Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 4, 2013

Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE CHROMATOGRAPHIC PURITY OF LERCANIDIPINE HYDROCHLORIDE IN TABLET DOSAGE FORM

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Received: 09 July 2013, Revised and Accepted: 20 Aug 2013

ABSTRACT

Objective: To simple, precise and accurate method and to perform validation for the chromatographic purity of Lercanidipine hydrochloride in tablets.

Methods: An isocratic HPLC analysis was developed on Waters Xterra RP18 column (150 cm x 4.6 mm, 5µ). The compound was separated with the mixture of potassium phosphate buffer pH 3.0 containing 0.1% triethylamine; the pH of the buffer solution was adjusted with ortho phosphoric acid and acetonitrile in the ratio of 55:45 v/v as the mobile phase at flow of 1.0 mL per minute. UV detection was performed at 240 nm using photodiode array detection.

Results: The retention time was found to be 9.2 minute. The system suitability parameters such as theoretical plate count, tailing and percentage RSD between six standard injections were within the limit. The method was validated according to ICH guidelines. Calibrations were linear over the concentration range of 0.04-150 µg per mL as indicated by correlation coefficient (r) of 0.999. The robustness of the method was evaluated by deliberately altering the chromatographic conditions.

Conclusion: The developed method can be applicable for routine quantitative analysis of Lercanidipine tablets in pharmaceutical formulations.

Keywords: Lercanidipine, Chromatographic purity, Validation.

INTRODUCTION

Lercanidipine hydrochloride, a calcium channel blocker, which is chemically 2[(3,3-diphenyl propyl)methylamino]-1,1-dimethylethylmethyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro pyridine-3,5-dicarboxylate (Fig. 1). Its molecular formula is $C_{36}H_{41}N_3O_6HCl$ and molecular weight 648.19 g/mol. Lercanidipine hydrochloride is used for treatment of angina pectoris and hypertension. It inhibits cellular influx of calcium leading to the maintenance of the plateau phase of the action potential [1].

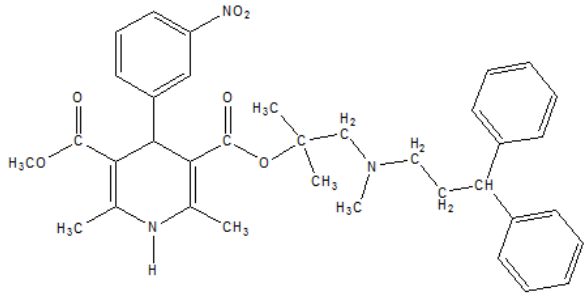

Fig. 1: Chemical Structure of Lercanidipine hydrochloride

There are few references to the analysis of Lercanidipine hydrochloride and its impurities in pharmaceutical dosage forms [2] and UV spectrophotometric determination of Lercanidipine hydrochloride in bulk and tablet are reported [3]. The development and validation of high performance liquid chromatographic method for estimation of Lercanidipine in rabbit serum [4] and determination of Lercanidipine and its impurities using DryLab software also reported [5]. Only one HPLC method reported in the literature for the assay determination of Lercanidipine hydrochloride in bulk and pharmaceutical dosage form [6].

The objective of this work was to develop and validate a stability-indicating liquid chromatographic analytical method for assay of Lercanidipine hydrochloride in tablet formulation. The tablets analysed in this work contain 10 mg Lercanidipine hydrochloride. The validation procedure followed the guidelines of ICH [7] and USP 35 [8].

MATERIALS AND METHODS

Instruments

HPLC system (Waters e2695 with 2998 PDA detector, USA) equipped with auto sampler with degasser and column component having temperature control was used. Column used for the analysis was Waters Xterra RP18, (150 mm x 4.6 mm), 5µmwere used. Chromatographic data acquired by Empower 3 software. Analytical balances (Mettler Toledo), Cyberscan pH meter, Grant sonicator, UV spectroscopy (Shimadzu), and Centrifuge (Eppendorf) were used.

Chemicals and reagents

All reagents and solvents were of analytical and HPLC grade including Methanol (HPLC-grade), Potassium dihydrogen phosphate, triethylamine, hydrochloric acid, ortho phosphoric acid (85%), sodium hydroxide and hydrogen peroxide were from Merck Laboratories, Mumbai. Water was purified by a Millipore (Bedford, MA, USA) Milli-Q water purification system and passed through a 0.45 µ membrane filter (Millipore) before use. Lercanidipine hydrochloride drug substance and Lercanidipine hydrochloride tablets 10 mg were available from Kemwell Biopharma Pvt Ltd., Bangalore, India. Standard and test samples were prepared in water and methanol in ratio of 30:70 as diluent.

Buffer preparation

Potassium dihydrogen phosphate (1.36 g) was dissolved in1000 mL of Milli-Q water, added 1.0 mL of triethylamine and adjusted the pH to 3.0 with O-phosphoric acid. It was filtered through 0.45 lm nylon membrane filter and degassed.

Method

Selection of wavelength

Accurately weighed and transferred about 10 mg of Lercanidipine Hydrochloride into a 100 mL volumetric flask, added about 70 mL of mobile phase and sonicated to dissolve the material completely. Diluted to volume with mobile phase and mixed well. The UV-spectra of Lercanidipine hydrochloride (Fig. 2) shows absorption bands at 238.2 nm and 358.1 nm. For analytical purposes the wavelength for the photodiode array detector was set at 240 nm which presents better reproducibility than the other UV-bands.

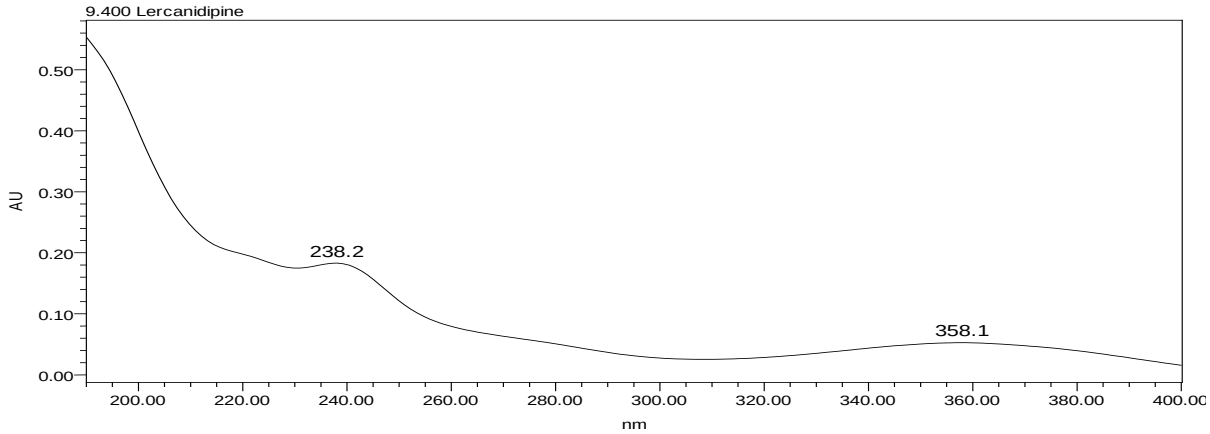

Fig. 2: UV absorbance spectrum of Lercanidipine hydrochloride

Chromatographic conditions

Chromatographic separation was achieved at ambient column oven temperature (25ºC), the detection was carried at 240 nm at a flow rate of 1.0 mL/min and run time was kept at 30 minute. Prior to the injection of drug solution, the column was equilibrated for 30 minutes with the mobile phase flowing through the system. The injection volume was 20 µL. Blank containing the mobile was injected to check the solvent interference.

Standard preparation

Accurately weighed and transferred about 25 mg of Lercanidipine Hydrochloride into a 50 mL volumetric flask, added about 35 mL of mobile phase and sonicated to dissolve the material completely. Diluted to volume with mobile phase and mixed well. Transferred 5.0 mL of the above standard stock solution in a 25 mL volumetric flask, diluted to volume with mobile phase and mixed well to get the final concentration of 100 µg/mL. A representative chromatogram of the standard was shown in Fig. 3.

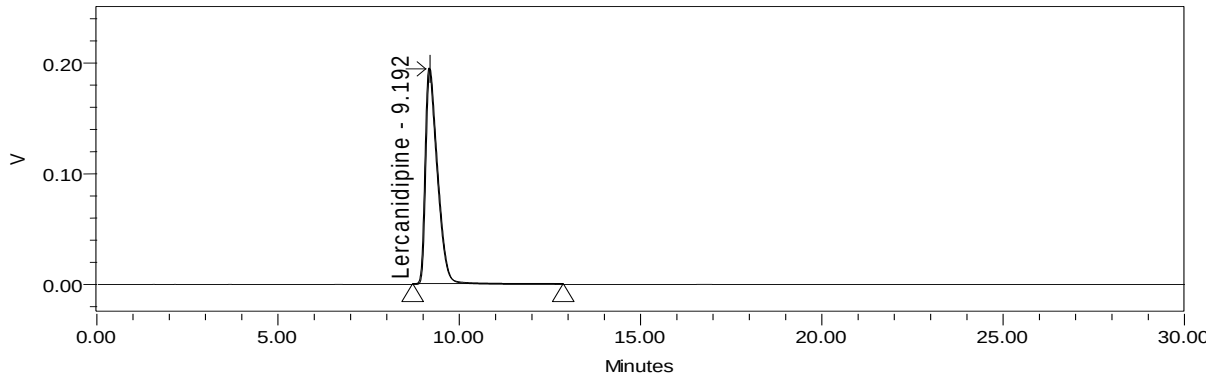

Fig. 3: Standard chromatogram

Sample preparation

Weighed 20 tablets and recorded the average weight. Crushed the tablets into a fine powder. Weighed and transferred the powder equivalent to about 10 mg of Lercanidipine hydrochloride into a 100 mL volumetric flask. Added about 70 mL of mobile phase and sonicated for 15 minutes with intermediate shaking. Diluted to volume with mobile phase and mixed well. Centrifuged a portion of the solution at 2500RPM for about 10 minutes in a capped centrifuge tube. Filtered a portion of the solution through 0.45 µm Nylon filter by discarding the first few mL of the filtrate. A representative chromatogram of the sample was shown in Fig. 4.

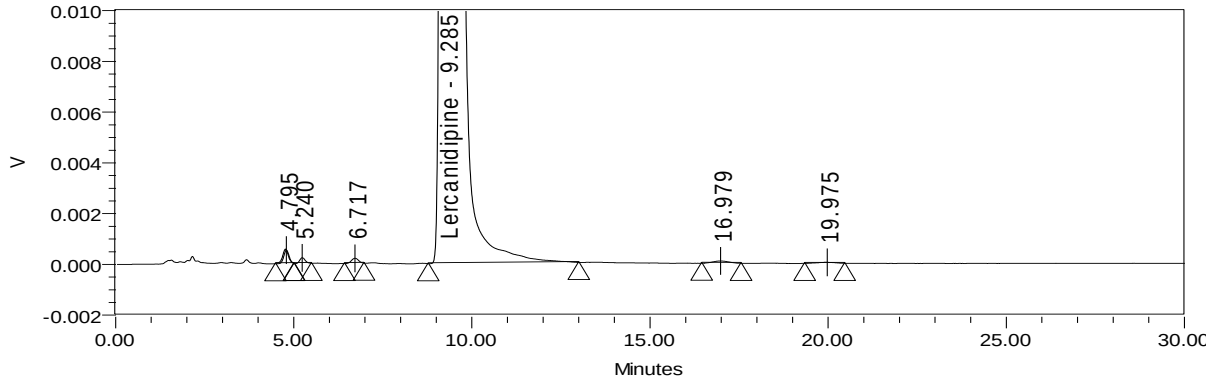

Fig. 4: Test chromatogram

Evaluation of system suitability

20µL of standard solution was injected in six duplicate at the start of the analysis and the chromatograms were recorded. System suitability parameters like column efficiency (plate count) and tailing factor were also recorded. The column efficiency determined was found to be more than 2000 USP plate count, USP Tailing for the same peak is not more than 2.0 and % RSD of six injection of the standard solution is not more than 2.0%. A representative chromatogram of system suitability was shown in Fig. 5 and result summarized in Table 1.

Analytical method validation

Specificity

Placebo solution was prepared as per the test solution using equivalent weight of the placebo in a portion. Placebo solution was injected into the HPLC system following the test conditions, the chromatogram was recorded and measured the responses of the peaks were noted for any interference of the excipient at the retention time of Lercanidipine hydrochloride (Fig. 6).

Table 1: System suitability study

Injection	Retention Time	Peak area	USP plate count	USP tailing factor
1	9.192	4482268	3497	1.7
2	9.311	4471981	3499	1.7
3	9.301	4464658	3499	1.7
4	9.299	4483185	3497	1.7
5	9.298	4464545	3493	1.7
6	9.295	4482091	3491	1.7
Mean	9.283	4474788	3496	1.7
SD	0.045	8890.463	-	-
% RSD	0.5	0.2	-	-

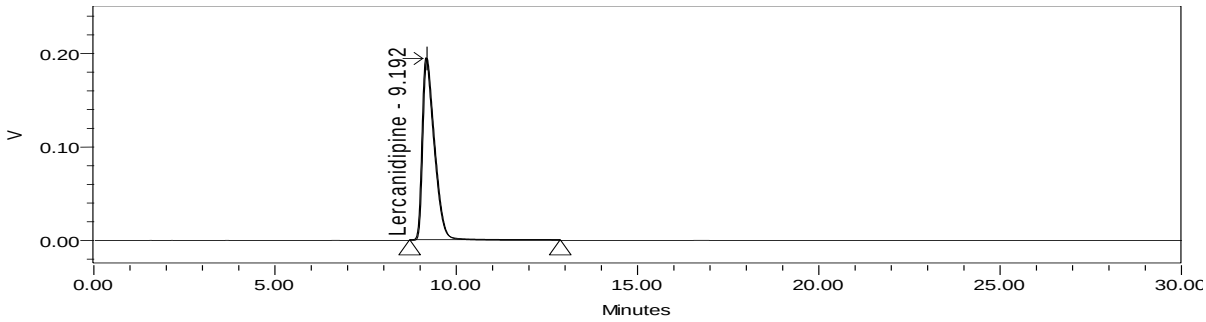

Fig. 5: System suitability chromatogram

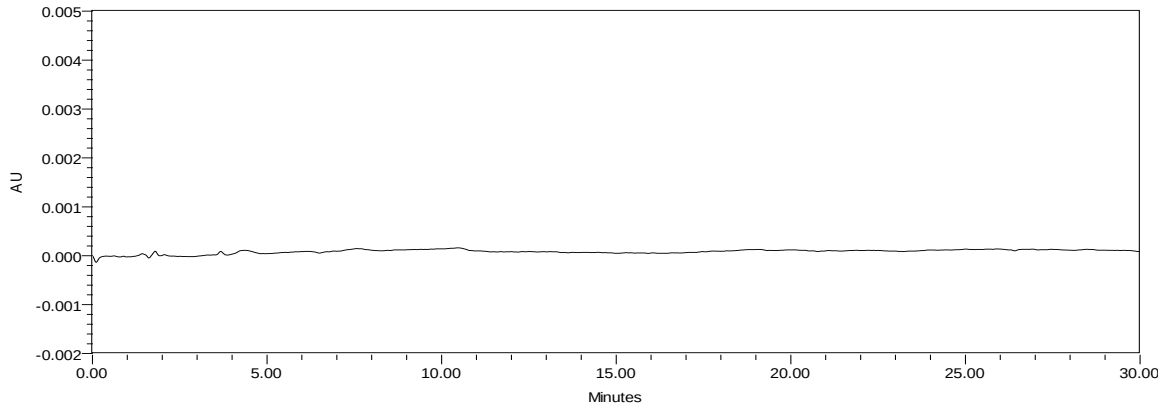

Fig. 6: Placebo (specificity) chromatogram

A forced degradation study was carried out on Lercanidipine hydrochloride tablets and its placebo under hydrolytic, oxidative, thermal and photolytic conditions. Using peak purity test, the purity of Lercanidipine peak was checked at every stage of the degradation study. The peak purity plots show that the Lercanidipine peak is homogeneous and has no co-eluting peaks indicating that the method is stability indicating and specific. The peak purity plots of unstressed and stressed samples are shown in figures 7 to 12. The results obtained from forced degradation studies are summarized in Table 2.

Table 2: Forced degradation study

Mode of degradation	Stress condition	% Degradation	Purity angle	Purity threshold	Purity Flag
Control	NA	0.37	0.013	0.212	No
Acid Stress Test	1N HCl 80°C for 2 hrs.	1.50	0.013	0.211	No
Base Stress Test	1N NaOH 80°C for 15 minutes	6.45	0.032	0.323	No
Peroxide Stress Test	3 % H_2O_2 80°C for 30 minutes	1.05	0.025	0.253	No
Thermal Stress Test	105°C / 12 hrs.	1.59	0.015	0.208	No
Photolytic Stress Test	1.2 million lux hrs / 200 watt hrs /square meter	2.70	0.013	0.207	No

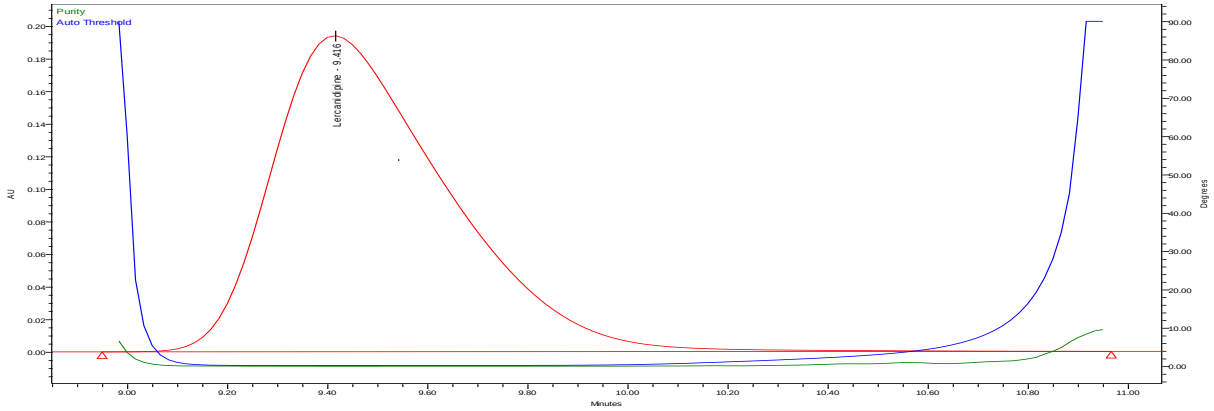

Fig. 7: Purity plot of unstressed sample

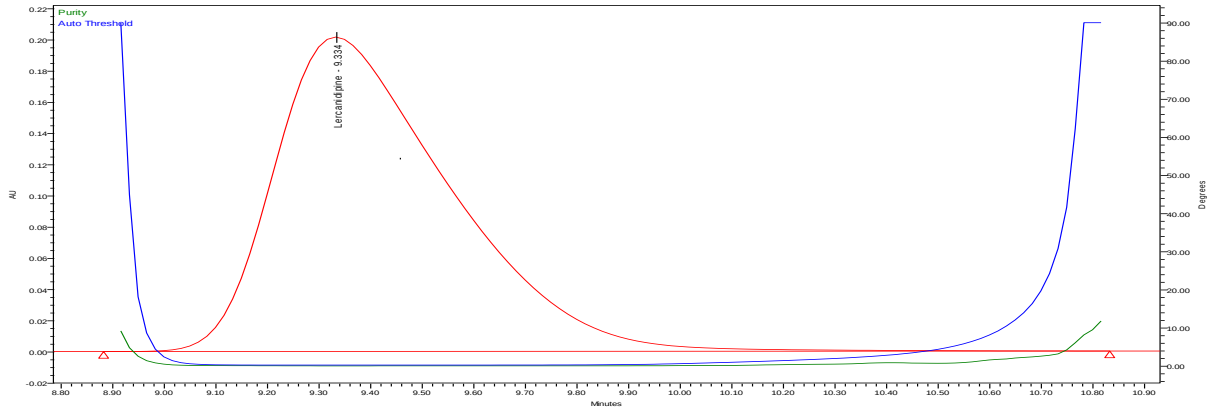

Fig. 8: Purity plot of Acid stressed sample

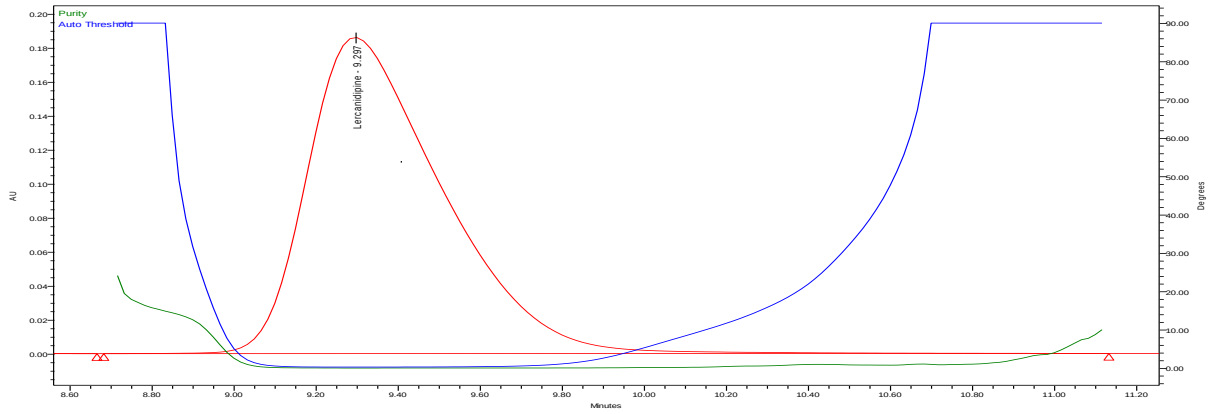

Fig. 9: Purity plot of Base stressed sample

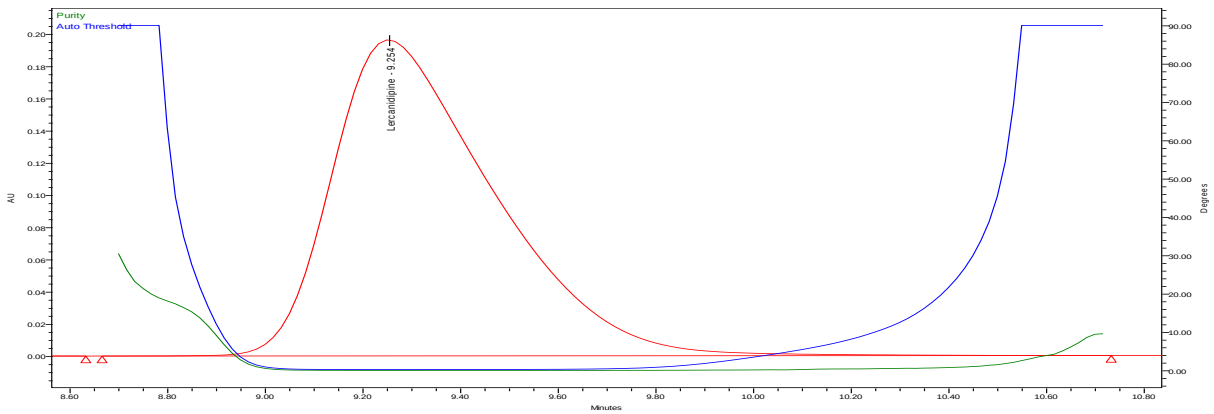

Fig. 10: Purity plot of Peroxide stressed sample

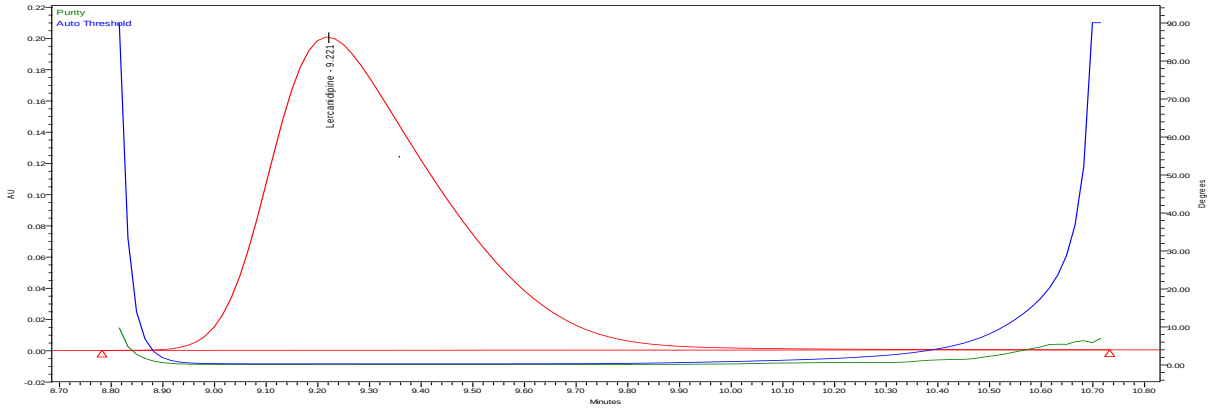

Fig. 11: Purity plot of Thermal stressed sample

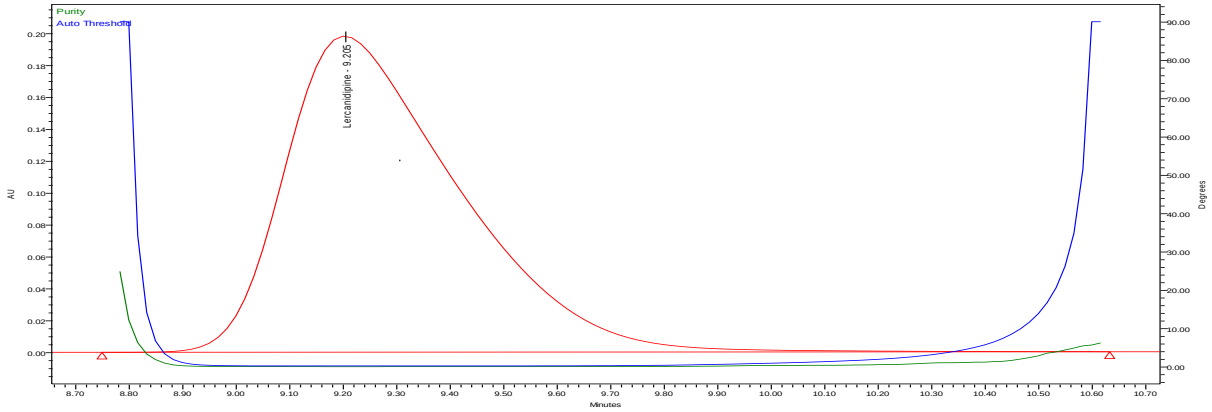

Fig. 12: Purity plot of Photolytic stressed sample

Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application (system precision) was carried out using six replicates of the standard injection (100 µg/mL). Repeatability of sample measurement (method precision) was carried out in six different sample preparations from the same homogenous blend of the sample (100 µg/mL). The RSD for repeatability of standard preparation was 0.2% whereas the RSD for repeatability of the sample preparation was 0.0%. This shows that the precision of the method is satisfactory as RSD is not more than 2.0 %. Representative chromatograms of Precision as shown in Figs. 13, 14 and result summarized in Table 3.

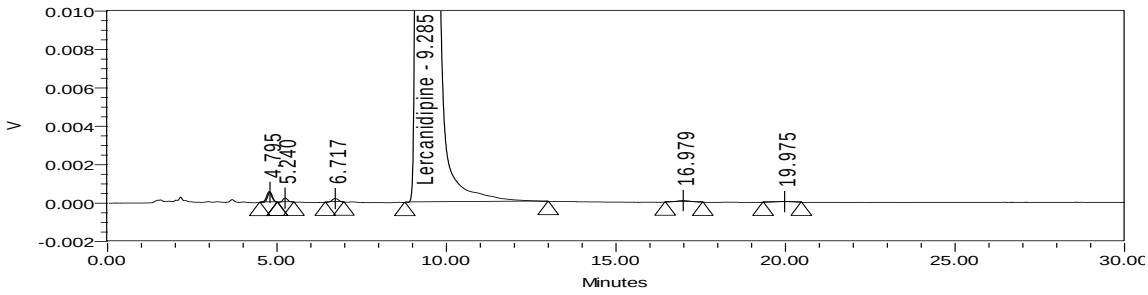

Fig. 13: Precision chromatogram

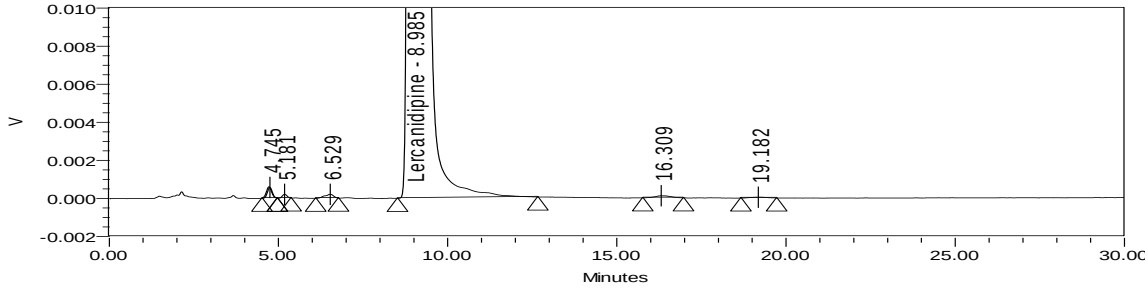

Fig. 14: Intermediate precision chromatogram

Table 3: Precision study

Sample No.	Chromatographic purity (%) for Method precision	Chromatographic purity (%) for Intermediate precision
1	99.68	99.65
2	99.68	99.66
3	99.67	99.68
4	99.68	99.68
5	99.68	99.68
6	99.68	99.52
Mean	99.68	99.65
SD	0.004	0.063
% RSD	0.00	0.06
Overall Mean	99.66	
Overall SD	0.046	
Overall % RSD	0.05	

Limit of Detection and Limit of quantification

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

The Limit of Detection and Limit of quantification of Lercanidipine hydrochloride was determined by preparing and injecting a series of solutions which will give a signal-to-noise ratio of about 10 for limit of quantification and a signal-to-noise ratio of about 3 for limit of detection. Precision at the Limit of quantification was determined in terms of repeatability of application and measurement. The result summarized in Table 4 and 5.

Linearity

The linearity of Lercanidipine hydrochloride was determined by preparing and injecting a series of solutions with a concentration of about limit of quantification to 150% of test concentration (0.04–150µg/mL). The calibration curve indicates the response is linear over the concentration range studied for Lercanidipine hydrochloride with a correlation coefficient (r) of 0.999. The calibration curve as shown in Fig 15 and result summarized in Table 6.

Table 4: Limit of Detection and Limit of quantification study

S No.	Parameter	Concentration (µg/mL)	signal-to-noise ratio	Peak Area response
1	Limit of Detection	0.013	3.2	532
2	Limit of quantification	0.040	10.3	1668

Table 5: Precision at Limit of quantification study

Injection Number	Peak Area response
1	1623
2	1861
3	1770
4	1721
5	1706
6	1875
Mean	1759
SD	96.680
% RSD	5.5

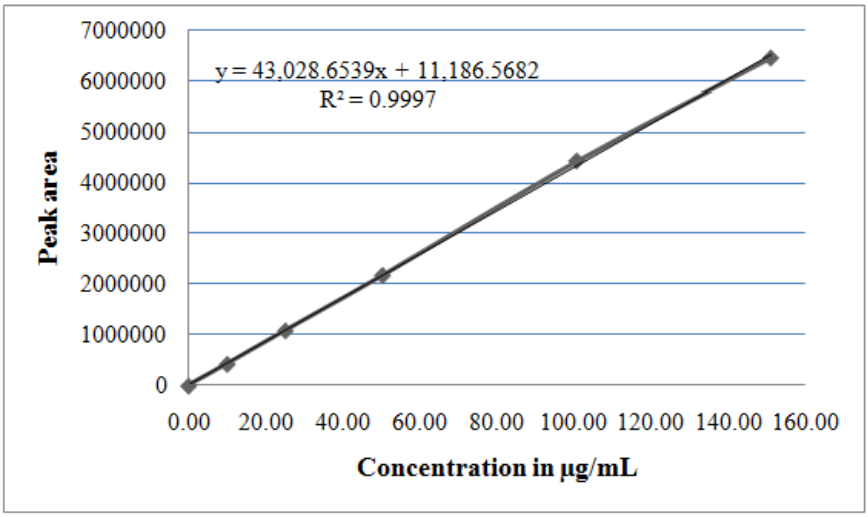

Fig. 15: Calibration curve

Table 6: Linearity study

Solution No.	% level	Actual Concentration (µg/mL)	Peak Area response
1	LOQ	0.0403	1782
2	10	10.0750	429892
3	25	25.1875	1090061
4	50	50.3750	2179040
5	100	100.7500	4432898
6	150	151.1250	6457889
Regression Coefficient (R^2)			0.9997
Slope			43028.6539
Intercept			11186.5682
% deviation of the Y- intercept			0.3

Accuracy

The percentage recovery experiments were performed by adding a known quantity of pure standard drug into the placebo sample. The placebo sample was spiked with standard at different levels namely at the limit of quantification, 50%, 100% and 150% of test concentration. The resulting spiked sample solutions were assayed in triplicate and the results were compared and expressed as percentage. The mean percentage recovery of Lercanidipine hydrochloride was found to be in the range between 90.0 and 107.5 which are within the acceptance limits as shown in Table 7.

Range

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.

The calibration curve indicates the response is linear over the concentration range of limit of quantification to 150% of test concentration (0.04–150µg/mL) for Lercanidipine hydrochloride with a correlation coefficient (r) of 0.999.

The accuracy study was performed at different levels namely at the limit of quantification, 50%, 100% and 150% of test concentration. The mean percentage recovery of Lercanidipine hydrochloride was found to be in the range between 90.0 and 107.5 which are within the acceptance limits. Precision was measured in terms of repeatability of application and measurement. Repeatability of sample measurement was carried out on six different sample preparations at the Limit of quantification and at 150% of test concentration. The RSD for repeatability of the sample preparation at the Limit of quantification was 6.1% and at 150% of test concentration was 0.3%. This shows that the precision of the method is satisfactory as RSD is not more than 10.0% at the Limit of quantification and not more than 2.0 % at 150% of test concentration. The results are summarized in Table 8, 9 and 10.

Table 7: Recovery study

Accuracy Level	Amount added (mg)	Amount found (mg)	%Recovery	Average % Recovery	%RSD
Accuracy solution LOQ-1	0.004	0.0036	90.0	97.5	6.1
Accuracy solution LOQ-2	0.004	0.0038	95.0		
Accuracy solution LOQ-3	0.004	0.0039	97.5		
Accuracy solution LOQ-4	0.004	0.0040	100.0		
Accuracy solution LOQ-5	0.004	0.0043	107.5		
Accuracy solution LOQ-6	0.004	0.0038	95.0		
Accuracy solution 50%-1	5.038	4.947	98.2	98.3	0.1
Accuracy solution 50%-2	5.038	4.960	98.5		
Accuracy solution 50%-3	5.038	4.953	98.3		
Accuracy solution 100%-1	10.075	10.056	99.8	100.0	0.2
Accuracy solution 100%-2	10.075	10.061	99.9		
Accuracy solution 100%-3	10.075	10.101	100.3		
Accuracy solution 150%-1	15.113	14.737	97.5	97.6	0.3
Accuracy solution 150%-2	15.113	14.721	97.4		
Accuracy solution 150%-3	15.113	14.744	97.6		
Accuracy solution 150%-4	15.113	14.830	98.1		
Accuracy solution 150%-5	15.113	14.740	97.5		
Accuracy solution 150%-6	15.113	14.760	97.7		

Table 8: Linearity study

Regression Coefficient (R^2)	0.9997
Slope	43028.6539
Intercept	11186.5682
% deviation of the Y- intercept	0.3

Table 9: Accuracy study

Accuracy Level	Average % Recovery
LOQ	97.5
50 %	98.3
100 %	100.0
150 %	97.6

Table 10: Precision at Limit of quantification and at 150% of test concentration study

Sample Number	%Recovery	
	At Limit of quantification	At 150% of test concentration.
1	90.0	97.5
2	95.0	97.4
3	97.5	97.6
4	100.0	98.1
5	107.5	97.5
6	95.0	97.7
Mean	97.5	97.6
SD	5.916	0.256
% RSD	6.1	0.3

Stability of standard and sample solutions

The standard solution and sample solutions were prepared as per the test method, and analysed initially at different time intervals by keeping the solution at room temperature. The % difference in peak area from initial and different time intervals for standard and % difference in chromatographic peak purity from initial and different time intervals for sample solution was calculated. It was observed that the standard and sample solutions were stable for at least 24 hours at room temperature (Table 11).

Robustness

Robustness of the method was determined by analysing the standard solution at normal operating conditions by changing some operating analytical conditions such as flow rate, column oven temperature, detection wavelength, pH and composition of the mobile phase etc. The conditions with variation and their results were shown in Table 12.The tailing factor and % RSD of five replicate injections of the standard was below 2.0 and theoretical plate counts were also above 2000.

Table 11: Stability study

Time in hours	Standard solution		Sample solution	
	Peak Area	% Difference from initial	Chromatographic purity (%)	% Difference from initial
0	4503363	NA	99.68	NA
1	4492815	0.2	99.67	0.01
2	4499441	0.1	99.68	0.00
4	4503748	0.0	99.68	0.00
8	4503745	0.0	99.68	0.00
12	4498167	0.1	99.68	0.00
16	4517121	-0.3	99.68	0.00
20	4525620	-0.5	99.69	-0.01
24	4536853	-0.7	99.69	-0.01

Table 12: Robustness study

Operating conditions	USP Tailing	Theoretical plates	% RSD	Chromatographic purity (%)	% Difference from control
Control (unchanged)	1.7	3499	0.1	99.65	NA
Variation in flow rate (0.8 mL/min)	1.8	3740	0.0	99.65	0.00
Variation in flow rate (1.2 mL/min)	1.6	3295	0.1	99.67	-0.02
Variation in column oven temperature (20 °C)	1.7	3499	0.1	99.65	0.00
Variation in column oven temperature (30 °C)	1.6	3612	0.1	99.61	0.04
Variation in organic content in mobile phase(- 2% absolute)	1.7	3621	0.0	99.68	-0.03
Variation in organic content in mobile phase (+ 2% absolute)	1.6	3428	0.0	99.64	0.01
Variation in pH of buffer (pH 2.8)	1.7	3469	0.1	99.69	-0.04
Variation in pH of buffer (pH 3.2)	1.7	3476	0.1	99.64	0.01
Variation in wavelength of detection (235 nm)	1.6	3488	0.1	99.67	-0.02
Variation in wavelength of detection (245 nm)	1.6	3488	0.1	99.68	-0.03

Filter validation

A study was conducted to verify the suitability of different filters for the filtration of the sample solution during sample preparation. A fraction of standard and test solutions were filtered through different filters (0.45 µm Nylon, 0.45 µm PVDF and 0.45 µm PTFE), and three sub-fractions were collected, by discarding 2 mL, 4 mL and 6 mL of solution passing through respective filters. All fractions of solutions were analysed. A fraction of sample solution was centrifuged and analysed as per the methodology. % chromatographic peak percentage of filtered samples was compared with the % chromatographic peak percentage of centrifuged sample solution to determine the selectivity of the filters. Area of Lercanidipine observed from the chromatograms of filtered standard solution was compared with the area of Lercanidipine observed from the chromatograms of unfiltered standard solution to determine the selectivity of the filters. The % difference in peak area percent from unfiltered and filtered solutions and the % recovery data shows that all the above mentioned filters are suitable for this method. Results obtained for filtered sub-fraction after discarding different volume of test solution and standard solution are summarised in table 13 and 14.

Table 13: Filter validation study for sample solution

Name	Peak area	Chromatographic purity (%)	% Difference from centrifuged solution
Unfiltered solution	4675358	99.67	NA
0.45µm Nylon filter 2 mL sub-fraction	4671716	99.67	0.00
0.45µm Nylon filter 4 mL sub-fraction	4677908	99.66	0.01
0.45µm Nylon filter 6 mL sub-fraction	4691713	99.68	-0.01
0.45µm PVDF filter 2 ml sub-fraction	4669480	99.67	0.00
0.45µm PVDF filter 4 mL sub-fraction	4671690	99.67	0.00
0.45µm PVDF filter 6 mL sub-fraction	4657800	99.68	-0.01
0.45µm PTFE filter 2 ml sub-fraction	4699881	99.68	-0.01
0.45µm PTFE filter 4 mL sub-fraction	4691418	99.69	-0.02
0.45µm PTFE filter 6 mL sub-fraction	4693181	99.67	0.00

Table 14: Filter validation study for standard solution

Name	Peak area	% Recovery
Unfiltered solution	4485303	NA
0.45µm Nylon filter 2 mL sub-fraction	4500323	-0.3
0.45µm Nylon filter 4 mL sub-fraction	4490790	-0.1
0.45µm Nylon filter 6 mL sub-fraction	4499895	-0.3
0.45µm PVDF filter 2 mL sub-fraction	4495825	-0.2
0.45µm PVDF filter 4 mL sub-fraction	4498367	-0.3
0.45µm PVDF filter 6 mL sub-fraction	4504099	-0.4
0.45µm PTFE filter 2 mL sub-fraction	4509601	-0.5
0.45µm PTFE filter 4 mL sub-fraction	4499682	-0.3
0.45µm PTFE filter 6 mL sub-fraction	4516949	-0.7

RESULTS AND DISCUSSION

A different combination of mobile phases and chromatographic conditions were tried and a mobile phase containing buffer solution of 10mM potassium dihydrogen phosphate with 0.1% triethylamine, pH 3.0 and acetonitrile (55:45v/v), Waters Xterra RP18 column (150 cm x 4.6 mm, 5µ), 1.0 mL/min flow rate, 20µL injection volume, 25ºC column oven temperature, 240 nm wavelength and 30 minute run time was found to be suitable for all combination.

These chromatographic conditions gave retention time of 9.2 minutes for Lercanidipine hydrochloride. Specificity of the method was checked by injecting the placebo solution, no peaks were found at the retention time of Lercanidipine hydrochloride. A forced degradation study was carried out on Lercanidipine hydrochloride tablets and its placebo under hydrolytic, oxidative, thermal and photolytic conditions. Using peak purity test, the purity of Lercanidipine peak was checked at every stage of the degradation study. The peak purity plots show that the Lercanidipine peak is homogeneous and has no co-eluting peaks indicating that the method is stability indicating and specific. The stability of the standard and sample solutions was evaluated by preparing a standard and sample solution as per the proposed method and analyzed initially and at 4 hour intervals up to 24 hours by keeping the sample solution at room temperature. The results of the stability studies showed that the solution of the drug was found to be stable for 24 hours at room temperature. System precision and method precision results showed the % RSD of 0.2 and 0.0, respectively. A good linearity relationship indicated by correlation coefficient (r) value 0.9999 was observed between the concentrations of LOQ at 0.04 µg/mL to 150µg/mLof Lercanidipine hydrochloride. Intermediate Precision was done by changing the analyst, column, with the same chromatographic conditions and the obtained results were within the limits. The accuracy of the method was determined and the percentage recovery was calculated. The data indicate 90.0 % to 107.5 % recovery of the standard sample. The range of the method was evaluated by demonstrating the suitable level of precision, accuracy and linearity between the concentrations of 0.04 to 150µg/mL. The Robustness method was evaluated by deliberately varying the chromatographic conditions of the method such as mobile phase acetonitrile content, pH of the buffer solution, flow rate, column temperature and wavelength. The parameters like tailing factor and retention time showed adherence to the limits. The suitability of different filters for the standard and test solutions was evaluated and it was observed that 0.45µm Nylon, PVDF and PTFE filters are suitable for sample filtration.

CONCLUSION

The method developed for the estimation of chromatographic purity of Lercanidipine hydrochloride in tablets was found to be a simple process. The validation results indicated that the method was specific, accurate, linear, precise, rugged, robust and stability indicating. The runtime was relatively 30 minutes which enabled a rapid quantification of many samples in routine and quality control analysis of tablet formulation.

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