Liquid Chromatographic Method for the Analysis of Brimonidine in Ophthalmic Formulations

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Abstract: A reverse phase LC method was developed for the determination of Brimonidine in eye drops. Chromatography was carried on an Inertsil ODS 3V column (C18) using a mixture of Octane 1- sulfonic acid sodium salt (0.02M) (pH 3.5 $\pm$ 0.05) and acetonitrile (64:36 v/v) as mobile phase at a flow rate of 1 mL/min with UV detection at 254 nm. The drug was eluted at 4.636 min. The detector response was linear in the concentration range of 0.4-72 μ g/mL. The limit of detection and limit of quantification were found to be 0.0561 and 0.1848 μ g/mL respectively. The proposed method was validated as per the ICH guidelines and can be applied for the routine analysis of Brimonidine in eye drops.

Keywords: Brimonidine, RP-HPLC, ICH and Eye drops.

Introduction

Brimonidine¹(BMD) (Figure 1) is an alpha adrenergic receptor agonist. It has a peak ocular hypotensive effect occurring at two hours post-dosing. Fluorophotometric studies in animals and humans suggest that Brimonidine has a dual mechanism of action by reducing aqueous humor production and increasing uveoscleral outflow. Brimonidine, chemically 5-Bromo-N-(4,5-dihydro-1H-imidazol-2-yl) quinoxalin-6-amine has a molecular formula C₁₁H₁₀BrN₅ and molecular weight 292.135 g/mol. Peripheral alpha 2 agonist activity results in vasoconstriction of blood vessels. This vasoconstriction may explain the acute reduction in aqueous humor flow. The increased uveoscleral outflow from prolonged use may be explained by increased prostaglandin release due to alpha adrenergic stimulation². This may lead to relaxed ciliary muscle and increased uveoscleral outflow.

Figure 1. Chemical Structure of Brimonidine (BMD).

Literature survey reveals that very few chromatographic³⁻⁶, LC-MS⁷⁻⁸ Spectrophotometric⁹ and HPTLC¹⁰ methods were available for the determination of Brimonidine. In the present study an isocratic RP-HPLC method has been developed and validated for the determination of Brimonidine in ophthalmic solutions as per the ICH guidelines.

Experimental

Brimonidine was obtained as a gift sample from Cipla Ltd (India). ALPHAGAN (2 mg/mL, 5 ml) and BRIMOPRESS eye drops (0.2%, 5 mL) were purchased from the local market. HPLC grade Acetonitrile (Merck), Octane-1 sulfonic acid sodium salt (Merck), *o*-phosphoric acid (Qualigens) and Milli-Q water were used during the study.

Instrumentation

The HPLC system consist of a Waters Alliance (Waters Corporation, MA, USA) equipped with a Waters 2695 separations module in a quaternary gradient mode and a Waters 2695 PDA detector. Data acquisition was performed by the Empower pro software operated on a Pentium® IV microprocessor. Analysis was carried out at 254 nm with a reversed phase Inertsil ODS 3V, (250 x 4.0 mm, 5 µm) at ambient temperature.

Chromatographic Conditions

Chromatographic separations were achieved using a Inertsil ODS 3V C18 (250 X 4.6 mm, 5µ) analytical column. The mobile phase consisted of (0.02M) Octane 1-sulfonic acid buffer : acetonitrile (64:36 v/v) and pH adjusted to (3.5 ± 0.05) with ortho phosphoric acid that was set at a flow rate of 1.0 mL/min. The mobile phase was passed through 0.45 µm membrane filter and degassed by ultra sonication before pumping into HPLC system .The flow rate was maintained at 1 mL/min and the measurements were made at 254 nm. The column and the HPLC system were kept in ambient temperature.

Preparation of Standard Stock solution: Accurately weighed 50 mg of Brimonidine standard was taken in 50 mL volumetric flask. This was dissolved in 25 mL of Acetonitrile and sonicated for 10 mins, and then diluted to 50 mL to get 1mg/mL standard stock solution. From the stock solution 5 mL was taken in 50 ml volumetric flask and thereafter made upto 50 mL with mobile phase to get a concentration of 100µg/mL.

Validation

Linearity

Several aliquots of standard stock solutions ranging from 0.4-72 µg/mL of Brimonidine were prepared in different 10 mL volumetric flasks by diluting with mobile phase. 20 µL of solutions were injected in to the HPLC system and was monitored with PDA detector at 254 nm. A representative chromatogram was shown in Figure 2.

Figure 2. Typical Chromatogram of Brimonidine by HPLC (20 µg/mL).

The peak area of the above solutions was recorded and a Calibration graph was plotted by taking the concentration on the x-axis and the corresponding peak area of Brimonidine on the y-axis. A straight line was obtained passing through the origin (Figure 3).

Figure 3. Calibration curve of Brimonidine by HPLC.

Assay of Commercial Formulations:

ALPHAGAN (2mg/ml, 5 ml) and BRIMOPRESS eye drops (0.2%, 5 ml) were purchased from the local market and required sample solutions were made using the mobile phase and 10 µL of these formulation solutions were injected into the HPLC system and the peak areas were recorded. The results are given in Table 1.

Table 1. Analysis of commercial formulations (Eye Drops).

Commercial Formulation	Labeled amount (%)	Amount found (%)	% Recovery
Brand I	0.2	0.1989	99.48 $\pm$ 0.25
Brand II	0.2	0.1997	99.85 $\pm$ 0.16

Precision Study:

The precision of the method was determined at three levels (10, 20 and 50 $\mu\text{g/ml}$) and each concentration was injected thrice (intra-day precision) on the same day and the mean, standard deviation and relative standard deviation were calculated. The inter-day precision was calculated by injecting each of the concentration (10, 20 and 50 $\mu\text{g/ml}$) thrice on three consecutive days and there by the mean, standard deviation and relative standard deviation were calculated (Table 2).

Table 2. Precision Study.

S No.	Conc. ($\mu\text{g/ml}$)	Intra-day precision		Inter-day precision	
		Mean* $\pm$ SD	%RSD	Mean* $\pm$ SD	%RSD
1	20	612778.33 $\pm$ 2637.639	0.431	611941.25 $\pm$ 2411.05	0.394
2	40	1226154.67 $\pm$ 4267.821	0.348	1223216.85 $\pm$ 3449.47	0.282
3	60	1839312 $\pm$ 16258.741	0.884	1849224.56 $\pm$ 18011.45	0.974
*Mean of three replicates.					

Accuracy Study:

Accuracy study was determined by the addition of a known amount of solution to the pre-analysed sample and the resultant samples were injected in to the HPLC system. Results of recovery study are shown in Table 3.

Table 3. Accuracy Study.

Amount (%) of drug added to analyte	Theoretical content ($\mu\text{g/mL}$)	Mean Conc. found ($\mu\text{g/mL}$) $\pm$ SD	% Recovery	% RSD
80	32	32.18 $\pm$ 0.0458	100.56	0.142
100	40	39.63 $\pm$ 0.10	99.13	0.252
120	48	47.89 $\pm$ 0.261	99.77	0.545
*Mean of three replicates.				

Results and Discussion

Brimonidine was eluted on an Inertsil ODS 3V column (C18) using mobile phase Octane 1-sulfonic acid sodium salt (0.02M) (pH 3.5 ± 0.05) and acetonitrile (64:36 v/v) at a flow rate of 1 mL/min with UV detection at 254 nm. Brimonidine was eluted at 4.636 min. The linearity was observed in the concentration range of 0.4-72 $\mu\text{g/mL}$ with a regression equation $30719x-362.1$ and correlation coefficient 0.999. The LOD and LOQ were found to be 0.0561 and 0.1848 $\mu\text{g/mL}$ respectively.

The system suitability test was performed to ensure that the complete testing system was suitable for the intended application. The theoretical plates were found to be 6939 and asymmetry 1.05. The method is precise (Interday 0.282-0.974) and Intra-day 0.348-0.884) and accurate (0.142-0.545) as the RSD is less than 2 %. The percentage of purity of Brimonidine in commercial formulations was found to be 99.48 and 99.85.

Conclusion

The proposed liquid chromatographic method is simple, rapid, reproducible and economical for the analysis of Brimonidine in pharmaceutical formulations and can be applied successfully for the quantitative analysis of Brimonidine in pharmaceutical formulations.

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