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Simultaneous RP HPLC determination of Latanoprost and Timolol Maleate in combined pharmaceutical dosage form

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ABSTRACT

A simple, fast and precise reverse phase high performance liquid chromatography method is described for simultaneous determination of latanoprost and timolol maleate using methyl paraben as an internal standard. Chromatographic separation of these two drugs was achieved on Waters symmetry C₁₈ column (250 mm x 4.6 mm, 5 μm) as stationary phase with a mobile phase comprising of 0.05% (v/v) trifluoro acetic acid in water : 0.05% (v/v) trifluoroacetic acid in acetonitrile (40:60 v/v) at a flow rate of 1ml / min and UV detection at 210 nm. The retention time of timolol, maleic acid, methyl paraben and latanoprost were 1.967 min, 2.342 min, 3.275 min and 7.633 min respectively. The proposed method was validated for system suitability, linearity, accuracy, precision, LOD, LOQ and solution stability. Linearity, accuracy and precision were found to be acceptable over the ranges of 2.5 – 7.5 μg/ml for latanoprost and 250 – 750 μg/ml for timolol maleate. It can be conveniently adopted for routine quality control analysis.

Keywords: Latanoprost, Timolol maleate, RP-HPLC, Pharmaceutical preparation.

INTRODUCTION

Latanoprost is chemically propan-2-yl 7-[3,5-dihydroxy-2-(3-hydroxy-5-phenyl-pentyl)-cyclopentyl] hept-5-enoate, is used in the treatment of glaucoma. Timolol maleate is chemically described as (-)-1-(tert-butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-2-propanol maleate (1:1). It is used in the treatment of elevated intraocular pressure in patients with ocular hypertension or open-angle glaucoma. Pharmaceutical preparation contains 0.005% (v/v) of latanoprost and 0.5% (v/v) of timolol maleate. The latanoprost is not official in U.S.P [1], B.P

[2], I.P [3] and E.P [4] but timolol maleate is official in U.S.P [1], I.P [3] and E.P [4]. The literature survey revealed that no pharmacopoeial method was available for simultaneous determination of these two drugs in pharmaceutical preparations by HPLC. Hence it is a novel HPLC [5] method for simultaneous determination of latanoprost and timolol maleate from their pharmaceutical dosage forms. The method described is simple, fast, precise and accurate for simultaneous determination of latanoprost and timolol maleate from pharmaceutical preparation.

EXPERIMENTAL SECTION

Materials

Reference Standards of timolol maleate and latanoprost were obtained with certificate of analysis. Trifluoroacetic acid and acetonitrile were used of analytical grade from SRL and Qualigens respectively. HPLC grade water was obtained using Millipore system. Sample and standard solutions were prepared in diluent 50:50 (v/v) acetonitrile : water.

Instrumentation

Chromatographic separation was performed using Shimadzu LC 2010 high performance liquid chromatography system in isocratic mode, equipped with auto sampler and a photo-diode array detector. Chromatograms and data were recorded by means of Class VP software.

Preparation of Standard Stock Solutions

The stock solution of latanoprost (50 µg/ml) was prepared by dissolving 5.02 mg of latanoprost (99.9 %) in diluent in a standard 100 ml volumetric flask (solution A). The stock solution of timolol maleate (5000 µg /ml) was prepared by dissolving 50.04 mg of timolol maleate (99.6 %) in diluent in a standard 10 ml volumetric flask (solution B). Internal standard (methyl paraben) stock solution (500 µg / ml) was prepared by dissolving 49.08 mg of methyl paraben in methanol in a 100 ml standard volumetric flask (solution C).

Working Standard Solution

About 1.0 ml of each stock solution A, B and C was transferred to a 10 ml volumetric flask and diluted up to the mark with diluent.

Sample Preparation

About 5.0 ml of sample solution is transferred in a 100 ml volumetric flask with 30 ml of diluent and sonicated for 2-3 minutes. The 10.0 ml of methyl paraben (500 µg /ml) was added to stock solution as internal standard and diluted up to the mark with diluent.

Chromatographic conditions

The criteria employed for selecting the mobile phase for the analysis of the drugs were cost involved, time required for the analysis and better separation of drugs. Chromatographic separation was performed on a reverse phase Waters symmetry C₁₈ (250 mm x 4.6 mm, 5 µm particle) column. The mobile phase consisted of 0.05% (v/v) trifluoro acetic acid in water : 0.05% (v/v) trifluoroacetic acid in acetonitrile 40:60 (v/v). The flow rate was set at 1ml /min. About 20 µl of standard and sample solutions were injected and detection wavelength was set at 210 nm (fig no.1) for simultaneous determination of latanoprost and timolol maleate.

Method Development

Different columns containing octyl and octadecyl silane stationary phase were tried for separation and resolution. Waters symmetry C₁₈ column was found satisfactory over the other columns. The UV spectrum of latanoprost and timolol maleate was scanned on photo diode array detector for selecting the optimum wavelength. Peak purity of latanoprost and timolol maleate was checked using photo diode array detector and 210 nm was considered satisfactory for detecting both the drugs with adequate sensitivity. A typical HPLC chromatogram for simultaneous determination of latanoprost and timolol maleate from pharmaceutical formulation is shown in fig 3.

RESULTS AND DISCUSSION**Method Validation**

The developed HPLC method was validated for parameters like system suitability, specificity, linearity, accuracy, precision etc.

System suitability

System suitability tests are used to verify that the reproducibility of the equipment is adequate for the analysis to be carried out. The test was carried out by injecting 20 µl standard solutions of 5 µg/ml of latanoprost and 500 µg/ml of timolol using 50 µg/ml of methyl paraben as an internal standard in five replicates. The RSD values of latanoprost and timolol maleate were 0.13 % and 0.11 % respectively. The RSD values were found to be satisfactory and meeting the requirements (RSD less than 2.0 %). Theoretical plates, resolution, tailing factor were determined and are presented in table 1.

Linearity

Linearity was evaluated by analysis of working standard solutions of latanoprost and timolol maleate of seven different concentrations. The range of linearity was from 2.5 – 7.5 µg/ml for latanoprost and 250 - 750 µg/ml for timolol. The peak area ratio and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained for latanoprost and timolol maleate is represented in table 2. The result shows that within the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively. The LOD and LOQ of latanoprost and timolol maleate were experimentally determined by injecting six injections of each drug. The LOD of latanoprost and timolol was found to be 0.2 µg /ml & 0.3 µg /ml respectively. The LOQ of latanoprost and timolol was found to be 0.6 µg /ml & 0.8 µg / ml respectively.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results of six replicate injections of the mixed standard solution. The relative standard deviation was less than 2%. Method precision was determined from results of six independent determinations at 100% of the test concentrations of latanoprost and timolol maleate in the

product. The %RSD was found to be 0.29 for latanoprost and 0.15 for timolol maleate. The results obtained are tabulated in table 3.

Accuracy

To study accuracy of the method, recovery experiment was carried out by applying the standard addition method. A known quantity of drug substance corresponding to 100%, 110%, 120% and 130% of the label claim of drug were added, to determine if there are positive or negative interferences from excipients present in the formulation. Each set of addition were repeated three times. The accuracy was expressed as the percentage of analytes recovered by the assay. The table 4 lists the recoveries of the drug from a series of spiked concentrations. The results indicate the method is highly accurate for simultaneous determination of latanoprost and timolol.

Table 1 – Results of System suitability

Parameters	Resolution	Tailing Factor	Theoretical
Timolol maleate	-	4.98	16.72
Methyl paraben	1.16	1.31	1.42
Latanoprost	2951	4265	9055

Table 2 – Results of Linearity

Analyte	Slope	Intercept	Correlation coefficient (r^2) (n=7)
Latanoprost	125.47	-597.69	0.9997
Timolol maleate	17537.67	59803.88	0.9994

Table 3 – Results of Precision study

	Latanoprost	Timolol maleate
Drug found in % ml ⁻¹ (mean)	0.00498	0.49971
Mean %	99.6	99.9
%RSD	0.60	0.09

Table 4 – Results of Accuracy experiment

Analyte	Initial conc. (ppm)	Conc. added (ppm)	Total conc. (ppm)	Conc. found (ppm)	RSD (%) n= 3	Recovery (%)
Latanoprost	5	0.50	5.50	5.52	0.54	100.4
	5	1.00	6.00	6.01	1.50	100.2
	5	1.50	6.50	6.52	0.46	100.3
Timolol maleate	500	50.0	550.0	549.91	0.15	99.9
	500	100.0	600.0	599.77	0.18	100.0
	500	150.0	650.0	650.89	0.27	100.1

Fig 1: Overlain spectra of latanoprost and timolol maleate

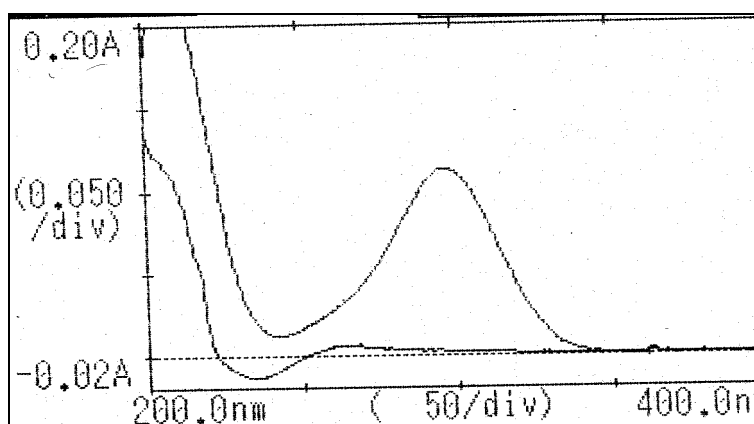
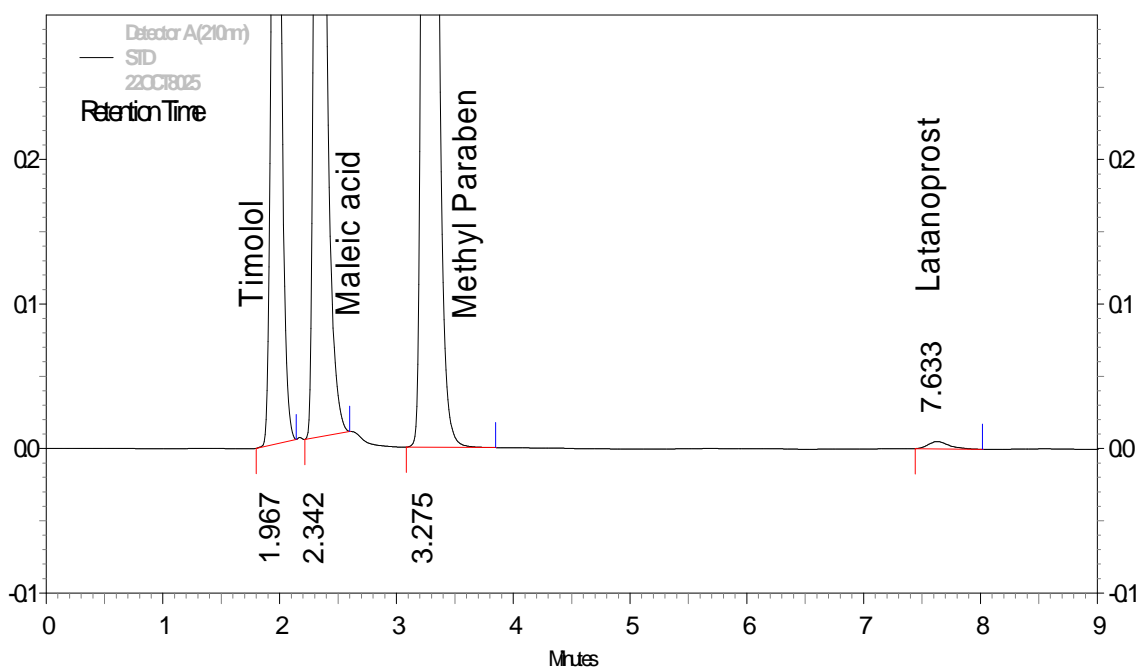


Fig. 2 chromatogram of Latanoprost and Timolol with Methyl paraben (internal standard) in standard preparation



Stability of Solution

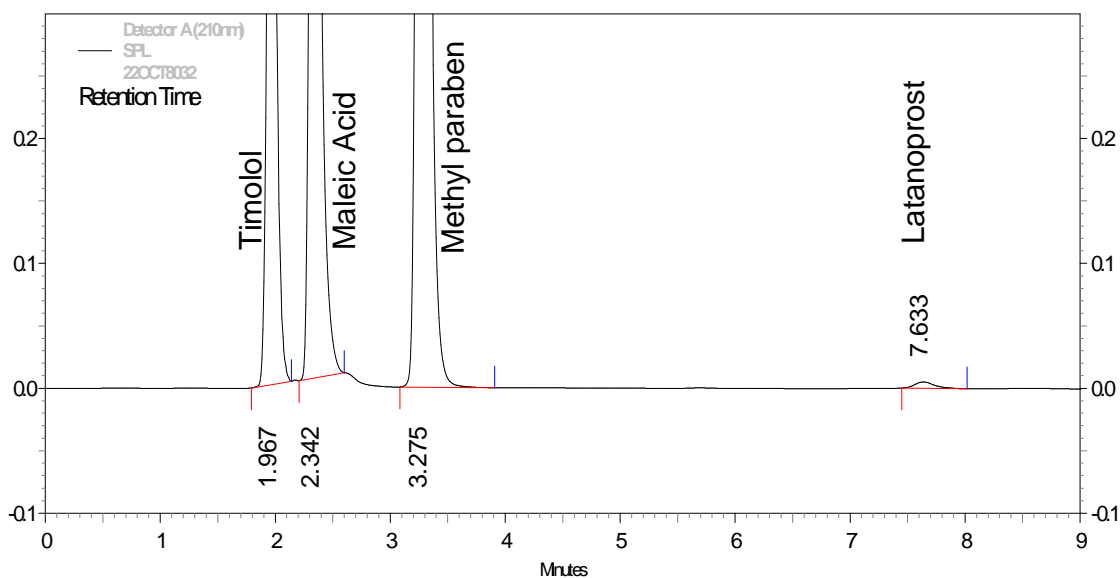
Stability of stock solutions were checked for 24 hours at room temperature. Stock solution of sample and standard contain 50 µg/ml latanoprost, 5000 µg/ml timolol maleate and 500 µg/ml methyl paraben. The drug solutions were found to be stable for the specified period.

Method Application

The validated high performance liquid chromatographic method was applied to simultaneous determination of latanoprost and timolol using methyl paraben as internal standard. About 5.0 ml of sample solution was dissolved in 30 ml of diluent, 10.0 ml of methyl paraben (internal standard) stock solution was added. It was mixed well and further diluted to get a solution of concentration of 5 µg / ml latanoprost, 500 µg / ml timolol and 50 µg / ml methyl paraben. The

20 μ l of this solution was injected into the chromatograph under the specified conditions. The analyte peaks were identified by comparison with observed retention times with those of respective standards. The peak areas obtained were used to calculate the drugs present. The results obtained are tabulated in table 3.

Fig. 3 chromatogram of Latanoprost and Timolol maleate with Methyl paraben (internal standard) in sample preparation



CONCLUSION

Several mobile phases such as water-methanol, water-acetonitrile in different ratios were tried but good peak shape and good resolution between latanoprost, timolol maleate and methyl paraben were observed using the mobile phase as 0.05% (v/v) trifluoro acetic acid in water: 0.05% (v/v) trifluoroacetic acid in acetonitrile (40:60). The method was completely validated. It showed satisfactory data for all the method validation parameters. The method was found to be specific. The low values of % RSD for method precision suggested that the method is precise. Linearity evaluated for the analyte peak showed a good linear response over a wide range of concentrations. The linearity, precision, accuracy of the method proved that the method is specific, accurate, easily reproducible and can be used for simultaneous determination of latanoprost and timolol from pharmaceutical preparations.

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