



ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR AZELASTINE HYDROCHLORIDE IN AZELASTINE HYDROCHLORIDE NASAL SPRAY BY RP-HPLC METHOD

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ABSTRACT

A simple, specific, accurate and precise RP-HPLC method has been developed for the Azelastine Hydrochloride in Azelastine Hydrochloride Nasal Spray. Linearity curve was linear with correlation coefficient of more than 0.999 for Azelastine Hydrochloride. The percentage recovery of the drug was found to be in the range of 99.0-101% indicating accuracy and reliability of method. The method was found to be precise as indicated by the repeatability of the analytical procedure, showing % RSD < 2%, that is 1.0%. RP-HPLC method was developed using Kinetex C18, 250 x 4.6 mm, 5 μ m, 100 \AA . The samples were analysed using mobile phase A: 50% buffer, 40% of methanol

and 10% of acetonitrile and mobile phase B: 10% buffer, 20% of methanol and 70% of acetonitrile as the mobile phase at the flow rate of 1.2 ml/min in gradient mode and detection wavelength is 239 nm, azelastine eluted about 6.4 minutes and gave sharp peaks with high theoretical plate count and low tailing factor. The proposed method was validated as per ICH guidelines. Hence, the developed analytical method can be utilized for the routine analysis of Azelastine Hydrochloride in Azelastine Hydrochloride Nasal Spray dosage form.

KEYWORDS: Azelastine Hydrochloride, Azelastine Hydrochloride Nasal Spray, UV detector, RP-HPLC method, Antihistaminic drug.

INTRODUCTION

Nasal sprays are used to deliver medications locally in the nasal cavities or systemically. They are applied locally to treat problems like allergic rhinitis and nasal congestion. Because it offers a pleasant alternative to injection or tablet, the nasal administration route is occasionally chosen for systemic therapy. Through the nose, substances can be directly and swiftly absorbed. Numerous pharmaceuticals are available as nasal sprays for systemic delivery, including sedative-analgesics, migraine, osteoporosis, and nausea medications. Nasal sprays are thought to be a more effective drug delivery method with the potential to pass the blood-brain barrier.

Antihistamine sprays:

How they work: Antihistamines work by blocking the effects of histamine, a substance that causes allergy symptoms like sneezing, itching, and runny nose.

Some examples: Azelastine (Astelin, Astepro), or olopatadine (Patanase).

Drug profile:

Azelastine is a selective, non-sedating H₁ antagonist with structural similarities to other antihistamines. As a phthalazinone derivative with a seven-membered ring, it is commonly used as an anhydrous monohydrochloride salt. The white, odorless, bitter-tasting crystalline powder is a racemic mixture of azelastine hydrochloride, with a high melting point of 225°C. Even as a hydrochloride salt, the small organic molecule is only sparingly soluble in methanol, propylene glycol, and water, but slightly soluble in glycerin, ethanol, and octanol. Although azelastine is soluble in dichloromethane and in chloroform, these solvents are not well tolerated in pharmaceutical preparations. Although azelastine is orally active, it is most often administered topically, either as a nasal spray or as a sterile ophthalmic solution.

Drug structure of azelastine hydrochloride: -

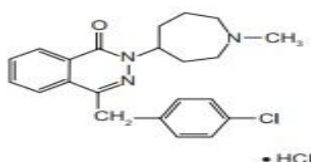


Fig. No. 1

Table 1

Drug name	:	Azelastine Hydrochloride
Therapeutic activity	:	Antihistaminic

pKa	:	8.88
Solubility	:	Soluble in methylene chloride, sparingly soluble in water, methanol, and propylene glycol, and slightly soluble in ethanol, octanol, and glycerine.
Melting point	:	about 225°C
pH	:	A saturated solution is between 5.0 and 5.4

MATERIAL AND METHOD

Details of the Chemical/reagents

Table 2

Name of the material	Make	Batch No. / Lot No.	Grade
Water	Milli-Q	NA	Chromatographic
Sodium hydrogen phosphate dihydrate	Rankem	F20A165	LR
Orthophosphoric acid	Rankem	CAIC700680	EMPLURA
Acetonitrile	Rankem	T050K20	HPLC
Methanol	Rankem	R036L20	HPLC

Equipment and Instrument details:

Table 3

Equipment/Instrument	Make
HPLC	Shimadzu
Analytical balance (Semi micro)	Radwag
Analytical balance (Micro)	Radwag
Ultrasonic bath	Labman
pH meter	Thermo Scientific
Vortex Mixture	ARD-VM-0012

Standards & Impurities details

Table 4

Standard & Impurities name	% Potency
Azelastine hydrochloride	100.0% w/w
Impurity A	98.93% w/w
Impurity B	90.16% w/w
Impurity C	99.22% w/w
Impurity D	96.38% w/w
Impurity E	97.33% w/w

Table 5: Column details.

Description
Phenomenex Kinetex, C18, 250 x 4.6 mm, 5µm, 100Å

Method development

Initiation of method development:

Since the drug product is not official in pharmacopoeias, the method development work was initiated by literature (Available in public domain) based nasal spray Assay method. The primary test was carried out to estimate the amount of Azelastine Hydrochloride in Nasal Spray solution formulation. Azelastine Hydrochloride was evaluated under various chromatographic conditions in both drug substance and drug product. The HPLC system's mobile phase was used to run drug substance for Azelastine Hydrochloride. Analysis was done on Sodium hydrogen phosphate dihydrate, acetonitrile, and methanol to determine the best conditions for separating Azelastine Hydrochloride and producing a good, symmetrical peak. Phosphate buffer was used in different concentration in water. Methanol and acetonitrile produced successful outcomes. Different ratios were attempted with this mobile phase system. Finally, phosphate butter (pH 3.1) with acetonitrile and methanol in a ratio of 50:40:10% v/v was selected as mobile phase A and Buffer, Methanol and Acetonitrile in the ratio of 10:20:70 %V/V as mobile phase B the ideal condition for the mobile phase for the study. The column temperature 30°C was used for all measurements. Utilizing several flow rate circumstances that were researched, the flow rate was optimized. For the current investigation, 1.2ml/min was the ideal flow rate.

Lambda max of azelastine hydrochloride by PDA detector:

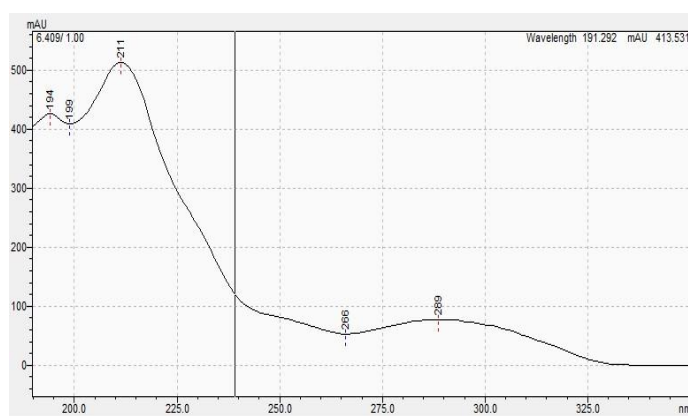


Fig. No. 2

Summary of method development:

Conditions	Procedure details
Optimization of Mobile phase:	Trimethylamine added in buffer preparation of mobile phase.

Optimization of Column:	No change in column concerning the reference method.
Optimization of Column temperature:	Column temperature has been changed from 50°C to 40°C.
Injection volume:	Injection volume has been changed from 10µL to 5µL.
Optimization of gradient program:	The gradient program has been changed from the reference method.
Optimization of Run time:	Run time has been changed from 35 mins to 20 mins.
Sample preparation optimization:	The ratio in diluent preparation has been changed from Water: Acetonitrile (25:75) to Water: Acetonitrile (60:40) (120ppm sample concentration)

Optimized method

Preparation of diluted Orthophosphoric acid (OPA):

Dilute 5 mL of conc. Orthophosphoric acid ($\geq 85\%$) to 50 mL with water and mix well.

Preparation of buffer:

Weigh about 3.0 g of Sodium dihydrogen phosphate dihydrate into 1000 mL of water dissolve and add 1.5 mL Trimethylamine (TEA) and mix well. Then adjust the pH to 3.5 ± 0.05 with diluted Orthophosphoric acid (OPA) and filter through 0.45µm PVDF membrane filter.

Preparation of mobile phase A:

Prepare mobile phase-A by mixing of Buffer, Methanol and Acetonitrile in the ratio of 50:40:10 % V/V/V, mix well and sonicate for about 5 mins to degas.

Preparation of mobile phase B:

Prepare mobile phase-B by mixing of Buffer, Methanol and Acetonitrile in the ratio of 10:20:70 % V/V/V, mix well and sonicate for about 5 mins to degas.

Preparation of diluent:

Prepare diluent by mixing of water and methanol in the ratio of 60:40 % V/V mix well and sonicate for about 5 mins to degas.

Chromatographic condition:

Column	Phenomenex Kinetex C18, 250 x 4.6 mm, 5µm, 100Å
Flow	1.2 mL/min.

Pump mode	Gradient
Detection	UV, 239nm
Column oven temperature	30°C
Sample temperature	10°C
Injection volume	10µl
Run time	20 min.

Gradient program:

Time (min)	Mobile Phase-A (%)	Mobile Phase -B (%)
0.00	90	10
6.00	95	5
10.00	60	40
10.01	0	100
15.00	0	100
15.01	95	5
20.00	95	5

Blank: Use diluent as blank.

Preparation of standard stock solution:

Weigh and transfer about 24.0 mg of Antihistaminic drug working Standard/reference standard/certified standard into 50 mL of the volumetric flask, add about 30 mL of diluent, sonicate for about 5 mins to dissolve, make up the volume with diluent and mix well.

Preparation of standard solution:

Further dilute 5.0 mL of Standard Stock solution to 20 mL volumetric flask and make up the volume with diluent and mix well, then filter the solution through 0.45µ PVDF syringe filter and by initial discarding of 3 mL filtrate.

Preparation of sample:

Weigh about 3.0 g of sample in 25 mL of the volumetric flask, add diluent to make up the volume and mix well, then filter the solution through 0.45µ PVDF syringe filter and by initial discarding of 3 mL filtrate.

Procedure:

Set the chromatographic conditions as described above and equilibrate the column with the mobile phase till a stable baseline is obtained. Inject diluent (blank) solution into the chromatograph and record the chromatogram. Inject standard solution (five injections), record the chromatograms, measure the peak areas. Inject Sample solution into the chromatograph in duplicate injection, record the chromatograms and measure the peak area.

Note:

- More than one blank can be injected based on requirements.
- The retention time of main peak is about 6.4 min (for information only).

System suitability parameters:

- No interference of blank peak at the retention time of main peak.
- USP Tailing factor for main peak in 1st injection of standard solution chromatogram should be NMT 2.0.
- USP Theoretical plates for main peak in 1st injection of standard solution chromatogram should be NLT 2000.
- %RSD of main peak area from five replicate injections of standard preparation should be NMT 2.0.

Note: RT may vary slightly based on system configuration and column usage

Method validation

Verification of method suitability

The developed method was further evaluated. The experimental details (Validation parameters) are given below:

Validation parameters:

Specificity

Linearity

Method precision

Filter study Accuracy

Robustness

Solution stability

accordance with ICH guidelines.

RESULTS AND DISCUSSION**Specificity**

No interference was observed due to the diluent, placebo, individual excipients, and impurity solutions at the retention Time of the Antihistaminic drug peak. Hence, the assay method was found to be specific concerning Diluent, Placebo, individual excipients, and impurities.

Preparation of solutions a)**Preparation of standard solution:**

Weighed and transferred 12.097mg of Antihistaminic drug HCl API into 100 mL of the volumetric flask, added about 70 mL of diluent, sonicated for about 5 mins to dissolve, made up the volume with diluent and mixed well.

Preparation of sample:

Weighed and transferred 3.02968 g of sample into 25 mL of the volumetric flask, added diluent to make up the volume and mixed well by vortexing.

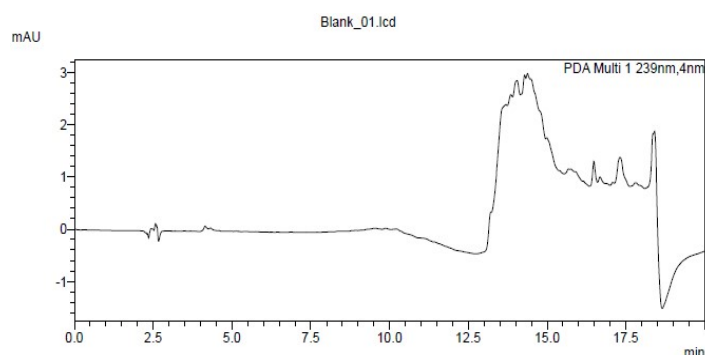
Preparation of diluent for impurity stock solution:**Placebo interference:**

Sample was prepared by taking the placebo equivalent to about the weight in portion of test preparation as per the test method and injected into the HPLC system.

Acceptance criteria:

Diluent, placebo, and individual impurity chromatograms should not show any peak(s) at the retention time of Antihistaminic drug peaks.

Peak purity index should be more than the single point threshold.

Blank:**Fig. No. 3****Standard:**

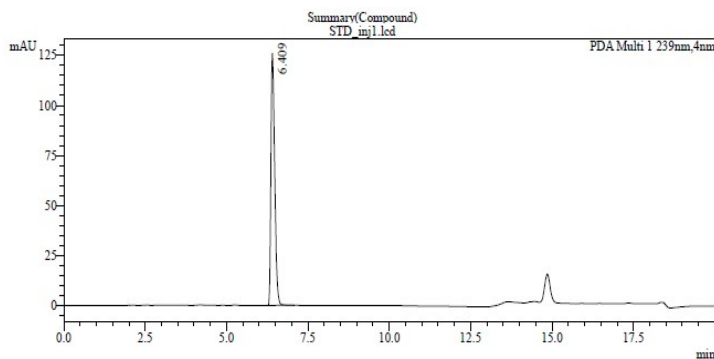


Fig. No. 4

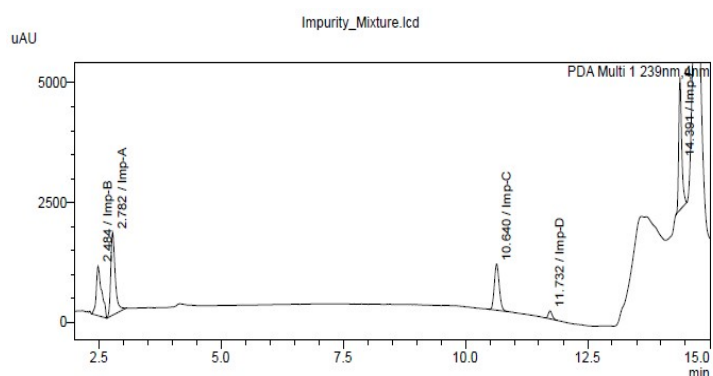
Impurity mix:

Fig. No. 5

Linearity**Preparation of linearity stock solution-1 (About 1000ppm):**

Weighed and transferred 100.10mg of Antihistaminic API into 100 mL of the volumetric flask, added about 70 mL of diluent, sonicated for about 5 mins to dissolve, made up the volume with diluent and mixed well.

Preparation of linearity stock solution-2 (About 100ppm):

Diluted 10 mL of Linearity stock solution-1 to 100 mL with diluent and mixed well.

Table 6: Linearity levels preparations.

% wrt sample concentration	ppm required	Volume took from	Vol -1	Dil-1	ppm
5	6	Linearity stock solution-2 (About 100ppm)	1	20	5
10	12		2	20	10
11	13.2		2.7	20	13.5
15	18		4	20	20
20	24		5	20	25

25	30	Linearity stock solution-1 (About 1000ppm)	6	20	30
50	60		6	10	60
75	90		0.9	10	90
100	120		1.2	10	120
150	180		1.8	10	180
200	240		2.4	10	240

Acceptance criteria: The correlation coefficient should be not less than 0.9990.

Table 7: Linearity levels of results.

%Level	Concentration ($\mu\text{g}/\text{mL}$)	Sample area/absorbance		Average Sample area/absorbance
		Inj-01	Inj-02	
5	4.976	44003	44005	44004
10	9.952	85948	85997	85973
11	13.435	114554	114824	114689
15	19.904	172623	172450	172537
20	24.88	215362	215058	215210
25	29.856	255030	255119	255075
50	59.712	508930	509357	509144
75	89.567	761603	762117	761860
100	119.423	1016950	1015940	1016445
150	179.135	1514577	1513408	1513993
200	238.847	2017174	2027303	2022239
			Slope	8453
			Intercept	3258
			% y-Intercept	0.3
			STEYX	2155
			CC	0.99999

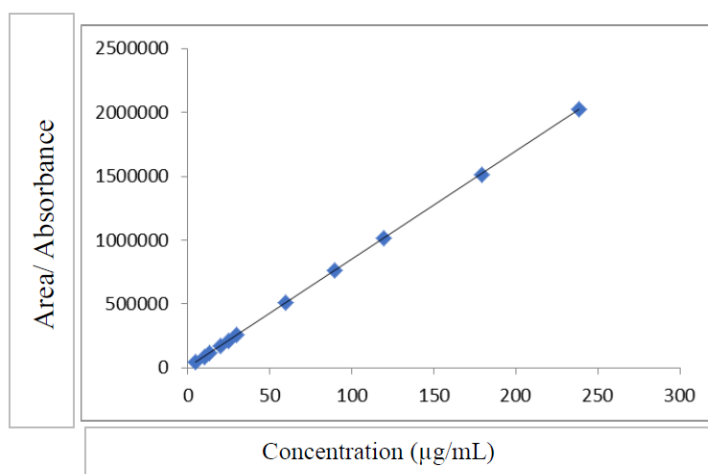


Fig. No. 6

Observation:

The correlation coefficient was found to be 0.99999 and % y-intercept was found to be 0.3. The linearity test meets the acceptance criteria.

Precision

Precision was the measure of the degree of repeatability of an analytical method under normal operation and it was normally expressed as the relative standard deviation for a statistically number of samples. Precision should be performed at three different levels: repeatability, intermediate precision and reproducibility.

System precision:

Acceptance criteria:

% RSD for an area of five replicate injections should be not more than 1.0%.

Table 8: System precision.

Title	Ret. Time	Area	NTP(USP)	Tailing Factor
STD_inj1	6.408	1013821	12885	1.5
STD_inj2	6.409	1013984	12924	1.5
STD_inj3	6.405	1013559	12967	1.5
STD_inj4	6.400	1014265	12949	1.5
STD_inj5	6.402	1013891	12978	1.5
Average	6.405	1013904	12941	1.5
Standard Deviation	0.0038	256.3	37.2	0.00
%RSD	0.1	0.0	0.3	0.0

Observation:

% RSD for Retention time, Area, NTP (USP) and Tailing factor of five replicate injections found to be 0.1%, 0.0%, 0.3% and 0.0% respectively.

Method precision:

Procedure

Six replicate Sample solutions were prepared as per the test method and injected as per the test procedure.

Acceptance criteria:

% RSD for six assay results should be not more than 2.0%.

Table 9: Method precision of sample.

Preparation	Amount of Sample(g)	Sample area/absorbance		Average Sample area/absorbance	Content in mg/Unit	% Labeled amount
		Inj-01	Inj-02			
Pre-1	3.04529	1029403	1028344	1028874	1.0027	100.3
Pre-2	2.98608	1033653	1033930	1033792	1.0275	102.8
Pre-3	3.02053	1029646	1029199	1029423	1.0114	101.1
Pre-4	2.99987	1033672	1033433	1033553	1.0225	102.3
Pre-5	3.01863	1043199	1044022	1043611	1.0260	102.6
Pre-6	3.03144	1035072	1035503	1035288	1.0136	101.4
				Average	1.0173	101.8
				SD	0.0097	1.0
				%RSD	1.0	1.0
				95% CI (±)	0.0102	1.0

Observation:

➤ % RSD for six assay results was found to be 1%.

Accuracy

Accuracy is the measure of exactness of an analytical method, or closeness of agreement between the measured value and the value that is accepted either as a conventional, true value or an accepted reference value. Accuracy is measured as the percentage of analyte recovered by assay, spiking samples in a blind study.

Preparation of accuracy stock solution-1 (About 1197ppm):

Weighed and transferred 120.39mg of Antihistaminic drug API into 100 mL of the volumetric flask, added about 70 mL of diluent, sonicated for about 5 mins to dissolve, made up the volume with diluent and mixed well.

Preparation of accuracy stock solution-2 (About 120ppm):

Diluted 5 mL of Accuracy stock solution-1 to 50 mL with diluent and mixed well.

Preparation of standard solution:

Accuracy stock solution-2 (About 120ppm) used as Standard solution.

Table 10: Accuracy solution preparations.

% wrt sample concentration	ppm required	Acetonitrile Added (mL)	Volume taken from	Vol -1	Dil-1	ppm
11	13	8.88	From stock 1	2.8	25	13
50	60	9.52	From	1.2	25	57

100	120	9.00	stock 2	2.5	25	120
200	240	8.00		5	25	239

Acceptance criteria:

Mean recovery should be between 95.0 to 105.0%.

Table 17: Accuracy results.

Sample ID	Sample area/absorbance		Average Sample area/absorbance	mg Added	mg Found	%Recovery	Average
	Inj-01	Inj-02					
11%-1	116762	117072	116917	0.335	0.339	101.2	101.1
11%-2	116639	116729	116684	0.335	0.338	100.9	
11%-3	116544	116954	116749	0.335	0.339	101.2	
50%-1	500139	500590	500365	1.436	1.451	101.0	100.9
50%-2	500684	500534	500609	1.436	1.452	101.1	
50%-3	499113	498541	498827	1.436	1.446	100.7	
100%-1	1022644	1023351	1022998	2.992	2.966	99.1	99.3
100%-2	1023893	1025070	1024482	2.992	2.971	99.3	
100%-3	1025125	1025109	1025117	2.992	2.973	99.4	
200%-1	2054041	2054813	2054427	5.985	5.957	99.5	99.5
200%-2	2055638	2055287	2055463	5.985	5.960	99.6	
200%-3	2053149	2053930	2053540	5.985	5.955	99.5	

Observation:

Mean recovery was found to be within 98.0 to 102.0% in all the preparations.

Table 18: Performed the following conditions for Robustness:

Flow rate	-10%	1.1 mL/min
	+10%	1.3 mL/min
Column oven temperature	-5°C	25°C
	+5°C	35°C
pH variation	-0.1	3.0
	+0.1	3.2

Acceptance criteria:

USP Tailing factor should be NMT than 2.0 and Theoretical plate count should be NLT than 2000.

The absolute difference in % assay results between as such and robustness test solutions should be not more than 2.0%.

RESULTS

Table 19: System suitability parameters from standard solution.

System suitability parameters	Variations						
	As such method	Flow Rate		Column Oven Temperature		pH Variation	
		-10%	+10%	-5°C	+5°C	-0.1	+0.1
USP Tailing Factor	1.4	1.4	1.4	1.4	1.4	1.5	1.5
USP Theoretical Plates	14921	15597	14197	14652	14755	14172	14007

Table 20: System suitability parameters from sample solution.

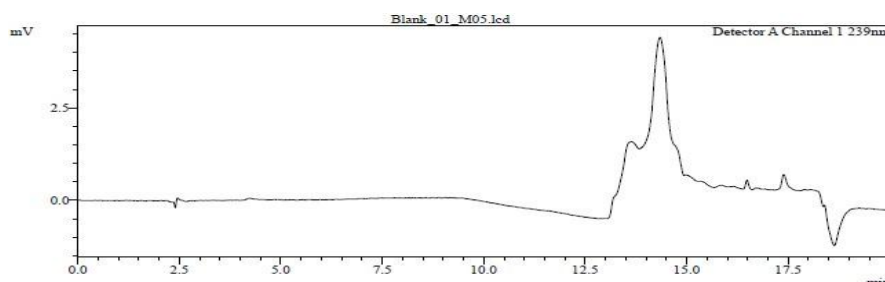
Variation		% Labeled amount	Abs % Difference
As such method		100.8	NA
Flow Rate	-10%	100.9	-0.1
	+10%	101.1	-0.3
Column Oven Temperature	-5°C	101.0	-0.2
	+5°C	101.1	-0.3
pH Variation	-0.1	100.9	-0.1
	+0.1	100.9	-0.1

Observation:

- The results of the robustness parameter meet the acceptance criteria.

Discussion:

The method is capable to remain unaffected by small deliberate variations in method parameters in HPLC. This indicates that the method is Robust for the determination of assay of HCl in Azelastine HCl Nasal Spray.

Chromatogram:**Blank chromatogram as such method:****Fig. No. 7****Standard chromatogram as such method:**

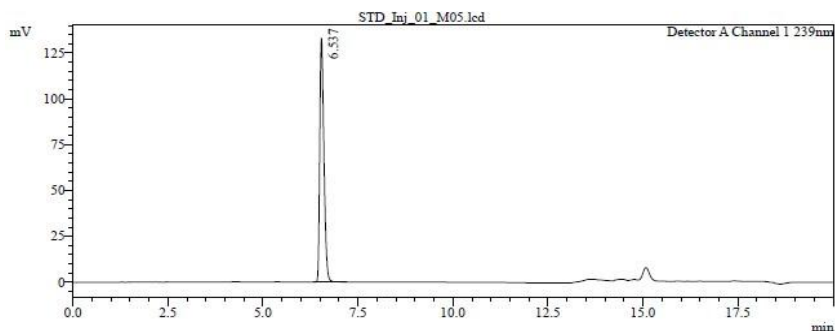


Fig. No. 8

Blank chromatogram at flow rate 0.9 mL/min:

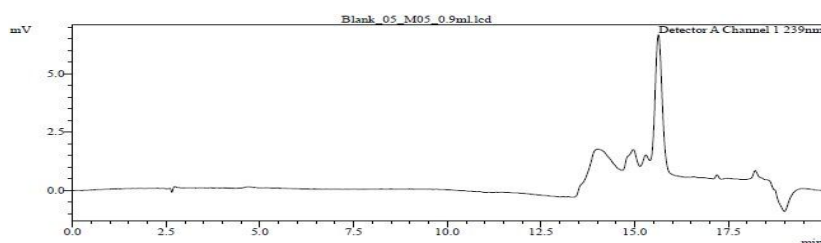


Fig. No. 9

Standard chromatogram at flow rate 0.9 mL/min

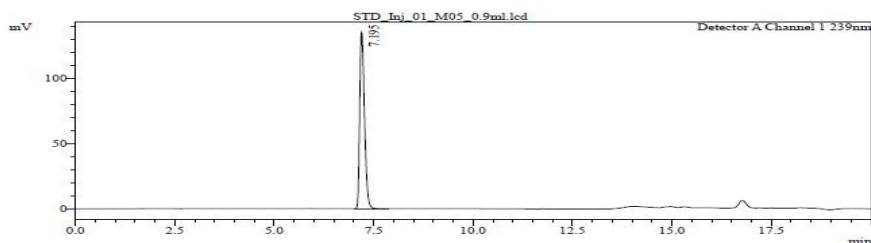


Fig. No. 10

Blank chromatogram at flow rate 1.1 mL/min

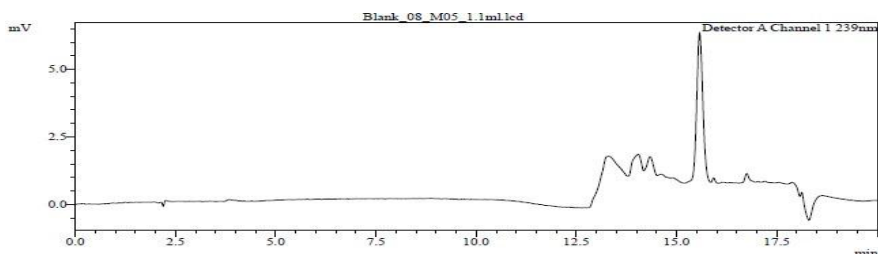


Fig. No. 11

Standard chromatogram at flow rate 1.3 mL/min

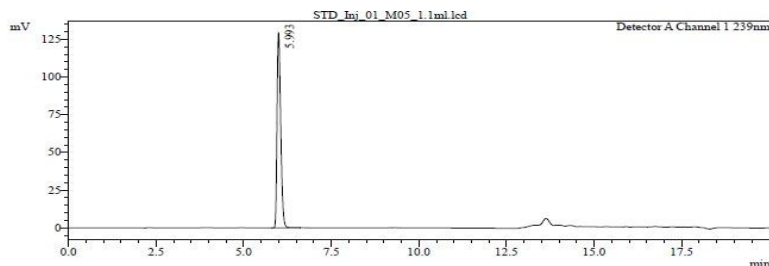


Fig. No. 12

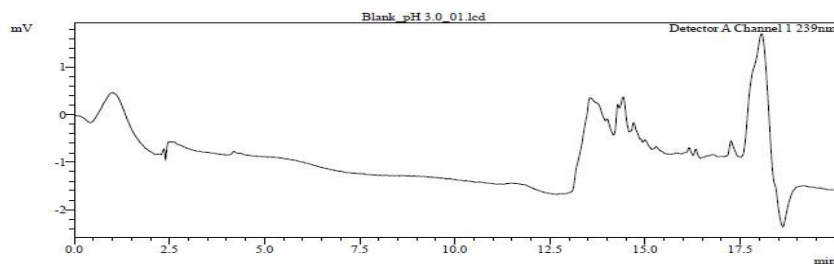
Blank chromatogram at buffer pH 3.0

Fig. No. 13

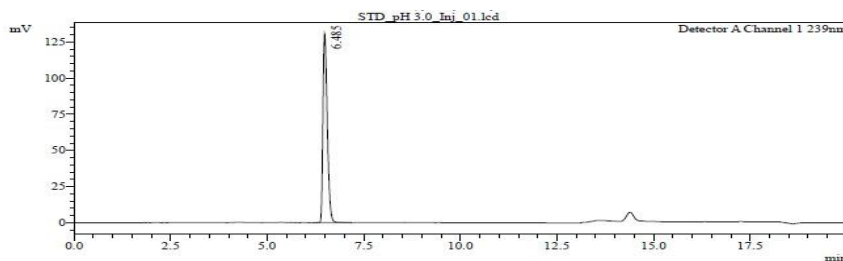
Standard chromatogram at buffer pH 3.0

Fig. No. 14

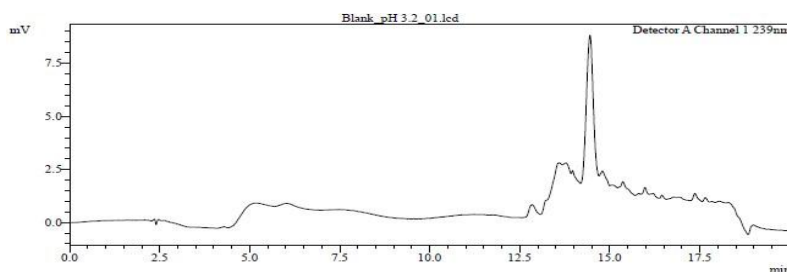
Blank chromatogram at buffer pH 3.2

Fig. No. 15

Standard chromatogram at buffer pH 3.2

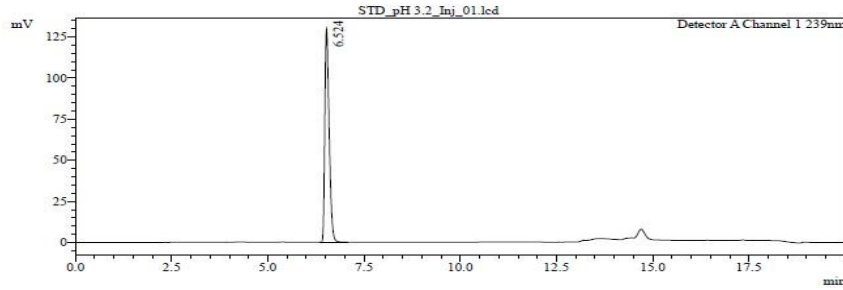


Fig. No. 16

Blank chromatogram at column temperature 25°C:

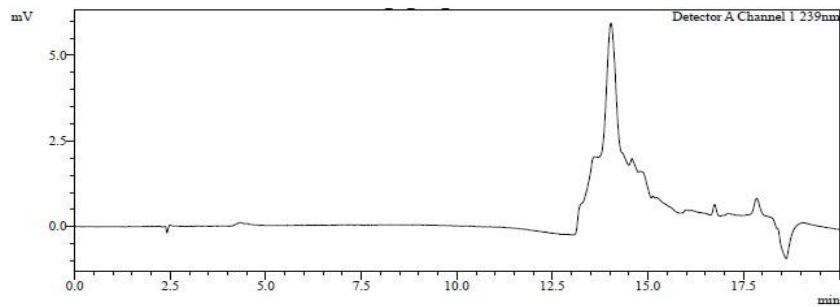


Fig. No. 17

Standard chromatogram at column temperature 25°C:

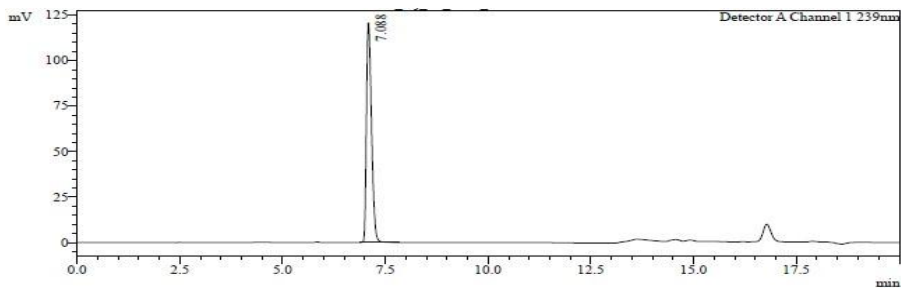


Fig. No. 18

Blank chromatogram at column temperature 35°C:

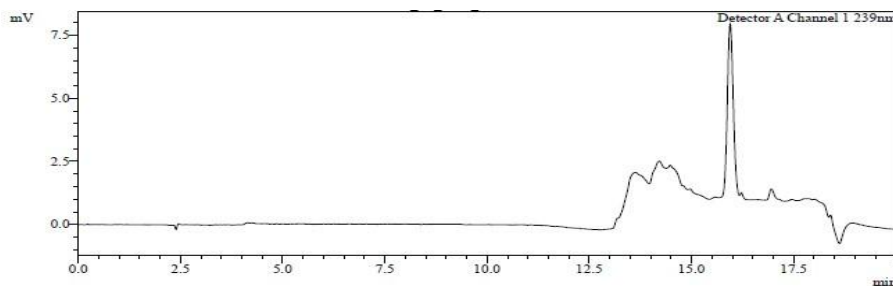


Fig. No. 19

Standard chromatogram at column temperature 45°C:

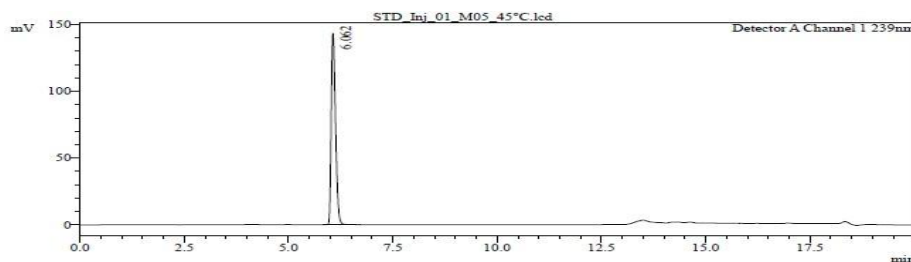


Fig. No. 20

Solution stability:**Acceptance criteria:**

% Difference between the responses obtained at initial and different time intervals should be no more than 2.0%.

Table 21: Standard-Solution stability results.

Standard-Solution stability		
Stage	h:mm:ss	% Difference
Initial	--	--
SS1	5:11:17	0.2
SS2	7:15:46	0.3
SS3	12:41:51	0.2
SS4	17:09:58	0.3
SS5	19:56:02	0.4
SS6	22:42:03	0.3
SS7	25:28:03	0.1
SS8	28:14:06	0.8
SS9	31:00:04	0.7
SS10	33:46:03	0.6
SS11	35:50:32	0.6
SS12	38:36:31	0.6
SS13	41:22:29	0.6
SS14	44:08:28	0.6
SS15	46:54:32	0.3
SS16	49:40:31	0.6
SS17	52:26:29	0.6
SS18	54:30:57	0.6
SS19	58:39:54	0.5
SS20	64:06:02	0.4
SS21	69:12:36	0.2

Table 22: Sample-Solution stability results.

Sample-Solution Stability		
Stage	h:mm:ss	% Difference
Initial	--	--
SS1	3:48:16	0.1
SS2	9:14:23	0.2
SS3	13:42:27	0.1
SS4	32:23:01	0.5
SS5	51:03:27	0.1
SS6	55:12:25	0.3
SS7	60:38:31	0.1
SS8	65:45:05	0.1

Observation:

- The results of the solution stability meet the acceptance criteria.
- The standard solution is stable up to 69 hours at 10°C.
- The sample solution is stable for up to 65 hours at 10°C.

DISCUSSION:

From the above data, it can be concluded that the standard solution and sample solutions were stable at least 69 hours and 65 hours at 10°C respectively.

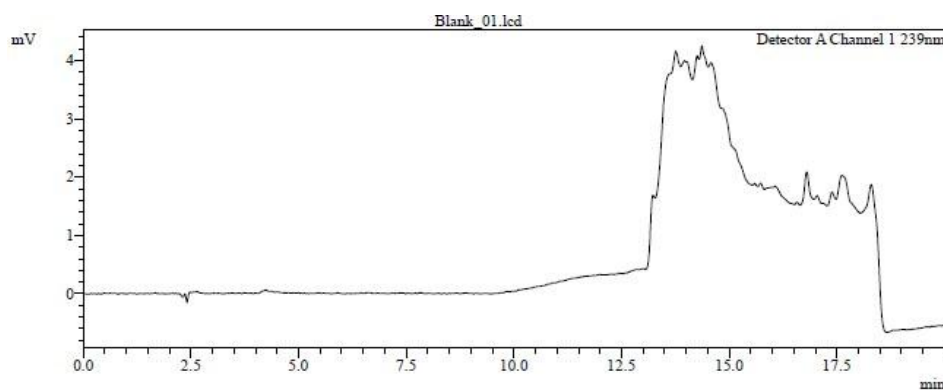
Chromatogram:**Blank:**

Fig. No. 21 Spectrum of blank for sample-solution.

Stability standard:

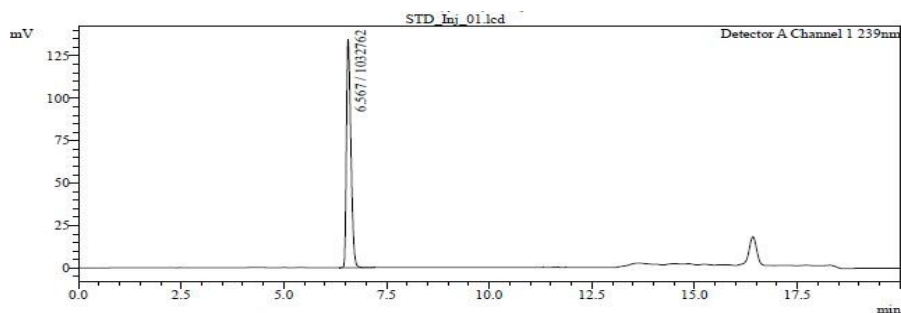


Fig. No. 22: Spectrum of Standard at 69 hours.

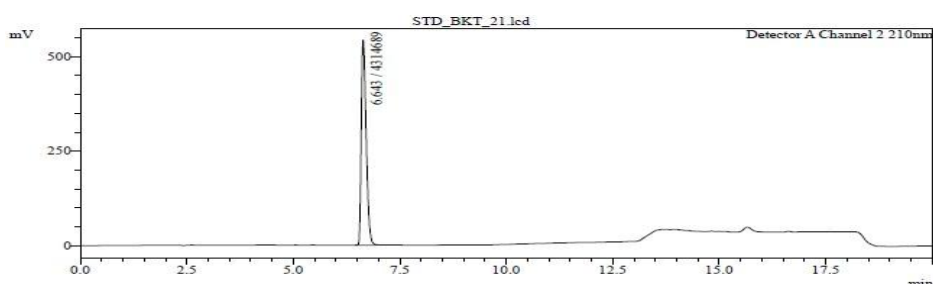


Fig. No. 23: Spectrum of standard BKT at 69 hours.

Sample initial:

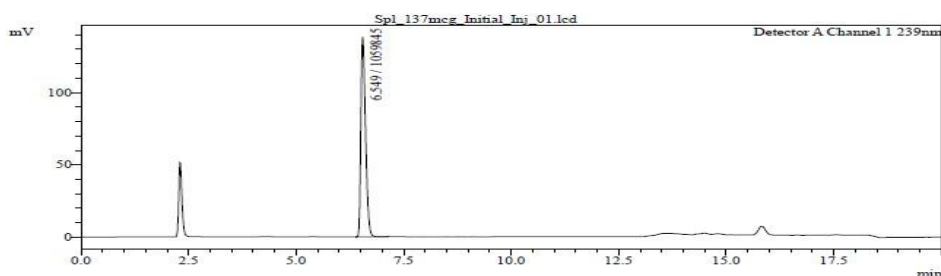


Fig. No. 24: Spectrum of sample initial.

Sample at 65 hours:

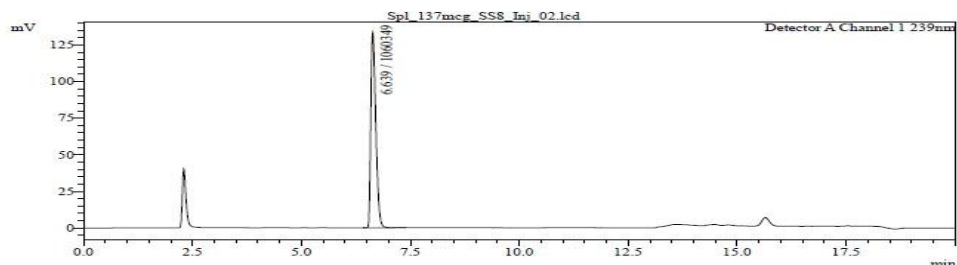


Fig. No. 25: Spectrum of sample at 65 hours.

CONCLUSIONS

The proposed method for estimating the amount of azelastine hydrochloride in azelastine hydrochloride nasal spray was specific, precise, accurate, and stable at room temperature. The

ICH guidelines were followed in the technique development and validation. The findings of the validation meet the established acceptance criteria.

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