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DEVELOPMENT AND VALIDATION BY RP-HPLC FOR THE *IN-VITRO* RELEASE OF LERCANIDIPINE HYDROCHLORIDE IN TABLET DOSAGES FORM.

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Abstract:

*A simple, accurate, precise and reproducible method was developed and validated by Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for estimation of Lercanidipine Hydrochloride in tablet dosage form. An isocratic RP-HPLC method was developed on C-18 Column, Cosmosil, (150 mm x 4.6mm) 5µm using Methanol, buffer (*Mix *1.0 ml of orthophosphoric acid in 1000 ml of water) and Tetrahydrofuran in the ratio of 890:100:10 as mobile phase. The flow rate was adjusted to 1.0 ml/min and the detection wavelength was 220 nm. The retention time for Lercanidipine Hydrochloride was found to be 6.52. Detection response for Lercanidipine Hydrochloride was found to be linear low in concentration range of 78-182 mcg/ml in the linearity study, regression equation and coefficient of correlation for Lercanidipine Hydrochloride was found to be (y = 4002.5x + 459.10, r2 =0.99996). Proposed method was validated for specificity, accuracy, precision, linearity, range, ruggedness & robustness. This developed method can be applicable for routine quantitative analysis.*

Keywords: *Lercanidipine Hydrochloride, RP- HPLC, Validation, IVR (In-Vito Release)*

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INTRODUCTION:

Lercanidipine hydrochloride is yellow powder and soluble in methanol, practically insoluble in water. Lercanidipine Hydrochloride Melting range is between 165.0°C and 180.0°C. Lercanidipine Hydrochloride has the structural formula shown below.

STRUCTURE FORMULA:

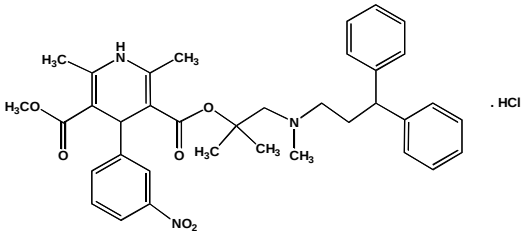

Fig.1: Lercanidipine hydrochloride

Chemical Name : 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid-2-[(3,3 diphenylpropyl)-methylamino]-1,1-dimethylethyl methyl ester hydrochloride; (±)-1,4-Dihydro- 2,6-dimethyl-4-(3-nitropheny l)pyridine-3,5-dicarboxylic acid- 2 - [N (3,3-diphenylpropyl)-N – ethylamino]-1, 1-dimethylethyl methyl diester hydrochloride

Molecular Formula : $C_{36}H_{41}N_3O_6$. HCl

CAS No. : [132866-11-6]

Molecular Weight : 648.19

Lercanidipine Hydrochloride is an active ingredient of Lercanidipine Hydrochloride Tablets for oral administration. Each Tablet contains 10 mg or 20 mg of Lercanidipine Hydrochloride. An HPLC method for determination of in-vitro release (IVR) for Lercanidipine Hydrochloride in Lercanidipine Hydrochloride Tablets has been developed and validated for routine use and testing the stability samples. Since the excipients used are proportionally same in all strengths and chromatographic conditions are same for all strengths of Tablets, This paper describes the experiments performed for in-vitro release (IVR) of Lercanidipine Hydrochloride in Lercanidipine Hydrochloride Tablets dosages form.

MATERIALS AND METHODS:

INSTRUMENTATION:

System 1: HPLC equipped with Quaternary pump, Auto sampler, Ultra-violet Detector and LC Solution software, make- SHIMADZU.

System 2: HPLC equipped with Quaternary pump, Auto sampler, Photo-Diode Array Detector and Empower 3 software, make- WATERS.

Electrolab Dissolution tester ETD-08Lx, Distek ezfill 4500 media degasser. Analytical balance (Mettler Toledo), Cyberscan pH meter, Grant sonicator.

Chemical		Grade
Tetrahydrofurone	:	Analytical reagent (AR)
Methanol	:	HPLC
Orthophosphoric acid (85%)	:	Analytical reagent (AR)
Sodium Lauryl Sulphate	:	Analytical reagent (AR)
Sodium Chloride	:	Analytical reagent (AR)
Purified water	:	Pharmaceutical
HPLC Water	:	Milli-Q

The solvents used were of HPLC/AR grade. Pure drug sample of Lercanidipine Hydrochloride was procured from Biocon Ltd. Capsule formulation containing Lercanidipine Hydrochloride (120 mg) purchased from market was manufactured by Glenmark pharmaceutical Ltd.

Chromatographic Conditions:

Column	:	C18, 150 mm × 4.6 mm, 5 µm, (Preferably Cosmosil)
Flow rate	:	1.2 ml/minute
Wavelength	:	220 nm
Injection volume	:	20 µl
Column temperature	:	30°C
Run time	:	10 minutes.
Sample Tray Temperature	:	25°C

Buffer Preparation:

Prepare the buffer by mixing 1.0 ml of orthophosphoric acid in 1000 ml of water.

Mobile Preparation:

Mix 900 volumes of methanol and 100 volume of buffer and add 10ml of Tetrahydrofurone mix it well and sonicate the mixture to degas and filter through 0.45µm Nylon filter.

Standard Preparation:

Weigh accurately about 130 mg of Lercanidipine Hydrochloride working standard into 100 ml volumetric flask. Add 70 ml methanol, sonicate to dissolve and make volume with methanol, and Dilute 5 ml of this solution to 50 ml volumetric flask and make volume with dissolution medium.

Sample Preparation:
Transfer 900 ml of dissolution media in the vessel carefully and allow the medium to equilibrate to a temperature of 37 ± 0.5ºC. Add capsule in each of the six Jars and operate the apparatus at 75 rpm. Withdraw 10 ml of the sample from each dissolution vessel after specified time, filter through 0.45µm Nylon filter.

Dissolution Parameters:

Use suitable Dissolution Apparatus:

Dissolution Media	3% SLS in 0.5% NaCl
Volume	900 ml
Apparatus	Paddle (USP Apparatus II)
Rotation Speed	75 rpm
Media Temperature	37 ±0.5°C
Sampling Time	60 min
Bath Temperature	37 ±0.5°C

Evaluation of system suitability:

1. The % RSD for the peak areas of Lercanidipine Hydrochloride from six replicate injections of standard Preparation should NMT 2.0.
2. Tailing factor of Lercanidipine Hydrochloride should NMT 2.0.

Calculation:

$$\% \text{ Release} = \frac{\text{Test Area}}{\text{Std Area}} \times \frac{\text{Std wt}}{100} \times \frac{5}{50} \times \frac{900}{1} \times \frac{\text{Potency}}{\text{Label Claim}}$$

RESULTS:

1. Linearity and Range:
To prepare the calibration curve for Lercanidipine Hydrochloride, 3.0, 4.0, 5.0, 6.0, 7.0 ml of Standard stock solution of Lercanidipine Hydrochloride was transferred to series of five, 50ml of volumetric flasks. The volume of each flask was adjusted to 50ml of mobile phase. Detection response for Lercanidipine Hydrochloride was found to be linear in concentration range of 78-182 mcg/ml. The slope, regression equation and coefficient of correlation for Lercanidipine Hydrochloride was found to be ($y = 4002.5x + 459.10$, $r^2 = 0.99996$).

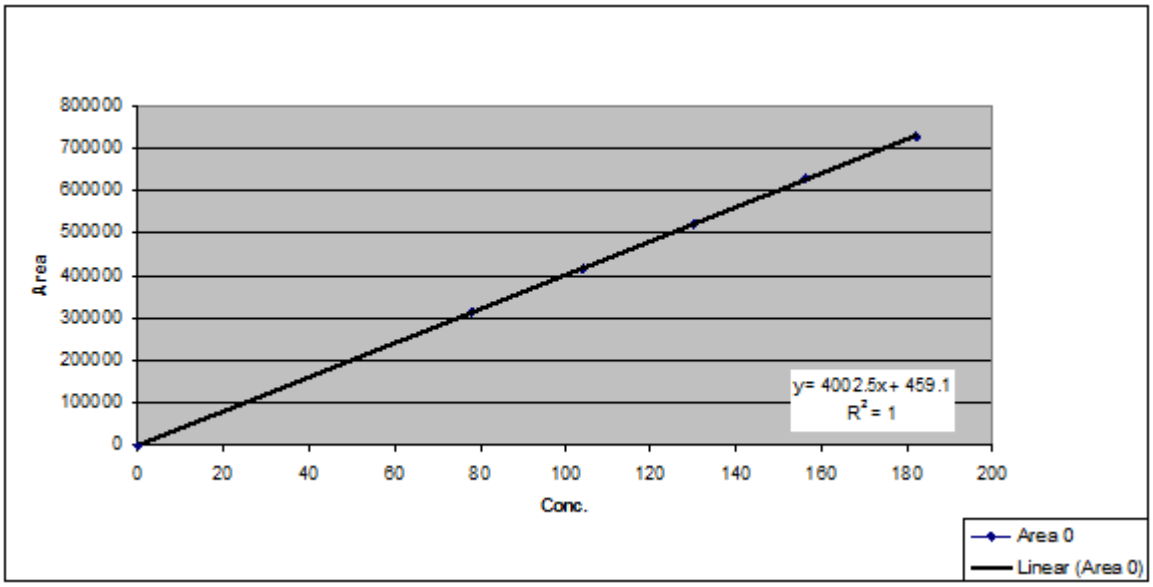

Fig.2: Linearity Plot of Lercanidipine Hydrochloride

2. Precision

2.1 System Precision for Lercanidipine Hydrochloride :

The system precision was evaluated by measuring the peak responses of Lercanidipine Hydrochloride for six replicate injections of standard solution, prepared as the proposed method. The results shown in the **Table – 1** indicate that the precision of the system is within the limit.
(Acceptance criteria: % RSD NMT 2.0%)

Table 1: System Precision

Sr. No.	Peak areas of Lercanidipine Hydrochloride
1.	164489
2.	165312
3.	166691
4.	164586
5.	167056
6.	167181
Mean	**165885.83**
SD	**1238.15**
RSD (%)	**0.75**

2.2 Method Precision for Lercanidipine Hydrochloride:

The method precision was determined by preparing a sample solution of single batch Lercanidipine Hydrochloride Capsule six times and analyzing as per the proposed method. The results shown in **Table 2** indicate that the proposed method is precise.
(Acceptance criteria: % RSD NMT 2.0%)

Table 2: Method Precision

Sr. No.	% Release of Lercanidipine Hydrochloride
1	101.79
2	101.35
3	101.74
4	100.69
5	100.91
6	101.40
Mean	**101.31**
SD	**0.44**
%RSD	**0.43**

3. Ruggedness for Lercanidipine Hydrochloride :

The ruggedness of the proposed method was determined by analyzing the same batch of Lercanidipine Hydrochloride Capsule by two different analysts using two different instruments, different columns on different days. The overall mean, standard deviation and %RSD of the assay values are shown in **Table- 3**.
(Acceptance criteria: Over all % RSD NMT 2.0%)

Table 3: Method Precision Lercanidipine Hydrochloride

Sample No.	% Assay for Lercanidipine Hydrochloride	
	Analyst - I	Analyst - II
1	101.79	99.48
2	101.35	98.32
3	101.74	99.34
4	100.69	98.95
5	100.91	98.55
6	101.40	98.35
Mean	101.31	98.83
SD	0.44	0.50
%RSD	0.43	0.51
Over all Mean	**100.07**	
Over all SD	**1.37**	
Over all RSD RRSDRSD%RSD	**1.37**	

4. Stability in analytical solution for Lercanidipine Hydrochloride :

The standard solution and sample solution of Lercanidipine Hydrochloride Capsule was prepared as per the proposed method and analyzed initially and also analyzed at different time intervals by keeping the solution at room temperature. The % Difference in peak area counts from initial and different time intervals of Lercanidipine Hydrochloride shown in **Table-4** indicates that solution is stable up to at least 48 hours.
(Acceptance criteria: % Difference should NMT 2.0%)

Table 4: Stability in analytical solution

Time (hours)	Standard solution	Sample solution
	Difference from initial	Difference from initial
Initial	--	--
6	-0.4	0.7
12	-0.6	-0.3
20	0.1	-0.8
30	0.4	0.7
36	-0.6	-0.1
48	-0.2	0.4

5. Accuracy (Recovery Study) for Lercanidipine Hydrochloride :

Known amounts of Lercanidipine Hydrochloride was spiked to placebo at 20%, 50%, 80%, 100% and 120% of specification in triplicate and analyzed as per the proposed method to determine the accuracy of the method. Percentage recovery was calculated from the amount found and amount added. The results are shown in **Table-5**. The percentage recovery is within the acceptance criterion, which indicates the accuracy of the method.
(Acceptance criteria: % Recovery should be between (95 – 105 %)

Table-5: Accuracy (Recovery Study) for Lercanidipine Hydrochloride

Level no/Spike level in %	Actual Amount of Lercanidipine Hydrochloride added in mg	Amount of Lercanidipine Hydrochloride found in mg	% Recovery	Average % Recovery	% RSD
Level – 1 (20%)	24.17	23.61	97.70	97.59	1.23
	23.65	23.35	98.74		
	24.77	23.86	96.34		
Level – 2 (50%)	60.53	59.55	98.39	97.28	1.01
	61.21	59.07	96.51		
	59.96	58.14	96.96		
Level – 3 (80%)	102.75	101.2	98.5	96.46	0.98
	102.58	100.7	98.2		
	102.67	101.3	98.6		
Level – 4 (100%)	128.56	129.0	100.3	98.91	0.19
	128.30	128.4	100.1		
	128.23	129.0	100.6		
Level – 5 (120%)	155.03	153.6	99.1	97.45	0.48
	155.89	154.6	99.2		
	155.01	153.4	99.0		

6. 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 excipients at the retention time of Lercanidipine Hydrochloride.

7. Robustness:

The robustness of the method was evaluated by deliberately varying the chromatographic conditions viz. pH of mobile phase by ±2, absolute flow rate by ±2ml, column oven temperature by ± 5°C, change in wavelength of detection by ± 2nm & change in organic phase by ± 5%. At these changed condition the standard and test preparation were injected. The system suitability was evaluated in each varied condition. The amount of Lercanidipine Hydrochloride was calculated from test preparation in each varied condition. The results were compared with the controlled data (Method Precision data). Results are tabulated in **Table – 6a** and **Table-6b** indicates that the method is robust under varied conditions.

(Acceptance criteria: Overall % RSD: NMT 2.0%)

Robustness Data: Lercanidipine Hydrochloride:

Table 6a: Robustness Data

Method Precision data		**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Set 6**	**Set 7**	**Set 8**	**Set 9**	**Set 10**
101.79	100.69	100.45	100.26	99.30	99.26	99.74	99.88	100.33	99.18	99.48	99.61
101.35	100.91										
101.75	101.40										
Mean	**101.31**										
SD	**0.44**										
RSD %	**0.43**										
Overall Mean		99.77	99.71	99.39	99.38	99.54	99.58	99.73	99.35	99.45	99.49
Overall SD		0.62	0.55	0.32	0.34	1.14	0.40	0.55	0.34	0.33	0.34
Overall RSD %		0.62	0.55	0.32	0.35	1.14	0.40	0.55	0.35	0.33	0.35

Robustness Data: Lercanidipine Hydrochloride

Table-6b: Robustness data under varied conditions

Varied conditions	% RSD	Tailing factor	Theoretical Plates
Method precision	0.39	1.30	14781.43
Set 1	0.37	1.32	14458.00
Set 2	0.40	1.31	14521.55
Set 3	0.08	1.28	13861.51
Set 4	0.26	1.34	15228.90
Set 5	0.34	1.32	14379.70
Set 6	0.25	1.32	14496.61
Set 7	0.16	1.31	14924.57
Set 8	0.20	1.30	14875.24
Set 9	0.24	1.32	14445.60
Set 10	0.26	1.32	14402.96

Where,

Set 1: Change in pH by + 0.2

Set 2: Change in pH by – 0.2

Set 3: Change in flow rate by + 0.2 ml/ min

Set 4: Change in flow rate by – 0.2 ml/min

Set 5: Change in wavelength by + 2 nm

Set 6: Change in wavelength by – 2 nm

Set 7: Change in. column temperature by + 5.0 units

Set 8: Change in column temperature by – 5.0 units

Set 9: Change in organic phase by + 5%

Set 10: Change in organic phase by – 5%

DISCUSSION:

Retention time of Lercanidipine Hydrochloride peak in sample preparation is comparable with respect to retention time of standard preparation and was found about 6.5 retention times. No interference was observed at the retention time of Lercanidipine Hydrochloride peak with placebo and diluent so method was found selective and specific. Detection response for Lercanidipine Hydrochloride was found to be linear in concentration range of 78-182 mcg/ml. The slope, regression equation and coefficient of correlation for Lercanidipine Hydrochloride was found to be (y = 4002.5x + 459.10, r2 =0.99996). Precision and accuracy was also found within limit, Sample and standard solution was also found stable upto 48 hours at room temperature. After deliberate variations in the chromatographic condition method was found suitable for stability and routine analysis. Intermediate precision was also found within acceptance criteria.

CONCLUSION:

The test method was validated for specificity, selectivity, precision, accuracy, linearity, ruggedness and robustness and method was found to meeting the predetermined acceptance criteria as per International Conference on Harmonization guideline entitled ‘Stability testing of new drug substances and new drug products’ (ICH) Q1A and ‘Analytical method validation of new drug substances and new drug product’ ICH Q2 R1 guideline. Hence this method can be introduced into routine use and testing of stability samples for the in-vitro release of Lercanidipine Hydrochloride in Lercanidipine Hydrochloride Tablets, 10mg and 20mg.

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REFERENCES:

1. ICH Q1A (R2) Stability Testing of New Drug Substances and Products, USFDA-Federal Register, USA < http://www.ich.org, 2003.
2. ICH Q2 (R1), Validation of Analytical Procedures; Text and Methodology, USFDA-Federal Register, USA < http://www.ich.org, 2005.
3. MIHALJICA S, RADULOVIC D, TRBOJEVIC J. Determination of Lercanidipine hydrochloride and its impurities in tablet. *Chromatographia*, 2005; 61; p. 25-29.
4. SWARTZ, M.E. UPLC, an Introduction and Review. *J. Liq. Chromatogr. R.T*, 2005; 28: p.1253-1263.
5. Raju S A, Karadi A B, Manjunath. A high performance liquid chromatographic assay for Lercanidipine hydrochloride. Int. J. Chem. Sci., 2008; 6; p.441-446.
6. DONG, M.W. *Handbook of pharmaceutical analysis by HPLC*. Elsevier: United Kingdom, 2005. V.6, p.2-3.
7. WREN, S.A.C; TCHELITCHEFF, P, Use of ultra-performance liquid chromatography in pharmaceutical development. *J.Chromatogr. A*, 2006; 1119: p. 140-146.
8. THE UNITED STATES PHARMACOPEIA, Thirty-fifth Edit, Validation of Compendial Methods, Section <1225>, Rockville, 2012.
9. SNYDER, L.R.; GLAJCH, J.L.; KIRKLAND, J.J. *Practical HPLC method development*. 2ed. New York: John Wiley & Sons, 1987. Chapter 1.2
10. BAKSHI, M.; SINGH, S. Development and validated stability indicating assay methods – critical review. *J.Pharm. Biomed. Anal.*, v.28, p.1011-2002.
11. P.L. GARCIA, E.BUFFONI, F.P. GOMES, J.L.V. QUERO, Wide spectra of quality control, in: I. Akyar (Ed.), Tech, Rijeka, 2011. p. 3-20.