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SIMULTANEOUS ESTIMATION AND VALIDATION OF LC-MS METHOD FOR THE DETERMINATION OF BARNIDIPINE IN HUMAN PLASMA: ITS STABILITY INDICATING

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ABSTRACT

Various LC-MS/MS and RP-HPLC methods are reported in the literature for the estimation of barnidipine individually. According to the literature survey, there is no regulatory method reported for the estimation of barnidipine by LC-MS/MS in human plasma either in literature and pharmacopeia. Hence, in this study a Novel LC/MS/MS method was optimized and validated for simultaneous estimation and validation of barnidipine in human plasma in accordance with the USFDA, EMEA and other relevant regulatory guidelines. Bamidipine is an anti-hypertensive drug belonging to the dihydropyridine (DHP) group of calcium antagonists. It is available in a modified-release formulation which has a gradual onset of action and is effective in a single daily oral dose of 10 to 20 mg. Bamidipine has selective action against cardiovascular calcium antagonist receptors and its anti-hypertensive action is related to the reduction of peripheral vascular resistance secondary to its vasodilatory action. The clinical anti-hypertensive efficacy of barnidipine is similar to that of other DHP calcium antagonists such as nitrendipine and amlodipine, and anti-hypertensives belonging to other drug classes such as atenolol and enalapril. Barnidipine has been found to be as efficacious and well tolerated as hydrochlorothiazide in the management of hypertension in elderly patients. Barnidipine is generally well tolerated. As with other DHP calcium antagonists, vasodilator adverse events such as a headache, flushing and peripheral oedema account for most of the adverse events reported with its use and are usually transient. Oedema is less frequent than with amlodipine and nitrendipine. Its use is not associated with reflex tachycardia. The validated regulatory bioanalytical method for estimation of barnidipine by LC-MS/MS in human plasma is useful for analysis of subject samples to support generic ANDA applications for different regulatory authorities for introducing new generic drugs in affordable rates for the patients. Also the validated regulatory method would be used as supporting literature for developing and validating better method in CROs and clinical research organizations to support new generic drugs.

Keywords: Bamidipine, LC-MS/MS, Di-hydropyridine (DHP), Human Plasma and Methanol.

INTRODUCTION

Barnidipine is an antihypertensive drug belonging to the di-hydropyridine (DHP) group of calcium antagonists. It is available in a modified-release formulation which has a gradual onset of action and is effective in a single daily oral dose of 10 to 20 mg. Barnidipine has selective action against cardiovascular calcium antagonist receptors and its antihypertensive action is related to the reduction of peripheral vascular resistance secondary to its vasodilatory action[1]. The clinical antihypertensive efficacy of barnidipine is similar to that of other DHP calcium antagonists such as nitrendipine and amlodipine, and anti-hypertensives belonging to other drug classes such as atenolol and enalapril. Barnidipine has been found to be as efficacious and well tolerated as hydrochlorothiazide in the management of hypertension in elderly patients. Barnidipine is generally well tolerated. As with other DHP calcium antagonists, vasodilator adverse events such as a headache, flushing and peripheral oedema account for most of the adverse events reported with its use and are usually transient. Oedema is less frequent than with

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amlodipine and nitrendipine[2]. Its use is not associated with reflex tachycardia. Barnidipine is a white crystalline powder with a molecular weight of 491.53. It is slightly soluble in water. The experimental water solubility for Barnidipine is 2.89 mg/mL. Within the physiologic pH range, barnidipine is an ionized compound pKa=7.91 as strong base and pKa=19.47 as strong acid[3]. Mechanism of action barnidipine (pure S, S isomer) is a liphophilic 1, 4-dihydropyridine calcium antagonist showing high affinity for the calcium channels of the smooth muscle cells in the vascular wall. Receptor kinetics of barnidipine are characterized by a slow onset of action and a strong and long-lasting binding. The reduction in peripheral resistance brought about by barnidipine results in blood pressure lowering. When using barnidipine, the anti-hypertensive effect remains during the entire 24-hour dose interval[4]. Use of barnidipine in chronic treatment does not lead to an increase in basic heart frequency. The impact of barnidipine on cardiovascular morbidity or mortality has not been studied. However, recently completed, controlled studies of other long-acting dihydropyridines indicate similar beneficial effects on morbidity and mortality compared to other anti-hypertensives in hypertension of the elderly[5]. After repeated administration of libradin 20 to healthy individuals, the concomitant intake of food did not have a statistically significant effect on AUC, Cmax, Tmax or t½. Maximum plasma levels are obtained 5 to 6 hours after oral administration of Libradin 20. Libradin shows an absolute bioavailability of 1.1%. Barnidipine plasma concentrations may show considerable interpersonal variation[6,7]. In-vitro studies show that barnidipine binds at the rate of 26-32% to human erythrocytes and to a high extent (89-95%) to plasma proteins. In-vitro analysis of protein components indicates that barnidipine mainly binds to serum albumin, followed by α1 acid glycoprotein and high-density lipoproteins.

To a much lesser extent binding to γ globulin takes place[8,9]. No drug interactions based on elimination of plasma protein binding have been observed in in-vitro studies. Barnidipine is to a great extent metabolized into inactive metabolites. No in-vivo chiral inversion of the pure S, S isomer takes place. Main reactions are N-debenzylisation of the side chain, hydrolysis of the N-benzylpyrrolidine ester, oxidation of the 1, 4-dihydropyridine ring, hydrolysis of the methyl ester and reduction of the nitro group. The metabolism of barnidipine seems mainly mediated by the CYP3A isoenzyme family.[10, 11]

The median terminal elimination plasma half-life of libradin was 20 hours after repeated administration, according to a two-compartment analytical model. Elimination mainly takes place through metabolism. Barnidipine and/or its metabolites are excreted in faeces (60%), urine (40%) and breath (less than 1%). No un-metabolized barnidipine is excreted in urine[12].

EXPERIMENTAL

Instrumentation

Chromatography was performed with HPLC coupled with MS/MS. All HPLC systems were equipped with a column compartment with temperature control and an on-line degasser. Data acquisition, analysis, and reporting were performed by analyst chromatography software.[13-15]

Reagents and Chemicals

A pharmaceutically pure sample of barnidipine reference standard was obtained from clear synthetics, Hyderabad as gift samples along with their analytical reports. HPLC grade methanol, acetonitrile, diethyl ether and ammonium acetate was obtained from Merck chemical division, Mumbai. HPLC Waters used. Human plasma lots were local blood banks.

Chromatographic Condition

The isocratic mobile phase consisted of acetonitrile: 5mm ammonium acetate at 90:10 ratios at a flow rate of 1.0 mL min^{-1}(with splitter out/in 70:30) and ACE C18 5µ, 100mm X 4.6mm was used as stationary phase. The Mass parameters for barnidipine is 492.2/315.1 (m/z), barnidipine D5 is 497.4/315.1 (m/z).[16, 17]

Preparation of Standard Stock Solution: Barnidipine

Weighed accurately 5.0000 mg barnidipine reference standard, transfer into a 5 mL volumetric flask. 3 mL of methanol was added and sonicated to aid dissolution. Volume was made up to 5 mL with methanol to achieve 1000.000 µg/mL concentration.

Preparation of Working Standard Solutions

Spiking solutions were made and with serial dilution, the spiking solutions were spiked in the screened pooled matrix to Standard concentrations.[18,19]

Table-1: Calibration Curve Standards Concentration for Barnidipine

S. No.	Barnidipine Concentration (pg/mL) A	CC ID
1	5047.800	STD 1
2	4416.820	STD 2
3	3224.280	STD 3
4	2224.760	STD 4
5	1201.380	STD 5
6	504.580	STD 6
7	131.200	STD 7
8	65.600	STD 8
9	25.580	STD 9

Diluent/ Rinsing solution

800 mL of methanol was measured in a reagent bottle. Separately 200 mL of water measured and transferred to above-mentioned reagent bottle. The bottle was swirled to mix and sonicated for 5 minutes. Used within 4 days of preparation.[20, 21]

5 mM Ammonium Acetate Solution

Weighed about 0.38540 gm of ammonium acetate and dissolved in 800 mL of water. Made up the volume to 1000 mL with water. Swirled the bottle to mix the contents. Sonicated for 5 minutes. Used within 4 days of preparation.

Mobile Phase

Measured100 mL of 5 mM Ammonium acetate buffer solution in a measuring cylinder. Separately measured 900 mL of Acetonitrile in a measuring cylinder. Transfer both the solution to a reagent bottle and swirl to mix the contents and sonicate for 5 minutes. Used within 4 days of preparation[22, 23].

Aqueous Standard Solution

Taken 20.0 µL of spiking solution standard SS (A) STD1, 855 µL of mobile phase and 125 µL ISTD dilution solutions in a poly-propylene tube and mix well. Provide the batch number. Prepared freshly as and when required.

Internal Standard Stock Solution Barnidipine D5 (1000.000 µg/mL)

Weighed accurately 2.000 mg Barnidipine D5 Reference standard transferred into a 2 mL volumetric flask. 1 mL of methanol was added and sonicated to aid dissolution. Volume made up to 2 mL with methanol to achieve the concentration 1000.000 µg/Ml.[24, 25]

Intermediate Internal Standard Stock Solution

Took 0.020 mL of ISTD stock solution (1000.000 µg/mL) into 10 mL volumetric flask and made up to volume with diluent to produce a concentration of 2.000 µg/mL. Stored in the refrigerator (2ºC to 8ºC).

Preparation of Internal Standard Dilution ((20.000 ng/mL))

Aliquoted volume 0.100 mL of ISTD INTM Stock solution into 10 mL volumetric flask and made up the volume with diluent. Provided a batch number. Stored in the refrigerator (2ºC to 8ºC).[26, 27]

Detection Method

Method Validation

Validation parameters like system suitability, specificity, linearity& range, accuracy, precision; recovery, Sensitivity, solution and matrix stability were estimated as per USFDA/ EMEA/ANVISA, GLP guidelines[28].

Table-2: Instrumentation

S. No.	Instrument	Model/Make
01	Detector	Sciex API 4000 Mass Detector
02	Ion source	Turbo ion spray
03	Roughing pump	Varian
04	Pump	LC-10ADvp , Shimadzu
05	Auto-sampler	SIL-HTC , Shimadzu
06	Degasser	DGU 20 A_3 , Shimadzu
07	Column Oven	CTO 10 AS VP, Shimadzu

Table-3: Chromatographic Conditions

S. No.	Parameters	Chromatographic Condition
01	Mobile Phase	90:10Acetonitrile :5 mm Ammonium Acetate
02	Flow Rate	1.000 mL/min (with splitter out/in 70:30)
03	Column	ACE C18 5µ, 100mm X 4.6mm
04	Injection Volume	15.000 µL
05	Retention Time	Drug: 1.750 ± 0.300 min
		ISTD: 1.730 ± 0.300 min.
06	Column Oven Temperature	40.0°C (± 1.0°C)
07	Auto Sampler Temperature	10.0°C (± 1.0°C)
08	Run Time	3.0 n.

Table-4: Mass Parameters

Drug	Q1/Q3- Mass 492.2/315.1 (m/z)*
ISTD	Q1/Q3- Mass 497.4/315.1 (m/z)*
Polarity	Positive

*Note: To optimize response these parameters may be changed up to ± 0.5 units

Table-5: LC/MS/MS Source/Gas Parameters

S. No.	Parameters	Drug (A)
1	Curtain Gas (CUR)	22.00
2	Collision Activated Dissociation (CAD)	10.00
3	Gas1(GS1)	40.00
4	Gas2(GS2)	45.00
5	Temperature (TEM)	450.00
6	Ionization Voltage(IS)	5500.00

Table-6: Compound Parameters

Scan Type		
Parameters	Drug	ISTD
Declustering Potential (DP)	100.00	105.00

Entrance Potential (EP)	10.00	10.00
Collision Energy (CE)	35.00	35.00
Collision Cell Exit Potential (CXP)	10.00	10.00
Dwell Time (Milli Second)	200.00	200.00

Analytical Batch Organization

Injected 15.000 µL of each sample in the following order:
AQS STD (Drug + ISTD)
RS
STD Blank (n=2)
STD Zero (n=2)
CC Standards
Quality Control Samples.

Table-7: Data Processing

Software used	Acquire chromatograms using the Analyst-software ver. 1.4.2.
Weighting	Linear regression with weighing factor ($1 / x^2$).
Analysis mode	Peak area ratio (Drug/ISTD) vs. the concentration of the drug.
Calculation	By using the following equation by Analyst software

Y = m X + C Where,
X = Concentration of analyte in pg/mL
Y = Peak area ratio of drug to ISTD
m = Slope of the Calibration Curve
C = Intercept on Y- axis

RESULTS AND DISCUSSION

Mass Spectroscopy

Various mobile phase combinations were tried initially to separate barnidipine on a C18 column. Preliminary experiments indicated that using different concentrations of acetonitrile or methanol with water was not able to separate the peaks of barnidipine or to obtain suitable retention and peak shape. In order to achieve acceptable peak shapes and get suitable run time for better separation, various buffer systems were tried systematically. The retention time of barnidipine obtained for different ratio of acetonitrile :5 mm Ammonium Acetate (60:40, 65:35, 70:30, 75:25& 80:20 v/v) in table 8.

Table-8: MD Trail Chromatographic Conditions for Barnidipine

S. No.	MD Trail No.	MD Trail Chromatographic Conditions
1	a	60:40 Acetonitrile :5 mm Ammonium Acetate , BDS, 1mL
2	b	65:35 Acetonitrile :5 mm Ammonium Acetate , Thermo, 1 mL
3	c	70:30 Acetonitrile :5 mm Ammonium Acetate , XDB, 1 mL
4	d	75:25 Acetonitrile :5 mm Ammonium Acetate , XDB, 1 mL
5	e	80:20 Acetonitrile :5 mm Ammonium Acetate , XDB, 1 mL
6	f	90:10Acetonitrile :5 mm Ammonium Acetate ACE C18

Thereafter, acetonitrile:5mm ammonium Acetate (90:10 v/v) at flow rate of1.0mL min^{-1}(with splitter out/in 70:30),ACE C18 5µ, 100mm X 4.6mm was used as the mobile and stationary phase respectively to improve resolution, get short run time and reducing tailing of both peaks close to1. The mass parameters for barnidipine is 492.2/315.1 (m/z), barnidipine D5 is 497.4/315.1 (m/z). The retention time was found to be 1.750 ± 0.300 min for barnidipine and 1.730 ± 0.300 min for barnidipine D5.

Method Validation: System Suitability Test

System suitability test was performed by acquiring six consecutive injections from a single sample of the Highest Standard. The system was found to be sensitive, specific and reproducible for the current analytical run. Results are shown in Table-9. A representative chromatogram is shown in Fig.-1 and 2.

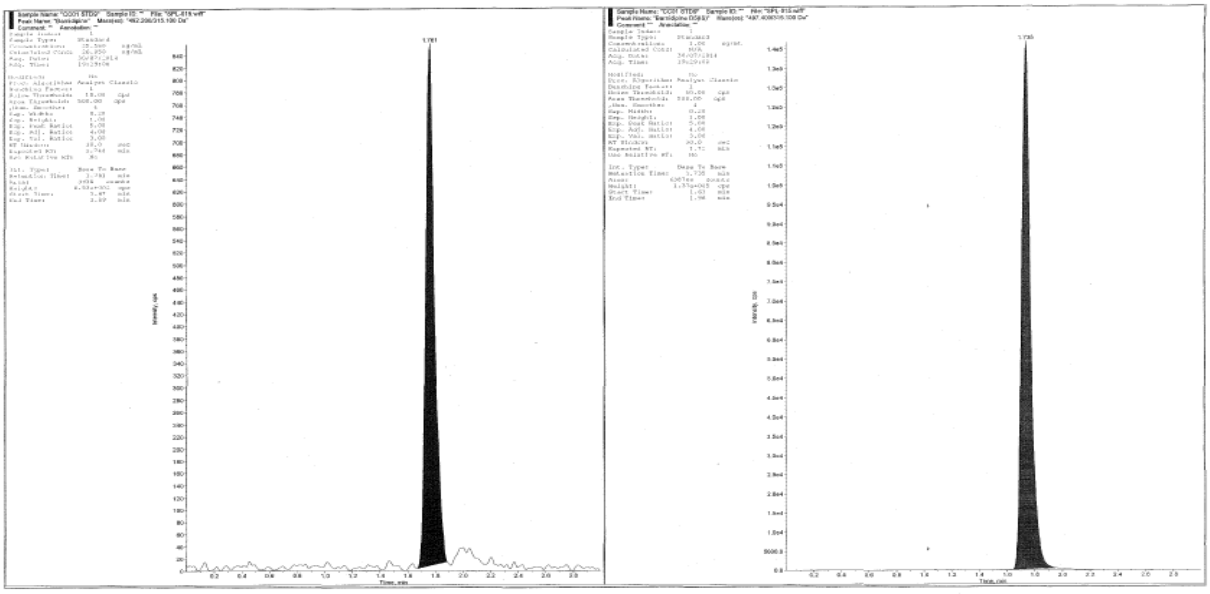

Fig.-1: Optimized Chromatogram of Barnidipine

Table-9: System Suitability Results for Barnidipine

Injection No.	Retention Time (min)		Area Ratio
	Analyte-Barnidipine	ISTD-Barnidipine D5	
1	1.748	1.725	1.211
2	1.749	1.727	1.213
3	1.748	1.725	1.215
4	1.749	1.727	1.228
5	1.748	1.725	1.226
6	1.748	1.724	1.218
Mean	1.7483	1.7255	1.2185
SD	0.00052	0.00122	0.00701
%CV	0.0	0.1	0.6

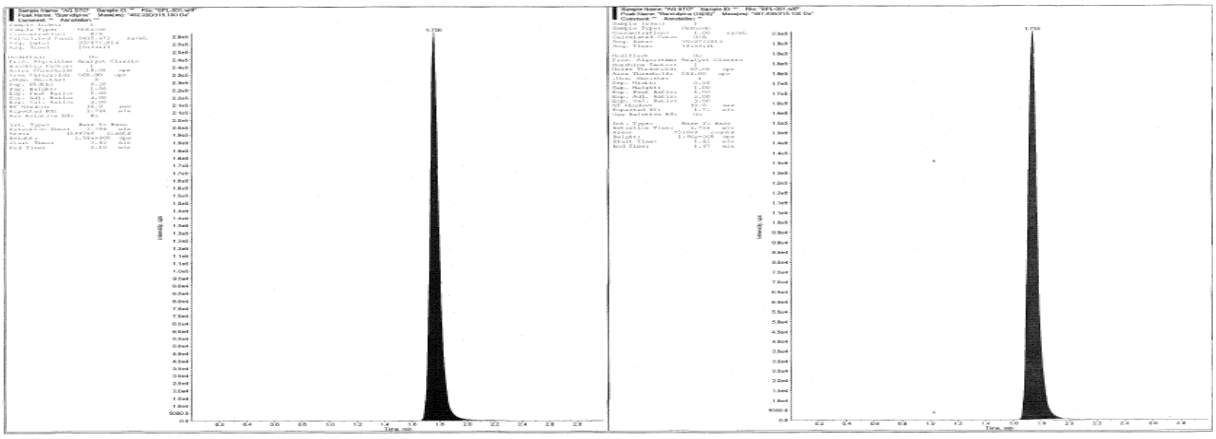

Fig.-2 : A Representative Chromatogram of Barnidipine for System Suitability Test Auto Sampler Carry Over

Barnidipine

Carryover was assessed by subsequently injecting reconstitution solution after an aqueous standard of highest concentration and standard blank (Extracted) after a calibration curve standard at the upper limit

of quantification. Aqueous and extracted LOQ samples were also injected. Carryover was not observed for barnidipine, and barnidipine D5 with respect to aqueous LOQ and extracted LOQ samples. Results are shown in table 10 for aqueous solution.

Table-10: ASCOT Results for Barnidipine

Aqueous	Response Barnidipine	% Carry Over	Response Barnidipine D5	% Carry Over
RS	0	-	0	-
AQS STD 1	1104161	-	907573	-
RS	0	0.0	0	0.0
AQS STD 1	1111994	-	898078	-
RS	0	0.0	0	0.0
AQ LOQ	9406	-	1451996	-

Selectivity and Specificity

Thirteen lots (A-314, A-332, A-347, A-348, A-350, A-351, A-354, A-363 including haemolytic (HA-123, HA-124) and lipemic (LT-3825, LA-030) lots) of plasma were evaluated for selectivity. No significant interference was observed at the retention time of Barnidipine and ISTD (barnidipine-D5) with respect to extracted LOQ shown in Table-11.

Table-11: Selectivity Results for Barnidipine

S. No .	Plasma Lot No.	Analyte - Barnidipine			ISTD - Barnidipine-D5		
		Area of interfering peak at RT of S-Barnidipine	Area observed for extracted LOQ	% interference at RT of S-Barnidipine	Area of interfering the peak at RT of ISTD	Area observed for extracted ISTD	Ok interference at RT of ISTD
1	A314	0	2139	0.0	0	374635	0.0
2	A-332	0	2629	0.0	0	441026	0.0
3	A-347	0	3048	0.0	0	488060	0.0
4	A-348	0	2780	0.0	0	493795	0.0
5	A-350	0	2877	0.0	0	467567	0.0
6	A-351	0	3076	0.0	0	493379	0.0
7	A-354	0	3192	0.0	0	489807	0.0
8	A-363	0	3029	0.0	0	510566	0.0
9	LT-3825	0	2578	0.0	0	377823	0.0
10	LA-030	0	3195	0.0	0	597203	0.0
11	HA-123	0	3350	0.0	0	524568	0.0
12	HA-124	0	3481	0.0	0	512201	0.0

H-Haemolyzed, L- Lipemic, Degree of Haemolysis: HA-123: 550 mg/dL HA-124: 1100 mg/dL.

Sensitivity

Six replicate injections at LOQ level were injected to evaluate the sensitivity of the method. The limit of quantification (LOQ) was 25.580 pg/mL. Accuracy and precision at LOQ were 95.6% and 6.3% respectively, which were within the acceptance criteria of 80 - 120% of nominal concentration for accuracy and 20% for precision shown in Table-12 and Fig.-3.

Table-12: Sensitivity of Results for Barnidipine

LLOQ		
Nominal concentration		25.580
Maximum limit (pg/mL)		30.696
Minimum limit (pg/mL)		20.464
Batch	S.No.	Back calculated concentration
P&A I	1	24.657
	2	26.840
	3	28.374
	4	27.309
	5	24.818
	6	27.524
	Mean	26.587
	SD	1.51729
	% CV	5.7
	% Accuracy	103.9
	% Bias	3.9

Sample Name	Signal to Noise Ratio
Sensitivity LOQ - 001	32.5
Sensitivity LOQ - 002	74.2
Sensitivity LOQ - 003	51.7
Sensitivity LOQ - 004	46.5
Sensitivity LOQ - 005	41.3
Sensitivity LOQ - 006	37.2
Mean	47.23

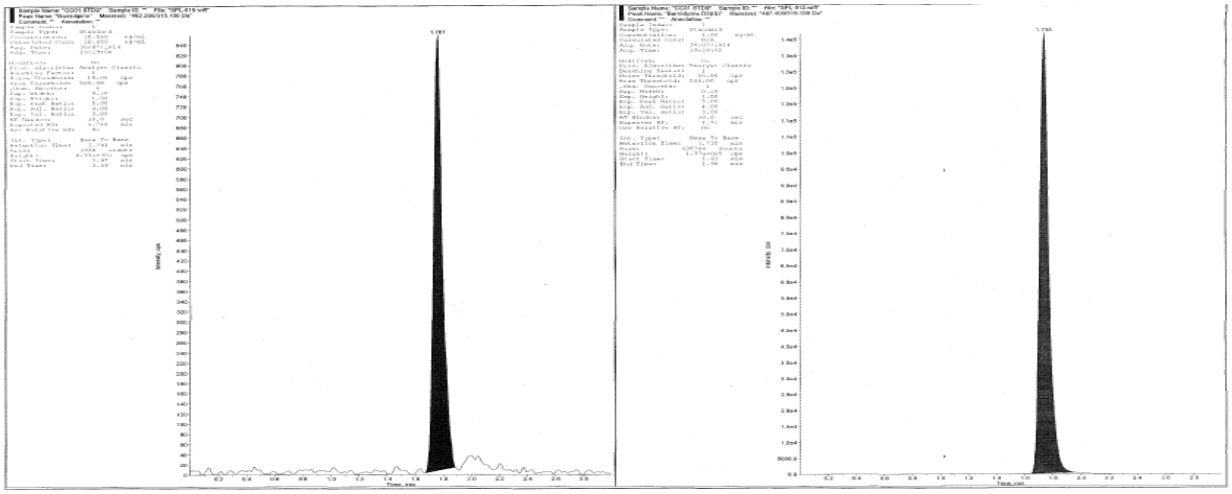

Fig.-3 : LOQ Chromatogram for Barnidipine

Precision and Accuracy

The Intra day (P & A I) precision (% CV) for the low, dilution, middle and high-quality control samples ranged from 0.9% to 6.5%. The inter day precision ranged from 1.8% to 4.3%, which were within the acceptance criteria of 15% in Table-13. The Intra day (P & A I) precision (% CV) for the limit of quantification quality control samples was 4.9%. The inter day precision was 5.0%, which were within the acceptance criteria of 20% in Table-13. The Intra day (P & A I) accuracy for the low, dilution, middle and high quality control samples ranged from 93.8% to 101.3%. The Inter day accuracy ranged from 92.7% to 99.1%, which were within the acceptance criteria of 85-115% of the nominal

concentration in table 13. The Intra day (P & A I) accuracy for the limit of quantification quality control samples was 101.2%. The Inter day accuracy was 99.3%, which were within the acceptance criteria of 80-120% of nominal concentration shown in Table-13 and Fig.-4.

Table-13: Precision and Accuracy Results of Barnidipine

Quality Control Samples		HQC	MQC	DQC	LQC	LOQ QC
Nominal concentration (pg/mL)		4082.980	2245.640	7581.200	67.360	25.600
Maximum limit (pg/mL)		4695.427	2582.486	8718.380	77.464	30.720
Minimum limit (pg/mL)		3470.533	1908.794	6444.020	57.256	20.480
Batch ID	S.No.	Back calculated concentrations (pg/mL)				
P&A I (Intra day)	001	4065.340	2252.421	7371.521	65.065	27.270
	002	4108.925	2267.472	7400.747	66.839	27.148
	003	4131.880	2277.174	7441.452	55.046	26.685
	004	4105.398	2276.132	7451.411	64.293	24.723
	005	3935.571	2261.490	7545.137	64.136	25.246
	006	4436.516	2309.926	7349.633	63.718	24.402
	Mean	4130.6050	2274.1025	7426.6502	63.1828	25.9123
	SD	165.52121	19.84944	70.01881	4.13632	1.27332
	% CV	4.0	0.9	0.9	6.5	4.9
	% Accuracy	101.2	101.3	98	93.8	101.2
	% Bias	1.2	1.3	-2.0	-6.2	1.2
P&A II	007	4044.2930	2128.8430	7395.5120	61.4630	23.9530
	008	3959.6870	2184.6970	7443.5320	58.9860	25.2140
	009	4061.6920	2196.2830	7530.8910	62.5890	24.8560
	010	4008.6120	2239.6830	7451.8380	62.9230	24.9370
	011	4027.1040	2233.7680	7446.2990	63.1900	22.9130
	012	3973.3900	2232.4260	7331.0070	60.0070	24.8980
	Mean	4012.4630	2202.6167	7433.1798	61.5263	24.4618
	SD	39.94456	42.51282	66.38228	1.70958	0.87120
	% CV	1.00	1.9	0.9	2.8	3.6
	% Accuracy	98.3	98.1	98	91.3	95.6
	°A Bias	-1.7	-1.9	-2	-8.7	-4.4
P & A III (Different column with different analyst)	013	3905.587	2202.257	7215.497	64.625	27.351
	014	4078.115	2178.173	7169.079	62.85	24.18
	015	3952.902	2158.665	7242.431	62.273	25.984
	016	4064.02	2185.581	7153.119	62.151	24.903
	017	3986.661	2173.777	7170.05	64.039	26.455
	018	4002.639	2210.537	7121.01	59.859	26.553
	Mean	3998.3207	2184.8317	7178.5310	62.6328	25.9043
	SD	65.57518	19.05889	43.73877	1.67625	1.16612
	% CV	1.6	0.9	0.6	2.7	4.5
	% Accuracy	97.9	97.3	94.7	93	101.2
	% Bias	-2.1	-2.7	-5.3	-7	1.2
Global Statistics (Inter day)	Mean	4047.1296	2220.5169	7346.1203	62.4473	25.4262
	SD	116.25987	48.27283	134.82345	2.68711	1.26190
	% CV	2.9	2.2	1.8	4.3	5.0
	% Accuracy	99.1	98.9	96.9	92.7	99.3

Quality Control Samples		HQC	MQC	DQC	LQC	LOQ QC
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Maximum limit (pg/mL)		4695.427	2582.486	8718.380	77.464	30.720
Minimum limit (pg/mL)		3470.533	1908.794	6444.020	57.256	20.480
	% Bias	-0.9	-1.1	-3.1	-7.3	-0.7

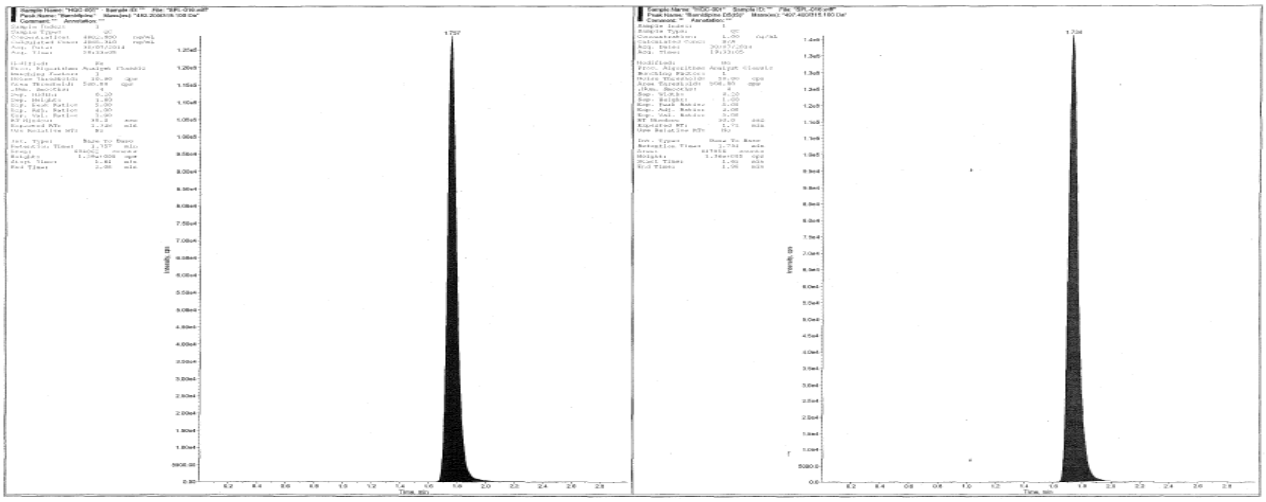

Fig.-4 : Precision and Accuracy Representative Chromatogram for Barnidipine

Recovery

The mean areas of extracted quality control plasma samples of Barnidipine were compared against the mean areas of post extracted quality control samples of HOC, MQC and LQC. The % recovery at HQC, MQC and LQC levels were 84.0%, 86.5% and 85.9% respectively. Global recovery for Barnidipine was 85.5%, which were within the acceptance criteria of 115% shown in Table-14.

The mean areas of extracted quality control plasma samples of ISTD (Barnidipine-D5) were compared to the internal standard mean areas of the post extracted quality control samples. Recovery for ISTD (Barnidipine-D5) is 48.8% which was within the acceptance criteria of 115% shown in Table-14 and 15 and Fig.-5.

Table-14: Recovery results for Barnidipine

	Post Extracted Area	Extracted Area
HQC	971775	636028
	981133	700040
	995580	650741
	967920	668484
	943023	626816
	988736	646470
Mean	974694.5	654763.2
SD	18613.56	26287.86
% CV	1.9	4.0
% Recovery	84.0	
MQC	504499	358899
	513774	365329

	520449	364127
	537683	348357
	544914	383332
	527796	359880
Mean	524852.5	363320.7
SD	15047.52	11492.93
% CV	2.9	3.2
% Recovery	86.5	
LQC	15860	11471
	14583	10736
	15439	10284
	15083	10224
	15015	10598
	14639	8926
Mean	15103.2	10373.2
SD	485.75	838.08
% CV	3.2	8.1
°A Recovery	85.9	
Global	85.5	

Table-15: Recovery Results for Barnidipine-D5

QC ID	Post Extracted Area	Extracted Area
HQC	982473	694009
	998443	731538
	1000492	701563
	972738	700986
	961165	670047
	977445	688298
MQC	936307	694553
	952420	714815
	959383	718902
	992172	679296
	999942	751557
	970321	693843
LQC	995880	759974
	973814	731470
	1007851	707164
	974274	704465
	981217	708605
	964939	638705
Mean	977848.7	704988.3
SD	19033.15	28533.7
% CV	1.9	4.0
% Recovery	90.1	

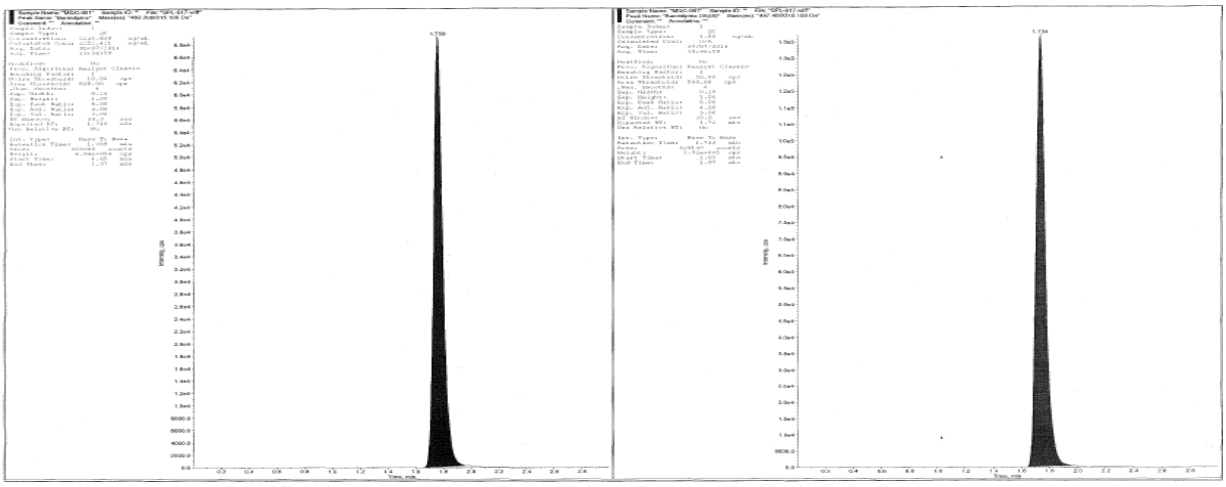

Fig.-5 : Recovery Representative Chromatogram for Barnidipine

CONCLUSION

The validated regulatory bioanalytical method for estimation of Barnidipine by LC-MS/MS in human plasma is useful for analysis of subject samples to support generic ANDA applications for different regulatory authorities for introducing new generic drugs in affordable rates for the patients.

Also the validated regulatory method would be used as supporting literature for developing and validating better method in CROs and clinical research organizations to support new generic drugs.

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