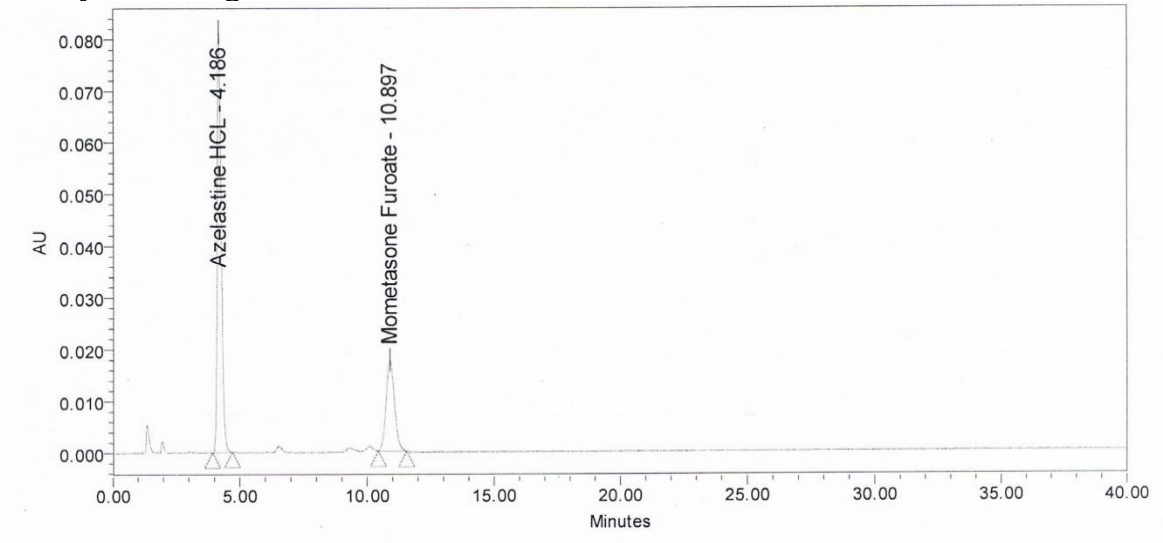


Figure 21: *HPLC Chromatogram of Acid Degradation Sample.*

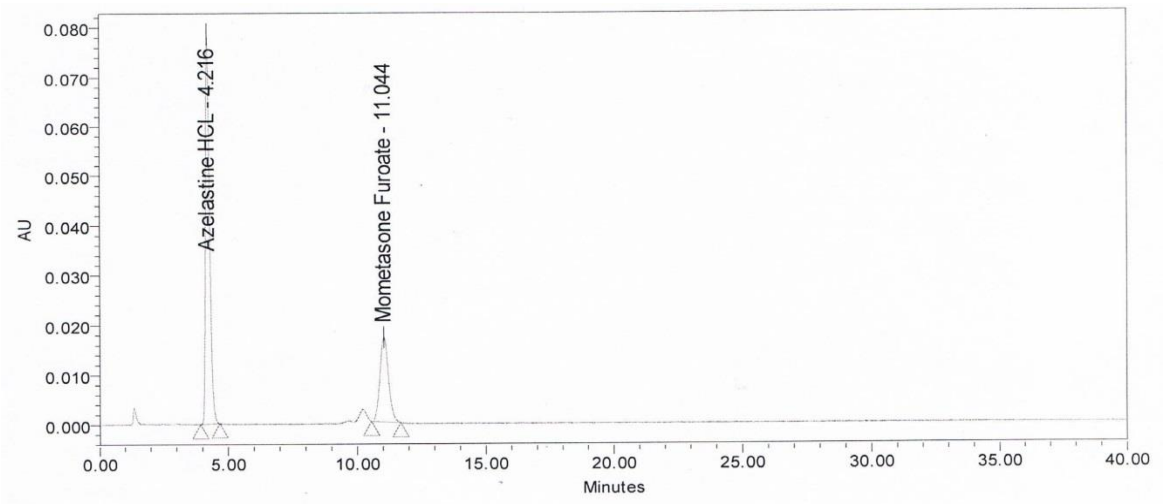


Figure 22: *HPLC Chromatogram of Base Degradation Sample.*

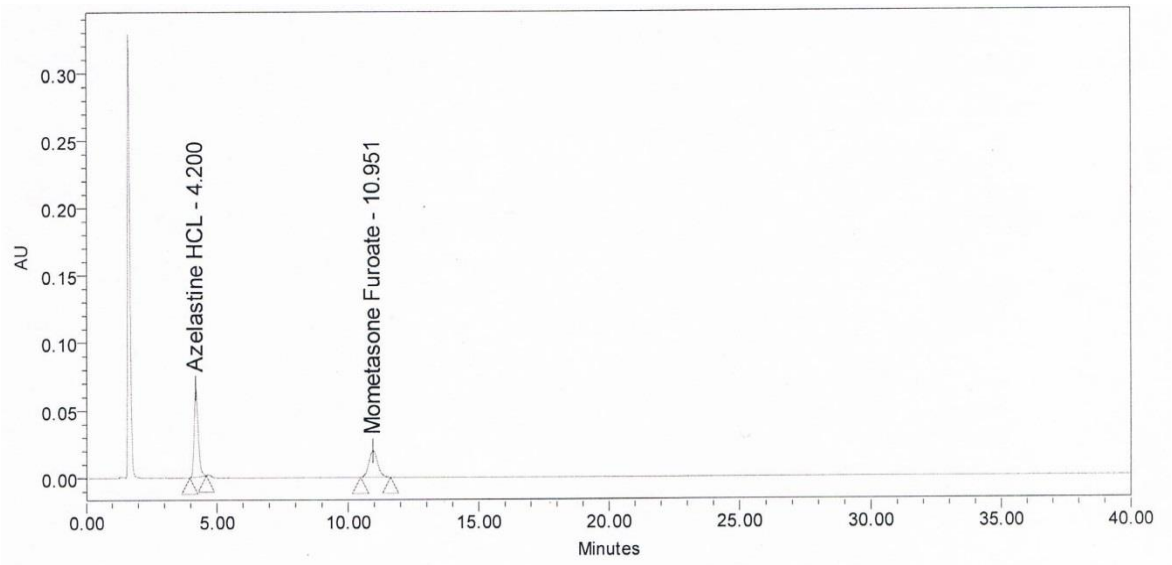


Figure 23: *HPLC Chromatogram of Peroxide Degradation Sample.*

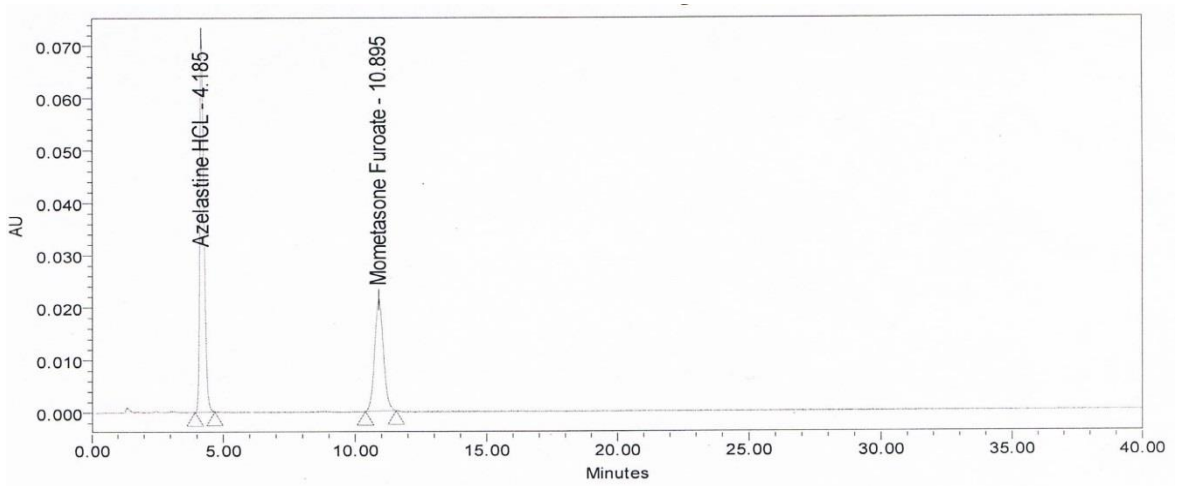


Figure 24: *HPLC Chromatogram of Thermal Degradation Sample.*

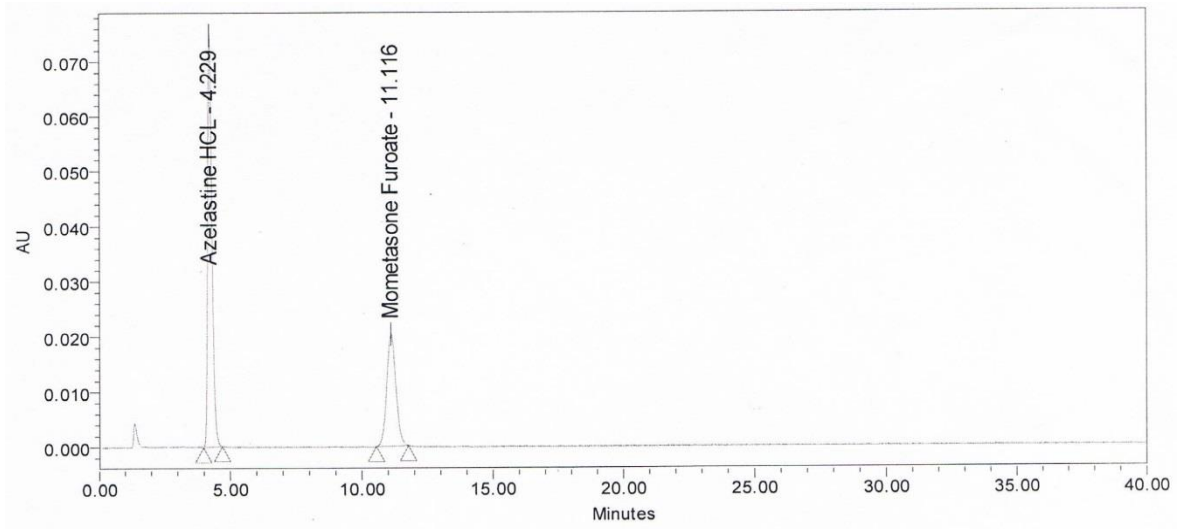


Figure 25: *HPLC Chromatogram of Humidity Degradation Sample.*

Table 14: *Data for Stability Study of Mometasone Furoate.*

Sr.No	Sample Identity	Average Area	Amount mcg/spray	% LC	% Degradation
1	Control sample	484897	50.989	102.0	
2	Acid degradation sample	401042	42.171	84.3	17.7
3	Base degradation sample	394042	41.435	82.9	19.1
6	Peroxide degradation sample	457178	48.074	96.1	5.9
7	Thermal degradation sample	493739	51.919	103.8	
8	Humidity degradation sample	492413	51.661	103.3	

Table 15: *Data for Stability of Azelastine HCl.*

Sr.No	Sample Identity	Average Area	Amount mcg/spray	% LC	% Degradation
1	Control sample	816202	137.607	98.3	
2	Acid degradation sample	886316	149.428	106.7	
3	Base degradation sample	859156	144.849	103.5	
6	Peroxide degradation sample	704207	118.725	84.8	13.5
7	Thermal degradation sample	780925	131.659	94.0	
8	Humidity degradation sample	840140	141.643	101.2	

Robustness

Table 16: *Robustness.*

Sr. No.	PARAMETER	INITIAL VALUE	CHANGE VALVE
1	**Change in pH**	3.0	2.8 and 3.2
2	**Change in flow rate**	1.0 ml	0.8 ml and 1.2 ml
3	**Change in temperature**	30º C	25º C and 35ºC
4	**Change in composition**	Buffer:ACN (55:45)	Buffer:ACN (57:43) and (53:47)

Table 17: *Data for Robustness of Mometasone Furoate on High pH 3.2 and Low pH 2.8.*

Sr. No	Sample Identity	Average Area	Amount mcg/spray	% LC
1	Robustness set-1	503836	50.910	101.8
2	Robustness set-2	511157	51.649	103.3
3	Robustness set-3	507758	51.306	102.6
4	High-pH Set-1	508307	50.853	101.7
5	High-pH Set-2	519973	52.021	104.0
6	High-pH Set-3	512447	51.268	102.5
7	Low-pH Set-1	508677	50.212	100.4
8	Low-pH Set-2	518802	51.211	102.4
9	Low-pH Set-3	513377	50.676	101.4

Table 18: *Compiled Data for Robustness of Mometasone Furoate on High pH 3.2 and Low pH 2.8.*

Sr.No	Sample Identity	pH 3.0	High-pH 3.2	Average	SD	% RSD
1	Robustness set-1	101.8	101.7	101.7	0.070	0.069
2	Robustness set-2	103.3	104.0	103.6	0.494	0.474
3	Robustness set-3	102.6	102.5	102.5	0.070	0.068
Sr.No	**Sample Identity**	**pH 3.0**	**Low-pH 2.8**	**Average**	**SD**	**% RSD**
1	Robustness set-1	101.8	100.4	101.1	0.989	0.979
2	Robustness set-2	103.3	102.4	102.8	0.636	0.618
3	Robustness set-3	102.6	101.4	102.0	0.848	0.831

Table 19: *Data for Robustness of Azelastine HCl on High pH-3.2 and Low pH-2.8.*

Sr.No	Sample Identity	Average Area	Amount mcg/spray	% LC
1	Robustness set-1	855042	140.244	100.2
2	Robustness set-2	872259	143.068	102.2
3	Robustness set-3	837854	137.425	98.2
4	High-pH Set-1	858783	139.618	99.7
5	High-pH Set-2	862713	140.257	100.2
6	High-pH Set-3	840889	136.709	97.6
7	Low-pH Set-1	864604	138.398	98.9
8	Low-pH Set-2	896444	143.494	102.5
9	Low-pH Set-3	844502	135.180	96.6

Table 20: *Compiled Data for Robustness of Azelastine HCl on High pH-3.2 and Low pH-2.8.*

Sr.No	Sample Identity	pH 3.0	High-pH 3.2	Average	SD	% RSD
1	Robustness set-1	100.2	99.7	99.9	0.353	0.353
2	Robustness set-2	102.2	100.2	101.2	1.414	1.397
3	Robustness set-3	98.2	97.6	97.9	0.424	0.433
Sr.No	**Sample Identity**	**pH 3.0**	**Low-pH 2.8**	**Average**	**SD**	**% RSD**
1	Robustness set-1	100.2	98.9	99.5	0.919	0.923
2	Robustness set-2	102.2	102.5	102.3	0.212	0.207
3	Robustness set-3	98.2	96.6	97.4	1.131	1.161

Table 21: *Data for Robustness of Mometasone Furoate on High flow 1.2 ml/min and Low Flow 0.8 ml/min.*

Sr. No	Sample Identity	Average Area	Amount mcg/spray	% LC
1	Robustness Set-1	503836	50.910	101.8
2	Robustness Set-2	511157	51.649	103.3
3	Robustness Set-3	507758	51.306	102.6
4	High-Flow Set-1	464894	52.280	104.6
5	High-Flow Set-2	458956	51.612	103.2
6	High-Flow Set-3	463713	52.147	104.3
7	Low-Flow Set-1	573915	52.821	105.6
8	Low-Flow Set-2	561635	51.690	103.4
9	Low-Flow Set-3	569972	52.458	104.9

Table 22: *Compiled Data for Robustness of Mometasone Furoate on high flow 1.2 ml/min and low flow 0.8 ml/min.*

Sr.No	Sample Identity	Flow-1.0ml/min	High Flow-1.2ml/min	Average	SD	% RSD
1	Robustness set-1	101.8	104.6	103.2	1.979	1.918
2	Robustness set-2	103.3	103.2	103.2	0.070	0.068
3	Robustness set-3	102.6	104.3	103.4	1.202	1.161
Sr.No	**Sample Identity**	**Flow-1.0ml/min**	**Low Flow-0.8ml/min**	**Average**	**SD**	**% RSD**
1	Robustness set-1	101.8	105.6	103.7	1.687	1.591
2	Robustness set-2	103.3	103.4	103.3	0.070	0.068
3	Robustness set-3	102.6	104.9	103.7	1.626	1.567

Table 23: *Data for Robustness of Azelastine HCl on High Flow 1.2 ml/min and Low Flow 0.8 ml/min.*

Sr.No	Sample Identity	Average Area	Amount mcg/spray	% LC
1	Robustness Set-1	855042	140.244	100.2
2	Robustness Set-2	872259	143.068	102.2
3	Robustness Set-3	837854	137.425	98.2
4	High-Flow Set-1	764586	139.779	99.8
5	High- FlowSet-2	766243	140.082	100.1
6	High- FlowSet-3	788740	144.195	103.0
7	Low-Flow Set-1	942906	140.769	100.5
8	Low-Flow Set-2	939722	140.293	100.2
9	Low-Flow Set-3	972886	145.244	103.7

Table 24: *Compiled Data for Robustness of Azelastine HCl on High Flow 1.2 ml/min and Low Flow 0.8 ml/min.*

Sr.No	Sample Identity	Flow-1.0ml/min	High Flow-1.2ml/min	Average	SD	% RSD
1	Robustness set-1	100.2	99.8	100	0.282	0.282
2	Robustness set-2	102.2	100.1	101.1	1.484	1.468
3	Robustness set-3	98.2	103.0	100.6	1.394	1.373
Sr.No	**Sample Identity**	**Flow-1.0ml/min**	**Low Flow-0.8ml/min**	**Average**	**SD**	**% RSD**
1	Robustness set-1	100.2	100.5	100.3	0.212	0.211
2	Robustness set-2	102.2	100.2	101.2	1.414	1.397
3	Robustness set-3	98.2	103.7	100.9	1.889	1.852

Table 25: *Data for Robustness of Mometasone Furoate on High Temp. 35^{0}C and Low Temp. 25°C.*

Sr.No	Sample Identity	Average Area	Amount mcg/spray	% LC
1	Robustness Set-1	503836	50.910	101.8
2	Robustness Set-2	511157	51.649	103.3
3	Robustness Set-3	507758	51.306	102.6
4	High-TempSet-1	520685	52.621	105.2
5	High-TempSet-2	508187	51.358	102.7
6	High-Temp Set-3	514949	52.042	104.1
7	Low-TempSet-1	523104	52.668	105.3
8	Low-TempSet-2	510612	51.410	102.8
9	Low-TempSet-3	515869	51.939	103.9

Table 26: *Compiled Data for Robustness of Mometasone Furoate on High Temp. 35^{0}C and Low Temp. 25°C.*

Sr.No	Sample Identity	Temp 30°c	High-Temp-35°c	Average	SD	% RSD
1	Robustness set-1	101.8	105.2	103.5	1.404	1.322
2	Robustness set-2	103.3	102.7	103	0.424	0.411
3	Robustness set-3	102.6	104.1	103.3	1.060	1.026
Sr.No	**Sample Identity**	**Temp 30°c**	**Low-Temp-25°c**	**Average**	**SD**	**% RSD**
1	Robustness set-1	101.8	105.3	103.5	1.474	1.390
2	Robustness set-2	103.3	102.8	103.1	0.353	0.343
3	Robustness set-3	102.6	103.9	103.2	0.919	0.890

Table 27: *Data for Robustness of Azelastine HCl on High Temp. 35^{0}C and Low Temp. 25°C.*

Sr.No	Sample Identity	Average Area	Amount mcg/spray	% LC
1	Robustness Set-1	855042	140.244	100.2
2	Robustness Set-2	872259	143.068	102.2
3	Robustness Set-3	837854	137.425	98.2
4	High-TempSet-1	854705	140.950	100.7
5	High-TempSet-2	847674	139.791	99.9
6	High-Temp Set-3	877576	144.722	103.4
7	Low-TempSet-1	868504	142.420	101.7
8	Low-TempSet-2	851974	139.710	99.8
9	Low-TempSet-3	874121	143.341	102.4

Table 28: *Compiled Data for Robustness of Azelastine HCl on High Temp.35^{0}C and Low Temp. 25°C.*

Sr.No	Sample Identity	Temp 30°c	High-Temp-35°c	Average	SD	% RSD
1	Robustness set-1	100.2	100.7	100.4	0.353	0.351
2	Robustness set-2	102.2	99.9	101.1	1.626	1.609
3	Robustness set-3	98.2	103.4	100.8	1.676	1.647
Sr.No	**Sample Identity**	**Temp 30°c**	**High-Temp-35°c**	**Average**	**SD**	**% RSD**
1	Robustness set-1	100.2	101.7	100.9	1.060	1.050
2	Robustness set-2	102.2	99.8	101	1.697	1.680
3	Robustness set-3	98.2	102.4	100.3	1.969	1.960

Table 29: *Data for Robustness of Mometasone Furoate on High Comp. (Buffer:ACN – 57:43) and Low Comp. (Buffer:ACN – 53:47).*

Sr.No	Sample Identity	Average Area	Amount mcg/spray	% LC
1	Robustness Set-1	503836	50.910	101.8
2	Robustness Set-2	511157	51.649	103.3
3	Robustness Set-3	507758	51.306	102.6

4	High-Comp Set-1	512788	49.924	99.8
5	High-Comp Set-2	529834	51.584	103.2
6	High-Comp Set-3	515380	50.566	101.1
7	Low-Comp Set-1	516787	49.651	99.3
8	Low-Comp Set-2	520243	49.983	100.0
9	Low-Comp Set-3	510948	49.090	98.2

Table 30: *Compiled Data of Rrobustness of Mometasone Furoate on High comp. (Buffer:ACN – 57:43) and Low comp. (Buffer:ACN – 53:47).*

Sr.No	Sample Identity	Comp 30ºc	High-Comp-35ºc	Average	SD	% RSD
1	Robustness set-1	101.8	99.8	100.8	1.414	1.402
2	Robustness set-2	103.3	103.2	103.2	0.070	0.068
3	Robustness set-3	102.6	101.1	101.8	1.060	1.041
Sr.No	**Sample Identity**	**Comp 30ºc**	**Low-Comp-25ºc**	**Average**	**SD**	**% RSD**
1	Robustness set-1	101.8	99.3	100.5	1.767	1.758
2	Robustness set-2	103.3	100.0	101.6	1.333	1.295
3	Robustness set-3	102.6	98.2	100.4	1.111	1.098

Table 31: *Data for robustness of Azelastine HCl on high comp. (Buffer:ACN –57:43) and low comp. (Buffer:ACN – 53:47).*

Sr.No	Sample Identity	Average Area	Amount mcg/spray	% LC
1	Robustness Set-1	855042	140.244	100.2
2	Robustness Set-2	872259	143.068	102.2
3	Robustness Set-3	837854	137.425	98.2
4	High-Comp Set-1	860049	137.541	98.2
5	High-Comp Set-2	900404	143.995	102.9
6	High-Comp Set-3	842821	134.786	96.3
7	Low-Comp Set-1	874657	134.689	96.2
8	Low-Comp Set-2	907503	139.747	99.8
9	Low-Comp Set-3	854235	131.544	94.0

Table 32: *Compiled Data for Azelastine HCl on high comp. (Buffer:ACN –57:43) and low comp. (Buffer: ACN – 53:47).*

Sr.No	Sample Identity	Comp 30ºc	High-Comp-35ºc	Average	SD	% RSD
1	Robustness set-1	100.2	98.2	99.2	1.414	1.425
2	Robustness set-2	102.2	102.9	102.5	0.494	0.482
3	Robustness set-3	98.2	96.3	97.2	1.343	1.381
Sr.No	**Sample Identity**	**Comp 30ºc**	**Low-Comp-35ºc**	**Average**	**SD**	**% RSD**
1	Robustness set-1	100.2	96.2	98.2	1.828	1.880
2	Robustness set-2	102.2	99.8	101.0	1.697	1.680
3	Robustness set-3	98.2	94.0	96.1	1.969	1.090

RESULTS AND DISCUSSION

A simple, specific, linear, precise, and accurate RP-HPLC method has been developed and validated for simultaneous determination of Mometasone Furoate and Azelastine HCl in Nasal Spray formulation. The HPLC method is very simple and specific as both peaks are well separated with total runtime of 20 min, which makes it especially suitable for routine quality control analysis work.

The HPLC analysis was performed on the Zorbax SB CN (150 mm ×4.6 mm) 5µm particle size, at temperature 30^0C using Potassium Dihydrogen Phosphate Buffer : Acetonitrile (55:45 *v/v*) as mobile phase; flow rate was set at 1.0 mL/min. The detection was carried out at 239 nm. The retention time for Mometasone Furoate and Azelastine HCl were found to be 10.9

min and 4.10 min, respectively. Mometasone Furoate and Azelastine HCl followed linearity in the concentration range of 5.07 – 20.28 µg/mL (r^2 = 0.999) and 14.14 – 56.58 µg/mL(r^2 = 0.999), respectively. The method has successively been applied for the determination of Mometasone Furoate and Azelastine HCl in marketed formulation. There was no interference from the excipients routinely present in the spray. The drug contents for Mometasone Furoate and Azelastine HCl were found to be between 98–102 % for each drug. Accuracy of the method was studied by the recovery studies at three different levels i.e. 40%, 80%, 100%, 120%, and 180 % level. The % recovery was found to be within the limits of the acceptance criteria within Range of 98−102 %.The precision of the method was studied as repeatability of sample application. The results were examined as %RSD values of concentration of drugs determined. The low value of %RSD (less than 2) indicates high precision of the method. The robustness of the method was studied by making deliberate variations in chromatographic conditions and the effects on the results were examined as %RSD, (less than 2). The low values of % RSD indicate robustness of the method.

Table 33: *Results with Acceptance Criteria.*

Sr. No.	Parameter	Acceptance Criteria	Results Obtained	
			Mometasone Furoate	Azelastine HCl
1	**Specificity**	Should not interfere with placebo	Pass	Pass
2	**Linearity**	Correlation coefficient not less than 0.999	0.999	0.999
3	**Precision**	R. S. D. NMT 2	1.207	1.173
4	**Accuracy**	R. S. D. NMT 2	1.33	0.43
		Recovery of the spiked drug (98-102 %)	100.6	100.4
5	**Robustness**	R. S. D. NMT 2		
	Low pH		0.809	0.763
	High pH		0.203	0.727
	Low Flow		1.075	1.153
	High Flow		1.049	1.041
	Low Temp.		0.874	1.563
	High Temp		0.919	1.202
	Low Comp.		1.383	1.550
	High Comp.		0.837	1.096

SUMMARY AND CONCLUSION

A simple, specific, linear, precise, and accurate RP-HPLC method has been developed and validated for simultaneous determination of Mometasone Furoate and Azelastine HCl in Nasal Spray. The HPLC method is very simple and specific as both peaks are well separated from with total runtime of 20 min, which makes it especially suitable for routine quality control analysis work.

The method provides selective determination of Mometasone Furoate and Azelastine HCl without interference from blank affirming its stability, indicating nature. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method was robust in the separation and determination of Mometasone Furoate and Azelastine HCl. This method can be used for the routine analysis of product during production. The information presented herein could be very useful for quality monitoring of bulk samples and as well employed to check the quality during stability studies.

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