STABILITY-INDICATING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR DETERMINATION OF ANTIHISTAMINE DRUG AZELASTINE

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ABSTRACT

Objective: The objective of this research was to develop a simple, precise, accurate, and stability-indicating reverse-phase high-performance liquid chromatographic method for estimation of azelastine hydrochloride (AZL) in nasal spray preparation.

Methods: Chromatography was performed on a 250 mm×4.6 mm, 5-µm particle size, Waters Spherisorb CN column using (50:50 v/v) mixture of potassium dihydrogen phosphate buffer and acetonitrile as mobile phase. The detection was carried out at 290 nm and flow rate employed was 1.0 ml/min. The degradation of AZL was studied under different ICH recommended stress conditions.

Results: The retention time was 4.34 min for AZL. Linearity was established in the concentration range of 5–120 µg/ml, with a correlation coefficient of 0.9996. Limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.81 µg/ml and 2.44 µg/ml, respectively. Percentage recovery was found between 99 and 102%. The values of percentage relative standard deviation (<2%) proved the high precision of the proposed method. The method was found to be robust regarding any small variation in the column temperature, pH of mobile phase, and mobile phase ratio. AZL was found stable in 5 M HCl at 80°C for 5 h, 5 M NaOH at 80°C for 5 h, 30% H_2O_2 at 80°C for 5 h, and in oven at 70°C for 8 h.

Conclusion: The results obtained in this research work clearly proved that the proposed HPLC method for the assay of AZL in nasal spray preparation is simple, precise, specific, accurate, and stability indicating. It indicates that the method is suitable for analysis of AZL in the raw material and the pharmaceutical product without interference from excipients.

Keywords: Azelastine hydrochloride, High-performance liquid chromatography, Degradation study, Stability-indicating method.

INTRODUCTION

Allergic reaction produces inflammation, and people, who suffer from nasal allergies such as hay fever, dust, mite, and pet allergies, tend to experience a variety of symptoms due to this inflammation. Inflammation of the nasal passage forces fluid out of the nasal tissues, resulting in a runny and blocked nose. The three main types of drugs available for anti-inflammatory and antiallergic effect are corticosteroids, antihistamines, and decongestants [1].

Azelastine hydrochloride (AZL), which is a second-generation H_1 receptor antagonist, chemically, it is known as 4-[(4-Chlorophenyl) methyl]-2-(hexahydro-1-methyl-1H-azepin-4-yl)-1(2H)-phthalazinone, hydrochloride. The chemical structure of AZL is shown in Fig. 1. AZL occurs as a white, almost odorless, crystalline powder with a bitter taste. It has a molecular weight of 418.37. It also inhibits lipoxygenase and platelet aggregation. It is bronchodilator agent also. It is indicated as antiallergic agent [2,3].

AZL is an official drug in British Pharmacopoeia [2], which recommends a titrimetric method for the determination of AZL in pure form using 0.1 M perchloric acid as a titrant. Various methods such as liquid chromatography (LC) [4], LC-electrospray ionization tandem mass spectrometry [5], high-performance LC (HPLC) [6,7], capillary HPLC [8], and electrokinetic capillary HPLC [8] had been reported for the determination of azelastine and its metabolite in biofluids. Visible spectrophotometric method [9], HP thin-layer chromatography (HPTLC) [10], reversed-phase HPLC (RP-HPLC) [11], and Fourier transform infrared [12] have been developed for estimation of AZL in formulation. RP-HPLC [13] method has been reported for simultaneous estimation of AZL and fluticasone propionate in formulation. Salama *et al.* [14] have established stability-indicating densitometry TLC method for quantitative determination of azelastine HCl in the presence of their acid and oxidative degradants produced by refluxing drug in 5 M HCl for 36 h and 30% H_2O_2 for 30 h, respectively. In the study performed by Rao *et al.* [13], the forced degradation was done by refluxing drug into individual flasks at 60° C for ½ h with 0.1 N HCl, 0.1 N NaOH, 1.0% H_2O_2, and water. None of the methods satisfy the stability protocol as per regulatory guidance. An ideal stability-indicating method is one which quantifies the drug and resolves it from its degradation products. These facts initiate the present study to establish an accurate, specific, repeatable, and stability-indicating HPLC method for analysis of AZL in the presence of its degradation products. The suggested method was designed to be suitable for routine analysis of the drug in pharmaceutical formulations. The proposed method was validated in accordance with ICH guidelines [15,16].
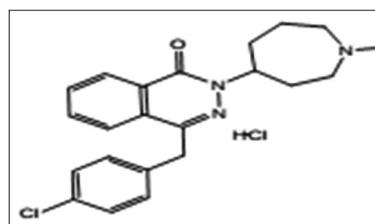

Fig. 1: Chemical structure of azelastine hydrochloride

METHODS

Instrumentation and chromatographic condition

The quantitative analysis was performed on Agilent HPLC 1260 equipped with quaternary solvent manager, autosampler, and photodiode array (PDA) detector integrated with Ez chrome software. Separation was achieved on Waters Spherisorb CN (250×4.6 mm, 5 µm) column. The standards and samples were separated using mobile phase consisting of acetonitrile:0.05 M potassium dihydrogen phosphate buffer pH 3.0 (50:50 %v/v). The flow rate was 1.0 ml/min and detection was set at 290 nm. The column temperature was set at 45°C, and the injection volume was 20 µl.

Materials and reagents

AZL (certified purity of 99.8%, batch no. 0301425316) was kindly gifted by Cadila Health Care Limited, Moraiya (Gujarat). Arzep nasal solution was purchased from local pharmacy. Acetonitrile and water (HPLC grade) were purchased from Merck, India. Potassium dihydrogen phosphate, O-phosphoric acid (OPA), sodium hydroxide, hydrochloric acid, and hydrogen peroxide used were of analytical grade and purchased from S.D. Fine Chemicals, India.

Standard stock solution preparation

A standard solution of AZL containing 1000 µg/ml was prepared in acetonitrile and diluted as appropriate with the same solvent to obtain the working concentration range. Solutions were found to be stable for at least 5 days when kept at room temperature.

Buffer preparation (pH 3.0)

About 6.805 g of potassium dihydrogen phosphate was dissolved in 600 ml HPLC water and then final volume was made up to 1000 ml with HPLC water. Buffer pH was adjusted to 3.0 with ortho-OPA.

Validation of the method

The method was validated for system suitability, specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and robustness as per the ICH guideline.

System suitability

The system suitability was checked by seven replicate analyses of 100 µg/ml solution of AZL and chromatographic parameters were evaluated.

Linearity

Accurately measured volumes of the AZL standard solution were successively transferred into a series of 10 ml volumetric flasks to obtain final concentrations of 5–120 µg/ml and diluted to the mark with acetonitrile and solutions were mixed properly. 20 µl aliquots of each solution were injected 3 times and were chromatographed. The average peak area of AZL was plotted against the drug concentration, and the regression equation was derived.

Precision

Repeatability of the method was assessed by analysis of seven injections of 100 µg/ml AZL solution. Percentage relative standard deviation (%RSD) for retention time and peak area of drug was calculated. The intraday and interday precision for analysis of AZL was performed with three concentrations (20, 40, and 60 µg/ml) for 3 times on the same day and for three different days, respectively.

Specificity - forced degradation studies

The forced degradation study was executed to find whether the analytical method was stability indicating and could determine the AZL in the presence of impurities and degradation products. AZL standard solution was stressed under acid, base, oxidative, thermal, and photolytic stress conditions. 2 ml of the standard solution of AZL (1000 µg/ml) was transferred to three 20 ml volumetric flask followed by 2 ml of 5.0 M HCl, 2 ml of 5.0 M NaOH, and 2 ml of 30% H_2O_2 to different flask and then heated in a thermostatic water bath at 80°C

for 5 h to induce degradation by acid, base, and oxidation, respectively. Then, the mixtures were diluted with acetonitrile up to the mark. 50 mg AZL powder was placed in convection oven and exposed to heat at 70°C for 8h and further preceded as per sample preparation for thermal degradation. To check photolytic degradation, AZL drug (50 mg) was taken in two Petri dishes. One Petri dish was wrapped with aluminum foil and other was not wrapped. Both the Petri dishes were kept in ultraviolet chamber (365 nm) for 24 h at room temperature and further preceded as per sample preparation. The photostability of a drug substance depends on the wavelength, time, and intensity of exposure [17]. All stressed samples were analyzed by proposed method.

Accuracy

Accurately measured 2 ml of nasal spray equivalent to 20 mg of AZL was taken in three different 10 ml volumetric flasks. 10 mg, 20 mg, and 30 mg of AZL standard was added to three different volumetric flasks containing nasal spray preparation and diluted up to mark with acetonitrile. Further, they were diluted to have 40 µg/ml of sample in each flask. The experiment was performed in triplicate. Percentage recovery was calculated for each level.

Robustness

To determine the robustness of the method, the experimental conditions were deliberately changed. The flow rate of the mobile phase (1.0±0.1ml/min), pH of mobile phase (3.0±0.2), mobile phase composition (±2%), and column temperature (45±1°C) was varied. In each case, the %RSD values were calculated for the obtained retention time and peak area. The number of theoretical plates and tailing factor was compared with that obtained under the optimized conditions.

LOD and LOQ

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

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

Analysis of the marketed formulation

To determine the AZL content of a nasal spray (label claim 1%w/v, 10 ml), solution of the container was taken out in 100 ml volumetric flask and mixed with acetonitrile (10 ml) and sonicated for 20 min, then it was diluted up to the mark with acetonitrile which made 1 mg/ml AZL concentration. 1 ml of this solution was transferred to a 10 ml volumetric flask and diluted to volume with acetonitrile which made 100 µg/ml. The analysis was repeated 3 times. The possibility of interference of excipients with the analysis was studied.

RESULTS

Development of method

To develop stability-indicating HPLC method for antihistamine drug AZL, mobile phases of various compositions were investigated and system suitability test was performed for method optimization. The mobile phase consisting of acetonitrile:0.05 M potassium dihydrogen phosphate buffer pH 3.0 (50:50 %v/v) was found to furnish sharp and well-defined peak with very good symmetry (1.43), theoretical plates (>10,000), and low retention time (Fig. 2). Other tried mobile phases gave too broad peak or asymmetric peak, so were not considered. A wavelength maximum of the AZL is 215 nm but to avoid interference from solvent 290 nm was chosen for detection of eluents. The pH of mobile phase was studied from 3.0 to 7.0. At higher pH, AZL peak lost the sharpness and precision of the retention time. Finally, the mobile phase of pH 3.0 was chosen as optimum. The ratio of acetonitrile and buffer was also studied. The mobile phase consisting of 50% acetonitrile was found optimum. The effect of flow rate was also optimized and 1.0 ml/ min was considered optimum for the separation method. The column temperature was set at 45°C.

Validation of method

The proposed method was validated according to the ICH guideline to prove its suitability for the intended purpose. Method validation data are summarized in Table 1.

Linearity

Peak area and concentration were subjected to linear regression analysis to calculate the calibration equation and correlation coefficient (Fig. 3). The regression data revealed a good linear relationship over the concentration range of 5–120 μg/ml.

Precision

The precision of an analytical method gives information on random error. The precision of the method was evaluated by repeatability, interday and intraday precision. The values of %RSD for all found <2% proved the high precision of the proposed method.
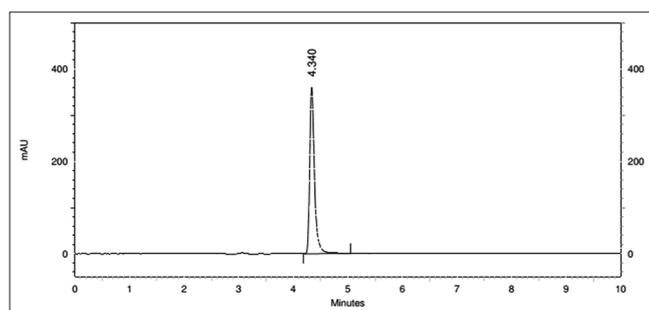

Fig. 2: Chromatogram of AZL standard (100 μg/ml)
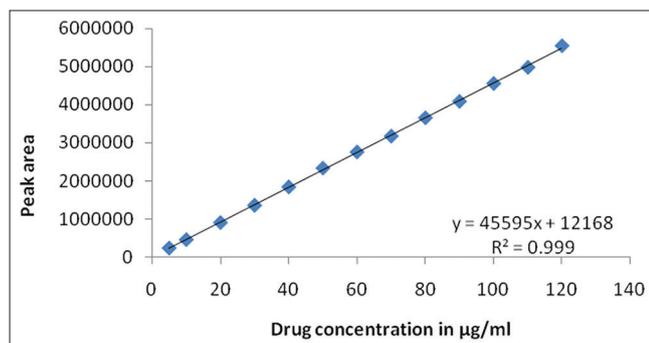
Fig. 3: Linearity curve

Table 1: Validation data

Data	Value
Linear range	5–120 μg/ml
Correlation coefficient ($r^2 \pm SD^a$)	0.9996±0.0002
LOD	0.81 μg/ml
LOQ	2.44 μg/ml
Accuracy	100.05±0.95
Precision (%RSD[b])	
Repeatability (n=7)	1.381
Interday (n=3)	0.800
Intraday (n=3)	0.372
Robustness	Robust in column temperature, pH of mobile phase (3.0±0.1), and mobile phase composition
Specificity	Specific

Note: [a] = standard deviation, [b] = relative standard deviation. LOD: Limit of detection, LOQ: Limit of quantitation, %RSD: Percentage relative standard deviation

Accuracy

When the method was used for subsequent analysis of AZL from the nasal spray solution after spiking of AZL standard at three different levels for 3 times, the recovery was found between 99 and 102% (Table 2). The percentage recovery was calculated from the amount recovered and actual amount added. Amount recovered was calculated from comparison of area before and after spiking the standard drug.

LOD and LOQ

LOD and LOQ were found to be 0.81 μg/ml and 2.44 μg/ml, respectively, from calculation.

Robustness

The method was found to be robust regarding any small variation in the column temperature (45±1°C), pH of mobile phase (3.0±0.2), and mobile phase composition (±2%) as revealed by the constancy of the retention time and the peak area. In case of flow rate of mobile phase (1±0.1 ml/min), where minor variation resulted in a significant change in the retention and peak area of the AZL.

Stability-indicating aspects

Any significant change in peak area of AZL and any additional peak were not observed when chromatographed after refluxing with 5.0 M HCl, 5.0 M NaOH, and 30% H_2O_2 for 5 h in separate flasks. No additional peak was observed and no significant change in initial concentration of AZL when solid AZL drug kept at 365 nm for 24 h. AZL samples kept under dry heat conditions furnished no additional peaks. Peak purity value of AZL was found 1.00 after exposure to forced degradation condition. Hence, AZL is stable under acid, alkali, oxidative, dry heat, and 365 nm for 24 h photolytic condition.

Assay of the pharmaceutical formulation

The proposed validated method was successfully applied to determine AZL in Arzep nasal spray solution. The recovery obtained was around 96.14%. In chromatograms of drug sample from nasal spray preparation, no interference was observed from excipients.

DISCUSSION

The present study was aimed at developing a precise, specific, robust, and accurate HPLC method for the analysis of AZL in bulk drug and in pharmaceutical dosage form. The author developed stability-indicating HPLC method and validated for the determination of AZL in bulk and nasal spray solution using Waters Spherisorb CN column (250 mm×4.6 mm, 5 μm). The mobile phase consists of potassium dihydrogen phosphate buffer (pH 3.0):acetonitrile (40:50 v/v) was used throughout the analysis. The flow rate 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 PDA detector. The retention time of AZL was found to be 4.34 min. The linearity was found satisfactory in the range of 5–120 μg/ml and showed good correlation coefficient values 0.9996. Less %RSD values showed good precision of the method. The results obtained by the forced degradation studies were enough to say that the drug is stable and method is stability indicating one. The comparison of the developed method with published methods shows the developed method is simple, robust, and economical one.

Table 2: Results of accuracy for AZL

% Addition	μg/ml added	μg/ml recovered	% recovery±SD (n=3)
50	20	19.91	99.54±1.29
100	40	40.46	101.14±0.70
150	60	59.67	99.46±0.72

AZL: Azelastine hydrochloride

CONCLUSION

The proposed HPLC method for the assay of AZL in nasal spray preparation is simple, precise, specific, accurate, and stability indicating. It proves that the method is suitable for analysis of AZL in the raw material and the pharmaceutical product without interference from excipients. A stress degradation study was conducted to investigate the degradation behavior of AZL under ICH recommended condition; it shows that AZL is stable drug.

ACKNOWLEDGMENT

AUTHOR'S CONTRIBUTION

Shital Patel has performed the work presented here. Dr. T. Y. Pasha has guided this project.

CONFLICTS OF INTEREST

All authors have none to declare.

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