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# SIMULTANEOUS QUANTIFICATION OF TRAVOPROST AND TIMOLOL MALEATE IN PHARMACEUTICAL FORMULATION BY RP-HPLC

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**Key words:**

Timolol maleate, Travoprost, RP-HPLC, Method development, Validation

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**ABSTRACT:** Travoprost and Timolol Maleate are used in treatment of glaucoma by decreasing intra ocular pressure. The present study was designed to develop and validate a simple, sensitive, precise, and specific reverse phase high-performance liquid chromatographic (HPLC) method for simultaneous determination of Travoprost and Timolol maleate in ophthalmic dosage form. Chromatographic separation of these two drugs was achieved on Hypersil BDS C18 column (250 x 4.6 mm, 5 µm) as stationary phase with a mobile phase Water (pH2 adjusted with OPA): Methanol (85:15% v/v) at a flow rate of 0.8 ml/min and PDA detection at 233 nm. The method was carried out at 40oC. The retention times of Timolol maleate and Travoprost were found to be 2.48±0.01 min, 5.10±0.1min respectively. The proposed method was validated for system suitability, linearity, accuracy, precision, LOD, LOQ and robustness. The calibration curves were linear in the concentration range of 25% to 150% of the working concentration (r2 = 0.999) for both the drugs in binary mixture. The LOD was found to be 0.002 µg/ml and 0.244µg/ml and LOQ was found to be 0.007µg/ml and 0.814µg/ml for Travoprost and Timolol maleate respectively. Hence the proposed RP-HPLC method of analysis can be used in quality control departments with respect to routine analysis of ophthalmic drops containing Travoprost and Timolol maleate.

**INTRODUCTION:** Travoprost is chemically [1*R*-[lα(*Z*),2β(l*E*,3*R**),3α,5α]]-7-[3,5-Dihydroxy-2 - [3-hydroxy-4[3-(trifluoromethyl) phenoxy]-1-butenyl] cyclopentyl]-5-heptenoic acid-1-methylethyleste, is used in the treatment of glaucoma. Timolol maleate is chemically as (*S*)-1-(*tert*-butylamino)-3-[(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl)oxy]propan-2-ol 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 Travoprost and 0.5% (v/v) of Timolol maleate. The Travoprost 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].

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The literature survey revealed that Timolol maleate is estimated with other combinations such as Brimonidine tartrate [6, 9, 17, 22], Brinzolamaide [11], Dorzalamide [7, 13, 14, 20], Latanoprost [12, 18, 23] and also with Rosuvastatin calcium and Diclofenac sodium by HPLC [19], Latanoprost by UV [15]. Timolol was also estimated with Dorzalamide by UPLC [16, 21] and UV spectrophotometric methods [8, 10] have been developed for estimation of Brimonidine tartrate and Timolol maleate. The RP HPLC method was developed by R. V. Valli kumari et al., for the estimation of Travoprost and Timolol maleate [5] using buffer as a mobile phase. So we planned to develop a better separation technique with economical mobile phase. The method described was simple, economical, fast, precise and accurate for simultaneous determination of Travoprost and Timolol maleate from pharmaceutical preparation.

## MATERIALS AND METHODS:

**Materials:** Travoprost and Timolol maleate were obtained from Chandra labs, Hyderabad. Tovaxo T

(Ajantha Pharma) has been taken for study, which contain 40 mcg of Travoprost and 5 mg of Timolol maleate. Methanol, Ortho phosphoric acid (OPA) and HPLC grade water were used of analytical grade from Merck.

**Standard Stock Solution:**
6.4mg of Travoprost working standard was accurately weighed and transferred into a 100ml clean dry volumetric flask and about 70ml of diluent is added and sonicated to dissolve it completely and the volume is made up to the mark with the same solvent.

**Working Standard Solution:**
Further 0.1ml of the above stock solution pipetted into a 100ml volumetric flask. To this 8mg of Timolol maleate working standard has been added and diluted up to the mark with diluent.

**Sample Preparation:**
4 ml of Travoprost and Timolol maleate ophthalmic drops transferred into 50 ml clean dry volumetric flask, about 40ml of diluent is added and sonicated to dissolve it completely and the volume was made up to the mark with the same solvent (Stock solution). Further 5ml of the above stock solution was pipetted into a 25ml volumetric flask 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 reverse phase Waters symmetry C18 (250 mm x 4.6 mm, 5µm) column. The mobile phase consisted of Water (pH 2 adjusted with OPA): Methanol (85:15%v/v) with PDA detection at 233 nm. The flow rate was set at 0.8 ml/min. About 20 µl of standard and sample solutions were injected and detection wavelength was set at 233 nm for simultaneous determination of Travoprost and Timolol maleate.

**Method Development:**
Different chromatographic conditions were tried for separation and resolution. Waters symmetry C18 column was found satisfactory. Peak purity of Travoprost and Timolol maleate was checked using photo diode array detector and 233 nm was considered satisfactory for detecting both the drugs with adequate sensitivity. A typical RP-HPLC chromatogram for simultaneous determination of Travoprost and Timolol maleate from standard preparation and from pharmaceutical formulation is shown in **Fig. 1** and **2.**

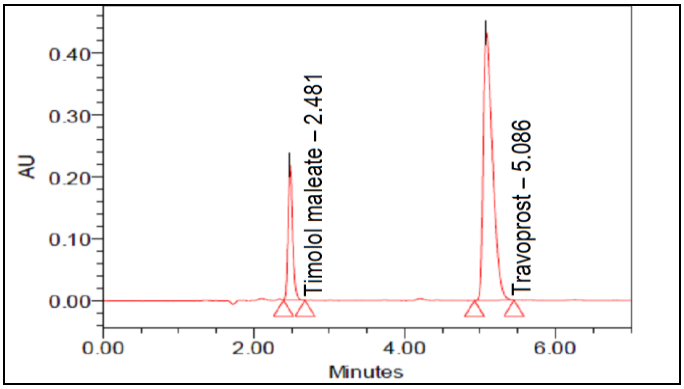


**FIG. 1: CHROMATOGRAM OF TRAVOPROST AND TIMOLOL MALEATE IN STANDARD PREPARATION**

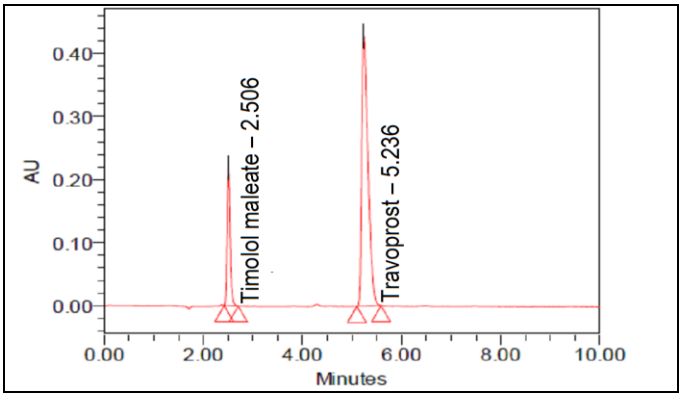


**FIG. 2: CHROMATOGRAM OF TRAVOPROST AND TIMOLOL MALEATE IN SAMPLE PREPARATION**

## RESULTS AND DISCUSSION:

**Method Validation:**
The developed RP-HPLC method was validated for parameters like system suitability, linearity, accuracy, precision, LOD, LOQ and robustness.

**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 0.64 µg/ml of Travoprost and 80 µg/ml of Timolol maleate in six replicates. The RSD values of Travoprost and Timolol maleate were 0.61 % and 0.73 % 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 were presented in **Table 1**.

**TABLE 1: RESULTS OF SYSTEM SUITABILITY**

| Analytes | Resolution | Tailing Factor | Retention times | Theoretical plates |
|---|---|---|---|---|
| Timolol maleate | - | 1.274 | 2.481 | 8489 |
| Travoprost | 15.227 | 1.768 | 5.086 | 7583 |

**Linearity:**

Linearity was evaluated by analysis of working standard solutions of Travoprost and Timolol maleate of seven different concentrations. The range of linearity was from 0.32 – 0.96 µg/ml for Travoprost and 40 - 120 µg/ml for Timolol maleate. 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 Travoprost and Timolol maleate was 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.

**TABLE 2: RESULTS OF LINEARITY**

| Analytes | Correlation coefficient ($r^2$) |
|---|---|
| Timolol maleate | 0.999 |
| Travoprost | 0.999 |

**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 Travoprost and Timolol maleate were experimentally determined by injecting six injections of each drug.

The LOD of Travoprost and Timolol maleate was found to be 0.002µg/ml & 0.244µg/ml respectively. The LOQ of Travoprost and Timolol maleate was found to be 0.007µg/ml & 0.814µg/ml respectively.

**Precision:**

Method precision was determined from results of six independent determinations at 100% of the test concentrations of Travoprost and Timolol maleate in the product. The %RSD was found to be 0.61 for Travoprost and 0.73 for Timolol maleate. The results obtained were tabulated in **Table 3**.

**TABLE 3: RESULTS OF PRECISION STUDY**

| Analyte | Mean % | %RSD |
|---|---|---|
| Timolol maleate | 100 | 0.73 |
| Travoprost | 100 | 0.61 |

**Accuracy:**

Accuracy of the method has been studied by recovery experiments by applying the standard addition method. A known quantity of drug substance corresponding to 50%, 100%, and 150% 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 Travoprost and Timolol maleate.

**TABLE 4: RESULTS OF ACCURACY EXPERIMENT**

| Analyte | Pre analysed sample conc. (ppm) | Amount added (ppm) | Amount found (ppm) | Recovery (%) |
|---|---|---|---|---|
| Travoprost | 0.32 | 0.323 | 0.330 | 101 |
| | 0.64 | 0.640 | 0.645 | 100 |
| | 0.96 | 0.991 | 0.970 | 98 |
| Timolol maleate | 40 | 39.600 | 40.390 | 101 |
| | 80 | 79.200 | 79.190 | 100 |
| | 120 | 122.76 | 124.150 | 102 |

**Robustness:**

Keeping the ratio of mobile phase constant, the chromatograms of drug solution were recorded with different flow rates such as 0.8 ml/min, 1.0 ml/min and 1.2 ml / min. At the flow rate of 1.0 ml / min, the peaks were sharp with good resolution, apart from above said flow rate, rest of the flow rates were found to be not satisfactory. But passed all system suitability parameters indicating the method is robust. The results were presented in **Table 5**.

Keeping the ratio of mobile phase constant, the chromatograms of drug solution were recorded with different column temperatures such as 35˚C, 40˚C, 45˚C. At the 40˚C, the peaks were sharp with good resolution, apart from above said temperature, rests of the temperatures were found to be not satisfactory, Although passed all system suitability

parameters indicating the method is robust. The results were presented in **Table 6**.

**TABLE 5: RESULTS OF ROBUSTNESS STUDIES (FOR VARIANT FLOW RATE)**

| Analyte | Flow rate (ml) | Plate count | Tailing |
|---|---|---|---|
| Travoprost | 0.6 | 7043 | 1.496 |
| | 0.8 | 7583 | 1.768 |
| | 1.0 | 6421 | 1.386 |
| Timolol maleate | 0.6 | 3908 | 1.666 |
| | 0.8 | 8489 | 2.506 |
| | 1.0 | 3454 | 1.120 |

**TABLE 6: RESULTS OF ROBUSTNESS STUDIES (FOR VARIANT COLUMN TEMPERATURE)**

| Analyte | Column temperature (˚C) | Plate count | Tailing |
|---|---|---|---|
| Travoprost | 35 | 7043 | 1.496 |
| | 40 | 7583 | 1.768 |
| | 45 | 6421 | 1.386 |
| Timolol maleate | 35 | 3908 | 1.666 |
| | 40 | 8489 | 2.506 |
| | 45 | 3454 | 1.120 |

**CONCLUSION:** The study was undertaken in order to develop and validate a simple analytical RP-HPLC method for estimation of Travoprost and Timolol maleate in pharmaceutical formulations. The method developed was validated by means of accuracy, precision, linearity, LOD and LOQ and robustness as per ICH guidelines. Thus resulted a simple, precise, accurate and sensitive RP-HPLC method for the simultaneous estimation of Travoprost and Timolol maleate in ophthalmic dosage form. The results of the study indicate that the proposed RP-HPLC method of analysis can be used in quality control departments with respect to routine analysis for the assay of the ophthalmic drops containing Travoprost and Timolol maleate.

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