SEPARATION AND SIMULTANEOUS DETERMINATION OF S-TIMOLOL MALEATE, ITS OPTICAL ANTIPODE AND RELATED SUBSTANCES BY LIQUID CHROMATOGRAPHY USING A CELLULOSE BASED CHIRAL STATIONARY PHASE

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A liquid chromatographic (LC) method was developed earlier for the determination of the enantiomeric purity of timolol maleate, a β-adrenergic blocker, used in therapeutics as a single enantiomer (S-timolol), and was recently introduced in the European Pharmacopoeia [1].
This method was based on the use of a column packed with a chiral stationary phase, cellulose tris-3,5-dimethylphenylcarbamate (5μm), in the normal phase mode, with a mixture of hexane, 2-propanol and diethylamine as mobile phase. The flow rate was set at 1.0 ml/min and UV detection was performed at 297 nm.
The objective of this study was to adapt this LC method in order to make it suitable also for the determination of other closely related impurities.
The concentration of the organic modifier, (2-propanol) and of the competing amine (diethylamine) as well as the column temperature (12-30° C) were optimized using a multivariate approach.
An adequate resolution of the critical pair (R-timolol/isotimolol) was obtained using a mobile phase containing n-hexane, 2-propanol and diethylamine in the proportions: 96/4/0.2 (v/v/v) and a column temperature of 20° C. Under these conditions, the resolution between the two enantiomers of timolol was still very high (about 4) and the latter were also well separated from the other potential impurities.
The developed method was validated. Very good results with respect to relationship between response and concentration (linearity), accuracy and precision were obtained. The limits of quantification for R-timolol and the other impurities were lower than 0.1 %.
The LC method was then applied to the purity testing of samples of S-timolol from different sources.

1. European Pharmacopoeia, 3rd Edition, Addendum 2001, p. 1520, Council of Europe, Strasbourg, France, 2000.