

An HPLC method development for simultaneous determination of metoprolol, propranolol and phenol red: application in perfusion studies

H. Tabatabayi^{1,*}, P. Zakeri-Milani², H. Valizade², Y. Azarmi², M. Barzegar Jalali², H. Tajerzadeh²

¹Students Research Committee, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

²Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

Background and Amis: Single-pass intestinal perfusion technique (SPIP) is the most used classic technique employed in the study of intestinal absorption of compounds in which a non-absorbable marker such as phenol red is used to correct the water flux.

Methods: A simple and rapid reversed-phase high performance liquid chromatographic method with UV detection at 227 nm was developed for simultaneous quantitation of propranolol and metoprolol along with phenol red for in-situ permeability studies. The mobile phase was a mixture of 55% methanol, 45% of 0.05 M KH₂PO₄ aqueous solution (adjusted to pH 6) and 0.2 % (v/v) triethylamine. Analysis was run at a flow rate of 1 ml/min with a 9 min run time.

Results: The calibration curves were linear for all three compounds ($r > 0.999$) across the concentration range of 7.5-125 µg/ml with a limit of detection of 4.24, 2.18 and 8.57 ng/ml and limit of quantification of 14, 7.2 and 28.3 ng/ml for metoprolol, propranolol and phenol red respectively. The coefficient of variation for intra-assay and inter-assay precision was less than 8% and the accuracy was between 93.6-107%.

Conclusions: Using the SPIP technique and the suggested HPLC method for sample analysis, the mean values of 0.49×10^{-4} (± 0.19) cm/sec and 0.32×10^{-4} (± 0.09) cm/sec were obtained for propranolol and metoprolol intestinal permeability coefficients respectively.

Keywords: Propranolol; Metoprolol; Phenol red; Liquid chromatography; Permeability