Stability Indicating High-Performance Liquid Chromatography Method for Determination of Antihistamine Drug Azelastine in Pharmaceutical Formulation

***[1]Shaikh Sameena, [1]Dhumal P.B, [1]Sameer Shafi.**

Shivlingeshwar College of Pharmacy, Almala-Latur (413520)
Maharashtra (MH), India.

Abstract : An innovative, sensitive, easy and fast methodology has been developed for estimation of Azelastine. Applied statistical analysis verified that the method is correct, selective, precise and duplicable. The straight forward procedure with high accuracy, wide dimensionality vary and sensitivity imply that the incontestable methodology to be acceptable for routine estimation and internal control assay of pharmaceutical formulations. The detection of the combined dose kind was allotted at 290 nm and rate used was 1.0ml/min. The mobile phase was used as a mixture of acetonitrile and buffer (adjusted to pH scale 3.0 with 5N NaOH) and was pumped up at a relentless rate of 1.5 cc per minute. This analysis study focuses on the event of the superior layer activity methodology mistreatment style of experiment approach for the determination of Azelastine coordination compound alongside forced degradation study. Full factorial style was applied on acid and base elicited degradation and applied mathematics analysis by analysis of variance was performed with interpretation of varied plots. The method was valid by determination of dimensionality, precision, accuracy, specificity and strength in keeping with ICH Guidelines. The target of this analysis was to develop a straight forward, precise, accurate, and stability-indicating high performance liquid chromatography methodology for estimation of Azelastine coordination compound (AZL) in pharmaceutical formulation.

Keywords: - Azelastine, Statistical Analysis, HPLC, ICH Guidelines etc.

INTRODUCTION

Azelastine (AZH) is a medicinal drug steroid that is on the market as a nasal spray formulation. Azelastine that may be a bitter style. Its chemical name is (±) 1(2H) phthalazinone, 4[(4chlorophenyl) methyl]-2-(hexahydro-1-methyl-1Hazepin-4-yl)-, monohydrochloride. Its formula is $C_{22}H_{25}C_{l2}N_3O$. It has a mass of 418.37 Azelastine complex (AZH). Azelastine was proprietary in 1971 and came into medical use in 1986. It's offered as a generic medication.

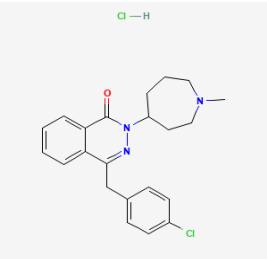

Figure 1

It is with chemicals referred to as second generation, selective, amino alkane antagonist. Azelastine nasal spray is employed to assist relieve symptoms (e.g., stuffy or fluid nose, itching, sneezing) of seasonal (short-term) or perennial (year-round) Coriz (hay fever), constriction inflammation, or alternative higher metabolism allergies. Azelastine is associate in nursing medicinal drug. It works by preventing the results of a substance referred to as amino alkane that is made by the body. Amino alkane will cause itch, sneezing, runny nose, and watery eyes. This medication is employed to alleviate nasal symptoms like runny/itching/stuffy nose, sneezing, and post-nasal drip caused by allergies or alternative conditions. Azelastine belongs to a category of medicine referred to as antihistamines. It works by obstruction sure natural substances referred to as histamines that are answerable for nasal symptoms. Azelastine coordination compound (AZL) that could be a second-generation H1 receptor antagonist. The chemical structure of AZL is shown in Fig.1. AZL happens as a white, virtually inodorous, crystalline powder with a bitter style. It additionally inhibits lipoxygenase and blood platelet aggregation. It's medication agent additionally. It's indicated as antiallergic agent. In current state of affairs, official technique for quantification of Azelastine coordination compound is out there which is of titrimetric technique that is very sensitive and time intense, however correct and precise reverse part HPLC technique isn't out there. Our interest of labor was to develop appropriate and fast HPLC technique needed for analysis and characterization of Azelastine coordination compound from nasal spray formulation. None of the ways satisfy the steadiness protocol as per restrictive steerage.

A perfect stability-indicating technique is one that quantifies the drug and resolves it from its degradation merchandise. These facts initiate the current study to determine associate in nursing correct, specific, repeatable, and stability-indicating HPLC technique for analysis of AZL within the presence of its degradation merchandise. The recommended technique was designed to be

appropriate for routine analysis of the drug in pharmaceutical formulations. The projected technique was valid in accordance with ICH tips. It's still a restricted range of analytical ways that are reported for the determination of Azelastine coordination compound as well as quantitative chemical analysis and spectrophotometric, Thin Layer Chromatography (TLC), capillary catachresis, High Performance Liquid Chromatography (HPLC), chemistry ways and thermal analysis are developed for the estimation of AZH separately or in indefinite quantity forms.

2. MATERIALS AND METHODOLOGY: -

2.1 Materials and Reagents: -

Azelastine complex operating normal and Placebo were a sort gift of Shree Industrial coaching Centre and research lab, Jalgaon, geographical region. Take a look at samples purchased from market store. HPLC grade Acetonitrile, atomic number 19 dehydrogenase phosphate and HPLC Water were purchased from Ranbaxy Fine Chemicals Ltd., India.

2.2 HPLC System: -

High-Performance Liquid Chromatography system (Youngling (S.K) (Gradient System) and equipped with UV– visible detector was used for the analysis. The information were recorded victimization Auto chrome -3000 package.

2.3 Preparation of Mobile Phase: -

Dissolved about 6.8 g of KH_2PO_4 in to a 1000 ml water and sonicated to dissolved (pH discovered 4.42), adjusted to pH scale 3.10 with diluted Orthophosphoric acid solution. Buffer filtered through a 0.45-µm PVDF membrane filter and sonicated to remove. Ready a combination of Buffer (pH 3.1): Acetonitrile (60:40), v/v, sonicated to remove.

2.4 Preparation of Standard Solution (50 PPM): -

Accurately weighed and transferred 50 mg of Azelastine complex operating normal in to 100 mL volumetric flask, added about 30 mL of Acetonitrile and sonicated to dissolve, wait to cool down and diluted up to mark with dilutant. 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 below isocratic condition. The mobile Phase was run at a rate of flow of 1.0 mL/minute for 10 min. The injection volume was 20 µL for standard and samples. Before analysis, every standard and sample were filtered through 0.45 µm Nylon syringe filter. The column eluent was monitored with UV detection at 239 nm.

3. METHOD VALIDATION: -

Validation of associate analytical procedure is that the method by that it's established by laboratory studies, that the performance characteristics of the procedure meet the necessities for the meant analytical applications. The target of validation of associate analytical procedure is to demonstrate that it's appropriate for its meant purpose. Typical parameters verified in validation of analytical technique area unit listed in Table No. 1. ICH Q2 (R1) and Limit of Detection (LOD), Limit of Quantification (LOQ) [54] is taken into account the first reference for recommendations and definitions on validation characteristics for analytical procedures.

Table No. 1: Typical Parameters verified in Method Validation

Sr. No.	Validation Parameters
1	System suitability
2	Specificity
3	Precision
4	Accuracy
5	Linearity
6	Robustness
7	LOD and LOQ

3.1 System suitability: -

System quality take a look at as Associate in nursing integral a part of technique development was accustomed guarantee adequate performance of the chromatographic system. To see system preciseness Azelastine complex common place resolution was ready and injected for 6 times into HPLC system. The mean, American state and a couple of RSD for peak areas of Azelastine was calculated. The system quality was checked by 7 replicate analyses of 100 µg/ml resolution of AZL and chromatographic parameters were evaluated. Consistent with USP system quality tests square measure Associate in nursing integral a part of chromatographic validation. The tests were accustomed verify that the liableness of the chromatographic system is adequate for analysis. To establish its effectiveness system quality tests were allotted on freshly ready common place resolution. 10µL of resolution was injected into the optimized chromatographic system. For system quality 6 replicates of operating common place samples were injected and therefore the parameters like Retention Time (RT), Theoretical Plate (N), Peak area, Tailing issue and determination of sample were calculated these results square measure conferred within the Table No.2. System quality tests square measure supported the construct that the instrumentation, physics, associate in nursing analytical operations and samples to be analyzed represent an

integral system that may be evaluated intrinsically. All calculated parameters were among the suitable limits indicating smart property of the ways and reassuring the system performance. See below Table No. 2

3.2 Specificity: -

The ICH documents outline specificity because the ability to assess without ambiguity the analysts within the presence of parts that will be expected to be gift, like impurities, degradation merchandise and matrix parts. The placebo resolution containing excipients while not Azelastine were injected. Specificity is that the ability to see the analysts of interest within the presence of drug parts which can be expected to be gift. Every of the answer like agent, customary resolution and sample resolution were injected on to HPLC equipped with detector and chromatograms were recorded. The specificity of methodology determined by analyzing the varied solutions like agent, customary resolution of Azelastine, placebo resolution and ophthalmic sample resolution of 100% level.

3.3 Precision: -

Repeatability of the strategy was assessed by analysis of 7 injections of 100 µg/ml AZL resolution. Percentage Relative Standard Deviation (%RSD) for retention time and peak space of drug was calculated. The intraday and interday preciseness for analysis of AZL was performed with 3 concentrations (20, 40, and 60 µg/ml) for 3 times on an equivalent day and for 3 completely different days, severally. The preciseness of associate analytical procedure is that the degree of agreement among individual take a look at results once the procedure is repeatedly applied to multiple samplings of a consistent sample. The preciseness of associate analytical procedure is typically expressed because the variance or relative variance (coefficient of variation) of a series of measurements. Preciseness is also a live of either the degree of reliability or of repeatability of the analytical procedure below traditional operative conditions. The preciseness of the assay methodology was assessed with regard to repeatability and reliability. The preciseness of the projected methodology was checked by ability of responses once replicate injections and expressed as %RSD among responses victimization the formula. Sample of one batch were ready 6-fold and analyzed as per take a look at methodology, diagnostic test of Azelastine for 6 samples calculated for methodology preciseness and instrument preciseness.

3.3.1. Instrument Precision: -

A standard resolution of Azelastine in agent having concentration 50µg/ml was ready and injected in 5 replicates to determine system preciseness. Completely different parameters like Retention Time, Tailing issue, theoretical plates and peak areas of all homolog's peaks were evaluated.

3.3.2. Methodology Precision: -

The preciseness of associate analytical procedure describes the closeness of agreement between a series of measurements obtained from multiple sample preparations of an equivalent consistent sample below the predefined bound conditions. To envision the system preciseness (repeatability) for peak response obtained with 5 replicates of normal at nominative concentration. The %RSD found to be among 2.0%. To envision repeatability (method precision) of the strategy 6 individual sample preparations kind same batch were ready and injected the half RSD with 6 samples found to be among 2.0%. The results obtained were given in Table No.3

3.3.3 Intermediate Precision (Ruggedness): -

Intermediate Precision expresses the within-laboratories variations: completely different days, completely different analysts, completely different equipment's, etc. Satisfactory results were obtained and are given in Table No.4

3.4 Accuracy: -

Accurately measured two cubic centimeter of nasal spray equivalent to 20 mg of AZL was taken in 3 completely different 10 ml volumetric flasks, 10 mg, 20 mg, and 30 mg of AZL customary was value-added to a few completely different volumetric flasks containing nasal spray preparation and diluted up to mark with acetonitrile. Further, they were diluted to own forty µg/ml of sample in every flask. The experiment was performed in triplicate. Share recovery was calculated for every level. The accuracy of associate analytical procedure is that the closeness of take a look at results obtained by that procedure to verity price. The accuracy of associate analytical procedure ought to be established across it's vary. Within the gift study, ordered analysis (n=3) for 3 completely different concentrations of normal mixtures (50, 100 and 150%) was disbursed to see the accuracy of projected methodology. The projected chromatographic methodology was easy, specific, precise, linear, correct and speedy for estimation of total Azelastine pharmaceutical formulation. Freshly developed and valid methodology differs from existing ways with regard to column, mobile section composition, retention time and separation of analysts. The validation study of developed methodology delineate that this methodology was right smart estimation of total Azelastine pharmaceutical formulations. The accuracy of associate analytical methodology is established across it's vary. Accuracy is performed in 3 completely different levels for Azelastine. The familiar amount of Azelastine at 500%, 100 % and 50 %level is analyzed for every level. The half recovery values for this medication were found to be in between 99.68% to 100.26% and %RSD values were found to be but 2.0%. The accuracy results were tabulated within the Table No. 5

3.5 Linearity: -

Accurately measured volumes of the AZL normal answer were in turn transferred into a series of 10ml volumetrically flasks to get final concentrations of 5–120 µg/ml and diluted to the mark with acetonitrile and solutions were mixed properly. 20 µl aliquots of every answer were injected 3 times and were chromatographed. The common peak space of AZL was aforethought against the drug concentration, and also the equation was derived. One-dimensionality was checked on completely different concentrations among 50–200% of the nominal normal concentration. The one-dimensionality of the projected methodology was evaluated by mistreatment standardization curve to calculate the constant of correlation, slope, and intercept values. The one-dimensionality of a way reveals the linear relationship of response against the chosen concentration of analysts. One-dimensionality of methodology was established as statistical regression analysis with least sq. methodology for Azelastine. 5 normal solutions containing Azelastine contains of 80% 90%, 100%, and 110% and 120% of 100 percent target level reminiscent of 40µg/ml to 60µg/ml. The one-dimensionality curve was obtained by plotting concentration of Azelastine normal answer versus the detector response. Curve was established by applied mathematics {least squares method of least squares statistical methodology statistical procedure} method.

The one-dimensionality of detector response to completely different concentration of those medication was studied with a series of operating normal answers ready by diluting the stock solution with diluents. 10 µL of every sample was injected below on top of chromatographically conditions and peak space was measured. The information of one-dimensionality curve was summarized within the Table No.6 and it had been found that parametric statistic (R2) and multivariate analysis were among the boundaries. The one-dimensionality of the projected ways was obtained within the concentration vary (6.25-50.0 µg/mL). The obtained coefficients of regression were 0.9994, severally, whereas the obtained slopes were 89358.23 severally. One-dimensionality results are shown in Table No. 6

3.6 Robustness: -

To determine the robustness of the tactic, the experimental conditions were deliberately modified. In every case, the %RSD values were calculated for the obtained retention time and peak space. The quantity of theoretical plates and tailing issue was compared therewith obtained below the optimized conditions. Robustness is a sign of the liableness of the analytical methodology throughout traditional usage. The impact of the subsequent deliberate changes in chromatographically conditions was monitored. The robustness of associate degree analytical methodology may be a live of its capability to stay unaffected by tiny however deliberate variations in methodology parameters and provides a sign of its robustness throughout traditional usage. Robustness was done by ever-changing the column temperature, rate and also the mobile phase. Robustness of associate degree analytical methodology may be a live of its capability to stay unaffected by tiny variations in methodology parameters. The variables taken under consideration were the share of organic modifier as acetonitrile composition (±5%), hydrogen ion concentration of the mobile phase (± 0.2), rate (±0.1 mL/min) and column action (40°C & 20°C). The low values of the half of RSD, as given in Table No.7 indicate the Robustness of the projected ways.

3.7 LOD and LOQ: -

The LOD and LOQ were calculable mathematically. The mathematical formulas used were as follows:

- LOD = 3.3 (SD of y-intercept/slope of the standardization plot)
- LOQ = 10 (SD of y-intercept/slope of the standardization plot).

These approaches are supported the quality deviation of the blank. Measuring of the magnitude of associate degree atypical background response is performed by analyzing an acceptable variety of blank samples and conniving the quality deviation of those responses. These ways were evaluated on the premise of signal-to-noise between3 or 2:1 is usually thought-about acceptable for estimating the detection limit. A typical signal-to-noise needed for LOQ is 10:1 in keeping with a formula given by miller, the limit of detection (LOD) and limit of quantification (LOQ) were calculated. The resulted are given in Table No.8

4. RESULTS: -

4.1 System suitability: -

The results of system suitability were within acceptable limits as shown in Table No.2

Sr. No.	Parameters	Azelastine (AZH)
1	Retention time (min)	3.1±0.3
2	Theoretical plate	2991±163.48
3	Tailing factor	1.01±0.117
4	Resolution	5.5

Table No. 2

4.2 Precision: -

4.2.1. Method Precision: -

The %RSD has been calculated for peak area response and retention time which were found under the acceptance limit as shown in table No.3

Sr. No	Injections	Azelastine (AZH)
1	1	1370930
2	2	1385998
3	3	1384522
4	4	1382733
5	5	1391190
6	6	1380626
Mean		1382667
SD		6769.1
% RSD		0.49

Table No. 3

4.2.2. Intermediate Precision (Ruggedness): -

The results of Intermediate Precision (Ruggedness) were within acceptable limits as shown in Table No.4

Parameter(%RSD)	Azelastine (AZH)

Intraday	1.685
Interday	0.526
Analyst to Analyst	1.158
Apparatus to Apparatus	1.184

Table No.4

4.3 Accuracy: -

The Accuracy results were tabulated in the Table No. 5

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

Table No.5

4.4 Linearity: -

The results of Linearity were within acceptable limits as shown in Table No.6

Parameters	Azelastine (AZH)
Linear range (µg/mL)	6.25-50
Slope	89358.23
Intercept	-53173.7844
Correlation coefficient	0.9994

Table No. 6

4.5 Robustness: -

The Robustness results were tabulated in the Table No. 7

Parameter (%RSD)	Azelastine (AZH)
Flow rate change (±0.1 mL/min)	2.470
pH changes of mobile phase (±0.2)	1.924
Organic (ACN) Composition change (±0.5%)	2.972
Column temperature change(40,20°C)	2.599

Table No.7

4.6 LOD and LOQ: -

The LOD and LOQ results were tabulated in the Table No. 8

Sr. No	Drugs	LOD (µg/ml)	LOQ (µg/ml)
1	Azelastine	0.46	1.41

Table No. 8

5. ASSAY OF THE PHARMACEUTICAL FORMULATION: -

The planned valid methodology was successfully applied to examine AZL in Pharmaceutical Formulation. The recovery obtained was around 96.14%. In chromatograms of drug sample from pharmaceutical preparation, no interference was resolute from excipients.

6. STABILITY-INDICATING ASPECTS: -

Any vital change in peak area of AZL and to any extent further peak weren't determined once chromatographed once refluxing with 5.0 M HCl, 5.0 M NaOH, and unit of time H_2O_2 for 5 h in separate flasks. No more peak was resolute and no vital change in initial concentration of AZL once solid AZL drug unbroken at 365 nm for 24 h. AZL samples unbroken beneath dry heat

conditions outfitted no more peaks. Peak purity price of AZL was found 1.00 once exposure to forced degradation condition. Hence, AZL is stable beneath acid, alkali, oxidative, dry heat, and 365 nm for 24 h photolytic condition.

7. DISSCUSSION: -

The present study was aimed towards developing a selected, specific, robust, and proper HPLC methodology for the analysis of AZL in bulk drug and in pharmaceutical formulations. The author developed stability indicating HPLC methodology and valid for the determination of AZL in pharmaceutical Formulations. The mobile phase consists of K dehydrogenase phosphate buffer (pH 3.1): acetonitrile (60:40 v/v) was used throughout the analysis. The flow was 1.0 ml/min, the injection volume was 20 µl, column temperature was 45°C, run time 6 min, and detection was performed at 290 nm using a personal digital assistant detector. The retention time of AZL was found to be 3.1±0.3 min. The spatial property was found satisfactory among the vary of 5–120 µg/ml and showed good correlation values 0.9994. Less %RSD values showed good truth of the strategy. The results obtained by the forced degradation studies were enough to say that the drug is stable and methodology is stability indicating one. The comparison of the developed methodology with discovered ways in which shows the developed methodology is straight forward, robust, and economical one. Development of associate in nursing analytical methodology for assessment of medication within the pharmaceutical quantity kind is of just about necessity to substantiate the standard of pharmaceutical formulations with reference to assay and content uniformity. Our developed HPLC analytical methodology for estimation of Azelastine in pharmaceutical formulations has used really a smaller number of organic solvents that's price effective and setting friendly. Also, the strategy was found to be simple and proper and was able to resolve the drug from excipients in an extremely short analytical run time. To optimize the mobile phase varied proportions of buffers with wood spirit and acetonitrile were tested. The numbers of theoretical plates obtained were 2991±163.48 for Azelastine severally that indicates the efficiency of the column. The high share recovery indicates that the planned methodology is very correct. There's no interference of filters with customary and sample solutions as a result of the excellence in responses is at intervals the limit.

8. CONCLUSION: -

The planned HPLC methodology for the assay of AZL in pharmaceutical preparation is straight forward, precise, specific, accurate, and stability indicating. It proves that the strategy is suitable for analysis of AZL among the fabric and additionally the pharmaceutical product whereas not interference from excipients and it shows that AZL is stable drug. An easy reversed-phase HPLC methodology for the analysis of Azelastine compound in pharmaceutical formulations was developed and valid. This technique was valid as per ICH Q2 (R2) so it square measure typically utilized by analytical department. The planned RP-HPLC methodology for the co-occurring estimation of Azelastine compound was administrated. The results of stress testing square measure beneath taken in step with the International Conference on Harmonization (ICH) tips and conspicuous that AZH was found to be stable below heat, light, acidic conditions and labile beneath AL calescent, oxidation. And reaction. Supported the on high of results, the analytical methodology is valid, acceptable use and should be applied for traditional routine analysis and stability study.

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10. CONFLICT OF INTEREST: -

Compliance with moral Standards: The authors declare that they need no conflict of interest.

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