



RP-HPLC DETERMINATION OF AZELASTINE IN PURE AND IN OPHTHALMIC FORMULATION

Boovizhikannan Thangabalan^{*1,2}, Palanirajan Vijayaraj kumar³

¹Department of Pharmaceutical Analysis, Southern institute of medical sciences, College of Pharmacy, Guntur, Andhra Pradesh, India.

²Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, Andhra Pradesh, India.

³Faculty of Pharmaceutical sciences, School of pharmacy, UCSI (University college of Sadaya International) University, Malaysia.

*Corresponding author's E-mail: bthangabalan@gmail.com

Accepted on: 19-10-2012; Finalized on: 30-11-2012.

ABSTRACT

A simple, rapid and reproducible reverse phase high performance liquid chromatographic method has been developed for the estimation of azelastine in pure form and in its ophthalmic dosage form using Kromosil C18, 150 x 4.6 mm, 5 μ m column. The mobile phase consists of phosphate buffer (pH 3) and acetonitrile in the ratio of 50:50 % v/v, and was pumped at rate of 1.0 mL/min at ambient temperature. The detection was carried out at 288 nm and the calibration curve was linear in the concentration range of 8 to 120 μ g/mL. The method was statistically validated for linearity, precision, accuracy, specificity, LOD and LOQ. Due to its simplicity, rapidness, good precision and accuracy, the proposed RP-HPLC method may be used for determining azelastine in pure form and in ophthalmic drops.

Keywords: Azelastine, RP-HPLC, validation, ophthalmic drops.

INTRODUCTION

Azelastine-HCl is 4-(4-chlorobenzyl)-2-[(4RS)-1-methylhexahydro-1H-azepin-4-yl] phthalazin-1(2H)-one hydrochloride¹. It is an intranasal antihistamine indicated as an appropriate medical treatment for patients suffering from the seasonal allergic rhinitis (SAR) and non-allergic vasomotor rhinitis (VMR). Reportedly, this medicament is also used topically in the symptomatic relief of both acute and chronic allergic conditions, including rhinitis and conjunctivitis^{2,3}. Literature survey reveals that azelastine can be estimated in human plasma by liquid chromatography coupled to tandem mass spectrometry method⁴. The present study aims to develop a simple, sensitive and accurate RP-HPLC method for the estimation of azelastine in pure form and ophthalmic dosage form.

MATERIALS AND METHODS

Instrumentation

Waters 2695 HPLC system equipped with Kromosil C18, 150 x 4.6 mm, 5 μ m column, Rheodyne injector with 20 μ L loop, 2996 PDA detector and Empower-2 software was used.

Reagents and chemicals

Potassium dihydrogen orthophosphate of analytical grade, HPLC grade milli-Q water and acetonitrile were used. Azelastine was a gift sample from Dr. Reddy's Laboratories Ltd. Hyderabad, India. The eye drops of azelastine were obtained from local pharmacy.

Preparation of buffer solution

Phosphate buffer was prepared by dissolving 6.8 gm of potassium dihydrogen orthophosphate in 1000 mL of

HPLC grade water and pH was adjusted to 3 with orthophosphoric acid solution.

Chromatographic condition

Azelastine was eluted in Kromosil C18, 150 x 4.6 mm, 5 μ m column using a mobile phase mixture of phosphate buffer and acetonitrile in the ratio of 50:50 % v/v at ambient temperature. The lambda max of the drug in mobile phase was 288 nm, so column outlet was monitored at 288 nm. The injection volume was 20 μ L. The total runtime was 8 min.

Procedure for standard solution preparation

100 mg of pure azelastine was weighed accurately and transferred in to a 100 mL volumetric flask. The content was dissolved by using HPLC grade water, after complete dissolution the volume was made up to the mark by using the same which gives 1000 μ g/mL of the drug.

Construction of calibration curve

The standard azelastine solution was further diluted in 10 mL volumetric flask to get various concentrations ranging from 8 to 120 μ g/mL of drug using mobile phase. From this each standard solutions 20 μ L was injected in to the HPLC system. The chromatograms were recorded. The concentrations of the azelastine in μ g/mL is taken in X axis and peak area of the individual concentrations of standard solution were taken in Y axis. The calibration graph was plotted. This is used for the estimation of azelastine in eye drops.

Estimation of Azelastine

2 mL of the test sample was transferred to a 10 mL volumetric flask containing mobile phase and mixed to dissolve and made up to the volume with the same. 4 mL



of the above solution was further diluted using the mobile phase to get the final concentration of working sample equivalent to 40 µg/mL of azelastine. The sample solution was injected in to the HPLC system and chromatogram was compared with area produced by standard and quantification of drug was carried out.

RESULTS AND DISCUSSION

Azelastine pure drug is soluble in water and acetonitrile. Different mobile phase compositions were tried to elute the drug from the column and adequate resolution is achieved with phosphate buffer (pH 3) and acetonitrile in the ratio of 50:50 % v/v at ambient temperature with Kromosil C18, 150 x 4.6 mm, 5 µm column and this solvent system was found to be most suitable for method development and validation with sufficient stability of drug in solution form. Azelastine shows the maximum absorbance at 288 nm in mobile phase, so the column outlet was detected at 288 nm in this method. A typical chromatogram of azelastine is shown in Fig.1. Chromatogram for mobile phase (blank) is shown in Fig. 2.

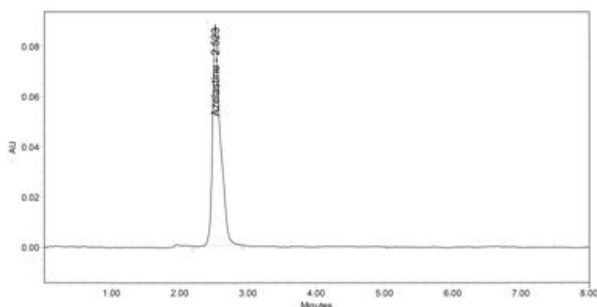


Figure 1: A typical chromatogram of Azelastine.

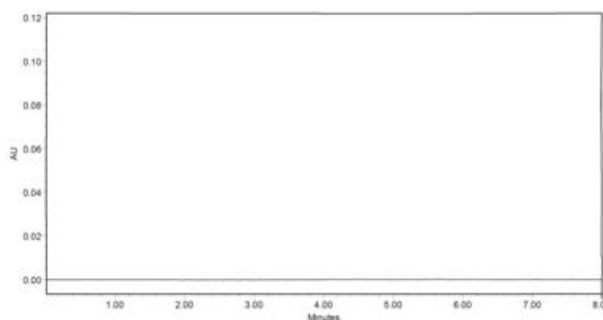


Figure 2: Chromatogram of blank

The retention time was 2.5 min. The system suitability tests were carried out on freshly prepared standard stock solution and summary is given in table 1. These parameters indicate good sensitivity of the developed method. In the present HPLC method, the standard and sample preparation involves simple and rapid extraction procedure and requires less time. A good linear relationship was obtained in the concentration range of 8-120 µg/mL. Linear regression analysis report is given in table 2. The proposed method was used to estimate the amount of azelastine in eye drops, assay results are in table 3.

The accuracy of the developed method was carried out by adding the known amount of azelastine pure drug to the pre analyzed samples and subjected to the proposed method. Results of recovery study are shown in Table 3. The study was done at 50, 100 and 150 % of 40 µg/mL concentration levels.

Table 1: System suitability study

Drug	USP Tailing	USP Plate Count	Retention time (min), (n=6)		Peak area, (n=6)	
			Mean± S.D	%CV	Mean± S.D	%CV
Azelastine 40 µg/mL	1.41	2048	2.57±0.0447	1.741	703210±1358	0.1930

Table 3: Assay and recovery results

Formulation	Labeled amount (mg/ 5 mL)	(%) label claim* ± S.D	% Recovery
Azelastine Eye Drop	2.5	100.11 ± 0.1503	99.67-100.61

* Average of six determinations.

Table 4: Precision Study

Repeatability (% RSD) (n=6)	Intermediate precision (% RSD) (n=6)			
	Day 1		Day 2	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2
0.1500	0.1957	0.2734	0.3597	0.5204

Table 2: Linear regression data for calibration curve

Parameters	Values
Concentration range, µg/mL	8- 120
Slope	18155
Intercept	-42524
Correlation coefficient	0.999

Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability refers to the use of the analytical procedure

within a laboratory over a short period of time that was evaluated by assaying six sample solutions, at the final concentration corresponding to 40 µg/mL of azelastine during the same day. Intermediate precision was assessed by comparing the assays on two different days and analysts (table 4). The azelastine concentrations were determined and the relative standard deviations (% RSD) were calculated. The limit of detection (LOD) and limit of quantification (LOQ) for azelastine was found to be 0.0767 and 0.2325 µg/mL, respectively.



CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of azelastine from pure form and eye drop dosage form. The mobile phase used in this method was simple to prepare and the run time was 8 min, so less time consuming method. The recovery study shows that there was no interference of additives used for the preparation of formulation. Hence, the method can be easily and conveniently applied for routine quality control of azelastine in its dosage form.

Acknowledgement: The authors express their sincere thanks to Spectrum Pharma Research Solutions, Hyderabad and the management, SIMS College of pharmacy, Guntur for providing the necessary facilities to carry out the research work.

REFERENCES

1. British Pharmacopoeia, Her Majesty's Stationary Office, London, 2010, 199.
2. S.C. Sweetman SC, Martindale: The Complete Drug Reference, 36th Ed., The Pharmaceutical Press, London, 2007, 568.
3. Horbal JM, Bernstein JA, Azelastine HCl: A Review of the Old and New Formulations, Clinical Medicine Insights: Therapeutics, 2, 2010, 427.
4. Park YS, Kim SH, Kim YJ, Yang SC, LeeMH, Shaw LM, KangJS, Determination of Azelastine in Human Plasma by Validated Liquid Chromatography Coupled to Tandem Mass Spectrometry (LC-ESI/MS/MS) for the Clinical Studies, International journal of Biomedical science, 6, 2010, 120.

