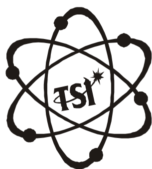
December 2009 Volume 8 Issue 4



Trade Science Inc.

Analytical CHEMISTRY
An Indian Journal

Full Paper

ACAIJ, 8(4) 2009 [506-510]

Development and validation of RP-HPLC method for estimation of felodipine in rat plasma

Minal Raghunath Narkhede[1,2,*], Bhanudas Shankar Kuchekar[2], Jitendra Yadav Nehete[3]

[1]C/o. R.G.Narkhede, 36 A., Vidyut colony, Jalgaon, Dist- Jalgaon (M S) (INDIA)

[2]Department of Biopharmaceutics, Govt. College of Pharmacy, Karad Dist-Satara (M.S) 415124 (INDIA)

[3]Head of Department Pharmaceutical Chemistry, Govt. College of Pharmacy, Karad Dist-Satara (M.S) 415124 (INDIA)

E-mail : minalnehete@rediffmail.com

Received: 22nd July, 2009 ; Accepted: 1st August, 2009

ABSTRACT

An attempt has been made to develop a simple, precise, sensitive, reproducible and rapid for estimation of felodipine in rat plasma by reverse phase HPLC. Chromatographic separation was achieved on a Spherisorb (250mm×4.6mm, 5 μm) ODS column using mobile phase (methanol and water) in the ratio 80:20 % v/v at a flow rate of 0.9 ml/min. The wavelength of detection was 260 nm. The proposed chromatographic method gave good resolution of the drug in plasma. Felodipine eluted at retention time of 9.94 min. The linearity of the method was established in the range 50 ng - 150 ng/ml with a correlation coefficient of 0.9943 The limit of detection of felodipine was 25 ng/ml and limit of quantification was 50 ng/ml. Excellent precision was evident with a coefficient of variance less than 15%. The developed HPLC method was found to be linear, precise, accurate and reproducible indicating method may be used for the quantitative estimation of felodipine in blood samples. © 2009 Trade Science Inc. - INDIA

KEYWORDS

Felodipine;
Rat plasma;
HPLC.

INTRODUCTION

Felodipine, 3-ethyl-5-methyl- 1,4-dihydro-2,6-dimethyl - 4 - (2,3 - dichlorophenyl) - 3,5 pyridinedicarboxylate, is a dihydropyridine calcium antagonist developed for use as a selective vasodilator in cardiovascular disorders, primarily arterial hypertension.[1] Individual determination of FLD has been carried out in human plasma by capillary gas chromatography[2], high selectivity gas chromatography[3], and HPLC[4-6], in pharmaceutical formulations by LC[7-9], reversed phase HPLC[10]. However, there are some problems with these reported methods. Gas chromatography with mass spectrometric detection or electron capture detector showed very high sensitivity and specificity[11-13], but might cause thermal decomposition of felodipine. The LC-MS method could be best choice considering sensitivity, specificity and stability, but in many laboratories LC-MS is not available for economic reasons. High-performance liquid chromatography (HPLC) using ultraviolet detection is widely available but shows low sensitivity and specificity compared with LC-MS (14). Several different methods have been reported for qualitative and quantitative analysis of felodipine in human plasma and biological samples; these include gas chromatography with mass spectrometric detection[15,16], gas chromatography with electron-capture detection,[17] high-performance liquid

chromatography with ultraviolet detection[18,19] high-performance liquid chromatography with amperometric detection,[20] and supercritical-fluid chromatography with electron capture and ultraviolet detection[21]. These methods are not ideal for pharmacokinetic work, however, because they are time-consuming, i.e. include derivatization steps, arduous sample preparation, and long chromatographic run times[22]. Heinig et al.[23] have reported the determination of felodipine in rat samples by column-switching liquid chromatography– tandem mass spectrometry concluding that this method is limited to rat plasma and tissue. A previous method describing the determination of felodipine involves liquid–liquid extraction and LC–ESI–MS–MS in Human and Dog Plasma[24]. The aim of this work was to optimize a simple RP- HPLC method for felodipine in rat plasma to obtain the best recovery.

MATERIALS AND METHODS

Chemicals

Felodipine obtained as gift sample from Cipla Ltd., Mumbai. HPLC grade methanol, acetonitrile and water were purchased from Merck. All other reagents were of analytical grade or higher.

Standard solutions

Stock solutions (1 mg/ml) of felodipine were prepared in methanol, protected from light and stored at -20 ^{0}C. Dilutions of the stock solution were made with methanol. Standard solutions of Felodipine in rat plasma were prepared by spiking an appropriate volume of the diluted stock solution in 100µl of rat plasma, giving final concentrations of 50, 60, 80, 100, 120, 140 and 150 ng/ml. Quality control samples were also prepared using the same method as standard solutions with three different concentrations containing 50,100,150 ng/ml felodipine, representing the low, middle and high concentration range of the calibration curve, respectively.

Sample preparation

Serum was spiked with felodipine dissolved in methanol to obtain final concentration 50ng-150 ng and treated with 500 µl acetonitrile as serum protein precipitating agent then volume was made with same serum sample. At 1500 g the tube vertexed for 5min, centrifuged for 10 min at 500 g for getting rid of protein residue. The supernant was taken carefully, serum samples including various concentrations of felodipine were injected to column. Felodipine peak area ratio in sample was used to calculate felodipine concentration from standard.

Chromatographic conditions

The reverse-phase HPLC system consisted of a Shimadzu HPLC system (LC-10Ai, Japan) consisting of a pump (LC-10Ai), a system controller (SCL-10AVP), an auto injector (SIL-10ADVP) and a diode array detector (SPD-M10 AVP). Data analysis and processing were done using class LC-10 software (version 1.6). Analysis was carried out using a Waters, Spherisorb ODS2 column (250mm×4.6mm, 5 µm). Elution was carried out using methanol and water (80:20 v/v) at a flow rate of 0.9ml/min.The felodipine peaks were monitored at 260 nm.

Validation studies

Selectivity and specificity of the method was determined from six different plasma samples for any interfering peaks at the retention time of felodipine. The calibration curve for linearity was obtained using seven standards of drug 50, 60, 80, 100, 120, 140 and 150 ng/ml. The concentration of samples was calculated from the equation using regression analysis, $y = mx + C$, where x = the concentration of felodipine, y = the peak ratio of felodipine, m = the slope of the calibration curve and C = the intercept of the calibration curve. The limit of detection (LOD) was set at peak with a signal-to-noise ratio of 3:1. Accuracy was determined as the absolute value of the ratio of the back-calculated mean values of the LOQ to their respective nominal values and was expressed as a percentage. Precision of the assay was measured by the percentage coefficient of variation (% CV) over the concentration range of LOQ, low, medium and high QC samples (50,100,150 ng/ml respectively) of felodipine during the course of validation. The plasma recovery was obtained from the peak areas of low, medium and high plasma extracts in comparison with those obtained with same concentration of drug in mobile phase solution directly injected.

Full Paper

RESULTS AND DISCUSSION

Drug concentration is monitored during various phases of pharmaceutical development, such as formulation and stability studies, quality control and pharmacological testing in animals and humans. All these investigations require reliable and validated analytical methods in order to measure drugs in pharmaceutical formulations and biological samples.

Sample preparation

Extraction of felodipine from plasma samples was carried out using a liquid extraction procedure with two different solvents, namely, dichloromethane and acetonitrile. Extraction recovery was higher with acetonitrile when compared with other organic solvents. The recoveries achieved with dichloromethane (92.65%) were significantly lower ($p < 0.05$) than that achieved with acetonitrile (99.03%).Hence for further studies, acetonitrile was used as the extracting solvent and showed higher extraction recovery with minimum interference.

Selectivity and specificity

Representative chromatogram of plasma spiked with 50 ng/ml (LOQ) felodipine is presented in Figure 1. In blank plasma samples chromatograms no interference was observed at the peaks of interest. The peaks were separated with retention timing 9.94 min for felodipine.

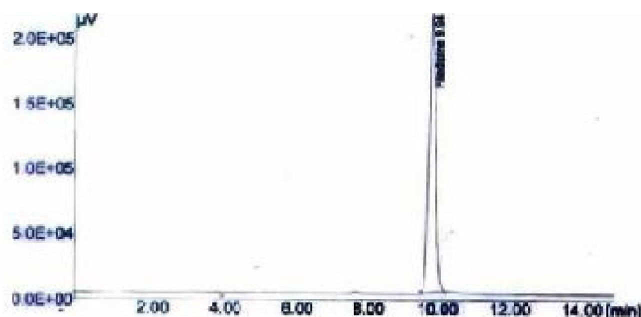


Figure 1 : Representative chromatograms of plasma spiked with felodipine.

Linearity

The felodipine calibration curve was obtained by plotting the peak area ratios (y) vs the concentrations (ng/mL) spiked in rat plasma samples (x). The linearity of the method was confirmed by assaying plasma standards in triplicate at seven separate concentrations over the concentration range 50ng - 150 ng/ml. Calibration curves were established on each day of analysis and a typical calibration curve (Figure 2) had the regression equation of $y=101827x+1801324$ with $r^2=0.9943$. The goodness of fit was consistently greater than 0.99 during the course of validation. As seen from TABLE 1, the range of accuracy and precision of the back-calculated concentrations of the standard curve points were 94.28–107.60% and 1.28– 2.80% respectively.

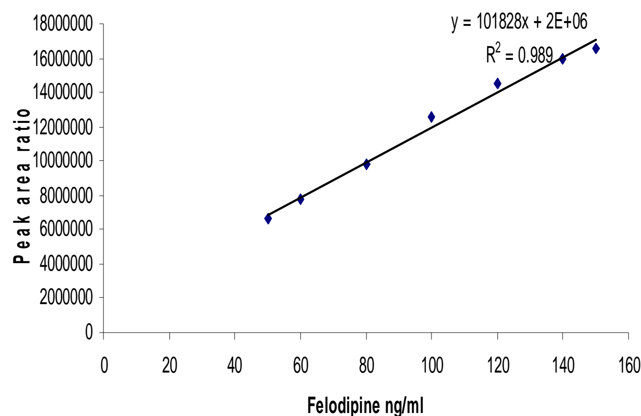


Figure 2 : Typical calibration curve of felodipine spiked in rat plasma.

TABLE 1 : Results of inter-day calibration curves of felodipine.

Nominal concentration (ng/mL)	Measured concentration (*n* ? 6; ng/mL, mean±SD)	Precision (%)	Accuracy (%)
50	53. 80± 1.12	1.45	107.60
60	60.89±0.5	2.80	101.48
80	75.43± 0.75	2.37	94.28
100	103. 53±1.45	2.27	103.53
120	123. 42±1.25	1.28	102.85
140	149. 57±2.01	2.41	106.83
150	160.96±1.12	1.48	100.60

Sensitivity

The lower limit of quantification, defined as the lowest quantifiable concentration on the calibration curve at which both accuracy and precision should be within the maximum tolerable CV of ±15%, was deemed to be 50 ng/mL. In the present conditions the limit of detection (LOD) was found to be 25 ng/mL. This LOQ was sufficient to determine felodipine concentrations in rat plasma obtained in the conduct of pharmacokinetic studies. The between-run accuracy

and precision for the drug at LOQ were 105.79% and 1.45 %, respectively.

Accuracy and precision

Intra day accuracy ranged from 92.15 to 107.2 % and that of inter-day was from 98.55 to 106.80%. The precision ranged from 1.95 to 7.23 % during intra-day and from 1.50 to 2.21% during the inter-day run throughout the study (TABLE 2).

TABLE 2 : Results of quality control samples obtained during the intra-day and inter-day Validation

QC sample	LOQ	Low	Medium	High
Nominal concentration (ng/mL)	50	50	100	150
Intra-day (n = 7)				
Mean (ng/mL)	53.20	53.6	93.19	138.23
SD	0.78	0.8	2.99	1.85
Precision (%)	9.13	7.23	2.45	1.95
Accuracy (%)	106.4	107.2	93.19	92.15
Inter-Day (n = 7)				
Mean (ng/mL)	53.5	53.4	98.65	148.2
SD	1.12	0.52	2.68	1.98
Precision (%)	6.10	2.21	1.50	2.13
Accuracy (%)	107.00	106.80	98.65	98.55

Extraction recovery

The liquid extraction procedure was simple, employing a single solvent with good reproducibility and high recovery. The analytical recovery for felodipine low, medium and high QC samples were found to be 107.66%, 105.34% and 104.59% respectively. A good recovery of felodipine indicated the suitability of acetonitrile for good extraction recovery of the drug from plasma (TABLE 3).

TABLE 3 : Results of the recovery for felodipine.

QC Samples	Low QC	Medium QC	High QC
Nominal concentration(ng/mL)	50	100	150
Measured concentration n=7			
Mean	52.83	105.34	156.89
SD	0.83	1.52	2.05
Precision (%)	2.83	1.55	1.16
Accuracy (%)	107.66	105.34	104.59

CONCLUSION

A specific and validated HPLC method for the determination of felodipine in rat plasma has been developed. Its specificity without interference from endogenous substances is very useful for qualitative and quantitative estimation of felodipine in rat plasma samples. The method was rapid, with a run time of 14 min, and had good reproducibility. The resolution between the peaks of felodipine was found to be good under the conditions of the study. This method required a small sample volume (100µL) for accurate and precise determination of felodipine in rat plasma. Also, this method employs a simple extraction procedure, which is reliable and consistent with good recovery. All other validation parameters were found to be within the acceptance limit, indicating the ruggdness and suitability of the method for routine estimation. This method can successfully utilize for the determination of plasma pharmacokinetic profile of felodipine.

REFERANCES

[1] M.Katoh, M.Nakajima, H.Yamazaki, T.Yokoi; Pharm.Res., **17(10)**, 1189 **(2000)**.

[2] M.Ahnoff; J.Pharm.Biomed.Anal., **2(3–4)**, 519 **(1984)**.

[3] M.Ahnoff, M.Ervik, L.Johnson; J.Chromatogr., **394(3)**, 419 **(1987)**.

[4] B.Lindmark, M.Ahnoff, B.Persson; J.Pharm.Biomed.Anal., **27**, 489 **(2002)**.

[5] J.A.Lopez, V.Martinez, R.M.Alonso, R.M.Jiminez; J.Chromatog.A, **870**, 105 **(2000)**.

[6] P.A.Soons, M.C.M.Roosemalen, D.D.Breimer; J.Chromatogr.Biomed.Appl., **93**, 343 **(1990)**.

[7] E.Bjorklund, M.Jaremo, L.Mathiasson, L.Karisson, J.Strode, J.Eriksson, A.Tostensson; J.Liq.Chromatogr.Rel.Technol., **21**, 533 **(1998)**.

[8] R.M.Cardoza, P.D.Amin; J.Pharm.Biomed.Anal., **27**, 711 **(2002)**.

[9] A.Karlsson, K.Pettersson, K.Hernquist; Chirality, **7**, 147 **(1995)**.

[10] J.S.Srinivas, A.B.Avadhanulu, V.Anjaneyulu; Indian Drugs, **35**, 18 **(1998)**.

[11] O.Beck, T.Ryman; Journal of Chromatography, 337 **(1985)**.

[12] C.Fischer, B.Heuer, K.Heuck, M.Eichelbaum; Biomedical and Environmental Mass Spectrometry, **13**, 645 **(1986)**.

[13] P.A.Soons, D.D.Breimer; Journal of Chromatography: Biomedical Applications, **428**, 362 **(1988)**.

Full Paper

[14] S.Y.Oh, K.Y.Kim, Y.G.Kim, H.G.Kim; Journal of Pharmaceutical and Biomedical Analysis, **32**, 387 (**2003**).

[15] T.Sakamoto, Y.Ohtake, M.Itoh, S.Tabata, T.Kuriki, K.Uno; Biomed Chromatogr., **7**, 99 (**1993**).

[16] J.D.Dru, J.Y.Hsieh, B.K.Matuszewski, M.R.Dobrinska; J.Chromatogr.B, **666**, 259 (**1995**).

[17] R.Nishioka, I.Umeda, N.Oi, S.Tabata, K.Uno; J.Chromatogr., **565**, 237 (**1991**).

[18] M.Gabrielsson, K.J.Hoffmann, C.G.Regardh; J.Chromatogr., **573**, 265 (**1992**).

[19] R.M.Cardoza, P.D.Amin; J.Pharm.Biomed.Anal., **27**, 711 (**2002**).

[20] J.A.Lopez, V.Martinez, R.M.Alonso, R.M.Jimenez; J.Chromatogr.A, **870**, 105 (**2000**).

[21] J.T.Strode, L.T.Taylor, A.L.Howard, D.Ip; Brooks MA J.Pharm.Biomed.Anal., **12**, 1003 (**1994**).

[22] L.H.Miglioranc, R.E.Barrientos-Astigarragac, B.S.Schugd, H.H.Blumed, A.S.Pereiraa, G.D.Nucci; J.Chromatogr.B, **814**, 217 (**2005**).

[23] K.Heinig, F.Bucheli; J.Chromatogr.B, **769**, 26 (**2002**).

[24] H.Kim, H.Roh, S.B.Yeom, H.J.Lee, S.B.Han; Chromatographia, **58**, 235 (**2003**).