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Chemistry

A VALIDATED LC METHOD FOR THE DETERMINATION OF CHIRAL PURITY OF (S) - (-) - 3-TERT BUTYLAMINO 1,2, PROPANE DIOL) : A KEY RAW MATERIAL OF TIMOLOL MALEATE

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ABSTRACT A simple and accurate normal phase liquid chromatographic method was developed for the determination of chiral purity of (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol, S-enantiomer used as key starting raw material in the manufacturing of timolol maleate bulk drug.
Chromatographic separation between (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol and its opposite enantiomer (R) - (-) - 3-Tert Butylamino 1,2, Propane Diol, R-enantiomer was achieved using a Chiralpak IC column using a mobile phase containing n-hexane, ethanol, formic acid and diethylamine (70:30:0.1:0.1 v/v/v/v). The resolution between the two enantiomers was found to be more than 5.0. The limit of detection (LOD) and limit of quantification (LOQ) of the R-enantiomer was 7.5 and 25.0 µg mL-1, respectively, for 20 µL injection volume. The percentage recoveries of the R-enantiomer ranged from 96.4 to 98.1 in the samples of (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol. The test solution and mobile phase was observed to be stable up to 24 h after the preparation. The developed method was validated as per International Conference on Harmonization guidelines in terms of LOD, LOQ, precision, linearity, accuracy, robustness and ruggedness.

KEYWORDS : Timolol maleate, Enantiomeric separation, Chiral Liquid chromatography, Validation and Quantification.

Introduction

Recently pharmaceutical companies have shifted their attention in developing enantiomerically pure API/drugs which carry a chiral carbon atom. Since, the ill effects of the presence of the other isomer is either not very well established or known, FDA is insisting that the molecules prepared have the least of the undesired isomers. Hence, a control and accurate quantification of undesired enantiomers in pharmaceuticals is essential [1] in this connection and LC is the most optimum tool for this purpose.

Timolol maleate [2] is a non-selective beta-adrenergic receptor blocking agent that lowers the ocular pressure in open angle glaucoma and ocular hypertension1. It is chemically described as (S)-1-(tert-butylamino)-3-[(4-morpholino-1,2,5-thiadiazol-3-yl)oxy]-propan-2-ol Maleate which is official in U.S.P, I.P and E.P [3]. Ever since its introduction for clinical usage in the management of glaucoma in 1978, none of the new generation beta blocker have been found more effective than Timolol Maleate. (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol, is a key raw material used for the synthesis of Timolol maleate. This key raw material controls the enantiomeric purity of Timolol maleate as the chirality in the API is because of this key starting material. Because of the stringent limits of the unwanted isomer 1.0% in pharmacopeia it is very important that we control the enantiomeric purity at the key starting material stage. To our present knowledge no chiral LC methods were reported in the literature for the chiral purity determination of (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol. Therefore, it was felt necessary to develop a chiral LC method for the accurate quantification of the undesired (*R*)-enantiomer.

The present research work focused on the development of a chiral LC method to determine the enantiomeric purity of (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol and also quantify the undesired (*R*)-enantiomer using various chiral LC columns. Very good resolution between *S* and *R*-enantiomers was observed on Chiralpak IC column. In the developed method, the *S* and *R*enantiomers were well resolved with a resolution >5.0 within a 20 min run time using a simple normal phase system containing n-hexane, ethanol, formic acid and diethylamine. This paper deals with the method development and validation of the developed method [5-6].

The determination of the stereo isomeric composition of pharmaceuticals is rapidly becoming the key issue in the development of new drugs. Among the methods currently used to achieve chiral separation of enantiomers, high resolution liquid chromatography systems based on chiral stationary phases (CSPs) are more rapid and are suitable for the resolution of racemic mixtures of pharmacologically active chemical entities [7].

EXPERIMENTAL SECTION

Chemicals and Reagents

Samples of *S* and *R*-enantiomers of 3-Tert Butylamino 1,2, Propane Diol confirmed by spectral characterization and SOR (specific optical rotation) were obtained from R & D synthetic Department of FDC Ltd, Mumbai, India. HPLC-grade n-hexane and ethanol was procured from Merck, Darmstadt, Germany. Formic acid and diethylamine were purchased from J.T BAKER USA. Analytical Reagent grade trifluoroacetic acid (TFA) was purchased from Fluka.

Instrumentation

The chromatographic separation was carried out on a Agilent HPLC (1200 model, Agilent. USA) system consisting of quaternary pump, Column oven, Autosampler, ELSD detector and data acquisition software ezchrom 4. Amylose based chiral stationary phase AD-3, modified cellulose based Chiralcel OD-RH, and cellulose based chiral stationary phase Chiralcel OJ-H(Diacel Chemical Industries, Ltd., Tokyo, Japan) was employed during the method development.

Chromatographic Conditions

The chromatographic conditions were optimized using a Chiralpak IC column (Daicel Chemical Industries, Ltd., Tokyo, Japan). The mobile phase, a mixture of n-hexane, ethanol, formic acid and diethylamine in the ratio of 70:30:0.1:0.1 mL with a flow rate of 1.0 mL min-1 was employed. The column temperature was maintained at 30 °C and the detection was monitored by using ELSD detector. The injection volume was 20 µL.

Preparation of Standard Solutions

The stock solutions of *S* and *R*-enantiomers of -3-Tert Butylamino 1,2, Propane Diol were prepared individually by dissolving an appropriate amount of the substances in diluent of mobile phase. Working

solutions were prepared in ethanol as diluent. The target analyte concentration was fixed as 10.0 mg mL-1.

RESULTS AND DISCUSSION

Method Development

The objective of this work was to evaluate the enantiomeric purity of the *S*-enantiomer of 3-Tert Butylamino 1,2, Propane Diol and accurate quantification of the undesired *R*-enantiomer. The preliminary trails carried out in reverse phase chiral columns were not fruitful in the separation of these isomers. Different chiral stationary phases were employed during the method development namely Chiralpak IA, Chiralpak IB, Chiralpak IC, Chiralpak AD-H, and Chiralcel ODH. Different trials were made during the method development with different composition of normal phase solvents and modifier. Chiralpak IC, an Cellulose base chiral stationary phase was found to be selective for the enantiomers of 3-Tert Butylamino 1,2, Propane Diol. Very good resolution was achieved on Chiralpak IC column using mobile phase contains the mixture of n-hexane, ethanol, formic acid and diethylamine (70:30:0.1:0.1 v/v/v/v).The addition of formic acid and diethylamine to the mobile phase plays an important role on enhancing the chromatographic efficiency and resolution between the enantiomers.

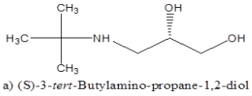
Optimized Chromatographic Conditions

Chromatographic base to base separation was achieved only on a Chiralpak IC (250 x 4.6 mm, 5 microns particle size) chiral column using the mobile phase, which contains the mixture of n-hexane, ethanol, formic acid and diethylamine (70:30:0.1:0.1 v/v/v/v). The flow rate of the mobile phase was 1.0 mL min-1. The column temperature was maintained at 30°C and the detection was done by ELSD. The injection volume was 20 µL. The total analysis time for each run was 20 min. Very good separation was observed within short runtime on Chiralpak IC column (resolution >5.0). The typical retention times of *R* and *S*-enantiomers of 3-Tert Butylamino 1,2, Propane Diol are 7.5 and 10.3. The USP tailing factor (T) was found to be 1.2 for *R*-enantiomers of 3-Tert Butylamino 1,2, Propane Diol. The system suitability results were given in Table 1.

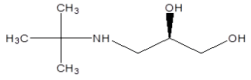
Table 1: System suitability criteria

Component	Retention time (min)	Relative retention time (min)	Resolution	Tailing factor
(R) - (-) - 3-Tert Butylamino 1,2, Propane Diol	7.5	0.7	--	1.2
(S) - (-) - 3-Tert Butylamino 1,2, Propane Diol	10.3	1.0	9.5	---

The structure and configurations of *R* and *S*-isomers of 3-Tert Butylamino 1,2, Propane Diol are displayed in Fig. 1. The typical chromatogram of the *S*-isomer of 3-Tert Butylamino 1,2, Propane Diol spiked with *R*-enantiomer of of 3-Tert Butylamino 1,2, Propane Diol displayed in Fig. 2.



a) (S)-3-*tert*-Butylamino-propane-1,2-diol



b) (R)-3-*tert*-Butylamino-propane-1,2-diol

Fig.1 Chemical structures of (S) and (R)-3-tert-Butylamino-propane-1,2-diol

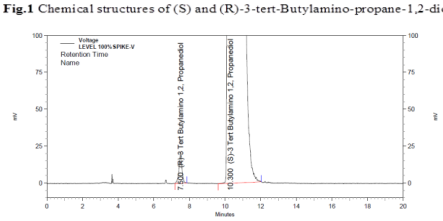


Fig. 2 A typical HPLC chromatogram of (*S*)- 3-Tert Butylamino 1,2, Propane Diol spiked with (*R*)- 3-Tert Butylamino 1,2, Propane Diol at specification level

Method Validation

Precision

The precision of an analytical procedure expresses the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogenous sample under prescribed conditions. The system and method precision for the *R*-enantiomer were checked at its specification level (i.e. 0.5% with respect to analyte concentration, 10.0 mg mL-1). The percentage RSD of method repeatability and system repeatability for the *R*-enantiomer were found to be 2.34 and 1.92, respectively, which confirms good precision of the method.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample. The linearity of the method for the *R*-enantiomer was checked at six concentration levels, i.e. from limit of quantitation (LOQ) (50%) to 150% of the undesired *R*-enantiomer specification level (0.5%), which is with respect to of (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol analyte concentration. The coefficient of regression of the calibration curve was found to be 0.9976, thus confirming the excellent correlation between the peak area and concentration of the *R*-enantiomer.

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantification were achieved by injecting a series of dilutions of *R*-enantiomer [7]. The precision of the developed enantioselective method for *R*-enantiomer at LOD and LOQ was checked by analyzing six test solutions prepared at the LOD and LOQ level and calculating the percentage relative standard deviation of area. The limit of detection and quantification for *R*-enantiomer was found to be 7.50 µg mL-1 and 25.2 µg mL-1 respectively for the 20 µL of injection volume.

Ruggedness and Robustness

The ruggedness [7] of a method was defined as degree of reproducibility of results obtained by analysis of the same sample under a variety of normal test conditions such as different laboratories, different analysts, different instruments and different days. The standard addition and recovery experiments carried out for the *R*-enantiomer in (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol bulk samples at the same concentration levels tested in Laboratory A were again carried out at laboratory B using a different instrument and analyst. The data obtained from Laboratory B was well in agreement with the results obtained in Laboratory A, thus proving the method ruggedness. The robustness [7] of an analytical procedure is measured by its capability to remain unaffected through small, but deliberate, variations in method parameters and provide an indication of its reliability during normal usage. In the varied chromatographic conditions like flow rate, mobile phase ratio and column temperature, the resolution between the peaks of *S* and *R*-enantiomers of 3-Tert Butylamino 1,2, Propane Diol was found to be >5.0 illustrating the robustness of the method.

Recovery of (*R*)-Enantiomer

Standard addition and recovery experiments were conducted to determine the accuracy of the present method, for the quantification of the (*R*)-enantiomer in samples of (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol. The study was carried out at 0.25, 0.50 and 0.75% of target analyte concentration of (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol. The percentage recoveries of the *R*-enantiomer ranged from 96.4 to 98.1 in samples of (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol.

Solution Stability and Mobile Phase Stability

Solution stability was studied by keeping the test solution in tightly capped volumetric flasks at room temperature on a laboratory bench for 24 h. The content of (*R*)-enantiomer was checked for every 6 h interval and compared with freshly prepared solution. No variation was observed in the content of the (*R*)-enantiomer for the study period and it indicates (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol sample solutions prepared in diluent were stable up to 24 h. Mobile phase stability was carried out by evaluating the content of (*R*)-enantiomer in (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol sample solutions, which were prepared freshly at every 6 h interval for 24 h. The same mobile phase was used during the study period. No variation was observed in the content of (*R*)-enantiomer for the study period and it indicated the prepared mobile phase was stable up to 24 h. The results obtained for

validation parameters are summarized in table 2.

Table 2 Summary of method validation data

Parameter	µg mL-[1]	r	% Mean recovery	% RSD
LOD	7.5	-	-	7.10
LOQ	25.2	-	-	2.88
Linearity				
(LOQ to 150%)	-	0.9976	-	-
Accuracy				
50 % spiking	-	-	96.8	0.12
100 % spiking	-	-	98.1	0.44
150 % spiking	-	-	96.4	0.18
Precision				
System precision	-	-	-	1.92
Method precision	-	-	–	2.34
Intermediate pre (Ruggedness)	-	-	-	3.02

CONCLUSION

A simple and accurate normal phase chiral LC method was developed for the quantitative determination of the (*R)*-enantiomer in (S) - (-) - 3-Tert Butylamino 1,2, Propane Diol, a key starting material of timolol maleate. Chiralpak IC, cellulose based chiral stationary phase was found to be selective for the enantiomers of 3-Tert Butylamino 1,2, Propane Diol. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method can be used for the quantitative determination of the undesired (*R)*- enantiomer in 3-Tert Butylamino 1,2, Propane Diol samples.

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