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Research Article

Development and Validation of Rp-Chiral Method for Quantification of (R)-Isomer in Lercanidipine Hydrochloride

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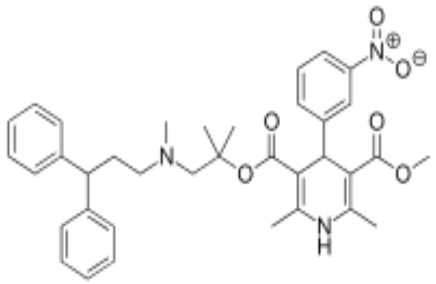
Abstract: ***Objective:*** The main objective of present study was to develop and validate a reverse phase chiral high performance liquid chromatographic method for R & S enantiomeric separation of Lercanidipine hydrochloride in API as well as tablet dosage form. ***Methods:*** The R-Isomer of Lercanidipine Hydrochloride was resolved on a Chiral OJ-H (150 x 4.0mm, 5um) column using mobile phase system containing 10mM Ammonium acetate and Acetonitrile in the ratio of (35:65 v/v). The mobile phase was set flow rate of 1.0ml and the volume injected was 10ul for every injection. The detection wavelength was set at 240nm. ***Results:*** The proposed method was productively applied for the quantitative determination of (R)-Isomer in Lercanidipine hydrochloride in API as well as tablet dosage form. The linear regression analysis data shows good linear relationship over a concentration range 0.5ug/ml to 4ug/ml for (R)-Isomer. The mean value of the correlation coefficient was 0.998 for (R)-Isomer. The method was validated as per the ICH guidelines. The detection limit (LOD) was about 0.05ug/ml and quantisation limit (LOQ) was about 1.0ug/ml for (R)-Isomer. The relative standard deviation was found to be 2.0% for (R)-Isomer of Lercanidipine Hydrochloride. ***Conclusion:*** The method is specific, rapid, precise and accurate for the separation and determination of (R)-isomer in (S) - Lercanidipine hydrochloride in API & tablet dosage form. The developed and validated HPLC method and the statistical analysis showed that the method is repeatable and selective for when compared to earlier published research articles for the estimation of (R)-Isomer of the Lercanidipine Hydrochloride drugs substances as well as in tablet dosage form.

Keywords: CHIRAL HPLC (R)- & (S)-Lercanidipine Hydrochloride.

INTRODUCTION



Lercanidipine (Figure 1)

The chemical name of Lercanidipine (LCD) is a 2[(3, 3-Diphenylpropyl) (methyl) amino]-1, 1-dimethylethyl methyl 2, 6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate and its empirical formula is $C_{36}H_{41}N_3O_6$ and Molecular weight is 651.21.

Lercanidipine is a yellow powder, which is soluble in methanol. The antihypertensive activity of lercanidipine is mainly due to its (S)-enantiomer. Lercanidipine is used for the treatment of essential hypertension (high blood pressure). It belongs to the dihydro - pyridine class of calcium channel blockers, which work by relaxing and opening the blood vessels allowing the blood to circulate more freely around the body.

LER has a chiral carbon atom in position 4 of the dihydropyridine ring (Figure 2 & 3). It is commercially available as racemic mixture of (R)- and (S)-enantiomer. The pharmacological effects of the enantiomers of LER essentially resides in the (S)-enantiomer. Studies in vitro showed that (S)-enantiomer has about 100-200 times higher affinity for calcium channels than the (R)-enantiomer. Consequently, the pharmacological effects of LER are mainly related to the (S)-enantiomer.

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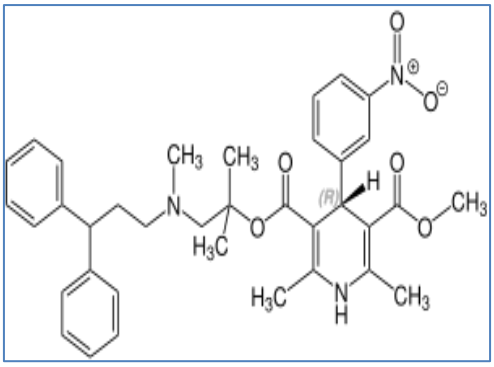
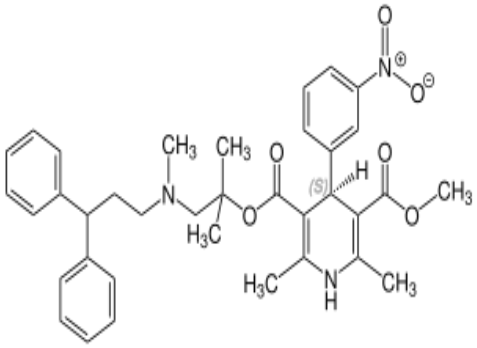


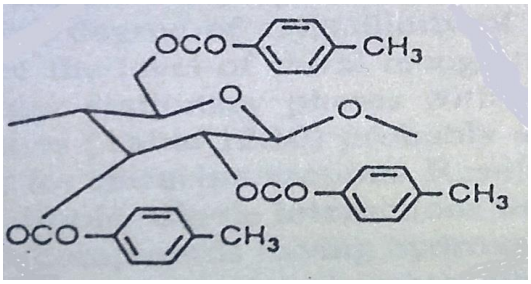
R-Lercanidipine (Figure 2)



S-Lercanidipine (Figure 3)

Since the fact that many enantiomers of recamic drugs may exhibit difference in pharmacological effects, there is a need for the development of enantioselective methods.

The selection of stationary phase is necessary for the separation to chiral analytes. OJ-H column is useful for separation of dihydropyridine class compound because it has a cellulose tri (4-methyl benzoate ring which form complex compound with dihydropyridine ring of LER.



[Chiral Cel OJ: - cellulose tri (4-methyl benzoate)] (Figure 4)

The literature survey reveals various HPLC methods were developed for analysis of LER not only in pharmaceutical formulations but also in biological fluids. No method has been cited in the literature for analysis of R-LER enantiomer by RP-HPLC.

In this paper, a simple, rapid, accurate stable and selective reverse phase HPLC method for the determination of R-LER enantiomer in API and Tablets dosage forms using OJ-H column. HPLC conditions were optimized and validated according to International Conference on Harmonization (ICH).

EXPERIMENTAL METHODOLOGY

Chemicals and reagents:

LER, active pharmaceutical ingredient (API), was supliied by Vivan Life Science, Mumbai and commercial tablets were purchased from the local market containing 20 mg of mixture racemic of LER per tablets. Acetonitrile (HPLC) and Ammonium Acetate (AR) were supplied by Rankem. Water was purified using Direct- Elix system from Milipore. All other chemicals employed were analytical or LC grade or other unless otherwise mentioned.

Instrumentation:

Waters Alliance HPLC system equipped with PDA detector and EMPOWER 2 software was used to monitor the data acquisitions and other proceedings. Chiral OJ-H (150mm X 4.6mm, i.d., 5µ) was used as stationary phase.

Sample Preparation

Racemic mixture solution of Lercanidipine and (R) – enantiomer (100ug/ml each) prepared in methanol was used in method development.

Stock solutions of (S)-Lercanidipine (0.1mg/ml) and (R)-Lercanidipine (0.1mg/ml) were prepared by dissolving an appropriate amount of the substance in methanol. The analyte concentration of Lercanidipine was fixed as 100ug/ml. Lercanidipine solutions spiked with low levels of (R)-enantiomer were prepared by transferring calculated amount of undesired enantiomer stock solution with a pipette into the calculated amount of Lercanidipine HCl stock solution, and then the solution was added to volume with methanol.

VALIDATION OF THE METHOD:

The method was validated for LER enantiomer analysis in standard solutions, API and tablet samples. Before starting the validation study, the conformity system was conducted. The system suitability test was also performed to evaluate the repeatability of the system. So, 6 replicate injection of standard solution containing 100ug/ml of LER (racemic mixture) in the optimized analytical condition were performed.

Table 1: Method Validated

Sr. No.	Ret. Time of (R)-Enantiomer	Plates of (R)-Enantiomer	Tailing of (R)-Enantiomer	Resolution between both Enantiomer
1	6.067	7116	1	1.8
2	6.067	6995	1	1.7
3	6.067	7014	1	1.8
4	6.071	7167	1	1.8
5	6.07	7122	1	1.8
6	6.073	6972	1	1.8
Avg.	6	7064	1	1.78
SD	0	81	0	0
%RSD	0.04	1.14	0	2.29

Detector response linearity was assessed by preparing six calibration sample solutions of (R)-enantiomer co-overing from 1.0ug/ml (LOQ) to 4ug/ml (0.5, 1.0, 1.5, 2.5, 3.0, and 4ug/ml) in methanol. The regration curve was obtained by plotting peak area versus concentrations, using the least square method. The percentage relative standard deviation of the slope and y-intercept of the calibration curve was calculated.

Table 2: Linearity of R-Lercanidipine HCl

S. No.	Concentration (µg/ml)	R-Isomer peak *(n = 6)
1	0.50	7966
2	1.00	13545
3	1.50	18325
4	2.00	23279
5	2.50	27315
6	3.00	32464
Correlation coefficient	0.9983	
Slope	4,821.5810	
Y-intercept	3,606.8	

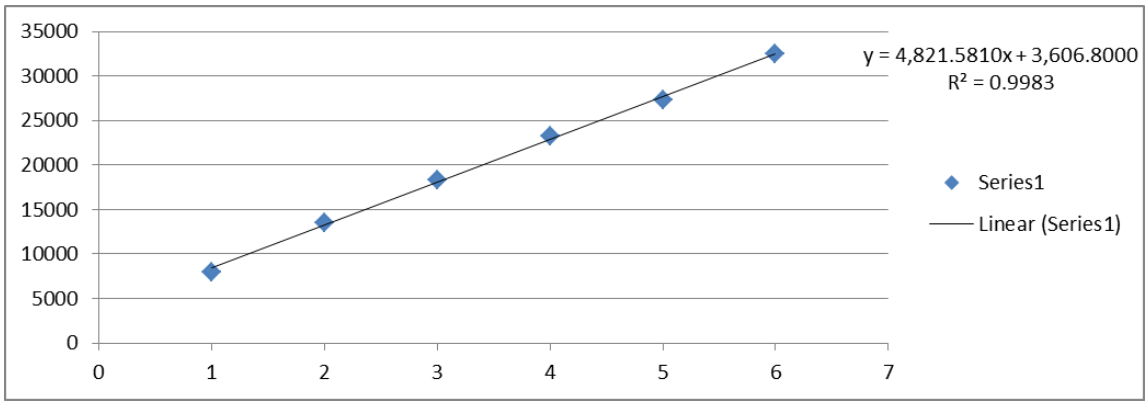


Fig.1

The limit of detection (LOD) and limit of Quantification (LOQ) for (R)-enantiomer was achieved by injecting a series of dilute solutions of by using standard deviation slope method (ICH Q2 (R1). The LOQ level precession of the developed chiral method for (R)-enantiomer was checked by analysing six solutions of (R)-enatiomer prepared at LOQ level and calculating the percentage relative standard deviation of the area.

The specificity of the method is performed by injecting both isomers and recemic mixture individually. There is no interference of placebo and process impurities.

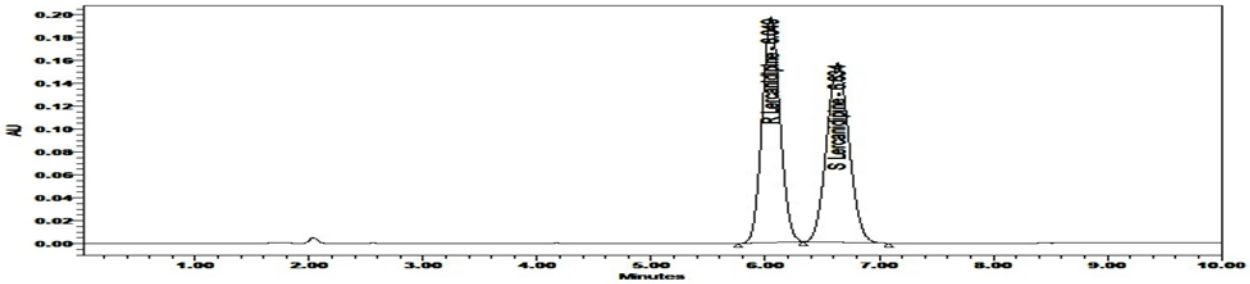


Fig.2

Method reproducibility was determined by measuring repeatability and intermediate precision of retention times and peak area for each enantiomer. The repeatability of the method was determined by analysing six replicate injections containing Lercanidipine HCl (100ug/ml) spiked with (R)-enantiomer (1.5, 2.0 & 2.5 ug/ml). The intermidate precision was determined over 2d by performed six successive injections (n=6) each day and also perfomed intermidate precision with different system, different analyst and with a different column by using six injections (n=6).

Table-3.

R- Isomer	
1	7116
2	6995
3	7014
4	7167
5	7122
6	6972
Average	7064
SD	81
%RSD	1.14

Table-4: Recovery amount

Added amount (ug)	**Peak area**	**Recover amount**	**Recovery (%)**	**% RSD (n=3)**
0.8	10151	0.8003	100.04	0.43
1	12658	0.998	99.8	0.45
1.2	15238	1.2015	100.12	0.05

The accuracy of the method was carried out by injecting a known concentration of (R)-enantomer to the Lercanidipine HCl. The accuracy was calculated in terms of recovery (%). The study was carried out in triplicate at covering from LOQ to 2.5ug/ml (1.5, LOQ & 2.5ug/ml) in methanol. The recovery of (R)-enantiomer was calculated by back-calculated concentration at each level in each preparation. The recovery is not less than 99.2% and not more than 102.8%. The recovery results are given in table 4.

Table 5: Ruggedness

Name of the Interval	*** %RSD (n = 6)**
Day-1	0.3
Day-2	0.4
Day-3	0.5
Diff system	0.7
Diff column	0.6
Diff analyst	0.2

As per ICH, the method robustness studies were demonstrated by adjusting flow rate, column temperature and mobile phase composition variations. The chromatographic resolution of Tenofovirdisoproxil fumarate and (S)-enantiomer was more than 2.0 under

all separation conditions. The robustness results were captured in table 6.

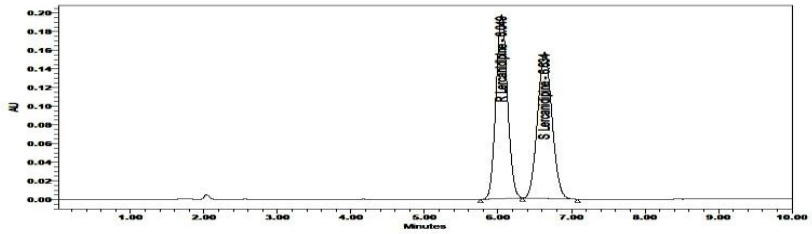
Table 6: Robustness

Description	USP tailing	USP Tangent	USP Resolution
Column flow: 0.80 ml/min	1.0	3829	2.5
Column flow: 1.20 ml/min	1.0	4362	2.2
Column Temp: 25˚C	1.1	4125	2.3
Column Temp: 35˚C	1.0	3829	2.3
Organic ratio: 110%	1.0	3242	2.1
Organic ratio: 90%	1.1	4457	2.3

The solution stability of Lercanidipine HCl at analyste concentration was studied by keeping the solution in a tightly capped volumetric flask at room temperature on a laboratory bench for 48hrs. The content of (R)-enantiomer was checked at 6hrs interval up to the study period. Mobile phase stability was carried out by evaluating the content of (R)-enantiomer in Lercanidipine HCl sample solutions prepared freshly at 6hrs interval of 48hrs. The same diluents were used during the study period.

RESULTS AND DISCUSSION

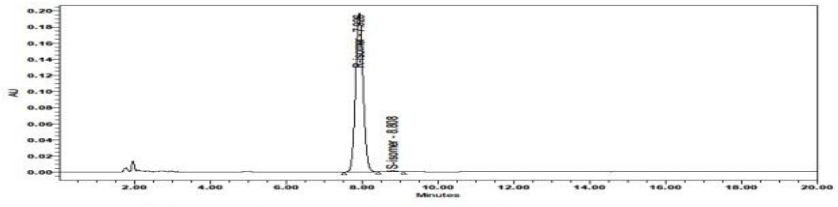
To develop a rugged and suitable reverse phase HPLC method for the separation of the two enantiomers, different stationary phases and mobile phases were employed. Chiral OJ-H column (150 × 4.0 mm, 5 µm) with a mobile phase consisting of 10mM ammonium acetate in water and Acetonitrile (35:65v/v) was used. Based on the data obtained from the method development and optimization activities, Chiral OJ-H (150 × 4.0 mm, 5 µm) column with a mobile phase of 10mM ammonium acetate in water and Acetonitrile (35:65 v/v) was selected from the method development. The flow rate of the final method was 1.0 ml/min with injection volume 10 µl. The column temperature was 40˚C, and the detection wavelength was 240 nm. Under these conditions, the two enantiomers were separated well and the peak of (R)-Lercanidipine eluted before the peak of (R)-Larcanidipine. In the optimized method, the typical retention time of (R)-Lercanidipine and (S)-Lercanidipine were 6.0 and 6.6 min respectively. The final optimized method was productively applied to separate (R)-isomer from Lercanidipine HCl and was proven to be reproducible and accurate for the quantitative determination of (R) - & (S)-isomer in Lercanidipine HCl drug substance as well as tablet dosage form.



	Peak Name	RT	Area	% Area	Height	USP Plate Count
1	R Lercanidipine	6.049	2142996	50.02	197349	6983
2	S Lercanidipine	6.634	2141695	49.98	156944	5328

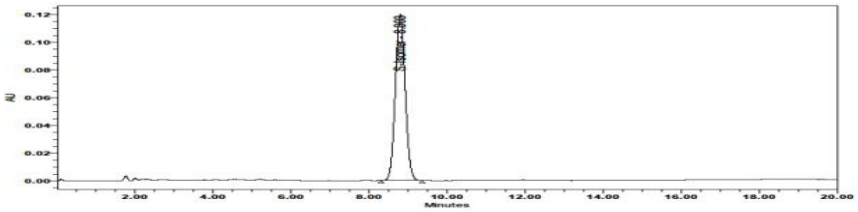
	USP Resolution	USP Tailing
1		1.1
2	1.8	1.1

Fig.3: Lercanidipine racemic



	Peak Name	RT	Area	% Area	Height
1	R-isomer	7.926	2735559	99.22	196900
2	S-isomer	8.808	21402	0.78	1217

Fig. 4: (R)-Isomer 0.1 mg/ml solution



	Peak Name	RT	Area	% Area	Height
1	S-Isomer	8.803	2093065	100.00	120144

Fig. 5: (S) Isomer 0.1 mg/ml solution

CONCLUSION

A simple, specific, linear, accurate and precise reverse phase chiral HPLC method was successfully developed, which was capable of separating the undesired enantiomer from Lercanidipine. chiral OJ-H column was found to be selective for the enantiomers of Lercanidipine HCl. The developed and validated method can be used for the chiral purity testing of Lercanidpine HCl. The developed method is also stable and can be used for the quantitative determination of chiral impurity in Lercanidipine HCl drug substance as well as tablet dosage form.

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