Method Development and Validation of Lercanidipine in Human Plasma by using LC-MS/MS and Comparison of Pkpd Parameters of Lercanidipine and its Enantiomer

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Abstract

Improved and Reliable Liquid Chromatography/ Tandem Mass Spectrometry (LC–MS/MS) method has been developed and validated for the determination of lercanidipine in human plasma. Plasma samples with lercanidipine-d3 as an internal standard (IS) were prepared by solid phase extraction on Lercanidipine in human plasma by using LC-MS/MS with symmetry C18 (75 x 4.6 mm, 3.5 µ) column. The developed method was extended to bio-equivalence studies in which pharmacokinetic parameters (PK) like concentration time profiles, Cmax, AUC0-t, AUC0-α, Tmax, T1/2, Kel, for lercanidipine were estimated. Statistical methods were used to analyze the log- transformed pharmacokinetic parameters AUC0-t, AUC0-α and Cmax for bioequivalence of each of these parameters. Statistical analysis was performed using WinNonlin software. The matrix effect was evaluated using standard-line slope, post-column infusion and post-extraction spiking techniques.

IS's mean extraction recovery was >94%. Five quality controls had an average accuracy (% CV) of 5.8% between batches and among batches. The results obtained for recemic and enantiomers lercanidipine were under the acceptance criteria. It shows both studies of lercanidipine under similar conditions and there is no in vivo conversion of enantiomers. When the concentrations of theR-enantiomer and S-lercanidipine were comparable, the R-enantiomer's presence protected the ester of S-lercanidipine from first-pass metabolism and the choice to produce the recemic version of the drug.

Keywords: Lercanidipine, LC-MS, Human plasma.

Introduction

The rate and extent to which the active ingredient or active moiety are absorbed from a drug product and become available at the site of action", is the definition of bioavailability. When given at the same molar dose under comparable circumstances in a suitably designed study, bioequivalence is defined as "the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents becomes available at the site of drug action. "Bioequivalence studies can be required in a number of circumstances[7] to establish links (bridging) between different formulations used during the development of a new product e.g. clinical trial formulations. The majority of bioequivalence studies are pharmacokinetic studies carried out using healthy volunteers.

Pharmacodynamic studies are only considered appropriate when a pharmacokinetic approach is not possible and a suitably validated pharmacodynamic model is available. Clinical studies as considered an insensitive (and expensive) approach to demonstrating bioequivalence. By creating a mass spectrum that represents the masses of the sample's constituent parts, is most frequently used to determine the composition of a physical sample[10].

The mass to charge ratio (m/z) of the ion effects this motion. Since the charge of an electron is known, the mass to charge ratio is a measurement of an ion's mass. Typical mass spectrometry research focuses on the formation of gas phase ions, the chemistry of ions and applications of mass spectrometry. The most popular use of APIs is electro spray ionization (ESI). Its application for LC/MS of high molecular weight and thermally labile chemicals has increased significantly in the past several years. Applying a high voltage between the metal intake needleand the first skimmer in an API source produces the electrospray. The mechanism for the ionization process is not well understood and there are several different theories that explain this ionization process. One theory is that as the liquid leaves the nozzle, the electric field induces a net charge on the small droplets.

(+/-) Lercanidipine is a new calcium-channel blocker structurally related to the 1,4–dihydropyridine. It has a chiral center, accounting for the presenceof two enantiomers, with S(+)lercanidipine being more active (eutomer) for antihypertensive activity. There is no *in vivo* conversion between enantiomers lercanidipine throughout the study. The goal of the HPLC approach is to make it possible to quantify pharmacological compounds in a range of stressful physical, chemical and photochemical environments[9].

Material and Methods

Standard Stock Solution: Store the stock solutions, intermediate solutions and working solutions of lercanidipine and lercanidipine-13C, d3 at 2-8°C

refrigerators in glass volumetric flask or poly propylene tubes.

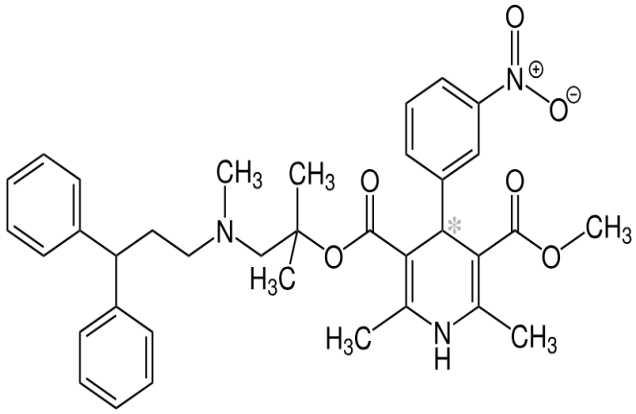

Fig. 1: Chemical structure of Lercanidipine

Lercanidipine Stock Solution Preparation: Weigh accurately lercanidipine hydrochloride working standard equivalent to 10.000 mg of lercanidipine and transfer it into a 10 mL volumetric flask. Add 5 mL of diluent-1. Dissolve it and make up the volume with same to prepare a solution of 1000.000 µg/mL of Lercanidipine[4]. Calculate the stock solution concentration by taking into account actual amount of lercanidipine weighed.

Lercanidipine Intermediate Solution (100.000 µg/mL): Transfer 500 µL of lercanidipine stock solution (1.000 mg/mL) into a 5 mL volumetric flask containing about 3 mL of diluent-2. Make up the volume with same to prepare a solution of 100.000 µg/mL of lercanidipine.

Internal Standard Stock Solution Preparation: Weigh accurately lercanidipine-13C, d3 hydrochloride working standard equivalent to 2.000 mg of Lercanidipine-13C, d3 and transfer into a 10 mL volumetric flask. Dissolve in diluent and make up the volume with same to prepare a solution of 200.000 µg/mL of lercanidipine-13C, d3. Calculated the stock solution concentration by taking into account actual amount of lercanidipine 13C, d3 weighed.

Internal Standard Intermediate Solution-1 (10.000 µg/mL): Transfer 250 µL of lercanidipine-13C, d3 Intermediate solution (200.000 µg/mL) into 5 mL volumetric flask containing about 3 mL of diluent. Make up the volume with same to prepare a solution of 10.000 µg/mL of lercanidipine.

Internal Standard Working Solution (120.000 ng/mL): Transfer 60 µL of internal standard stock solution (10.000 µg/mL) into 5 mL of a volumetricflask containing about 3 mL of diluent. Make up the volume with same to prepare a solutionof 120.000 ng/mL of lercanidipine-13C, d3.

Screening: Blank plasma samples from 8 different lots were processed according to the extraction procedure and check the interference at the retention times of analytes and internal standard. The 6 free interference lots were selected.

Specificity: Prepare the LLOQ standard including the spiking of the internal standard's operating range, in one of the screened blank plasma samples. Six LLOQ standards and eight distinct sets of blank plasma samples were processed in accordance with the extraction protocol[5].

The LLOQ standard of the two analytes and the internal standard were compared with the results for the blank plasma from eight distinct lots. No significant response (≤20% for the analyte response and ≤5% of the internal standardresponse.) was observed at the retention times of the analytes or the internal standard in blank plasma as compared to the LLOQ standard.

Optimized Extraction Procedure: 150 µL of plasma samples was taken, 20 µL of WIS, vortex (Lercanidipine-d3, 120.000 ng/mL) was added to 150 µL of 0.1% acetic acid in water solution and vortexed for few seconds, then add 4.0mL of Methyl-tert-Butyl Ether (TBME), keep the samples on Multipulse Vortexer for shaking for 10 mins at medium frequency[3], 5 mins with and without pulse. Centrifuge the above samples at 3000rpm at ≤ 10 ºC for 5 min. Then transfer the supernatant layer into pre labeled Ria vials or glass tubes and evaporated it to complete dryness under a stream of nitrogen at ≤ 50ºC. Reconstitute it with150µL of mobile phase. Load the samples into auto sampler vials and inject 10µL on to the LCMS/MS system.

Results and Discussion

Data Collection and Computer System: All integrations were performed by Applied Biosystems Analyst Software version 1.4.2. Using the ratios of drug/internal standard peak areas of calibration curve standards, least squares linear regression analysis was used to calculate the slopes, intercepts and correlation coefficients. A weighing factor of $1/X^2$ ($1/conc^2$) was used in the calculation of the linear regression line. All the QC samples were also calculated by Applied Biosystems Analyst Software version 1.4.2. The concentrations were exported directly to the Microsoft Excel and from the excel sheet, the data was transferred into individual tables with 100% data check. The report was prepared using Microsoft Word and all the entries were verified against the raw data.

Pre-method Validation: Following experiments were performed during the pre-method validation.

Precision and Accuracy batch: One calibration curve and 6 replicates each of theLLOQ, LQC, MQC, HQC were spiked in plasma and processed by using solid extraction procedure and these samples were analyzed in LC-MS/MS. All QC's were within the acceptance criteria.

Extraction Recovery: Take out 6 spiked samples each of LQC, MQC, HQC and extract as per the extraction technique. The same amount of analyte was added to the reconstituted solution at low, medium, high concentrations and analyzed together with extracted samples.

Table 1
Details of Preparation of Calibration standards and Quality control standards of Lercanidipine

Working solutions			Plasma blank	Sample	
ID	**Conc. (µg/ml)**	**Volume (ml)**	**Volume (ml)**	**Plasma Conc. ng/ml**	**ID**
LER -WCS1- # *	0.005	100	5.00	0.100	CS-1
LER -WCS2- # *	0.010	100	5.00	0.200	CS-2
LER -WCS3- # *	0.030	100	5.00	0.600	CS-3
LER –WCS4- # *	0.090	100	5.00	1.800	CS-4
LER –WCS5- # *	0.200	100	5.00	4.000	CS-5
LER –WCS6- # *	0.500	100	5.00	10.000	CS-6
LER –WCS7- # *	0.750	100	5.00	15.000	CS-7
LER –WCS8- # *	1.000	100	5.00	20.000	CS-8
LER –WCS9- # *	1.250	100	5.00	25.000	CS-9
LER -WLLOQ- # *	0.005	100	5.00	0.100	LLOQC
LER -WLQC- # *	0.015	100	5.00	0.300	LQC
LER –WM1QC- # *	0.150	100	5.00	3.000	M1QC
LER –WM2QC- # *	0.400	100	5.00	8.000	M2QC
LER -WHQC- # *	0.900	100	5.00	18.000	HQC

Developed methods

METHOD SUMMARY

Biological matrix	Human plasma
Anticoagulant	Heparin
Required sample volume	150 µL
Analyte	Lercanidipine
Calibration range	0.100 to 25.420 ng/Ml
Analytical technique	Liquid chromatography
Detection mode	Mass spectrometer
Extraction procedure	Liquid-Liquid Extraction
Quantitation method	Peak area
Weighing method	1/x2
MW of Internal standard	Lercanidipine 13C, d3: 615.73 g/mol

CHROMATOGRAPHIC CONDITONS

Chromatographic mode	LC/MS/MS-API-2000
Mobile phase	Buffer solution 0.1 % Formic acid: Acetonitrile (10:90)
Column	Symmetry-C18 (75x4.6mm, 3.5µ)
Isocratic/gradient mode	Isocratic
Mobile phase flow rate	0.500 ml/min
Auto sample temperature	5°C
Splitter	50:50
Probe Position	X axis = 5 Y axis = 5
Rinsing volume	400µl
Column temperature	40°C
Injection volume	10µl

RETENTION TIMES

Total Run Time	1.21 min
Lercanidipine:	1.24 min
Lercanidipine 13C, d3	1.95 min

DETECTION PARAMETERS

Drug name	**Lercanidipine**	**Lercanidipine13C, d3**
Parent mass	612.60	616.60
Product mass	280.50	284.50

Matrix Effect: Spike LQC, MQC, HQC samples in duplicate in each of six different batches of screened matrix and extract as per the extraction technique and analyze in LC- MS/MS. The values of QC samples were calculated using a calibration curve[6]. All QC's are within the acceptance criteria.

Blank plasma samples from eight distinct lots were treated in accordance with the extraction protocol during validation in order to assess interference at the analyte and internal standard retention durations[8]. Out of the eight lots, the six free interference lots were chosen. Table 1 presents the findings. By examining one calibration curve and six replicates of the LLOQ, LQC, MQC and HQC from precision and accuracy batch-1, the intra-batch accuracyand precision were evaluated:

- The Intra batch percentage of nominal concentrations for lercanidipine ranged between 88.20% to 104.39% and 1.40% to 14.17%
- The Intra batch percentage of coefficient of variation is presented in table 4.

Inter batch accuracy and precision evaluation were assessed by analyzing 5 sets of calibration curves for lercanidipine and 5 sets of QC samples and 6 replicates each of the LLOQ, LQC, MQC, HQC.

- The inter batch percentage of nominal concentrations for lercanidipine ranged between 97.70% to 104.54% and 1.68% to 13.32%.
- The Inter batch percentage of coefficient of variation for lercanidipine is 1.68% to 13.32% presented in table 5.

Calibration Curve: Calibration curve is found to be consistently accurate and precise for lercanidipine in the range 0.100 to 25.200 ng/mL. The correlation coefficient is greater than or equal to 0.9970 for lercanidipine. Back calculations were made from the calibration curves to determine lercanidipine concentrations of each calibration standard[11]. Results are presented in table 6.

Extraction Recovery: The percentage recovery of lercanidipine was determined by comparing the mean peak area of lercanidipine in extracted LQC, MQC, HQC samples with freshly prepared un-extracted LQC, MQC, HQC samples respectively.

- The mean % recovery for LQC, MQC, HQC samples of lercanidipine was 66.59%,68.12%, 83.05%.
- The mean recovery of lercanidipine across QC levels is 72.59%.
- The mean recovery of % CV recovery of lercanidipine across QC levels is 12.53 %.
- For the internal standard, mean peak area of 18 extracted samples was compared to the mean peak area of 18 un-extracted IS solution. The mean % recovery is 79.33%.
- The %CV recovery of IS for extracted is 11.14%.

Ruggedness Analysis: To evaluate ruggedness experiment with different analysts, one P and A batch was processed by different analyst. The run consisted of a calibration curve standard and 6 replicates of each LLOQ, LQC, MQC, HQC samples.

- The accuracy of lercanidipine QC samples is within the range of 97.24% to 101.04%.
- The precision of lercanidipine QC samples is within the range of 1.13% to 3.69%.
- These results indicated that the method is rugged and reproducible by different analyst.

To evaluate ruggedness experiment with different column, samples of P and A batch-5 were re-injected on different columns with same specifications. Concentrations were calculated to determine precision and accuracy.

- The accuracy of lercanidipine QC samples is within the range of 89.45% to 105.31%.
- The precision of lercanidipine QC samples is within the range of 1.55% to 5.66%.
- These results indicated that the method is rugged and reproducible by different analyst.

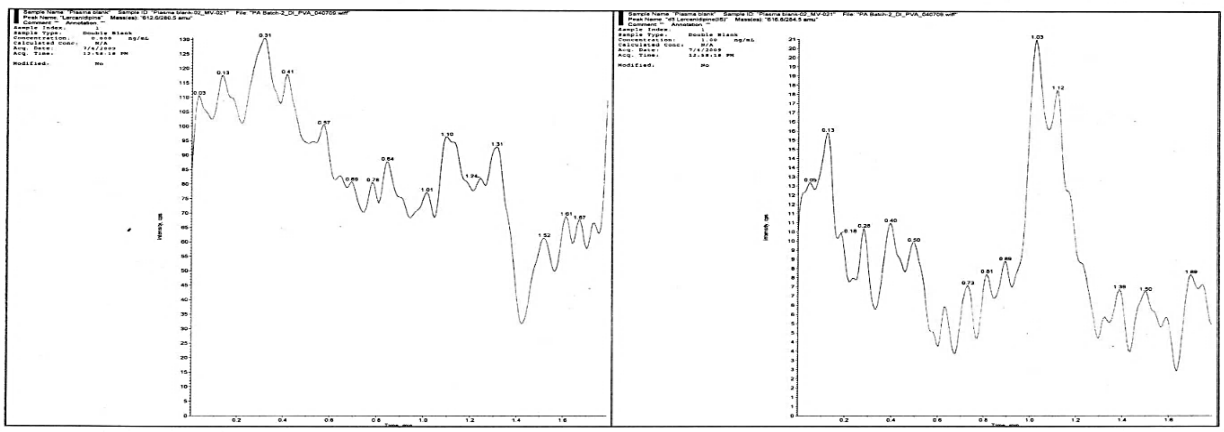

Fig. 2: Chromatogram of blank human EDTA plasma sample

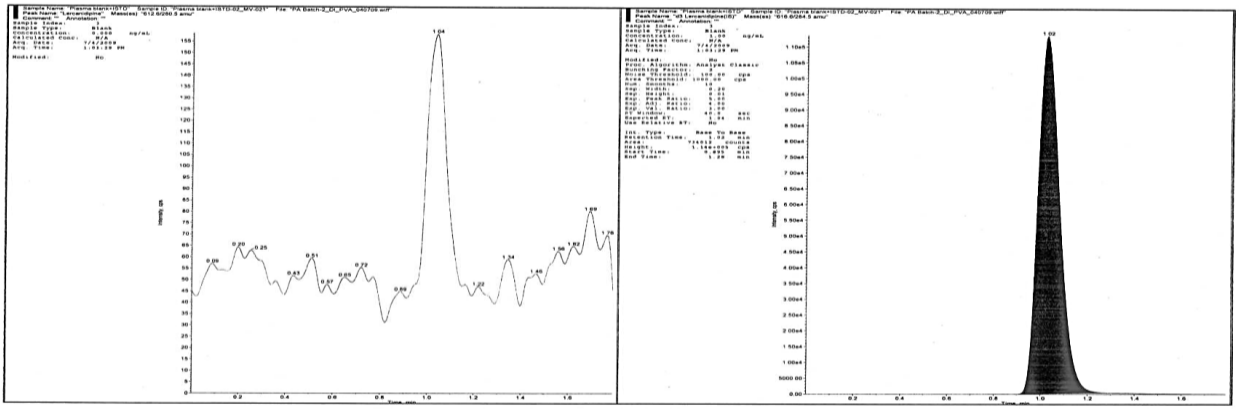

Fig. 3: Chromatogram of Placebo + Internal standard sample

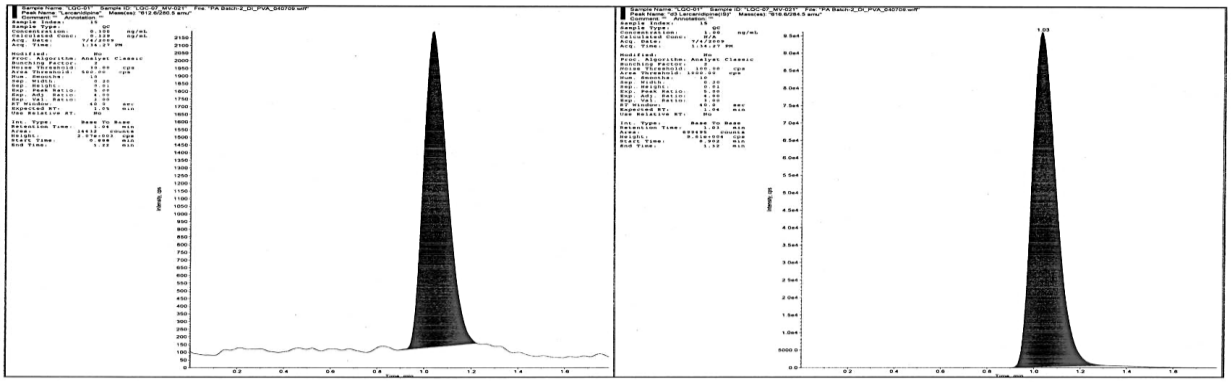

Fig. 4: Chromatogram of lower Limit of Quantification (LOQ) sample

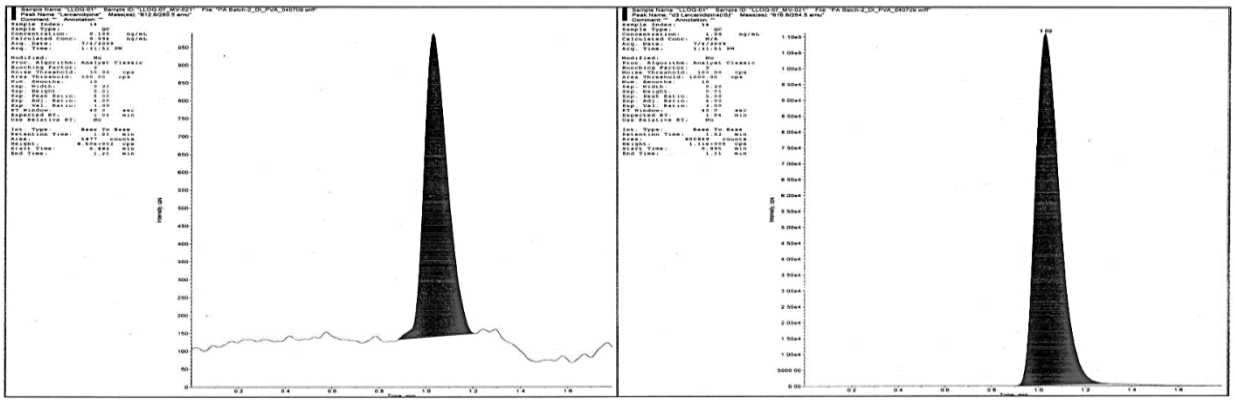

Fig. 5: Chromatogram of Low Quality control sample

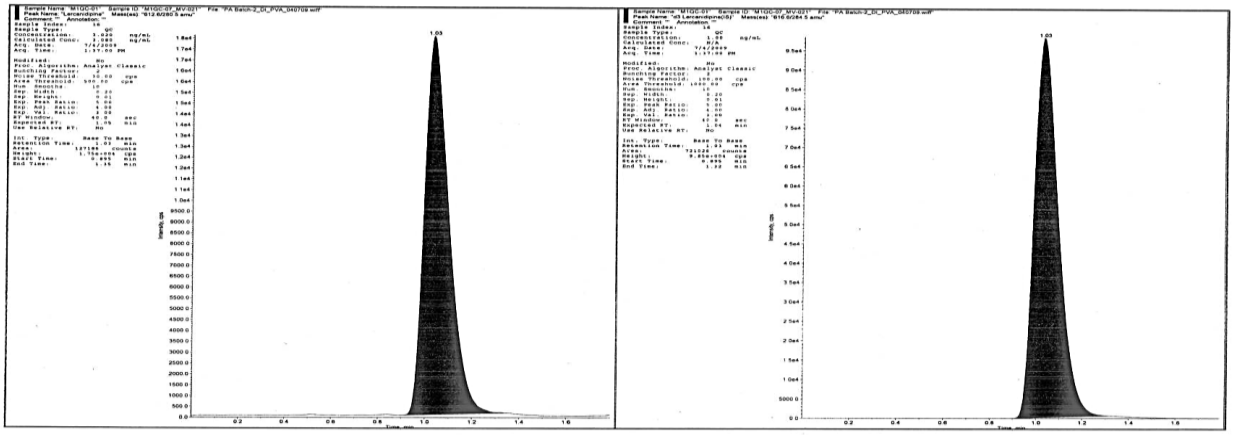

Fig. 6: Chromatogram of Medium Quality control (M1QC) sample

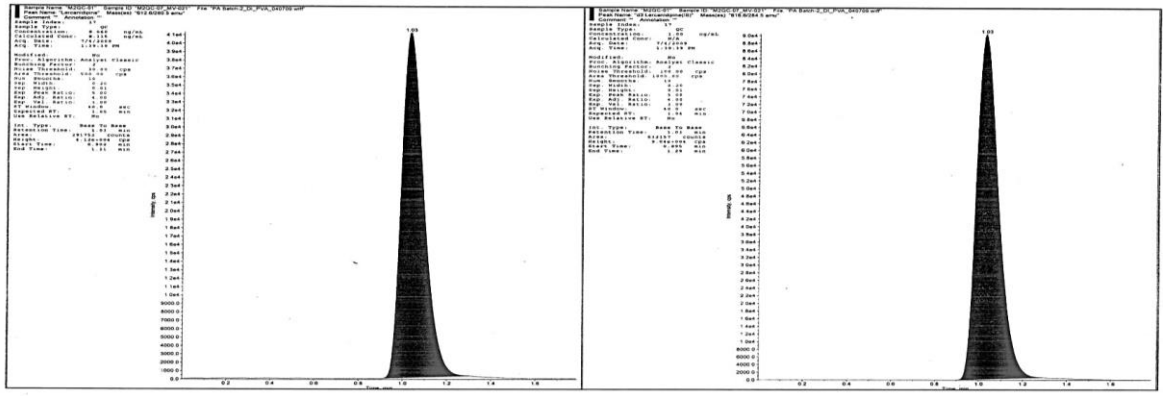

Fig. 7: Chromatogram of Medium Quality control (M2QC) sample

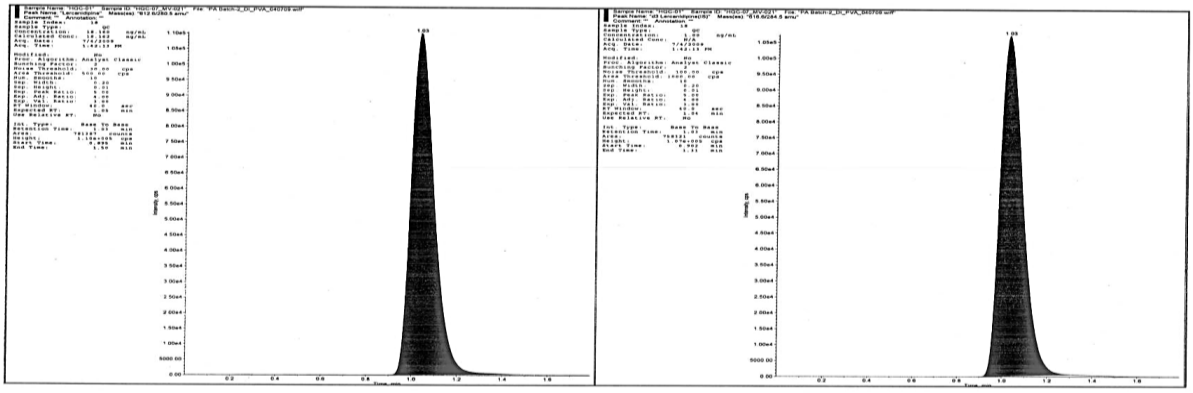

Fig. 8: Chromatogram of High-Quality control sample

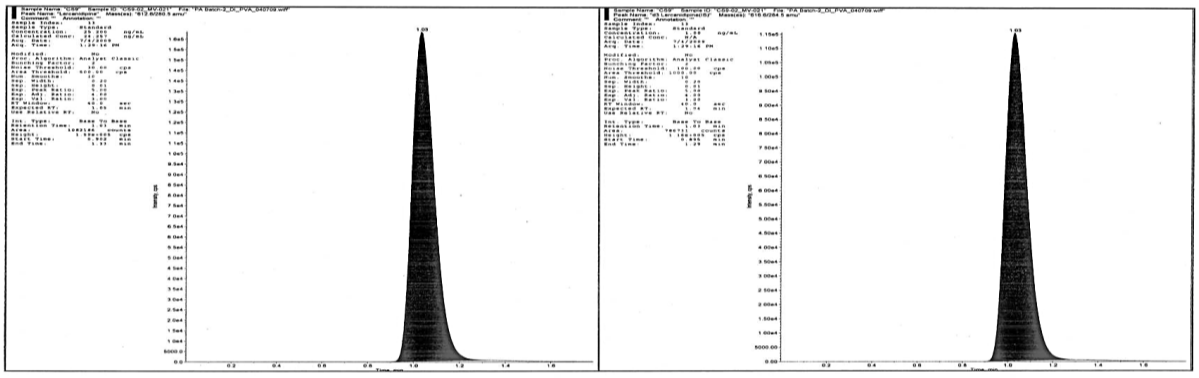

Fig. 9: Chromatogram of Upper Limit of Quantification sample

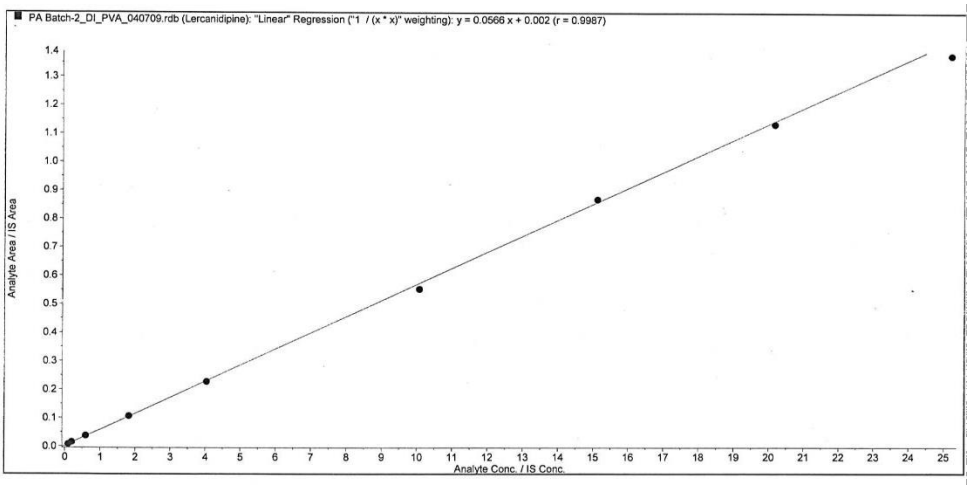

Fig. 10: Calibration curve of Lercanidipine

Table 2
Back-Calculated Standard Concentrations from Each Calibration Curve for Lercanidipine in Human Plasma

Analytical Run ID	**Nominal Concentration (ng/mL)**				
	CS1	**CS2**	**CS3**	**CS4**	**CS5**
	0.100 (0.080-0.120)	**0.200 (0.170-0.230)**	**0.600 (0.510-0.690)**	**1.820 (1.547-2.093)**	**4.040 (3.434-4.646)**
PA Batch-1	0.100	0.199	0.604	1.925	3.991
PA Batch-2	0.094	0.220	0.617	1.845	3.974
PA Batch-3	0.099	0.202	0.620	1.904	4.183
PA Batch-4	0.099	0.204	0.614	1.838	4.017
N	**4**	**4**	**4**	**4**	**4**
Mean	0.098	0.206	0.614	1.878	4.041
SD (±)	0.0027	0.0094	0.0069	0.0431	0.0961
CV (%)	2.76	4.56	1.12	2.29	2.38
% Nominal	**98.00**	**103.15**	**102.30**	**103.19**	**100.03**

Analytical Run ID	**Nominal Concentration (ng/mL)**			
	CS6	**CS7**	**CS8**	**CS9**
	10.080 (8.568-11.592)	**15.120 (12.852-17.388)**	**20.160 (17.136-23.184)**	**25.200 (21.420-28.980)**
PA Batch-1	9.665	14.607	19.825	26.329
PA Batch-2	9.711	15.290	19.963	24.257
PA Batch-3	9.959	14.702	18.526	25.419
PA Batch-4	10.036	14.898	19.914	25.153
N	4	4	4	4
Mean	9.843	14.874	19.557	25.290
SD (±)	0.1824	0.3025	0.6897	0.8528
CV (%)	1.85	2.03	3.53	3.37
% Nominal	97.65	98.38	97.01	100.36

Acceptance criteria:
1. Mean % Nominal (100 ±15%) except lowest calibration standard
2. Mean % Nominal (100 ± 20%) for lowest calibration standard (CS1)
3. % C.V ≤ 15% except lowest calibration standard (CS1) for which it is ≤ 20%

Table 3
Selectivity of Different Batches of Blank Matrix (Human Heparin Plasma).

Sample ID	**Peak Area Analyte**	**Peak Area IS**
Plasma blank+d3-LER(ISTD)-1	0	668455
Plasma blank+d3-LER(ISTD)-2	0	669269
Plasma blank+d3-LER(ISTD)-3	0	657438
Plasma blank+d3-LER(ISTD)-4	0	690274
Plasma blank+d3-LER(ISTD)-5	0	671255
Plasma blank+d3-LER(ISTD)-6	0	658528
Plasma blank+LER(CS9)-1	801953	0
Plasma blank+LER(CS9)-2	649243	0
Plasma blank+LER(CS9)-3	958702	0
Plasma blank+LER(CS9)-4	804543	0
Plasma blank+LER(CS9)-5	915783	0
Plasma blank+LER(CS9)-6	912229	0
N	6	6
Mean	840409	669203

Acceptance Criteria:
1. Analyte response should be ≤ 20% of mean LOQ response.
2. Internal standard response should be ≤ 5% of mean internal standard response oneanalyte to other.

Table 4
Specificity and Limit of Quantitation for Lercanidipine

Matrix Identification	LLOQ Response (Analyte)	Internal Standard Area	LLOQ* S/N Ratio (Analyte)
Plasma blank-01	0	0	NA
Plasma blank-02	0	0	
Plasma blank-03	1628	0	
Plasma blank-04	0	0	
Plasma blank-05	0	0	
Plasma blank-06	0	0	
Plasma blank-07	0	0	
Plasma blank-08	0	0	
MD_HEP-BM	5940	602969	87
	4928	503387	75.5
	7267	681125	77.8
	7013	715181	61.5
	7526	741173	40.5
	7119	722093	25.5
N	6	6	6
Mean	6632	660988	61.3
SD (±)	997	91335	NA
%CV	15.03	13.82	NA

Acceptance Criteria:
1. %CV of LLOQ response shall not be more than 20%
2. S/N Ratio of mean LLOQ should be ≥

Table 5
Intra Batch (Within- Batch) Accuracy and Precision for determination ofLercanidipine levels in Human Plasma

Analytical Run ID	LLOQ (ng/mL) 0.100 (0.080-0.120)		Low QC(ng/m)0.300 (0.255-0.345)		M1QC (ng/mL)3.020 (2.567-3.473)		M2QC (ng/mL)8.060 (6.851-9.269)		High QC(ng/mL) 18.160 (15.436-20.884)	
	LLOQQC		QC LOW		QC MED		QC MED		QC HIGH	
	Conc. Found	% Nom	Conc. Found	% Nom	Conc. Found	% Nom	Conc. Found	% Nom	Conc. Found	% Nom
						103.0				
	0.067	*67.21*	0.301	100.19	3.112	5	8.553	106.11	19.013	104.70
	0.099	99.45	0.319	106.44	2.875	95.21	8.396	104.17	18.398	101.31
						104.4				
	0.079	*79.07*	0.307	102.44	3.155	8	8.275	102.67	18.605	102.45
PA Batch										
						102.6				
	0.093	93.16	0.297	99.13	3.101	8	8.464	105.01	18.617	102.52
						104.5				
	0.095	95.15	0.286	95.30	3.158	8	8.191	101.63	18.264	100.57
						101.3				
	0.096	95.59	0.313	104.21	3.060	4	8.606	106.78	18.722	103.10
N	6		6		6		6		6	
Mean	0.088		0.304		3.077		8.414		18.603	
	0.012		0.011		0.105					
SD (±)	5		8		4		0.1600		0.2605	
Min	0.067		0.286		2.875		8.191		18.264	
Max	0.099		0.319		3.158		8.606		19.013	
%CV	**14.17**		**3.88**		**3.43**		**1.90**		**1.40**	
%NOM	**88.20**		**101.27**		**101.88**		**104.39**		**102.44**	

Acceptance criteria:
1. % C.V ≤ 15% except LLOQ for which it is ≤ 20%
2. Mean % Nominal (100 ±15% & For LLOQ 100 ±20%

Table 6
Inter Batch (Between- Batch) Accuracy and Precision for determination of Lercanidipine Levels in Human Plasma

Analytical Run ID	LLOQ(ng/mL)0.100 (0.080-0.120)		Low QC(ng/mL) 0.300 (0.255-0.345)		M1QC (ng/mL)3.020 (2.567-3.473)		M2QC (ng/mL)8.060 (6.851-9.269)		High QC(ng/mL) 18.160 (15.436-20.884)	
	Conc. Found	% Nominal	Conc. Found	% Nominal	Conc. Found	% Nominal	Conc. Found	% Nominal	Conc. Found	% Nominal
Batch-1	*0.067*	*67.21*	0.301	100.19	3.112	103.05	8.553	106.11	19.013	104.70
	0.099	99.45	0.319	106.44	2.875	95.21	8.396	104.17	18.398	101.31
	0.079	*79.07*	0.307	102.44	3.155	104.48	8.275	102.67	18.605	102.45
	0.093	93.16	0.297	99.13	3.101	102.68	8.464	105.01	18.617	102.52
	0.095	95.15	0.286	95.30	3.158	104.58	8.191	101.63	18.264	100.57
	0.096	95.59	0.313	104.21	3.060	101.34	8.606	106.78	18.722	103.10
PA Batch-2	0.094	94.24	0.329	109.66	3.080	101.98	8.115	100.68	18.162	100.01
	0.081	81.29	0.320	106.81	3.075	101.83	8.379	103.96	18.173	100.07
	0.093	93.46	0.338	112.73	3.116	103.18	8.215	101.93	17.979	99.00
	0.108	108.10	0.324	108.09	3.071	101.69	8.293	102.89	17.929	98.73
	0.102	102.39	0.322	107.31	3.067	101.54	8.179	101.47	17.915	98.65
	0.107	106.65	0.335	111.51	3.062	101.40	8.108	100.60	18.087	99.60
PA Batch-3	0.114	114.39	*0.359*	*119.72*	2.883	95.48	7.721	95.80	17.883	98.47
	0.119	118.86	0.288	96.09	2.842	94.10	7.774	96.45	17.915	98.65
	0.106	106.42	0.304	101.47	2.860	94.69	7.815	96.96	18.028	99.27
	0.123	*122.72*	0.297	99.07	2.852	94.45	7.874	97.69	18.039	99.33
	0.101	101.08	0.292	97.43	2.859	94.68	7.852	97.42	17.825	98.15
	0.107	106.50	0.287	95.77	2.825	93.53	7.884	97.82	17.813	98.09
PA Batch-4	0.103	103.38	0.314	104.62	2.723	90.17	7.293	90.48	18.177	100.09
	0.094	94.05	0.314	104.51	2.688	89.02	7.013	87.00	18.197	100.20
	0.086	86.07	0.317	105.65	2.845	94.22	7.465	92.61	17.965	98.93
	0.085	85.22	0.321	106.99	2.959	97.97	7.088	87.95	18.088	99.60
	0.288	288.49	0.327	108.86	2.843	94.15	7.540	93.55	18.059	99.44
	0.095	95.26	0.316	105.35	2.741	90.77	7.405	91.88	18.268	100.59
N	23		24		24		24		24	
Mean	0.098		0.314		2.952		7.937		18.172	
SD(±)	0.013		0.018		0.147		0.455		0.305	
CV (%)	**13.32**		**5.68**		**4.98**		**5.74**		**1.68**	
Min.	0.067		0.286		2.688		7.013		17.813	
Max.	0.123		0.359		3.158		8.606		19.013	
% Nominal Conc.	**97.70**		**104.54**		**97.75**		**98.48**		**100.06**	

Acceptance Criteria:
1. % C.V ≤ 15% except LLOQ for which it is ≤ 20%
2. Mean % Nominal (100 ±15% & For LLOQ 100 ±20%

Table 7
Summary of Calibration Curve Parameters for Lercanidipine (Analyte) in Human Plasma

Analytical Run ID	Y-Intercept	Slope	Coefficient of regression(r)
PA Batch-1	0.00250	0.0529	0.9993
PA Batch-2	0.00200	0.0566	0.9987
PA Batch-3	0.00146	0.0546	0.9991
PA Batch-4_Differentanalyst	0.00110	0.0594	0.9999
N	4	4	4
MIN	0.00110	0.0529	0.9987
MAX	0.00250	0.0594	0.9999

Acceptance Criteria
1. Coefficient of regression (r) ≥ 0.9800

Table 8
Recovery of Analyte (Lercanidipine) from Human Plasma

Standard	Unextracted	Extracted
	Lercanidipine PeakResponse	**Lercanidipine PeakResponse**
High QC: 18.160 ng/mL	1175876	894086
	1088150	910804
	1078369	895436
	1037314	895295
	1061700	914914
	1073796	900211
N	6	6
Mean	**1085868**	**901791**
SD (±)	**47435**	**8921**
%CV	**4.37**	**0.99**
% Recovery	**83.05**	
Medium QC (M2QC): 8.060 ng/mL	448141	304083
	462252	289102
	453886	321557
	453236	337646
	454197	303467
	453420	300407
N	6	6
Mean	**454189**	**309377**
SD (±)	**4543**	**17332**
%CV	**1.00**	**5.60**
% Recovery	**68.12**	
Low QC: 0.300 ng/mL	20807	15673
	24864	13894
	20072	14690
	20774	13388
	20571	13221
	19992	13760
N	6	6
Mean	**21180**	**14104**
SD (±)	**1838**	**923**
%CV	**8.68**	**6.54**
% Recovery	**66.59**	
Mean Recovery of Lercanidipine across QC levels		**72.59**
SD (±) of Mean Recovery of Lercanidipine across QClevels		**9.09**
The Mean % CV Recovery of Lercanidipine across QClevels		**12.53**

Acceptance Criteria: 1. % CV for Mean Recovery of analyte across QC levels should be <25%

Table 9
Assessment of Matrix Effect on determination of Lercanidipine levels in Human

Identification Matrix	Low QC (ng/mL)0.300 (0.255-0.345)		M2QC (ng/mL)8.060 (6.851-9.269)		QC (ng/mL)18.160 (15.436-20.884)	
	Conc.found	**% Nom**	**Conc.found**	**% Nom**	**Conc.found**	**% Nom**
(HEP-BM/693)	0.332	110.68	8.312	103.13	18.239	100.43
(HEP-BM/693)	0.331	110.45	8.290	102.86	18.156	99.98
(HEP-BM/694)	0.331	110.33	8.340	103.48	18.282	100.67
(HEP-BM/694)	0.324	107.99	8.196	101.69	18.023	99.24
(HEP-BM/697)	0.331	110.37	8.240	102.23	18.232	100.40
(HEP-BM/697)	0.327	108.88	8.317	103.19	17.939	98.78
(HEP-BM/698)	0.336	111.93	8.458	104.94	19.713	108.55
(HEP-BM/698)	0.339	112.86	8.399	104.20	19.753	108.77
(HEP-BM/699)	0.340	113.24	8.463	105.00	19.724	108.61

(HEP-BM/699)	0.334	111.28	8.452	104.86	19.780	108.92
(HEP-BM/700)	0.340	113.38	8.512	105.60	18.344	101.01
(HEP-BM/700)	0.334	111.21	8.369	103.83	18.018	99.22
N	12		12		12	
Mean	**0.333**		**8.362**		**18.684**	
SD (±)	0.0050		0.0973		0.7908	
CV (%)	**1.50**		**1.16**		**4.23**	
Min.	0.324		8.196		17.939	
Max.	0.340		8.512		19.780	
%Nominal Conc.	**111.10**		**103.75**		**102.88**	

(Heparin) Plasma
Acceptance criteria:
1. % Nominal should be within 85-115.
2. %CV≤15

Table 10
Ion Suppression and Enhancing Effect of Lercanidipine

UnextractedSample ID	**Unextracted Analyte Response**	**Post ExtractedSample ID**	**Post Extracted Analyte Response**
Un extracted (M2QC)-1	527174	PE MQC(HEP-BM/693)-01	660079
Un extracted (M2QC)-2	605812	PE MQC(HEP-BM/693)-02	691803
Un extracted (M2QC)-3	666011	PE MQC(HEP-BM/694)-01	710052
Un extracted (M2QC)-4	625408	PE MQC(HEP-BM/694)-02	692044
Un extracted (M2QC)-5	624972	PE MQC(HEP-BM/697)-01	728069
Un extracted (M2QC)-6	653730	PE MQC(HEP-BM/697)-02	837501
		PE MQC(HEP-BM/698)-01	784972
		PE MQC(HEP-BM/698-02	785864
		PE MQC(HEP-BM/699)-01	649822
		PE MQC(HEP-BM/699)-02	546249
		PE MQC(HEP-BM/700)-01	489500
		PE MQC(HEP-BM/700)-02	808091
N	6		12
Mean	**617185**		**698671**
SD (±)	**49151**		**103679**
CV	**7.96**		**14.84**
% Ion Suppression /Enhancing	**13.20**		

Table 11
Assessment of Dilution Integrity for Lercanidipine at DQC Concentration(ng/mL)

	DQC			
	Dilution Factor: 1/2 Nominal Conc: 18.860 ng/mL		**Dilution Factor: 1/4 Nominal Conc.: 9.430 ng/mL**	
	Conc.Found	**% Nominal**	**Conc.Found**	**% Nominal**
	19.924	105.64	3.059 (O)	32.44
	19.825	105.12	9.623	102.05
	19.823	105.11	8.359	88.64
	19.683	104.36	8.356	88.61
	19.656	104.22	8.423	89.32
	19.415	102.94	8.433	89.43
N	6		5	
Mean	19.721		8.639	
SD (±)	0.1799		0.5513	
CV (%)	0.91		6.38	
%NOM	104.57		91.61	

Acceptance criteria
1. % Nominal should be within 85-115.
2. %CV≤15%

Table 12
Assessment of Effect of using Partial volume of plasma Samples duringestimation of Lercanidipine in Human Plasma

	High QC (ng/mL)18.160			
	½ Quantity		**¼ Quantity**	
	9.080		**4.540**	
	Conc. found	**% Nominal**	**Conc.found**	**% Nominal**
	8.413	92.65	3.956	87.14
	8.350	91.96	4.746	104.54
	8.362	92.09	3.973	87.51
	8.325	91.68	4.682	103.13
	8.365	92.12	4.674	102.96
	8.368	92.16	4.710	103.75
N	6		6	
Mean	8.364		4.457	
SD (±)	0.0288		0.3822	
CV (%)	**0.34**		**8.58**	
Min.	8.325		3.956	
Max.	8.413		4.746	
%Nominal Conc.	**92.11**		**98.17**	

Acceptance criteria:
1. % C.V $\leq$ 15
2. Mean % Nominal (100 ±15%)

Table 13
Assessment of Injector Carry-Over effect for Analyte (Lercanidipine) and Internal Standard

Sample Identification	**Analyteresponse**	**Internalstandard response**	**Carry over observed with Analyte**	**Carry over observed withInternal Standard**
Mean of CS1 &IS	8180	751567	NA	NA
RS	0	0	Nil	Nil
PB	0	0	Nil	Nil
Aqs MIX	644090	1266288	NA	NA
RS	0	0	Nil	Nil
RS	0	0	Nil	Nil
PB	0	0	Nil	Nil
EXH	972304	689560	NA	NA
RS	0	0	Nil	Nil
RS	0	0	Nil	Nil
PB	0	0	Nil	Nil

Acceptance Criteria:
1. The Injector carry over of EXH and SS Mix at the retention time of the analyte and internal standard in the RS and PB sample shall not be more than 20% and 5% of mean area response of extracted analyte and internalstandard of LLOQ samples (CS1).

Table 14
Ruggedness of the method (Different analyst) for estimation of Lercanidipine Plasma levels in Human Plasma with different analyst.

Analytical Run ID:	**LLOQ(ng/mL) 0.100 (0.080-0.120)**		**Low QC(ng/mL)0.300 (0.255-0.345)**		**M1QC (ng/mL) 3.020 (2.567-3.473)**		**M2QC (ng/mL) 8.060 (6.851-9.269)**		**High QC(ng/mL) 18.160 (15.436-20.884)**	
	Conc. Found	**% Nom**	**Conc. Found**	**% Nom**	**Conc. Found**	**% Nom**	**Conc. Found**	**% Nom**	**Conc. Found**	**% Nom**
PA Batch-4	0.103	103.38	0.314	104.62	2.723	90.17	7.293	90.48	18.177	100.09
	0.094	94.05	0.314	104.51	2.688	89.02	7.013	87.00	18.197	100.20
	0.086	86.07	0.317	105.65	2.845	94.22	7.465	92.61	17.965	98.93
	0.085	85.22	0.321	106.99	2.959	97.97	7.088	87.95	18.088	99.60
	0.288	288.49	0.327	108.86	2.843	94.15	7.540	93.55	18.059	99.44
	0.095	95.26	0.316	105.35	2.741	90.77	7.405	91.88	18.268	100.59
N	5		6		6		6		6	

Mean	0.093		0.318		2.800		7.301		18.126	
SD (±)	0.0074		0.0050		0.1011		0.2113		0.1092	
%CV	**7.99**		**1.57**		**3.61**		**2.89**		**0.60**	
Min	0.085		0.314		2.688		7.013		17.965	
Max	0.103		0.327		2.959		7.540		18.268	
%NOM	**92.60**		**106.07**		**92.71**		**90.58**		**99.81**	

Acceptance criteria

1. % C.V ≤ 15% except LLOQ for which it is ≤ 20%
2. Mean % Nominal (100 ±15% & For LLOQ 100 ±20%)

Table 15
Ruggedness of the method (With different Analytical column) for determination of Lercanidipine levels in Human Plasma

Analytical Run ID:	**LLOQ(ng/mL) 0.100 (0.080-0.120)**		**Low QC(ng/mL) 0.300 (0.255-0.345)**		**M1QC (ng/mL)3.020 (2.567-3.473)**		**M2QC (ng/mL) 8.060 (6.851-9.269)**		**High QC(ng/mL) 18.160 (15.436-20.884)**	
	Column ID (LC/163)	**Column ID (LC/283)**	**Column ID (LC/163)**	**Column ID (LC/283)**	**Column ID (LC/163)**	**Column ID (LC/283)**	**Column ID (LC/163)**	**Column ID (LC/283)**	**Column ID (LC/163)**	**Column ID (LC/283)**
PA Batch-1	*0.067*	0.106	0.301	0.315	3.112	3.084	8.553	8.555	19.013	19.024
	0.099	0.106	0.319	0.334	2.875	2.880	8.396	8.263	18.398	18.409
	0.079	0.101	0.307	0.313	3.155	3.122	8.275	8.275	18.605	18.629
	0.093	0.102	0.297	0.309	3.101	3.067	8.464	8.479	18.617	18.499
	0.095	0.113	0.286	0.295	3.158	3.110	8.191	8.208	18.264	18.349
	0.096	0.107	0.313	0.334	3.060	3.071	8.606	8.659	18.722	18.701
N	6	6	6	6	6	6	6	6	6	6
Mean	0.088	0.106	0.304	0.317	3.077	3.056	8.414	8.407	18.603	18.602
SD (±)	0.0125	0.0043	0.0118	0.0151	0.1054	0.0887	0.1600	0.1835	0.2605	0.2451
%CV	14.17	**4.06**	3.88	**4.77**	3.43	**2.90**	1.90	**2.18**	1.40	**1.32**
Min	0.067	0.101	0.286	0.295	2.875	2.880	8.191	8.208	18.264	18.349
Max	0.099	0.113	0.319	0.334	3.158	3.122	8.606	8.659	19.013	19.024
%NOM	88.20	**105.80**	101.27	**105.57**	101.88	**101.18**	104.39	**104.30**	102.44	**102.43**

Acceptance criteria

1. %CV≤15% except LLOQ for which it is ≤ 20%
2. Mean % Nominal (100±15% & For LLOQ 100 ±20%)

Table 16
Assessment of Stability of Analyte (Lercanidipine) in Biological Matrix atRoom temperature.
Storage Duration: Approx. 25 hrs 25 min

	Low QC (ng/mL)0.300 (0.255-0.345)				**High QC (ng/mL)18.160 (15.436-20.884)**			
	Comparison Samples (0.00hrs)		**Stability Samples (25 hrs 25 min)**		**Comparison Samples (0.00 hrs)**		**Stability Samples (25 hrs 25 min)**	
	Conc. Found	**% Nominal**	**Conc. Found**	**% Nominal**	**Conc. Found**	**% Nominal**	**Conc. Found**	**% Nominal**
	0.309	103.02	0.306	102.03	18.492	101.83	18.560	102.20
	0.308	102.81	0.304	101.20	18.398	101.31	18.379	101.20
	0.316	105.26	0.317	105.77	18.250	100.50	18.226	100.37
	0.309	103.00	0.315	105.12	18.383	101.23	18.405	101.35
	0.311	103.71	0.299	99.68	18.205	100.25	18.438	101.53
	0.312	103.94	0.313	104.33	18.584	102.33	18.398	101.31
N	6		6		6		6	
Mean	0.311		0.309		18.385		18.401	
SD (±)	0.0029		0.0071		0.1427		0.1075	
CV(%)	**0.93**		**2.30**		**0.78**		**0.58**	
Min.	0.308		0.299		18.205		18.226	
Max.	0.316		0.317		18.584		18.560	
% Nominal Conc.	**103.60**		**103.00**		**101.24**		**101.33**	

% Change	**-0.58**	**0.09**

Acceptance Criteria

1. %Change should be ±15 or% Ratio (Stability/Comparison) should be within 85-115%2.
2. C.V ≤ 15%.
3. Mean % Nominal (100 ±15%)

Table 17
Assessment of Stability of Lercanidipine in Biological Matrix in Dry Ice(Coolant)
Storage Duration: Approx. 47 hrs 41 min

	Low QC (ng/mL)				**High QC (ng/mL)**			
	0.300				**18.160**			
	(0.255-0.345)				**(15.436-20.884)**			
	Comparison		**Stability Samples**		**Comparison**		**Stability Samples**	
	Samples (0.00 hrs)		**(47 hrs 41 min)**		**Samples (0.00 hrs)**		**(47 hrs 41 min)**	
	Conc. Found	**% Nominal**	**Conc. Found**	**% Nominal**	**Conc. Found**	**% Nominal**	**Conc. Found**	**% Nominal**
	0.320	106.52	0.306	101.87	18.194	100.19	18.373	101.17
	0.322	107.31	0.311	103.53	18.134	99.86	18.225	100.36
	0.311	103.79	0.316	105.25	18.171	100.06	18.404	101.35
	0.311	103.79	0.320	106.69	18.088	99.60	18.301	100.78
	0.318	106.13	0.316	105.45	18.229	100.38	18.301	100.78
	0.310	103.31	0.320	106.70	17.914	98.65	18.061	99.46
N	6		6		6		6	
Mean	0.315		0.315		18.122		18.278	
SD (±)	0.0053		0.0055		0.1128		0.1232	
CV(%)	**1.68**		**1.75**		**0.62**		**0.67**	
Min.	0.310		0.306		17.914		18.061	
Max.	0.322		0.320		18.229		18.404	
%Nominal Conc.	**105.10**		**104.93**		**99.79**		**100.65**	
% Change	**-0.16**				**0.86**			

Table 18
Assessment of Stability of Analyte (Lercanidipine) as Dry Extract. Storage Duration: Approx. 22 hrs 30 min

	Low QC (ng/mL)0.300 (0.255-0.345)				**High QC (ng/mL)18.160 (15.436-20.884)**			
	Comparison Samples (0.00 hrs)		**Stability Samples (22 hrs 30 min)**		**Comparison Samples (0.00 hrs)**		**Stability Samples (22 hrs 30 min)**	
	Conc. Found	**% Nominal**	**Conc. Found**	**% Nominal**	**Conc. Found**	**% Nominal**	**Conc. Found**	**% Nominal**
	0.309	103.02	0.312	104.00	18.492	101.83	18.326	100.91
	0.308	102.81	0.318	106.12	18.398	101.31	18.477	101.75
	0.316	105.26	0.310	103.40	18.250	100.50	18.540	102.09
	0.309	103.00	0.306	101.85	18.383	101.23	18.257	100.53
	0.311	103.71	0.302	100.82	18.205	100.25	18.193	100.18
	0.312	103.94	0.313	104.47	18.584	102.33	18.538	102.08
N	6		6		6		6	
Mean	0.311		0.310		18.385		18.389	
SD (±)	0.0029		0.0056		0.1427		0.1500	
CV(%)	**0.93**		**1.81**		**0.78**		**0.82**	
Min.	0.308		0.302		18.205		18.193	
Max.	0.316		0.318		18.584		18.540	
% Nominal Conc.	**103.60**		**103.40**		**101.24**		**101.26**	
% Change	**-0.19**				**0.02**			

Acceptance Criteria

1. %Change should be ±15 or
   % Ratio (Stability/Comparison) should be within 85-115%
2. % C.V ≤ 15%.
3. Mean % Nominal (100 ±15%)

Table 19
Assessment of Short-term Stock Solution Stability of Internal Standard at Room Temperature.
Storage Duration: Approx. 15 hrs 35 min, Storage condition: Room Temperature

	Comparison Stock Solution (IS) Response (0.00 hrs)	**Stability Stock Solution (IS) Response (15 hrs 35 min)**
	1541831	1469078
	1435798	1446867
	1444307	1490332
	1540091	1520361
	1467522	1439622
	1417939	1467701
	1451727	1453580
	1415934	1386346
	1375313	1376545
	1360946	1379823
	1407410	1459636
	1425310	1380290
N	12	12
Mean	1440344	1439182
SD (±)	55674	47963
CV (%)	3.87	3.33
% Ratio	99.92	
% Change	-0.08	

Acceptance Criteria:
1. %Change should be ±5 or
2. % Ratio (Stability/Comparison) should be within 95 -105%

Table 20
Mean Values Pharmacokinetic data derived from plasma Lercanidipine Concentrations (ng/ml).

	R-Lercanidipine		***S-Lercanidipine***		***Rec.-Lercanidipine***	
	A	*B*	*A*	*B*	*A*	*B*
Tmax(*h*)	2.104	2.101	2.142	2.101	2.086	2.112
Cmax (ng/mL)	4.768	5.133	4.803	5.071	8.95	9.37
AUCo-t	24.265	26.565	220681	24.451	41.72	44.9

Table 21
Comparison of Mean Concentrations Enantiomers (integrated values) with Recemic form of Lercanidipine.

	Case 1		**Case 2**	
	S+R / Rec.		***R / S***	
	A	*B*	*A*	*B*
Tmax(*h*)	1.017	0.99	0.98	1.069
Cmax (ng/mL)	1.069	1.089	0.99	1.01
AUCo-t	1.125	1.125	1.069	1.08

Acceptance criteria: The Mean ratios of Recemic form Lercanidipine and its enantiomersare near to 1(0.85-1.15).

Lercanidipine Hydrochloride tablets 20 mg vs. Zanidip® (Lercanidipine hydrochloride) tablets 20 mg (Fasting State)							
Pharmacokinetic data derived from plasma **Lercanidipine** Concentrations(ng/ml)							
Subject No.	**Sequence**	**R-Lercanidipine**		**S-Lercanidipine**		**Rec.-Lercanidipine**	
		Tmax (h)		***Tmax (h)t***		***Tmax (h)***	
		Formulation		***Formulation***		***Formulation***	
		A	**B**	**A**	**B**	**A**	**B**
1001	ABAB	2.16	1.83	2.16	1.50	2.17	1.67

1002	BABA	1.50	2.33	1.50	2.33	1.5	2.34
1003	BABA	1.50	2.00	1.50	2.16	1.83	2
1004	ABAB	1.50	3.25	1.66	3.08	1.5	3.34
1005	BABA	2.50	1.33	2.50	1.50	2.5	1.33
1006	ABAB	1.16	4.33	1.33	4.33	1.67	4.17
1007	BABA	4.00	3.66	4.00	3.66	4	4
1008	ABAB	2.16	1.50	2.16	1.50	2.17	1.5
1009	BABA	2.33	2.16	2.33	2.16	2.34	2.17
1010	BABA	2.66	1.66	2.66	1.66	3	1.5
1011	ABAB	2.33	2.50	2.33	2.50	2.33	2.67
2012	ABAB	2.00	1.16	2.00	1.33	1.84	1.17
1013	BABA	2.33	1.83	2.33	1.83	2.33	1.83
1014	ABAB	2.00	1.66	2.00	1.66	2	1.67
1015	ABAB	2.33	4.00	3.08	4.0	2.34	3.75
1016	BABA	2.33	2.58	2.33	2.75	2	2.59
1017	ABAB	2.16	2.16	2.16	2.16	2.002	2.17
1018	BABA	2.33	2.16	2.33	2.16	2.17	2
1019	BABA	2.58	2.83	2.58	2.83	2.59	3
1020	ABAB	2.50	1.50	2.50	1.83	2.5	2
1021	ABAB	2.16	2.50	2.33	2.50	2.17	2.33
1022	BABA	1.50	1.16	1.66	1.00	1.17	1
1023	ABAB	2.33	1.16	2.33	1.16	2.33	1.34
1024	BABA	4.16	1.16	4.16	1.50	3.34	1.33
1025	ABAB	1.16	1.16	1.16	1.16	1.17	1.17
1027	BABA	1.33	2.33	1.33	2.50	1.33	2.59
1029	ABAB	2.33	2.66	2.33	2.66	2.34	2.5
1030	BABA	1.50	1.00	1.66	1.00	1.5	1
1031	ABAB	2.00	1.16	2.00	1.16	2	1.17
1032	BABA	2.00	1.66	1.83	1.66	2	1.84
1033	ABAB	1.66	4.50	1.66	3.66	1.67	3.5
1034	ABAB	2.00	1.83	2.00	1.83	2.17	1.84
1035	BABA	2.50	1.50	2.50	1.50	2.34	2.5
2036	BABA	1.83	2.16	1.83	2.16	1.84	2.34
1037	ABAB	2.00	1.50	2.00	1.50	2	1.5
1038	BABA	2.33	1.66	2.33	1.66	2.33	1.67
1039	BABA	1.16	2.16	1.16	2.1	1.5	2
1040	ABAB	1.83	2.00	1.83	2.00	1.84	1.83
N		75	76	75	76	75	76
Mean		2.104	2.101	2.142	2.101	2.086	2.112
SD		0.876	1.203	0.869	1.067	0.754	1.109
Min		0.667	0.667	1.000	0.667	0.67	0.67
Median		2.000	1.667	2.000	1.833	--	--
Max		6.000	8.000	6.000	6.000	6	6
CV%		41.7	57.3	40.6	50.8	36.1	52.5
Geometric Mean		1.954	1.869	2.004	1.901	1.969	1.894

Lercanidipine Hydrochloride tablets 20 mg vs. Zanidip® (Lercanidipine hydrochloride) tablets 20 mg (Fasting State)							
Pharmacokinetic data derived from plasma Lercanidipine Concentrations(ng/ml)							
Subject No.	**Sequence**	**R-Lercanidipine**		**S-Lercanidipine**		**Rec.-Lercanidipine**	
		Cmax(ng / mL)		***Cmax(ng / mL)***		***Cmax(ng / mL)***	
		Formulation		***Formulation***		***Formulation***	
		A	**B**	**A**	**B**	**A**	**B**
1001	ABAB	3.09	3.00	3.29	3.49	6.51	6.85
1002	BABA	9.32	8.6	11.06	8.92	19.64	18.25

1003	BABA	3.91	5.96	3.57	5.56	7.35	11.71
1004	ABAB	3.18	2.38	3.042	2.26	6.39	4.51
1005	BABA	3.93	2.49	3.69	2.39	7.40	4.80
1006	ABAB	2.49	2.05	3.19	1.67	6.16	3.43
1007	BABA	3.00	5.42	2.74	5.13	4.97	9.11
1008	ABAB	5.86	6.13	6.12	5.85	11.02	10.82
1009	BABA	6.51	5.73	6.78	5.69	11.34	10.99
1010	BABA	2.58	2.97	2.94	3.65	5.23	5.98
1011	ABAB	5.45	6.38	6.04	7.00	11.08	12.56
2012	ABAB	2.02	1.73	2.30	1.92	4.30	4.13
1013	BABA	8.61	7.26	6.31	5.19	14.18	12.07
1014	ABAB	3.00	4.25	2.78	3.99	5.88	8.22
1015	ABAB	4.22	5.95	3.72	5.94	7.05	9.08
1016	BABA	2.51	2.87	2.57	2.91	5.30	5.68
1017	ABAB	3.75	2.80	4.81	3.84	9.23	6.02
1018	BABA	4.15	3.93	4.42	4.23	10.24	9.51
1019	BABA	10.71	13.85	10.08	11.5	17.55	22.60
1020	ABAB	6.99	7.55	6.35	7.75	10.37	12.24
1021	ABAB	9.12	10.81	10.12	11.18	18.68	21.16
1022	BABA	1.18	0.45	1.39	0.53	2.56	0.95
1023	ABAB	3.71	2.13	3.67	2.31	6.90	4.00
1024	BABA	3.66	3.07	3.71	3.48	6.01	4.88
1025	ABAB	3.98	3.84	5.55	5.45	8.64	8.20
1027	BABA	4.52	6.66	4.29	5.99	8.50	12.52
1029	ABAB	8.10	8.30	7.76	7.52	16.79	13.27
1030	BABA	1.93	1.61	1.99	1.69	3.80	3.37
1031	ABAB	4.88	9.01	4.89	9.94	9.29	17.65
1032	BABA	5.19	6.35	5.22	5.89	11.08	12.54
1033	ABAB	0.86	0.68	0.79	0.54	1.63	1.29
1034	ABAB	7.54	5.03	7.94	5.309	14.63	10.15
1035	BABA	4.03	4.76	3.73	4.37	6.58,2	7.58
2036	BABA	2.52	4.41	2.36	3.82	4.21	7.14
1037	ABAB	6.89	7.41	6.96	8.31	12.77	14.18
1038	BABA	7.54	6.76	7.08	6.65	10.41	11.04
1039	BABA	4.51	5.95	4.13	5.15	7.39	9.35
1040	ABAB	4.46	6.39	4.05	5.54	7.13	8.08
N		75	76	75	76	75	76
Mean		4.76	5.13	4.80	5.07	8.95	9.37
SD		2.59	3.07	2.57	2.88	4.71	5.46
Min		0.639	0.215	0.684	0.282	1.36	0.49
Median		4.178	4.707	4.223	4.650	--	--
Max		11.484	14.411	11.837	14.091	20.33	25.69
CV%		54.5	59.8	53.7	57.0	52.70	58.30

Conclusion

Since, results of the analytical method development and validation parameters studied fell in the acceptance levels of each criterion, the developed method can be deemed legitimate and can be applied for the routine estimation of the concentration of Lercanidipine in plasma in a single analytical run for any given dosage form.

The ruggedness efficiency of the liquid- liquid extraction method provides exceptional sample clean up and high recoveries using 200µl of plasma. The high extraction efficiency, low limit ofquantitation and wide linear dynamic range make this a suitable method for use in clinical samples from bioequivalence studies following oral administration of Lercanidipine fixed dose(10mg or 20 mg) tablets in healthy human subjects.

The results obtained for recemic and enantiomers lercanidipine were under the acceptance criteria. Results show both studies of lercanidipine under similar conditions and there is no *in vivo* conversion of enantiomers. The presence of the R-enantiomer and S-lercanidipine concentration was similar.

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