Development and Validation of a RP-HPLC Method for Simultaneous Quantification of Pilocarpine Hydrochloride and Timolol Maleate in Ophthalmic Formulations

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Purpose
The purpose of this study was to develop and validate a simple, cost effective method to simultaneously detect and quantify Pilocarpine and Timolol in ophthalmic formulations.

Methods
A Shimadzu HPLC solution Chromatography Data System with a Photo-Diode-Array detector was used in this study. A 1.0mg/mL standard stock solution of the drugs were prepared in mobile phase (MeOH:buffer) and filtered through a 0.22µ filter. Standard curve is plotted from a series of working solutions ranging from 2 - 400µg/mL for both drugs. Separation was achieved using an Alltech platinum C18 analytical column (150mm X 4.6mm, 5µm). The mobile phase system was a methanol: buffer (60:40 v/v), at a total flow rate of 1.0ml/min and a runtime of 15mins. With an injection volume of 20µl, the sample was detected at a predetermined wavelength of 215nm and 295nm for pilocarpine and Timolol respectively. The analysis was carried out at ambient temperature.

Results
The method was very selective. Preliminary results for timolol consistently produced an average retention time of 7.6mins. The method was also highly sensitive and linear. Preliminary results for Timolol showed a correlation coefficient (r2) of 0.99 over the test concentration range 2 - 400µg/mL. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.21µg/ml and 0.68µg/ml. Intra-day and inter-day repeatability and precision values (as mean percent RSD) were less than 2%. The accuracy of the method (percent recovery) was 99.3 – 101.3% of true values over the concentration range of 5 - 20µg/mL.

Conclusion
A reliable, sensitive and cost effective RP-HPLC method was successfully developed and validated. This method can be employed to determine drug entrapment efficiency and to quantify drug release of pilocarpine and timolol from ophthalmic formulations.