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Research Article

A study of method development and validation for estimation of Azelastine hydrochloride in nasal spray formulations by RP-HPLC method

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ABSTRACT

A simple reverse-phase HPLC method for the estimation of Azelastine hydrochloride in nasal spray formulations has been developed. The method is simple, accurate, precise, specific and linear over the analysis range. This developed method has been validated according to International Conference on Harmonization (ICH) guideline with respect to system suitability, specificity, precision, linearity, accuracy, and robustness. An isocratic condition of mobile phase comprising Phosphate buffer (pH 3.1): Acetonitrile in a ratio of 60:40, v/v at a flow rate of 1.0 mL/minute over RP C18 (octadecylsilane (ODS), 250 × 4.6 mm, 5 µm, CHROMOSIL) column at ambient temperature was maintained. Besides, the chromatographic peak was observed sharp & symmetric. The proposed method was successfully applied for the estimation of the Azelastine hydrochloride in nasal spray formulation.

Keywords: Azelastine hydrochloride, ICH, Nasal Spray, RP-HPLC and Validation

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INTRODUCTION

Inflammation mostly occurs due to an allergic reaction, and people who suffer from nasal allergies, such as hay fever, dust and mite, tend to experience a variety of symptoms due to this

Inflammation¹. Inflammation is not a synonym for infection. Infection describes the interaction between the action of microbial invasion and the reaction of the body's inflammatory response². Simply, Inflammation is part of the body's immune response. Inflammation of the nasal passage forces fluid out of the nasal tissues, resulting in a runny and blocked nose. Other symptoms include interfere with hearing and speech, sneezing, watery and itchy eyes.

The three main types of drugs available for anti-inflammatory and anti-allergic effect are corticosteroids, antihistamines³ and decongestants. Corticosteroid drugs include budesonide, flunisolide, fluticasone furoate, fluticasone propionate and mometasone furoate, antihistamine drugs include azelastine and olopatadine. Two common decongestants available in the nasal sprays or drops form are oxymetazoline and phenylephrine.

Azelastine hydrochloride is an antihistamine formulated as a metered-spray solution for intranasal administration. It works by blocking certain natural substances called histamines that are responsible for nasal symptoms. This medication issued to relieve nasal symptoms such as runny/itching/stuffy nose, sneezing, and post-nasal drip caused by allergies or other conditions¹.

Azelastine has a triple mode of action: Antihistamine effect, Mast-cell stabilizing effect and Anti-inflammatory effect⁴.

Its chemical name is (\pm)-1-(2H)-phthalazinone,4-[(4-chlorophenyl) methyl]-2-(hexahydro-1-methyl-1H-azepin-4-yl)-, monohydrochloride. Its molecular formula is C₂₂H₂₄ClN₃O•HCl with the following chemical structure:

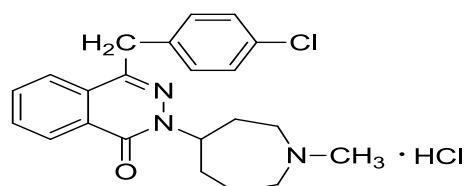


Figure 1: Chemical structure of Azelastine hydrochloride

Azelastine hydrochloride occurs as a white, almost odorless, crystalline powder with a bitter taste. It has a molecular weight of 418.37. It has a melting point of about 225 °C. It is sparingly soluble in water, methanol, and propylene glycol⁵.

In current scenario, official method for quantification of Azelastine hydrochloride is available and that is of titrimetric method⁶ which is highly sensitive and time consuming, but accurate and precise reverse phase HPLC method is not available. Our interest of work was to develop suitable and rapid HPLC method required for analysis and characterization of Azelastine hydrochloride from nasal spray formulation.

2. MATERIALS AND METHODS

2.1 Materials and Reagents

Azelastine hydrochloride working standard and Placebo were a kind gift of Shree Industrial Training Centre And Research Laboratory, Jalgaon, Maharashtra. Test samples purchased from market store. HPLC grade Acetonitrile, Potassium dihydrogen phosphate and HPLC Water were purchased from Ranbaxy Fine Chemicals Ltd., India.

2.2 HPLC system

High-performance liquid chromatographic system (Younglin (S.K) Gradient System) and equipped with UV-visible detector was used for the analysis. The data were recorded using Autochro -3000 software.

2.3 Preparation of mobile phase

Dissolved 6.8 gm of KH₂PO₄ in to 1000 ml water and sonicated to dissolved (pH observed 4.42), adjusted to pH 3.10 with diluted Orthophosphoric acid solution. Buffer filtered through a 0.45-μm PVDF membrane filter and sonicated to degas. Prepared a mixture of Buffer (pH 3.1) : Acetonitrile (60:40), v/v, sonicated to degas.

2.4 Preparation of standard solution (50 PPM)

Accurately weighed and transferred 50 mg of Azelastine hydrochloride working standard in to 100 mL volumetric flask, added about 30 mL of Acetonitrile and sonicated to dissolve, wait to cool and diluted up to mark with diluent. Transferred 5 mL of this solution in to 50 mL volumetric and diluted up to mark with Mobile Phase.

2.5 Preparation of sample solution (50 PPM)

Brand Name:- ARZEP(CADILA HEALTH CARE)

Transferred 2.5 mL of Sample solution in to 50 mL volumetric flask, added about 30 mL of mobile phase and sonicated to mixed properly, diluted up to mark with mobile phase, and the samples were analyzed using the proposed analytical methods.

2.6 Chromatographic conditions

The analysis was carried out at ambient temperature under isocratic condition. The mobile phase was run at a flow rate of 1.0 mL/minute for 10 min. The injection volume was 20 μL for standard and samples. Before analysis, every standard and sample was filtered through 0.45 μm Nylon syringe filter. The column eluent was monitored with UV detection at 239 nm⁷.

2.7 Method validation

Validation of an analytical procedure is the process by which it is established by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications. The

objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. Typical parameters verified in validation of analytical method are listed in table 1. ICH Q2(R1)⁸ is considered the primary reference for recommendations and definitions on validation characteristics for analytical procedures.

Table 1: Typical parameters verified in method validation

Sr No	Validation parameter
1	System suitability
2	Specificity
3	Precision
4	Accuracy
5	Linearity
6	Robustness

2.7.1 System suitability

System suitability test as an integral part of method development was used to ensure adequate performance of the chromatographic system. To determine system precision Azelastine Hydrochloride standard solution was prepared and injected for six times into HPLC system. The mean, SD and % RSD for peak areas of Azelastine was calculated.

2.7.2 Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components. The placebo solution containing excipients without Azelastine were injected.

To evaluate the specificity of the method blank, placebo and test solution were injected.

2.7.3 Precision

The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is repeatedly applied to multiple samplings of a homogeneous sample. The precision of an analytical procedure is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. Precision may be a measure of either the degree of reproducibility or of repeatability of the analytical procedure under normal operating conditions. The precision of the assay method was assessed with respect to repeatability and reproducibility. The precision of the proposed method was checked by ability of responses after replicate injections and expressed as %RSD among responses using the formula. Sample of a single batch were prepared six times and analyzed as per test method, % assay of Azelastine for six samples calculated for method precision.

2.7.4 Accuracy

The accuracy of an analytical procedure is the closeness of test results obtained by that procedure to the true value. The accuracy of an analytical procedure should be established across its range. In the present study, successive analysis (n=3) for three different concentrations of standard mixtures (50, 100 and 150%) was carried out to determine the accuracy of proposed method.

2.7.5 Linearity

Linearity was checked on different concentrations within 50–200% of the nominal standard concentration. The linearity of the proposed method was evaluated by using calibration curve to calculate the coefficient of correlation, slope, and intercept values.

2.7.6 Robustness

Robustness is an indication of the reliability of the analytical method during normal usage. The effect of the following deliberate changes in chromatographic

conditions was monitored: Detector wavelength ± 2 nm, Flow rate $\pm 10\%$, Temperature ± 2 °C, and pH of Buffer solution ± 0.1 .

3. RESULTS AND DISCUSSION

3.1 System suitability

The results of system suitability were within acceptable limits as shown in table 2.

Table 2: Results from the determination of system precision for determination of Azelastine

Standard No	Peak area of Azelastine
1	1233
2	1256
3	1240
4	1242
5	1249
6	1239
Mean	1243
SD	8.13
%RSD	0.65

Values are expressed for six replicate (n=6)

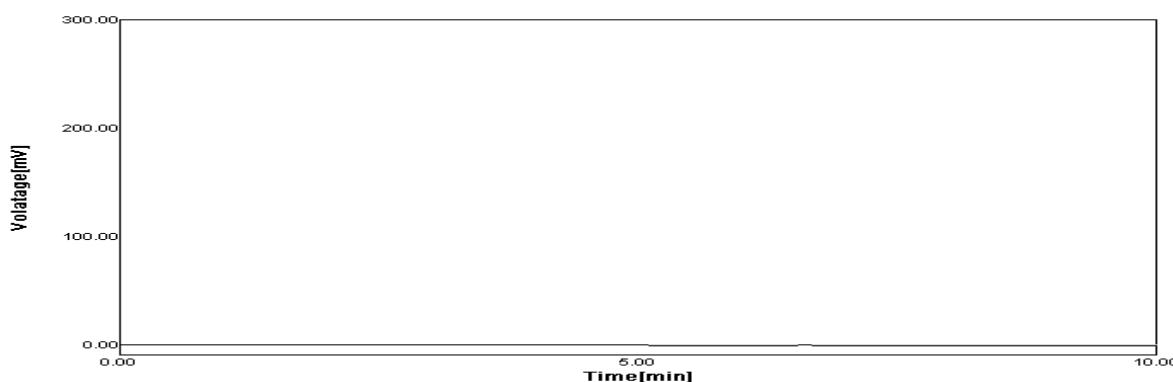
Table 3: System suitability data from Method precision

Sample No	% Assay of Azelastine
1	99.7
2	99.3
3	99.7
4	100.2
5	100.1
6	99.3
Mean	99.7
SD	0.35
% RSD	0.35

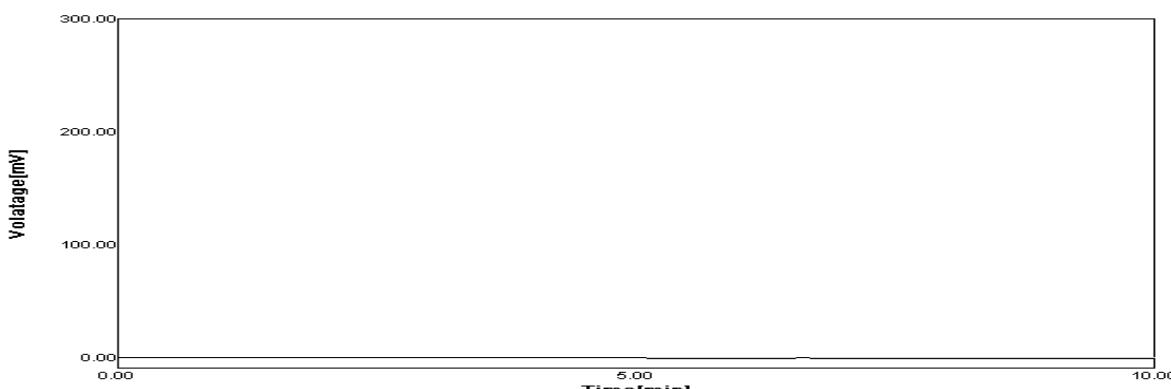
Values are expressed as mean \pm standard deviation of six samples (n=6)

3.2 Specificity

a) Blank



b) Placebo



c) Sample solution

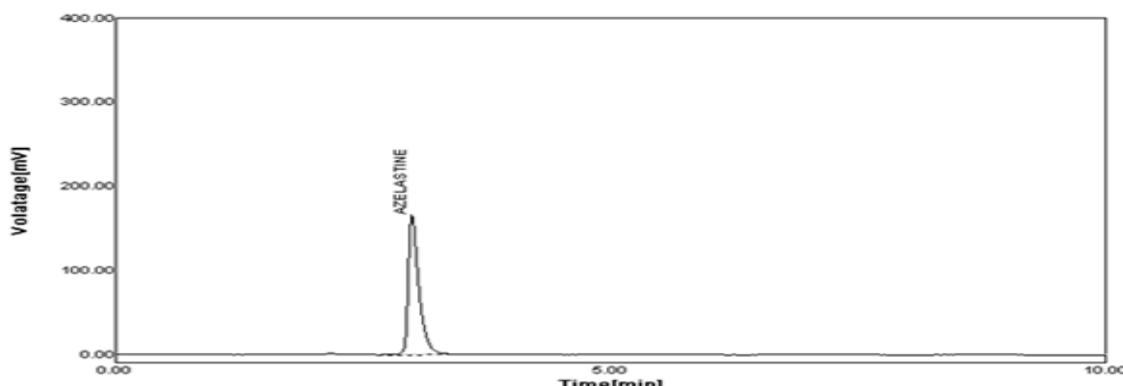


Figure 2: HPLC Chromatogram a) Blank, b) Placebo and c) Sample solution

3.3 Accuracy

Known amount of Azelastine hydrochloride was spiked in placebo at about 50,100 and 150% of test concentration. The amount of Azelastine hydrochloride recovered was quantified as per developed method. The % recovery was

calculated from the amount found and actual amount added. The results are tabulated in table 4. The overall recovery of Azelastine hydrochloride in the samples was in between 98.0 to 102.0% (RSD<2%) which is sufficient for quantification of Azelastine hydrochloride in nasal spray formulations.

Table 4: Evaluation of accuracy of the proposed method for quantification of Azelastine

Spiked level (%)/ Sample No	Actual amount of API added(mg)	Amount of Azelastine found (mg)	% Recovery	Mean	SD	%RSD
50% Sample-1	1.2549	1.2532	99.9	99.8	0.33	0.33
50% Sample-2	1.2549	1.2472	99.4			
50% Sample-3	1.2549	1.2552	100.0			
100% Sample-1	2.5098	2.4964	99.5	99.4	0.22	0.22
100% Sample-2	2.5098	2.4894	99.2			
100% Sample-3	2.5098	2.5004	99.6			
150% Sample-1	3.7646	3.7436	99.4	99.4	0.10	0.10
150% Sample-2	3.7646	3.7376	99.3			
150% Sample-3	3.7646	3.7446	99.5			

Values are expressed as mean± standard deviation of replicate (n=3)

3.4 Linearity

A graph was plotted with concentration (in $\mu\text{g}/\text{ml}$) of Azelastine hydrochloride on X-axis and peak areas of Azelastine on Y-axis. The results are tabulated in table 5 and graphically represented in figure 3. The method showed excellent linear response with correlation coefficient (R^2) values of 0.999, which was within the limit of the correlation coefficient ($R^2=0.995$).

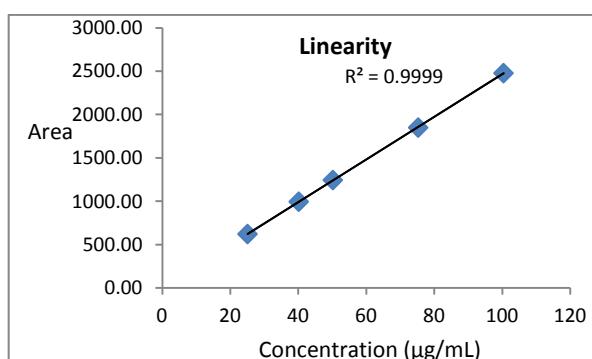


Figure 3: Linearity plot for Azelastine

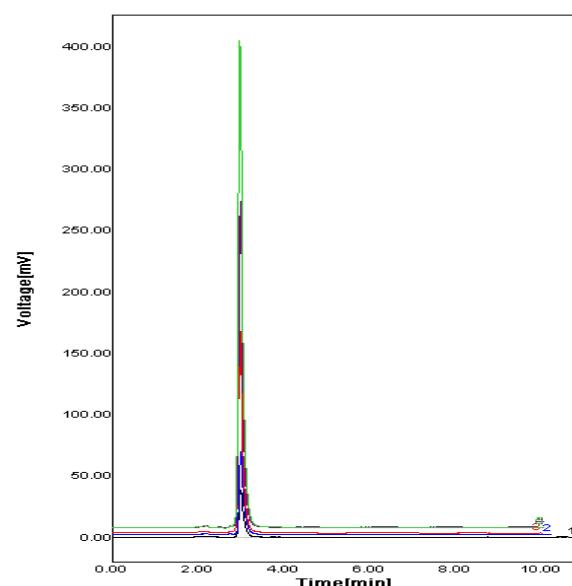


Figure 4: Overlay graphs of linearity plot.

Table 5: Results of linearity

Spike level in %	Concentration (mcg/mL)	Average Area (N=2)
50	25.10	622
80	40.16	995
100	50.20	1245
150	75.29	1850
200	100.39	2478
Slope		24.59
Y-Intercept		6.24
Correlation Coefficient ®		0.99998

5 Robustness results

Robustness of the method was verified by deliberately varying the following chromatographic conditions as shown in table 5 i.e.

- By changing the wavelength by ± 2 nm

- By changing the flow rate by $\pm 10\%$
- By changing the column oven temperature by ± 2 °C
- By changing the pH of buffer used for mobile phase by ± 0.1 unit

Table 6: Robustness experiment

Sr. No.	Robustness Parameter	Retention Time (min)	Tailing factor	Theoretical plates	% RSD of Standard solution
1	Wavelength 237 nm	2.98	1.3	4268	0.17
2	Wavelength 241 nm	2.98	1.3	4170	0.23
3	Flow rate (0.90 mL/min)	3.04	1.3	4527	0.10
4	Flow rate (1.10 mL/min)	2.88	1.2	4649	0.06
5	Column Temp 28°C	2.98	1.3	4200	0.11
6	Column Temp 32°C	2.98	1.3	4270	0.34
7	Buffer pH 3.0	2.98	1.2	3819	0.17
8	Buffer pH 3.2	2.98	1.2	3945	0.11

3.6 Estimation of formulations

The assay values of Azelastine hydrochloride in nasal spray formulations ranged from 99. % to 100.2%, with a standard deviation of not more than 0.35%. The assay values for the formulations were same as mentioned in the label claim, indicating the suitability of the proposed analytical method. The estimated drug content with low values of standard deviation established the precision of the proposed method.

3.7 DISCUSSION

Development of an analytical method for assessment of drugs in the pharmaceutical dosage form is of utmost necessity to confirm the quality of nasal formulations with respect to assay⁶ and spray content uniformity.

Our developed HPLC analytical method for estimation of Azelastine in nasal spray formulations has used very less amount of organic solvents which is cost effective and environment friendly. Also, the method was found to be simple and accurate and was able to resolve the drug from excipients in a short analytical run time.

4.0 CONCLUSIONS

A simple reversed-phase HPLC method for the analysis of Azelastine hydrochloride in nasal spray formulations was developed and validated. The proposed method is simple, accurate, precise, specific and linear over the analysis

ranges and was able to resolve the drug from excipients in a short analytical run time.

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CONFLICT OF INTERESTS

Declared none

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