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Research Article

ESTIMATION OF NIFEDIPINE IN K_2EDTA HUMAN PLASMA USING UHPLC-MS/MS METHOD

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ABSTRACT

Nifedipine is a dihydropyridine calcium channel blocker extensively used for hypertension. The present study has been designed to develop and validate a highly specific, selective, accurate, precise, stable method for a new estimation of Nifedipine using UHPLC-MS/MS method in human plasma. In this method Nifedipine D6 acts as internal standard and K_2EDTA acts as an anticoagulant. Nifedipine and Nifedipine D6 were extracted from human plasma by liquid-liquid extraction method using ethyl acetate as extraction solvent. They are separated on UHPLC column (Phenomenex, Gemini 3µ, $C_{18,}$ 50×4.6mm) using mobile phase composed of Acetonitrile:5mM Ammonium Formate pH 3.50 (60:40V/V) at a flow rate of 1.0ml/min. Both the compounds were detected in mass spectrometer (API-4000) by using Positive polarity, Electro spray ionization (ESI) with MRM scan mode. The retention times of Nifedipine and Nifedipine D6 were 1.22min and 1.20min respectively. The linearity range was in between 0.802ng/ml-241.037ng/ml. The regression coefficient was found to be 0.9994. The Q1/Q3 values for Nifedipine and Nifedipine D6 were 347.100/315.200 and 353.200/318.200 respectively. Stock solutions and working solutions were stable for 6days 2hrs and 3days respectively at 2-8ºC. The developed method was accurate, precise and stable throughout the process and the results are within the acceptable limits.

1. INTRODUCTION

Nifedipine, a widely used antihypertensive drug, chemically it is 3,5-Dimethyl-2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. Nifedipine belongs to the class of dihydropyridine calcium channel blocker. It acts by inhibiting the transmembrane influx of extracellular calcium ions across the vascular smooth muscle cells and myocardial membrane without affecting the plasma calcium concentrations, therefore reduce peripheral vascular resistance, relaxes arteriolar smooth muscle thereby lowering arterial blood pressure. Nifedipine has a bioavailability of 50% with 96% plasma binding and half-life of 4hrs[1-4]. Only few methods were reported on individual estimation of Nifedipine by UPLC-MS/MS[5] and by LC-MS/MS[6]. Various analytical methods have been illustrated for the estimation of Nifedipine with other combinations in biological samples or in pure form effectively, which includes UHPLC-MS/MS, HPLC-MS/MS and HPLC[7-14]. Whereas LC alone without MS coupling was found to be more time consuming with sample deposition, further did not comply with the requirement of LLOQ of Nifedipine pharmacokinetic studies.

The developed UHPLC–MS/MS technique involves short retention time and less LLOQ value which were much lower than the other techniques. The present study has been designed to develop a highly specific, selective, accurate, precise and stable method

for estimation of Nifedipine in K_2EDTA human plasma using UHPLC-MS/MS method. Nifedipine D6 is used as an internal standard.

2. MATERIALS AND METHODS

2.1. Chemicals and instruments:

Nifedipine (Figure 1A) and Nifedipine D6 (Figure 1B) were purchased from Vivan Life Sciences Pvt Ltd Mumbai. Methanol, Water, Formic acid and Ethyl acetate of HPLC grades are from E-Merck India. Human plasma was purchased from National Institute of Nutrition, Hyderabad. API 4000 model mass spectrometer system was made from AB SCIEX and UHPLC was made of Shimadzu.

2.2. Preparation of stock solutions, Calibration curve (CC) standards and quality control (QC) samples

The stock solution of Nifedipine (1000µg/ml) in methanol (50%V/V) was prepared and stored at 2-8ºC. The stock solution was spiked with blank K_2EDTA human plasma to attain a set of eight non-zero concentrations of calibration curve(CC) standards of Nifedipine ranging from 0.801ng/ml-241.347ng/ml and stored at 2-8ºC till use. The Quality control (QC) samples were prepared by spiking Nifedipine stock solution with blank K_2EDTA human plasma to achieve the final concentrations of 192.672ng/ml, 96.336ng/ml, 2.168ng/ml and 0.802ng/ml and labelled as HQC, MQC, LQC and LLOQ QC respectively and stored at 2-8ºC till use. Nifedipine D6 stock solution of 450µg/ml in diluent methanol was prepared and then diluted with acetonitrile to achieve final concentration of 300ng/ml, vortexed and stored at 2-8ºC.

Aliquot 0.300ml or an appropriate volume of each CC standards and QC samples were taken into prelabelled polypropylene vials and added 0.300ml of screened blank plasma into prelabelled polypropylene vials for standard blank and zero samples separately and stored the samples in deep freezer at -70±15ºC and -20±5ºC. All the stock solutions, spiking solutions, CC standards, QC samples were prepared under yellow chromatic light due to light sensitivity of Nifedipine.

2.3. Sample extraction and preparation

Required number of samples containing CC standards and QC samples were collected from deep freezer and thawed in water bath to room temperature. 50µl of Nifedipine D6 dilution (300ng/ml) was added to each pre-labelled polypropylene tubes except blank. To all polypropylene tubes 0.2ml of plasma sample, 0.2ml of 0.1N HCl and 2.0ml of Ethyl acetate to be added and vortexed for 20mins at 2000rpm. Further all sample tubes were centrifuged at 3500rpm for 10mins at 5ºC. 1.5ml of supernatant was collected into pre-labelled tubes subjected for evaporation under nitrogen gas at 40ºC till dryness. The dried samples were reconstituted with 0.4ml of mobile phase, vortexed transferred to vials kept in auto sampler for injection. Unextracted or post extracted samples are prepared same as above extraction method for matrix effect and recovery.

2.4. Chromatographic conditions and UHPLC-MS/MS instrumentation-parameters

Both Nifedipine and Nifedipine D6 were separated on UHPLC using column (Phenomenex, Gemini C18, 3µ, 50x4.6mm) and mobile phase (Acetonitrile:5mM ammonium formate pH 3.50, {60:40V/V}) with a flow rate of 1.0ml/min, isocratic flow and with run time of 2.20mins. Auto-sampler temperature and column oven temperature were maintained at 10ºC and 35ºC respectively. The retention times for Nifedipine and Nifedipine D6 were 1.22min and 1.20min (Figure 2A and Figure 2B) respectively.

Mass spectrometric detections was performed on API-4000 mass spectrometer with analyst software version 1.6.2. Electro spray ionization was performed in the MRM scan mode with positive polarity using nitrogen gas as collision gas. The spray voltage and source temperature were 5500V and 450ºC respectively. The source parameters were curtain gas(CUR), collision gas(CAD), nebulizer gas(GS1) and turbo gas(GS2) of nitrogen were optimized to 30.0psi, 6.0psi, 40.0psi and 45.0psi respectively. The optimized compound parameterswere declustering potential(DP), entrance potential(EP), collision energy(CE) and collision cell exit potential(CXP) were 65.0V, 10.0V, 15V and 10.0V respectively for both Nifedipine and Nifedipine D6. The Q1/Q3 for Nifedipine were 347.100/315.200 and Nifedipine D6 was 353.200/318.2 which were monitored with a dwell time of 200 msec.

2.5. Calibration curve

An eight point calibration curve was constructed by taking (Nifedipine area/Nifedipine D6 area) area ratio on Y-axis and concentration of Nifedipine on X-axis. The calibration curve (Figure 3) was found to be linear over a concentration range from 0.802ng/ml-241.037ng/ml for Nifedipine. A linear equation was established to provide the best fit for the concentration versus detector response using $1/X^2$ as weighing factor. The goodness of fit was consistently greater than 0.99 during the course of validation. In the

current method the LLOQ of Nifedipine was found to be 0.802ng/ml.

2.6. Method Validation[15-16]

The method was validated over a linear concentration range of 0.802ng/ml-241.037ng/ml. The method was validated for system suitability, selectivity, specificity, accuracy, precision, matrix effect, recovery and stability as per standard validation guidelines.

2.7. System suitability

System suitability was determined by injecting six consecutive injections of AQ STD (equivalent to MQC concentration) of Nifedipine and Nifedipine D6. The system was found to be sensitive and reproducible when the retention times of Nifedipine and Nifedipine D6 and area ratios were within the limits.

2.8. Selectivity

Ten plasma lots were evaluated for selectivity which includes two lipidemic matrix lots and two hemolyzed matrix lots. All of these 10 plasma lots were spiked with Nifedipine D6 and Nifedipine at LLOQ level. All the lots were estimated for percentage of interfering peaks present at retention time of Nifedipine and retention time of Nifedipine D6.

2.9. Specificity

Specificity for Nifedipine and Nifedipine D6 was evaluated at LLOQ levels by injecting replicate injections of STD blank, Concomitant blank and STD ZERO samples. The response of interfering peaks at retention times of Nifedipine and Nifedipine D6 in presence of other analytes were compared with the response of Nifedipine and Nifedipine D6 in absence of other analytes at LLOQ level.

2.10. Accuracy and Precision

Six replicate analysis of quality control (HQC, MQC, LQC and LLOQ QC) samples were used to determine intra-day and inter-day precision and accuracy. Accuracy was measured by % difference of back calculated mean concentrations of quality control samples to their respective nominal values. Precision was measured by %CV over the concentration range of quality control samples during validation.

2.11. Matrix Factor

The matrix effect was performed to make sure selectivity and precision were not compromised by the matrix screened. Ten lots (includes 2 lipidemic and 2 hemolyzed lots) of screened blank plasma spiked with HQC and LQC were evaluated for matrix effect. Six replicate injections of aqueous samples of LQC and HQC concentration levels and Post extracted samples of biological matrix at concentrations equivalent to LQC and HQC were injected to estimate matrix factor. The ISTD normalized matrix factor was calculated based upon the matrix factor of Nifedipine and Nifedipine D6.

Matrix factor and ISTD Normalized matrix were calculated by

$$\text{Matrix factor} = \frac{Peak\ area\ in\ presence\ of\ matrix\ ions}{Mean\ peak\ area\ in\ absence\ of\ matrix\ ions}$$

$$\text{ISTD Normalized factor} = \frac{Matrix\ factor\ of\ Nfedipine}{Matrix\ factor\ of\ Nifedipine\ D6}$$

2.12. Recovery

The recovery of the method was evaluated by comparing the mean areas of extracted quality control (HQC, MQC and LQC) samples of Nifedipine with the mean areas of post extracted quality control (HQC, MQC and LQC) samples of Nifedipine. Similarly the mean areas of extracted middle quality control samples of Nifedipine D6 were compared against mean areas of post extracted middle quality control samples of Nifedipine D6.

$$\%\ \text{Mean Recovery} = \frac{\text{Mean Extracted peak area}}{\text{Mean Post extracted peak area}} \times 100$$

$$\%\ \text{Recovery with factor} = \frac{\text{Extraction solvent added to sample}}{\text{Supertant transfered for evaporation}}\ \text{X \%Mean Recovery}$$

2.13. Stability of Nifedipine in aqueous solutions

The short term stock solutions stabilities of Nifedipine and Nifedipine D6 were evaluated at ULOQ levels stored at 25±5ºC for 7hours 21mins and 7hours 17mins respectively. Similarly the short term working solutions stabilities of Nifedipine was estimated at ULOQ and LLOQ levels stored at room temperature for 6hours 55mins and for Nifedipine D6 at 25±5ºC working solution stored for 6hours 47mins. The long term stock solutions stabilities of Nifedipine and Nifedipine D6 was determined by stock solutions at ULOQ levels stored at 2-8ºC for 6days 2hours. Similarly the long term working solutions stabilities of Nifedipine was carried out by working solutions at ULOQ and LLOQ levels stored at 2-8ºC for 3days and for Nifedipine D6 working solution stored at 2-8ºC

for 6days. The mean responses obtained from stability samples were compared with the mean responses obtained from Nifedipine and Nifedipine D6 stored at 2-8ºC.
The stabilities of short term stock, working solutions, long term stock, working solutions were calculated by formula:

$$\%\text{Mean Stability} = \frac{\text{Mean peak area of stability sample}}{\text{Mean peak area of comparison sample}} X\ 100$$

2.14. Stability of Nifedipine in biological matrix

Freshly prepared CC standards used for the assessment of bench top stability, freeze thaw stability, stability in extract-ambient, stability in extract- auto sampler and dry extract stability. Bench top stability was estimated at high and low quality control samples stored at ambient temperature (25±5ºC) for 7hrs 13mins after reconstitution. Freeze stability was determined after completing 5 freeze thaw cycles at -70±5ºC and at -20±5ºC separately accessing at LQC and HQC levels. Stability in extract-ambient or wet extract stability was estimated at LQC and HQC levels stored at 25±5ºC for 7hrs 2mins. Auto sampler stability was estimated at low and high quality control samples by storing for about 52hrs 57mins in auto sampler. Dry extract stability was estimated after 47hrs 16mins at LQC and HQC levels stored at 2-8ºC.

3. RESULTS and DISCUSSION

3.1. Method validation

The method was validated for entire linearity concentration range. According to standard validation guidelines the validation parameters includes system suitability, selectivity, specificity, matrix effect, linearity, precision, accuracy, recovery and stability.

3.2. System suitability

Aqueous standard equivalent to MQC concentration level was evaluated for system suitability by injecting six consecutive injections. The %CV for mean retention times of Nifedipine and Nifedipine D6 were 0.3% and 0.0% respectively and the %CV for mean area ratio was 1.2%.

3.3. Selectivity

Ten plasma lots didn't show any interfering peaks at retention times of Nifedipine and Nifedipine D6.

3.4. Specificity

No interference peaks were observed with standard blank, concomitant blank and standard zero samples at the retention times of Nifedipine and Nifedipine D6 at LLOQ quality control levels.

3.5. Accuracy and Precision

The intra-day accuracies were in between 104.0% to 110.7% and the inter-day accuracies were in between 103.9% to 109.8% at HQC, MQC and LQC levels to the nominal concentration levels. For LLOQ QC the intra-day accuracy and inter-day accuracy were 103.8% and 103.7% respectively. The intra-day precision (%CV) was in between 1.1% to 1.6% and inter-day precision (%CV) was in between 1.2% to 1.5% for HQC, MQC and LQC levels and for LLOQ QC the intra-day precision and inter-day precision was 1.4% and 2.0% respectively (Table 1).

3.6. Matrix Factor

The matrix factor for Nifedipine at HQC and LQC samples was found to be 1.059 and 1.072 respectively, similarly matrix factor for Nifedipine D6 at HQC and LQC samples was found to be 1.053 and 1.05 respectively. The %CV for ISTD normalized matrix factor for both HQC and LQC levels were 0.4% and 2.1% respectively (Table 2).

3.7. Recovery

The % recovery of Nifedipine with factor at HQC, MQC, LQC levels were 97.7%, 100.7% 99.1% respectively. The mean areas of extracted MQC samples of Nifedipine D6 were compared against mean areas of post extracted MQC samples of Nifedipine D6. The % recovery of Nifedipine D6 with factor at MQC level was 103.8% (Table 3).

3.8. Stability of Nifedipine in aqueous solutions

The short term stock solution stabilities of Nifedipine and Nifedipine D6 at ULOQ level and at ambient temperature were 101% and 99.7% respectively. The long term stock solution stabilities of Nifedipine and Nifedipine D6 at ULOQ level and at 2-8ºC were 98.9% and 96.7% respectively. The short term working solution stabilities of Nifedipine at ULOQ and LLOQ levels and at ambient temperature were 101.2% and 96.7% respectively and for Nifedipine D6 it was 99.5%. The long term working solution stabilities for Nifedipine at ULOQ and LLOQ levels and at 2-8ºC were 100.8% and 101.6% respectively and for Nifedipine D6 it was 94.6% (Table 4).

3.9. Stability of Nifedipine in biological matrix

The accuracy and precision for bench top stability at ambient temperatures for Nifedipine at HQC level were found to be 104.6% and 1.5% and for LQC level they were 107.4% and 6.2% respectively. The % freeze thaw stability of Nifedipine after five freeze thaw cycles at-70±15°C at HQC and LQC levels were found to be 104.7% and 104.7% and the %CV were 0.6% and 1.0% respectively. The % freeze thaw stability of Nifedipine at -20±5°C and at HQC and LQC levels were found to be 104.6% and 105.2% and the %CV were 1.1% and 1.8% respectively. Stability of extract at ambient temperature at HQC and LQC levels were 105.7% and 106.8% and the %CV were 1.0% and 6.7% respectively. Stability of Nifedipine at HQC and LQC levels in auto-sampler at 10±1ºC was found to be 105% and 103.4% and the %CV were 1.1% and 0.6% respectively. Dry extract stability of Nifedipine at HQC and LQC levels at 2-8ºC was found to be 105.1% and 104.9% and the %CV was 1.0% and 0.9% respectively (Table 5).

4. CONCLUSION

The analytical method is validated for the analysis of Nifedipine in K_2EDTA human plasma over the range of 241.037ng/ml-0.802ng/ml using Phenomenex, Gemini C_{18}, 3µ, 50x4.6mm column on UHPLC-MS/MS using Nifedipine D6 as internal standard. The LLOQ of the method was 0.802ng/ml. The regression coefficient was found to be 0.9998. Nifedipine was stable over a period of minimum of 6 days in stock solutions. By using liquid-liquid extraction method, electro spray ionization, positive polarity multi reaction monitoring (MRM) scan mode a highly sensitive, specific, selective, accurate, precise stable method was developed validated as per standard guidelines. The m/z values for Nifedipine (Q1/Q3) were 347.100/315.200 (m/z) and for Nifedipine D6 (Q1/Q3) were 353.200/318.200 (m/z).

5. ACKNOWLEDGEMENT

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Table 1: Accuracy and precision

Q conc. level	Nominal conc.	Mean conc. found	±SD	%CV	%Accuracy	%Bias
Intraday						
HQC	193.568	207.9763	2.27045	1.1%	107.4%	7.4
MQC	96.784	107.1648	1.13053	1.1%	110.7%	10.7
LQC	2.168	2.2557	0.03699	1.6%	104.0%	4.0
LLOQ QC	0.804	0.8347	0.01159	1.4%	103.8%	3.8
Interday						
HQC	193.568	207.3316	2.48391	1.2%	107.1%	7.1
MQC	96.784	106.2660	1.62872	1.5%	109.8%	9.8
LQC	2.168	2.2517	0.03215	1.4%	103.9%	3.9
LLOQ QC	0.804	0.8335	0.01645	2.0%	103.7%	3.7

ISTD =Internal standard; SD =Standard deviation; CV =Coefficient of variance

Table 2: Matrix effect

Aqueous Samples	Biological Matrix Lot No	Analyte Matrix Factor		ISTD Matrix Factor		ISTD Normalized Matrix factor	
		HQC	LQC	HQC	LQC	HQC	LQC
1	BM-01	1.09	1.10	1.09	1.08	1.00	1.02
2	BM-02	1.08	1.10	1.07	1.10	1.01	1.00
3	BM-03	1.08	1.14	1.08	1.06	1.00	1.07
4	BM-04	1.08	1.09	1.07	1.06	1.01	1.03
5	BM-05	1.07	1.08	1.07	1.05	1.01	1.02
6	BM-06	1.06	1.07	1.06	1.06	1.01	1.02
-	BM-L1	1.05	1.05	1.05	1.03	1.00	1.02
-	BM-L2	1.03	1.03	1.02	1.02	1.01	1.00
-	BM-H1	1.02	1.01	1.01	1.01	1.00	1.00
-	BM-H2	1.02	1.05	1.02	1.03	1.00	1.02
Mean	**-**	**1.059**	**1.072**	**1.053**	**1.050**	**1.006**	**1.021**
SD	**-**	**-**	**-**	**-**	**-**	**0.0044**	**0.0218**
%CV	**-**	**-**	**-**	**-**	**-**	**0.4**	**2.1**

BM =Biological matrix; L1 &L2 = Lipidemic lots; H1&H2 = hemolyzed lots

Table 3: Recovery

Sample	Analyte			ISTD
	HQC	MQC	LQC	
Post extracted mean peak area	2814362.0	1392054.3	30600.8	1083037.5
Extracted mean peak area	2062390.2	1051322.3	22753.7	842751.8
%Recovery	73.3	75.5	74.4	77.8
%Recovery with factor	97.7	100.7	99.1	103.8

HQC =High quality control; MQC =Medium quality control; LQC =Low quality control

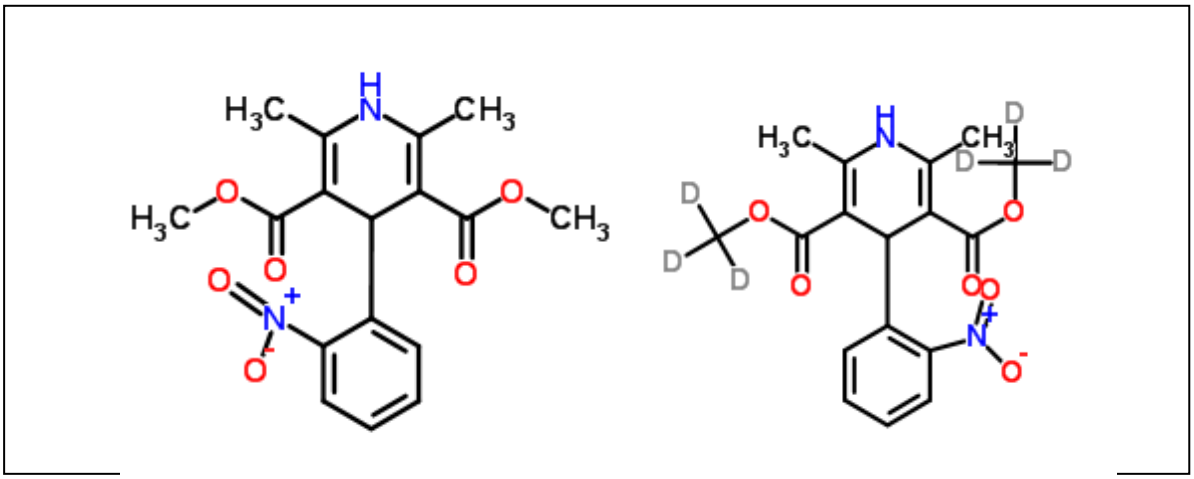
Table 4: Stability data of Nifedipine in aqueous solutions (n=6)

Type of Stability	Level	Storage duration	Comparison stock %CV	Stability stock %CV	% Stability
Short term stock solution	ULOQ	7hrs 21mins	0.7%	0.5%	101.0%
Long term stock solution	ULOQ	6days 2hrs	0.3%	0.4%	98.9%
Short term working solution	ULOQ LLOQ	6hrs 55mins 6hrs 55mins	0.5% 3.2%	0.6% 2.2%	101.2% 96.7%
Long term working solution	ULOQ LLOQ	3days 00hrs 3days 00hrs	1.9% 3.5%	1.1% 3.5%	100.8% 101.6%

Table 5: Stability data of Nifedipine in biological matrix (n=6)

Type of Stability	Levels	Temp	%CV	% Stability
Bench Top Stability	HQC LQC	25±5ºC 25±5ºC	1.5% 6.2%	104.6% 107.4%
Freeze thaw stability	HQC LQC HQC LQC	-70±15ºC -70±15ºC -20±5ºC -20±5ºC	0.6% 1.0% 1.1% 1.8%	104.7% 104.7% 104.6% 105.2%
Stability In Extract– Ambient	HQC LQC	25±5ºC 25±5ºC	1.0% 6.7%	105.7% 106.8%
Stability in Extract–Auto sampler	HQC LQC	10±1ºC 10±1ºC	1.1% 0.6%	105.0% 103.4%
Dry Extract Stability	HQC LQC	2-8ºC 2-8ºC	1.0% 0.9%	105.1% 104.9%

HQC=high quality control, LQC =Low quality control;
CV =Coefficient of variance.



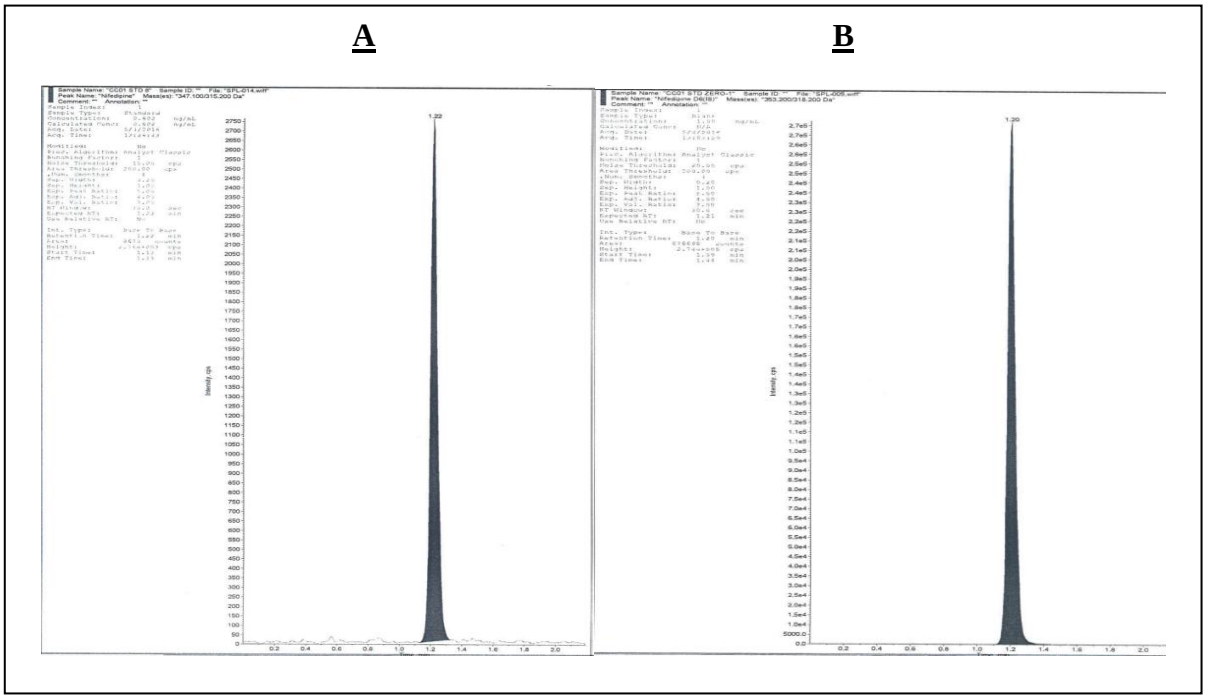


Fig. 2: Representative chromatograms of (A) Nifedipine (B) Nifedipine D6

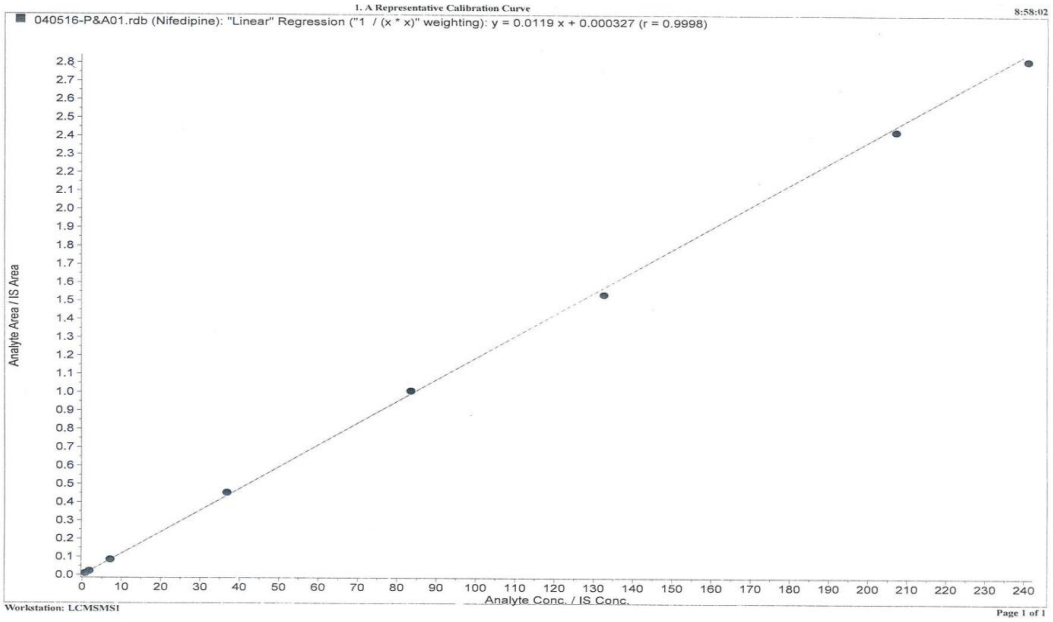


Fig. 3: Calibration curve

6. REFERENCES

1. Encyclopedia of molecular pharmacology edited by Offermanns S, Rosenthal W. Springer-Verlag Berlin Heidelberg, New York. 2008;2:295-299.
2. The Gale encyclopedia of medicine edited by Laurie J Fundukian, Gale Cengage Learning, New York. 2011;4(1-6):377-379.
3. Modern pharmacology with clinical applications edited by Charles R. Craig and Robert E. Stitzel, Lippincott Williams & Wilkins. 2004;5:148-268.
4. Martindale: the complete drug reference edited by Sweetman SC, Press P, London. 2009; 36:940-946.
5. Wang D, Jiang K, Yang S, Qin F, Lu X and Li F. Determination of nifedipine in human plasma by ultra-performance liquid chromatography–tandem mass spectrometry and its application in a pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci. 2011;879(20):1827-32.
6. Reddy S, Ahmad I, Nayak N, Thangam S and Mukhopadhyay A. Estimation of nifedipine in human plasma by LCMS/MS. Asian J Pharm Cli Res. 2013;6(1):83-86.
7. De Nicolo A, Avataneo V, Rabbia F, Sciandra M, Tosello F, Cusato J, Perlo E, Mulatero P, Veglio F, Di Perri G and D'avolio A. UHPLC–MS/MS method with sample dilution to test therapeutic adherence through quantification of ten antihypertensive drugs in urine samples. J Pharm Biomed Anal. 2017;142:279-285.
8. Chen R, Huang J, Lv C, Wei C, Li R, Yuan G, Liu X, Wang B and Guo R. A more rapid, sensitive, and specific HPLC-MS/MS method for nifedipine analysis in human plasma and application to a pharmacokinetic study. Drug Research. 2013 63(01):38-45.
9. Ezzeldin E, Abo-Talib NF, Tammam MH and Shahat AA. Development and validation of LC/MS/MS method for the simultaneous determination of montelukast, gliclazide, and nifedipine and its application to a pharmacokinetic study. Chemistry Central Journal. 2014; 8(17):01-09.
10. Kallem RR, Inamadugu JK, Ramesh M and Seshagirirao JV. Sensitive LC-MS/MS-ESI method for simultaneous determination of nifedipine and atenolol in human plasma and its application to a human pharmacokinetic study. Biomed Chrom. 2013;27(3):349-355.
11. Wang XD, Li JL, Lu Y, Chen X, Huang M, Chowbay B and Zhou SF. Rapid and simultaneous determination of nifedipine and dehydronifedipine in human plasma by liquid chromatography–tandem mass spectrometry. Application to a clinical herb–drug interaction study. J Chrom B. 2007;852(1-2):534-544.
12. Guo Y, Dai J, Qian G, Guo N, Ma Z and Guo X. Determination of nifedipine in human plasma and its use in bioequivalence study. Int J Pharm. 2007;341(1-2): 91-96.
13. Niopas I and Daftsios AC. Determination of nifedipine in human plasma by solid-phase extraction and high-performance liquid chromatography: validation and application to pharmacokinetic studies. J Pharm Biomed Anal. 2003;32(6):1213-1218.
14. Suzuki H, Fujiwara S, Kondo S and Sugimoto I. Determination of nifedipine in human plasma by high-performance liquid chromatography with electrochemical detection. J Chrom B: Biomed Sci Appli. 1985;341:341-347.
15. Goswami D, Gurule SJ, Singh P, Goru NS, Modhave Y, Khuroo AH and Monif T. Method development challenges and regulatory expectation in nifedipine. Int J Pharm Kin. 2017; 2(1):21-37.
16. DHHS U and FDA C. Guidance for industry, Bioanalytical method validation. US Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CV). 2001;2015.