Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(1):586-591

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Liquid Chromatographic method for the Determination of Enantiomeric Purity of Levobetaxolol by Chiral Chromatography

G. Anantha Ram[a], M. N. K. Harish[c], Y. Arthob Naik[c], J. Keshavayya[c] and K. R. Venugopala Reddy[b*]

[a]*Central Drugs Testing Laboratory, G.M.S.D Compound, Belasis Road, Mumbai Central, Mumbai, Maharashtra, India*
[b]*Department of P.G. Studies and Research in Industrial Chemistry, Sahyadri Science College (Autonomous), University College, Shivamogga, Karnataka, India*
[c]*Department of Studies in Chemistry, Kuvempu University, Jnana, Sahyadri, Shankaragatta, Shimoga District, Karnataka, India*

ABSTRACT

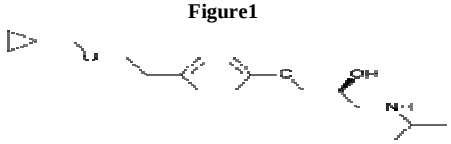
A simple precise, accurate Normal phase High performance liquid chromatographic method has been developed for the Determination Of Enantiomeric Purity of Levobetaxolol by Chiral Chromatography. In this method a Chiral OD-H 25 cm x 4.6 mm x 5.0µ (Cellulose based) column with mobile phase Hexane: Ethanol: Diethyl amine (95:5:0.1v/v),was used. The detection wavelength is 220 nm and the flow rate is 1.0 ml/min. The linearity of Levobetaxolol shows regration coefficient of 0.9999. The proposed method is sufficiently selective to distinguish the parent drugs from its other enanatiomer.

Keywords: HPLC. Levobetaxolol, Betaxolol, Hexane & Ethanol.

INTRODUCTION

Levobetaxolol (Fig.1) is chemically known as S)-1-[2-(cyclopropylmethoxy) ethyl]phenoxy]-3-(isopropylamino)-2-propanolhydrochloride. Levobetaxolol is a cardioselective β-blocker that has been demonstrated to reduce intraocular pressure in patients affected with primary open-angle glaucoma and ocular hypertension [1, 2]. Levobetaxolol (43-fold beta1-selective) exhibited a higher affinity at cloned human β-1(Ki=0.76 nM) than at β-2(Ki=32.6 nM) receptors, while dextrobetaxolol is much weaker at both receptors.

Drug profile of Levobetaxolol

Chemical name	S)-1-[2-(cyclopropylmethoxy)ethyl]phenoxy]-3-(isopropylamino)-2-propanol hydrochloride
2) Formula A) Empirical	C_6 H_6 N_2 O_3
B) Structure	**Figure1** 
C) Molecular Weight	307.427 g/mol
3) Description	White powder.

4) Solubility Soluble in water & in methanol
5)Category Antiglaucoma

EXPERIMENTAL SECTION

Instrumentation

The HPLC instrument consisted of E. Merck Hitachi system equipped with a model L-7100 pump, an automatic sample injection device L-7200, a variable wave length UV-Visible detector L-7400 controlled by interface module with HSM soft ware.

Materials and reagents

- Hexane HPLC Grade
- Ethanol HPLC Grade
- Diethyl amine
- Betaxolol reference standard
- Levobetaxolol raw material

Development of analytical method

Analytical method was developed by optimization of chromatographic condition[3] and optimization of standard and sample preparation procedure. And UV_{max} found at 220 nm after scanning [4] standard solution prepared in mobile phase, the Enantiomeric separation was achieved on Chiral OD-H 25 cm x 4.6 mm x 5.0 µ (Cellulose based) column and different concentration of mobile phase were tried to optimize the total run time and finally the mobile phase fixed in the ratio Hexane: Ethanol: Diethyl amine (95:5:0.1v/v). Flow of 1ml/min was found to be optimum. Most commonly used 10µl injection volume was selected for the study. The chromatographic conditions summarized in the Table I

Table I : Chromatographic conditions

Chromatograph	MERCK HITACHI & SHIMADZU Class M10A
Column	Chiral OD-H 25 cm x 4.6 mm x 5.0 µ
Column temperature	Ambient $25^0 \pm 2^0$C
Mobile phase	Hexane: Ethanol: Diethylamine (95:5:0.1v/v)
Injection volume	10µl
Flow rate	1 ml/min
Detectorwave length	220nm

System suitability study showed that the method is precise and accurate. The linearity study (in the range of 10µg/ml-30µg/ml) showed that linear regression was found to be 0.9999 and low values of RSD and coefficient of variation indicated that the proposed method is precise and accurate

Optimization of standard and sample preparation

The drug is very soluble in mobile phase and hence the mobile phase is selected as diluents for preparation. Five different concentrations of Levobetaxolol (10–30µg/ml) were prepared from stock solution (10.0 mg to 100 ml with mobile phase) into a series of $10cm^3$ volumetric flasks and volume was made up to the mark with the mobile phase. 10µl of each solution was injected in to chromatograph and the peak areas were recorded for all the chromatograms (n=3). Calibration graph[5,6] was constructed by plotting amount of Levobetaxolol in µg/ml on X-axis against peak area on Y- axis and linear relationship for the components was evaluated by the method of least square.

System suitability solution

To determine system suitability test[7], prepared working standards solution of Betaxolol Standard (20µg/ml), were injected into the chromatograph and relative standard deviation of peak areas were calculated. Other SST parameters such as tailing factor, and resolution between Levobetaxolol and D-Betaxolol also calculated. The results are given in Table II, Fig 2&3.

Table-II

System Suitability Test (SST)	Response
Theoretical plates (N) Tailing factor Resolution (peak to peak separation)	5085 & 5145 1.43 & 1.46 More than 5.0
Linearity range Coefficient of variation(r 2)	10 to 30mcg/ml
Accuracy (in purity of drug)	99.81
Limit of quantitation(LOQ) (mcg/ml)	10 mcg/ml
Limit of detection (LOD) (mcg/ml)	1mcg/ml

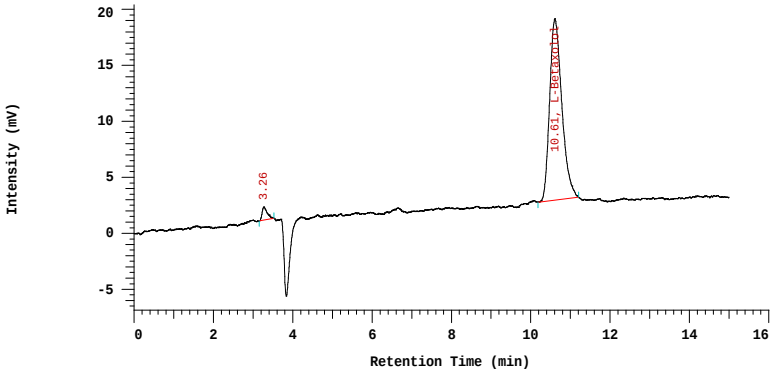


Fig 2: Chromatogram of Levobetaxolol

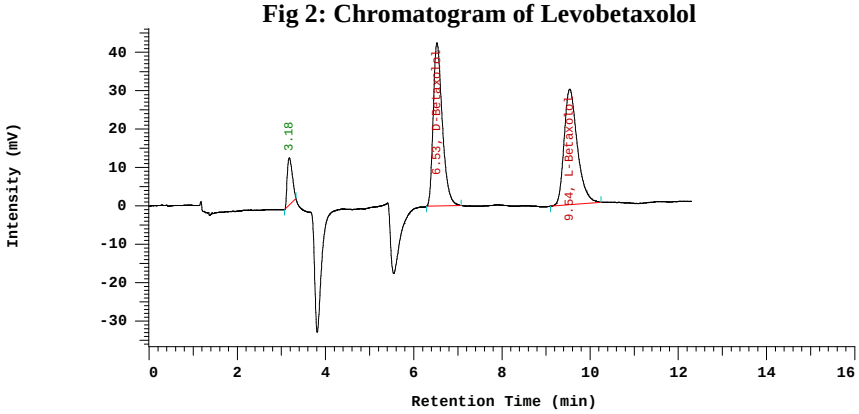


Fig.3 Chromatogram of Betaxolol (Recimic mixture) standard

The RT of D-Betaxolol: 6.53 minutes and RT of L-Betaxolol: 9.54 minutes

Calculations

Chiral purity calculated by area normalization method, the results are in Table III

Table-III

Experiment	Chiral purity (in area %) (by Area normalization method)
I	99.86
II	99.75
III	99.80
Average	99.80
SD	0.055
% of RSD	0.06

Method Validation

The developed method has been widely validated [8] for chiral separation of Levobetaxolol using the following parameters. Linearity, experiments were also conducted to determine the accuracy of the proposed method.

Linearity study

Five different concentration of Levobetaxolol from 10-30µg/ml was prepared for linearity studies [8, 9] and chromatographed (n=5). Calibration curves were constructed for Levobetaxolol by plotting the peak area of the drug on (Y-axis) against the amount of drug concentration in µg/ml on (X-axis) and the calibration curve obtained shows linearity of Levobetaxolol in above concentration range. The results are in Table-IV & Fig 4

Table-IV

%Level of standard	Levobetaxalol	
	Concentration (µg/ml)	Area
50	10	62797
75	15	93974
100	20	125139
125	25	156753
150	30	186665
Correlation Coefficient(r)		0.9999

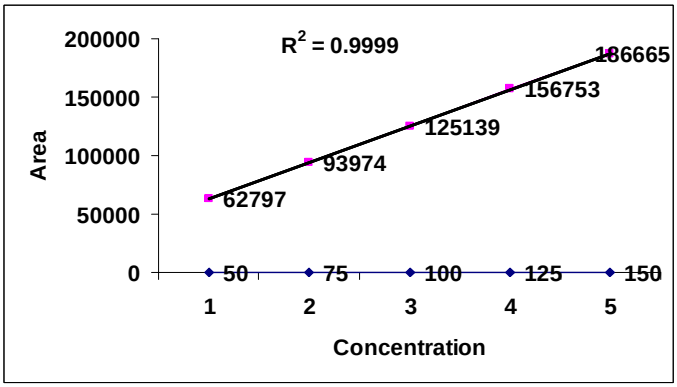


Fig 4: Linearity graph

Limit of detection (LOD) and Limit of quantification (LOQ)

For the proposed method limit of detection and limit of quantitation for Levobetaxolol was found to be 1-10µg/ ml. The signal to noise ratio (noises in the chromatogram) obtained for above parameters are well within the criteria of ICH guidelines indicating good quantitation capability of the method**.**

Precision

The precision of the method was established by following parameters.

Table-V Precision (Injection Repeatability)

No of Injections	Retention time of D-Betaxolol	Retention time of Levobetaxolol
1	6.53	9.54
2	6.42	9.50
3	6.49	9.56
4	6.58	9.49
5	6.43	9.49
6	6.49	9.55
Mean	6.49	9.52
S.D	0.055	0.029
% RSD	0.84	0.3

Injection repeatability: The system suitability solution was injected on the HPLC system six times and relative standard deviation of the peak retention times were calculated, which was found to be less than 2% indicating good injection repeatability. The result obtained is as given Table V.

Intermediate precision (Ruggedness): The sample was analyzed using two different instruments (E.Merck and Shimadzu) and relative standard deviation of the purity of Levobetaxolol were calculated, RSD with both instruments were found to be less than 2% indicating that the method is rugged. The results obtained are as given in Table VI

Table VI-- Intermediate Precision (Ruggedness)

Name of the Instrument	Day	%Chiral purity of Levobetaxolol
E. Merck Classic	1	99.80
Shimadzu	2	99.72
Mean		99.76
SD		0.0565
%RSD		0.06

Accuracy:
The accuracy of the method was recognized by doing the chiral purity of three different concentration of the drug. The mean purity of the drug is well within the acceptance limit hence the method is accurate. (Results are in Table III)

Specificity:
It was observed that solutions showed a clear base line at the retention time of Levobetaxolol and D-Betaxolol

Table VII: Stability in solution

Sample preparation	% of Chiral purity
Fresh	99.80
After 24 hrs	99.65
Mean	99.73
SD	0.106
% RSD	0.11

Stability study:
The stability of Levobetaxolol in solution containing mobile phase have been determined by keeping same sample in a tightly capped volumetric flask placed at ambient temperature under normal lighting Conditions[10,11]. The samples were checked for chiral purity after 24 hrs of storage and compared with freshly prepared sample. The RSD values of Experiments were found to be below 2.0% in both cases. This indicated that the Levobetaxolol is stable in the solution. (Results are in Table VII).

RESULTS AND DISCUSSION

In this method Enantiomeric separation was achieved on Chiral OD-H 25 cm x 4.6 mm x 5.0 µ (Cellulose based) column and different concentration of mobile phase were tried to optimize the total run time and finally the mobile phase fixed in the ratio Hexane: Ethanol: Diethylamine (95:5:0.1v/v).System suitability study , the linearity study showed that linear regression was found to be 0.9999, low values of RSD and coefficient of variation indicated that the proposed method is precise and accurate.

CONCLUSION

Precise, accurate method was developed and validated for the Chiral separation (Purity) of Levobetaxolol in bulk. Analytical data indicates that the developed method is selective and specific for monitoring chiral purity of levobetaxolol.

REFERENCES

[1] H.Bishoff, *Eur. Clinical investigation* .24(suppl.3) (**1994**)3
[2] P.J.Lefebvre, J.Scheen, *Eur.J.Clin.Invest*.3 (Suppl.3) (**1994**)40.
[3] W.Puls,U.Keup,H.P.Krause, G.Thoamas,F.Halfmiester, *Naturwissenschaften* 64(**1977**)536
[4] The Merck Index. X111th edition Page no 201(1186)
[5] A.A Gordus, Theory and Problems of Analytical Chemistry, Schaums Outline Series, McGraw Hill, **1997**, pp. 22-30.
[6] Parris, N.A; Instrumental Liquid Chromatography. Elsevier, Amsterdam, Chhapte –4, **1976**.
[7] USP -31 2008 Page no 628.
[8] "Pharmaceutical Process Validation", Second edition, Ira Berry and Robert Nash,Marcel Decker,New York (**1993**
[9] Henry, R.A.; Modern Practice of Liquid Chromatography. (Ed.J.J.Kirkland) Wiley-Interscience, NY, Chapter – 1, **1971**
[10] International conference on Harmonization, Guidelines on Validation of Analytical Procedures;Methodology, Federal Register **1996**,61,59-62
[11] Done, J. N.; Practical High Performance Liquid Chromatography. (Ed.C.F.Simpson), Heyden,