Original Article

DETERMINATION OF NIFEDIPINE IN RAT PLASMA USING HPLC-UV DETECTOR: A SIMPLE METHOD FOR PHARMACOKINETICS AND ORAL BIOAVAILABILITY STUDIES

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ABSTRACT

Objective: To develop and validate a high-performance liquid chromatographic method (HPLC) for the determination of nifedipine (NFD) concentration in rat plasma.

Methods: 1.5 mol of sodium hydroxide solution was added to each plasma sample, followed by the addition of an extraction solvent based on n-hexane and dichloromethane (70: 30, v/v). The organic layer was transferred and evaporated to dryness under nitrogen flow. The residue was reconstituted with 0.5 mol of acetic acid, followed by the addition of n-hexane. After centrifuging the mixture, the supernatant organic layer of n-hexane was discarded, and the aqueous solution was injected onto the HPLC using A Phenomenex Luna-C18 reversed phase analytical column (250 x 4.6 mm, 5 μm). The mobile phase consisted of 0.01 mol aqueous ammonium formate: methanol: acetonitrile (55: 43: 2, v/v) with pH adjusted to 4.9 using formic acid. The flow rate was 0.8 ml/min; UV detector set at 235 nm and the samples were quantified using the peak area.

Results: A well-resolved NFD peak was achieved free of interference from endogenous compounds in rat plasma. Recovery of NFD was more than 93 % over concentrations ranged from 5.00 to 200 ng/ml. The limit of quantification (LOQ) of this assay was 6 ng/ml and, intra-and inter-day coefficient of variation (CV) were 5.75 % and 7.93 %, respectively. NFD was found to be stable in rat plasma after being stored at -30 °C over 90 d.

Conclusion: The stability, sensitivity, specificity and reproducibility of this method make it suitable for the determination of NFD plasma concentration in pharmacokinetics and oral bioavailability studies.

Keywords: Nifedipine, HPLC-UV assay, Validation, Stability, Plasma concentration

INTRODUCTION

Nifedipine (NFD), 1,4-dihydro-2,6-dimethyl-4(2-nitrophenyl)-3,5-pyridine dicarboxylic acid dimethyl ester [1], is a dihydropyridine calcium channel blocker commonly used in the treatment of hypertension, angina pectoris and atherosclerosis [2]. The empirical formula for NFD is $C_{17}H_{18}N_2O_6$ and its molecular weight is 346.33 g/mol (fig. 1). In the market, NFD is available in the form of retard or prolonged-release (Adalat®) tablets containing 20 or 60 mg of NFD, respectively. According to the Biopharmaceutical Classification System (BCS), NFD is a class II drug, poorly soluble in water (<10 mg/l) [3], but rapidly absorbed from the GIT due to its lipophilic nature [4]. However, NFD has very low plasma concentration related to the presystemic metabolism resulting in a very low oral bioavailability (up to 70 %) [5] and inter/intra individual pharmacokinetic variations [6, 7]. Following absorption, NFD is further metabolized via the major hepatic metabolizing enzyme (CYP3A4) into inactive polar metabolites [8]. The elimination half-life is approximately 2-5 h [9, 10]. On the other hand, a 96 % degradation of NFD in methanol solution has been reported upon it's exposure to normal light for 2 h [11]. Besides, a decomposition of NFD upon injection to the injection port has been reported as a result of thermal instability [12–15]. The low profile of plasma concentration of NFD based on low oral bioavailability, photo-degradation due to light sensitivity and thermal instability during analysis requires a very sensitive, simple and practical method of analysis.

Several analytical methods have been published for the determination of NFD in biological samples. They were based on gas chromatography (GC) with different detectors [16-20], HPLC with UV detection [21-26], voltammetric method [8], electrochemical detection and LC-MS [27-28]. However, some of these methods were not completely validated and applying peak height quantification rather than peak area quantification. Furthermore, they required long running times for drug detection using large amounts of blood samples (up to 1 ml). Besides, most of these sensitive HPLC methods

reported so far were using tandem mass spectrometry [29-30]. Some of these NFD CG analytical methods using a split–splitless injector port might cause thermal degradation of NFD as mentioned earlier, lead to oxidation of the dihydropyridine moiety of NFD, and result in formation of the NFD metabolites (dehydronifepinine) by oxidative dehydrogenation [16].

In this paper, the use of HPLC-UV was highlighted as an alternative method for GC analysis to avoid the possibility of thermal degradation by controlling the temperature of the injector port and column during NFD separation. Thus, a sensitive, selective, reproducible and stabile HPLC-UV assay of NFD concentration in rat plasma was developed and validated for accuracy, precision, recovery, linearity and long-term stability. This simple method was verified by analyzing plasma samples from rats after oral administration of NFD solution. Only the collection of a few blood samples (100 μl) was required. The concentration was quantified by peak area.

MATERIALS AND METHODS

Materials

Chemicals (suppliers) were as follows: NFD and diazepam (Sigma Aldrich Co., St. Louis, USA); Pharmaceutical grade of poly-(ethylene glycol) 400 (PEG) (Merck KGaA, Darmstadt, Germany); HPLC grade formic acid and ammonium formate (Fluka, Darmstadt; Germany); HPLC grade n-hexane, dichloromethane, sodium hydroxide, acetonitrile and methanol (Merck, Darmstadt; Germany); Glacial acetic acid (BDH Laboratory Supplies Poole, England).

Methods

HPLC system for plasma samples

The analytical instrument used was a Shimadzu HPLC system (Shimadzu Corporation, Japan) consisting of an LC-10A vp Intelligent HPLC pump and an SPD-10A vp detector set 235 nm and a Rheodyne 7725i injector fitted with a 50 μl sample loop. A

Phenomenex Luna-C18 reversed phase analytical column (250 x 4.6 mm, 5 μm) fitted with a Refillable Phenomenex guard column (10 x 2 mm, 5 μm) was used. The mobile phase (MP) was a mixture of 0.01 mol aqueous ammonium formate: methanol: acetonitrile (55: 43: 2, v/v) adjusted to pH 4.9 using formic acid. The flow rate of MP was running at 0.8 ml/min and the column temperature was set at 37 °C. Data were collected and processed using Shimadzu Class VP version 7.4 software and samples were quantified using the peak area.

Pharmacokinetic study design

NFD preparation (1 mg/ml) for oral administration was prepared by dissolving a 50 mg aliquot of NFD in 50 ml of PEG 400 (co-solvent) and mili-Q water (1:1, v/v) and stirred thoroughly at room temperature for 1 h. The drug solution was protected from light at preparation time and stored immediately after in the fridge to be used within 12 h of preparation. NFD was administered to the rats orally by gavage in the dose of 5 mg/kg. Five male Wister rats (age 2-3 mo, weight 290±30 g) were used in the experiment. They were kept in the experimental animal facility and given standard diet and water *ad libitum*. Temperature and light were controlled mimicking the natural habitat. The study was approved by Animal Ethics Committee. Blood samples were collected into heparinized tubes from the tip of the tail pre-dose and at 15, 30, 60, 90, 120, 150, 180, 240, 300 and 360 min after the dose. Blood samples were centrifuged at 3,000 rpm for 15 min to collect the plasma, which was stored at-30 °C prior to HPLC analysis. Immediately after the last blood sample, rats were sacrificed by carbon dioxide asphyxiation. Samples were analyzed within 6 d of collections.

Sample preparation

Prior to analysis, NFD was extracted from plasma samples as follow: 80 μl of plasma aliquot was transferred into a micro-eppendorf tube followed by the addition of 15 μl of internal standard (15 μg/ml of diazepam) and 20 μl of 1.5 mol sodium hydroxide solution. The mixture was vortex-mixed for 30 s and 400 μl of n-hexane: dichloromethane (70: 30, v/v) was added as an extraction solvent. The resultant organic layer was transferred into another glass tube and evaporated to dryness at 40 °C under nitrogen gas. The dry residue was reconstituted with 200 μl of 0.5 mol acetic acid followed, by the addition of 800 μl hexane. The mixture was again vortex-mixed for 30 s and centrifuged at 5.000 rpm for 10 min. The n-hexane layer was completely discarded and only 100 μl of the aqueous solution was injected into the HPLC system.

Assay validation

Based on a number of previous reported methods [21-26, 31-33], The HPLC assay validation method was developed as following: A stock solution of an aliquot NFD (25 μg/ml) was prepared by dissolving 2.5 mg aliquot of NFD in 100 ml of a mixture containing mili-Q water and methanol (20: 80, v/v) and then NFD (25 μg/ml) was further diluted to 250 ml with mili-Q water and methanol to obtain a 100 ng/ml of NFD calibration curve solution. Three different batches of six standard curves each were prepared by spiking rat plasma with NFD calibration curve solution, spiking MP with NFD calibration curve solution and spiking PEG with NFD calibration curve solution to give concentrations in the range over 5 to 100 ng/ml. QC samples (6, 12, 24 and 48 ng/ml of NFD) were prepared by spiking blank rat plasma with NFD calibration curve solution. The calibration curve solutions were stored at-30 °C until used for assay validation and sample analysis. Linearity was assessed by least–square regression and lack-of-fit analysis. Accuracy was expressed as the percentage of the spiked
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concentration and precision as the coefficient of variation (CV). LOQ is defined as the concentration with signal-to-noise ratios of 10: 1. For inter-day, accuracy and precision determination analyses of each concentration were carried out daily for 6 consecutive days whereas for intra-day accuracy and precision evaluations analyses were carried out 6 times on the same day. Recovery was estimated by comparing peak areas in QC samples (n = 6) with drug solutions at corresponding concentrations. The stability of NFD was tested in plasma at 4 different concentrations (12.5, 25, 50 and 100 ng/ml NFD). Short term stability at -30 °C was tested for freshly prepared samples and over 2 d, while long term stability was tested at-30 °C over 30 and 90 d. Another stability test was carried on after 5 freeze-thawing cycles for each sample at-30 and 25 °C.

Statistical analysis

Analysis of variance (one-way ANOVA) was used to compare mean values of variables determined for different calibration curves spiked with different materials. Differences were considered statistically significant when *p<0.05. The analysis was performed using the Minitab program (Version 15; Minitab Inc, USA).

RESULTS

The NFD peak with a retention time of 4.8 min was well-resolved and free from the interference of endogenous compounds in rat plasma. The total run time for each sample was 6 min. At least six calibration curve standards of rat plasma were tested. Thus, blank plasma was used in the preparation of the calibration curves. NFD was found to be stable in plasma after being protected from light for at least 24 h at room temperature with no noticeable changes in NFD concentrations in plasma.

The calibration curve of NFD spiked with rat plasma, PEG and MP (n = 6) reported in table 1 were linear over the concentration range from 5.00 to 100 ng/ml (r^2 0.9997±0.0016) with a mean intercept of 0.0132±0.0509. Similarly, the calibration curve of NFD spiked with PEG and MP (table 1) was linear over the same concentration range (r^2 0.9998±0.0023 and 0.9996±0.0064) with a mean intercept of-0.0904±0.0281 and+0.0176±0.0371, respectively. It is evident that a linear correlation is existed between the peak area and the concentrations of NFD spiked with rat plasma, PEG and MP. This result also demonstrates that no considerable interference exists as no significant difference was found (p>0.05).

Measured NFD, inter-and intra-day accuracy and precision values for different NFD concentrations are presented in table 2 and table 3, respectively. The lowest concentration was used in the construction of QC samples was 6 ng/ml at which intra-and inter-day CV were 5.75 % and 7.93 % with a recovery of more than 93 %. This verifies that the present method is very sensitive. Furthermore, the present experimental setup was testing the NFD stability in plasma before extraction. NFD was found to be stable in rat plasma after 5 freeze-thawing cycles for each sample at-30 and 25 °C and after storage at -30 °C over 90 d (table 4). These results proof that the assay is stable, selective and sufficiently sensitive for oral bioavailability and pharmacokinetic studies in rats.

For verification, this developed method was used to analyze plasma samples from five rats after an oral administration of 5 mg/kg NFD solution. Fig. 2 shows the plasma concentration-time profiles for NFD over 6 h after the oral dose (5 mg/kg) of NFD solution. It is apparent that the presented HPLC method is sensitive enough to detect the plasma concentration-time profiles for a drug with well-known low oral bioavailability.

Table 1: Comparison between r², slopes and intercepts of different standards curves (NFD–HPLC assay)

St Curves	Regression (r²)	Slope (mean±SD)	Intercept (mean±SD)
Plasma spiked with NFD	0.9997±0.0016	1.0029±0.32	+0.0132±0.0509
PEG spiked with NFD	0.9998±0.0023	1.0371±0.14	-0.0904±0.0281
MP spiked with NFD	0.9996±0.0064	1.0083±0.52	+0.0176±0.0371

Data are means±SD, n=6.

Table 2: Measured NFD, Intra-day accuracy and precision of the HPLC method for the analysis of NFD in rat plasma samples

Nominal [NFD] (ng/ml)	Measured [NFD] (ng/ml)	Accuracy (%)	Precision (CV) (%)
6.00	5.05±0.30	93.40±1.01	5.75±0.52
12.0	11.4±1.70	94.18±1.26	6.41±1.16
24.0	23.2±1.35	95.77±0.97	3.87±1.74
48.0	47.6±2.11	96.17±0.98	2.17±0.48

Data are means±SD, n=6.

Table 3: Measured NFD, Inter-day accuracy and precision of the HPLC method for the analysis of NFD in rat plasma samples

Nominal [NFD] (ng/ml)	Measured [NFD] (ng/ml)	Accuracy (%)	Precision (CV) (%)
6.00	5.15±0.02	93.73±0.56	7.93±0.28
12.0	11.2±0.10	92.16±0.93	6.58±0.94
24.0	23.5±0.70	97.15±1.39	5.79±1.06
48.0	47.3±0.60	98.17±0.56	4.18±0.85

Data are means±SD, n=6.

Table 4: NFD stability in rat plasma after 5 Freeze-thaw cycles and at 2, 30 and 90 d at-30 °C

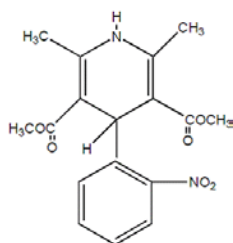| Nominal [NFD] (ng/ml)	[NFD] at 1 h (ng/ml)	[NFD] at 2 d (ng/ml)	[NFD] at 30 d (ng/ml)	[NFD] at 90 d (ng/ml)	[NFD] at 5 freeze-thawing cycles (ng/ml)
12.5	12.4±0.25	12.4±0.25	11.4±0.16	11.3±0.53	11.5±1.03
25.0	24.8±0.21	24.8±0.51	24.6±0.74	24.5±0.86	24.0±0.68
50.0	49.6±0.52	49.6±0.52	49.5±0.85	49.4±0.92	48.9±1.26
100	99.8±1.31	99.8±1.31	99.5±1.08	99.3±1.95	98.4±3.81

Data are means±SD, n=6

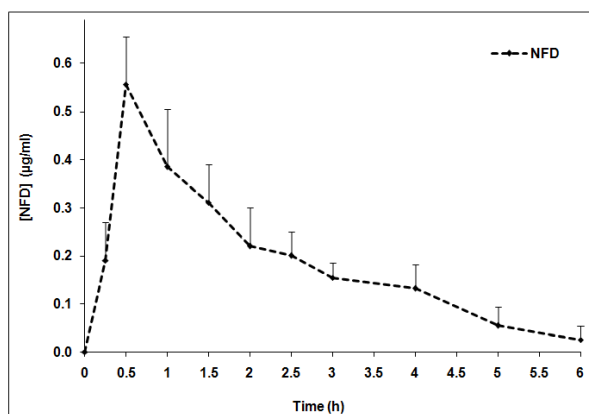**Fig. 1: Chemical structure of nifedipine (NFD)**

Fig. 2: Plasma concentration-time profile of NFD after oral administration (5 mg/kg) of NFD solution to male rats (data are means±SD, n=5)

DISCUSSION

A number of analytical methods have been reported for determination of NFD in plasma [16-28]. The HPLC method developed here is based on a number of previously reported methods [21-26, 31-33], but with modifications to the organic modifier ratios and concentrations in the MP, the flow rate, the pH strength and the volume of the extraction agent. The ratio of organic solvent in the MP is essential to retain the drug molecule on the stationary phase of the column. Thus, the assessment of MP containing buffer solution with different pH strength in combination with several organic solvents such as acetonitrile, methanol and triethylamine with different volume were tested.

To determine an optimal amount of organic solvent, which can be used in the MP for the separation of the analyte, 35 to 65 % of the organic solvent mixture (methanol and acetonitrile) was used. The best peak separation with short analysis time was obtained, when the percentage of the organic solvent mixture (methanol and acetonitrile) in the MP was 45 %. The addition of 43% methanol and 2% acetontrile were found to be necessary to produce a well-resolved peak, free of interference from adjacent endogenous compounds.

The effect of different pH strength and buffer concentration in MP on NFD retention time and resolution were assessed. Several concentrations of the aqueous ammonium formate in the buffer solution varying from 0.01 to 0.05 mol were tested. As a result of this assessment, no significant change in retention and resolution of NFD was observed, when the concentration of buffer has increased from 0.01 to 0.05 mol. For pH assessment, previous studies showed that NFD exhibits maximum stability in solutions with low pH value, as NFD is a weak acid drug molecule having low pKa value of 3.93 [34]. Therefore, different pH values with acidic range as mentioned earlier were assessed for NFD peak resolution and retention. The retention of NFD was significantly unchanged in the buffered solution over the tested pH ranges (3.5 to 6.5) with clear peak resolution appearance. However, a mobile phase with pH 4.9 was chosen to protect the used column from the low pH effect on column validity. As a result of this MP assessment, a good NFD retention with less than 6 min running time for analysis was obtained.

For normal light protection, a complete NFD degradation has been reported upon exposure to normal light for 2 h [11]. Based on these findings, an appropriate protection from light during sample preparation was taken into account to avoid any possibility of drug photo-degradation for the plasma sample during treatments. A number of organic solvents have been reported for NFD extraction,

such as dichloromethane [35], diethyl ether [13], a mixture of hexane and dichloromethane [36], a mixture of isooctane and methyl-tert-butyl ether [37], ethyl acetate [38], a mixture of n-pentane and dichloromethane [39] and chloroform [40]. Based on that, many organic solvent mixtures with different ratios were assessed such as a mixture of diethyl ether and ethyl acetate, a mixture of isooctane and methyl-tert-butyl ether and a mixture of n-hexane with dichloromethane. The result from this assessment study showed that less cloudy samples with an optimum recovery (up to 70 %) for NFD could be obtained by using a mixture of n-hexane and dichloromethane (70: 30, v/v). Therefore, the addition of 20 μl of 1.5 mol sodium hydroxide solution prior to the addition of organic solvent mixture was necessary for complete extraction to eliminate any sample turbidity and to increase the recovery of NFD up to satisfactory level. Thus, the used extraction solvents were found to be essential to give satisfactory recoveries for NFD and to provide distinctive chromatograms. Besides, a linear correlation existed between the peak area ratio of plasma, MP and PEG spiked NFD. Under the experimental conditions used, LOQ of this assay was found to be 6 ng/ml, whereas LOD was found to be 2 ng/ml. This points out that the present method was found to be very sensitive stable.

It is evident that the plasma concentration of NFD after the oral administered dose was low indicating low oral bioavailability of NFD when administered orally. However, the presently developed method is highly sensitive to detect the plasma concentration-time profiles for a drug with well-known low oral bioavailability (0.47-0.82%) [41]. HPLC method developed in this paper is applicable for the determination of NFD *in vivo* studies of different kind of NFD drug delivery dosage forms [42].

CONCLUSION

In conclusion, the HPLC method described here is simple, sensitive, selective, reproducible, linear, precise, accurate with short retention time and stable requiring only a small blood collection volume from rat plasma. The method was fully validated and is applicable for the determination of NFD *in vivo* as well as for the assessment of pharmacokinetic and oral bioavailability studies of the different type of drug dosage forms.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Stone PH, Antman EM, Muller JE, Braunwald E. Calcium channel blocking agents in the treatment of cardiovascular disorders. Part II: hemodynamic effects and clinical applications. Ann Intern Med 1980;93:886-904.
2. Sorkin EM, Clissold SP, Brogden RN. Nifedipine: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy, in ischaemic heart disease, hypertension and related cardiovascular disorders. Drugs 1985;30:182–274.
3. Cao QR, Cui JH, Park JB, Han HK, Lee J, Oh KT, Park I, Lee BJ. Effect of food components and dosing times on the oral pharmacokinetics of nifedipine in rats. Int J Pharm 2010;396:39-44.
4. Abrahamsson B, Alpsten M, Bake B, Jonsson UE, Eriksson-Lepkowska M, Larsson A. Drug absorption from nifedipine hydrophilic matrix extended release (ER) tablet-comparison with an osmotic pump tablet and effect of food. J Controlled Release 1998;52:301-10.
5. Filgueira GC, Filgueira OA, Carvalho DM, Marques MP, Moisés EC, Duarte G, et al. Analysis of nifedipine in human plasma and amniotic fluid by liquid chromatography-tandem mass spectrometry and its application to clinical pharmacokinetics in hypertensive pregnant women. J Chromatogr B: Anal Technol Biomed Life Sci 2015;993-994:20-5.
6. Raemsch KD, Sommer J. Pharmacokinetics and metabolism of nifedipine. Hypertension 1983;5:18–24.
7. Eichelbaum M, Echizen H. Clinical pharmacology of calcium antagonists: a critical review. J Cardiovasc Pharmacol 1984;6:S963-7.
8. Ozaltin N, Yardimci C, Suslu I. Determination of nifedipine in human plasma by square wave adsorptive stripping voltammetry. J Pharm Biomed Anal 2002;30:573-82.
9. Guengerich FB, Martin MV, Beaune PH, Kremers P, Wolff T, Waxman DJ. Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. J Biol Chem 1986;261:5051–60.
10. Challenor VF, Waller DG, Renwick AG, Gruchy BS, George CF. The trans-hepatic extraction of nifedipine. Br J Clin Pharmacol 1987;24:473-7.
11. Rosseel MT, Bogaert MG. Determination of nifedipine in human plasma by capillary gas chromatography with nitrogen detection. J Chromatogr 1983;279:675-80.
12. Abou-Auda HS, Najjar TA, Al-Hadiya KI, Ghilzai NM, Al-Fawzan NF. Liquid chromatographic assay of nifedipine in human plasma and its application to pharmacokinetic studies. J Pharm Biomed Anal 2000;22:241-9.
13. Goldnik A, Gajewska M, Marszałek D, Przyzyçki M. Determination of nifedipine in serum by HPLC. Acta Pol Pharm 1996;53:7-8.
14. Jankowski A, Lamparczyk H. Evaluation of chromatographic methods for the determination of nifedipine in human serum. J Chromatogr A 1994;668:469-73.
15. Grundy JS, Kherani R, Foster RT. Sensitive high-performance liquid chromatographic assay for nifedipine in human plasma utilizing ultraviolet detection. J Chromatogr B: Biomed Appl 1994;654:146-51.
16. Schmid BJ, Perry HE, Idle JR. Determination of nifedipine and its three principal metabolites in plasma and urine by automated electron-capture capillary gas chromatography. J Chromatogr 1988;425:107-19.
17. Patrick KS, Jarvi EJ, Straughn AB, Meyer MC. Gas chromatographic mass spectrometric analysis of plasma nifedipine. J Chromatogr 1989;495:123-30.
18. Le Guellec C, Bun H, Giocanti M, Durand A. Determination of nifedipine in plasma by a rapid capillary gas chromatographic method. Biomed Chromatogr 1992;6:20-3.
19. Horváth V, Hrabéczy-Páll A, Niegreisz Z, Kocsi E, Horvai G, Gödörházy L, et al. Sensitive high-performance liquid chromatographic determination of nifedipine in dog plasma using an automated sample preparation system with laboratory robot. J Chromatogr B: Biomed Appl 1996;686:211-9.
20. Streel B, Zimmer C, Sibenaler R, Ceccato A. Simultaneous determination of nifedipine and dehydronifedipine in human plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B; Biomed Sci Appl 1998;720:119-28.
21. Kostewlcz E, Sansom L, Flshlock R, Morella A, Kuchel T. Examination of two sustained release nifedipine preparations in humans and in pigs. Eur J Pharm Sci 1996;4:351-7.
22. Yritia M, Parra P, Iglesias E, Barbanoj JM. Quantitation of nifedipine in human plasma by on-line solid-phase extraction and high-performance liquid chromatography. J Chromatogr A 2000;870:115-9.
23. Zhiping L, Mingkang Z, Xiaojin S, Zhongdong L. A solid phase extracted-HPLC method for determining nifedipine in human plasma. J Clin Pharm 2005;14:115-7.
24. Niopas I, 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;32:1213-8.
25. Vertzoni MV, Reppas C, Archontaki HA. Sensitive and simple liquid chromatographic method with ultraviolet detection for the determination of nifedipine in canine plasma. Anal Chim Acta 2006;573-574:298-304.
26. Zendelovska D, Simeska S, Sabinovska O, Kostova E, Milosevska K, Jakovski K, *et al.* Development of an HPLC method for the determination of nifedipine in human plasma by solid-phase extraction. J Chromatogr B: Anal Technol Biomed Life Sci 2006;839:85-8.
27. Guo Y, Dai J, Qian G, Guo N, Ma Z, Guo X. Determination of nifedipine in human plasma and its use in bioequivalence study. Int J Pharm 2007;341:91-6.

28. Wang XD, Li JL, Lu Y, Chen X, Huang M, Chowbay B, *et al*. 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 Chromatogr B: Anal Technol Biomed Life Sci 2007;852:534-44.

29. Dankers J, van den Elshout J, Ahr G, Brendel E, van der Heiden C. Analytical determination of nifedipine in human plasma by flow-injection tandem mass spectrometry. J Chromatogr B: Biomed Sci Appl 1998;710:115-20.

30. Baranda AB, Mueller CA, Alonso RM, Jimenez RM, Weinmann W. Quantitative determination of the calcium channel antagonists amlodipine, lercanidipine, nitrendipine, felodipine, and lacidipine in human plasma using liquid chromatography-tandem mass spectrometry. Ther Drug Monit 2005;27:44-52.

31. Golocorbin-Kon S, Mikov M, Arafat M, Lepojevic Z, Mikov I, Sahman-Zaimovic M. Cefotaxime pharmacokinetics after oral application in the form of 3alpha,7alphadihydroxy-12-keto-5beta-cholanate microvesicles in rat. Eur J Drug Metab Pharmacokinet 2009;34:31-6.

32. Arafat M, Golocorbin Kon S, Mikov M. The measurement of cefotaxime sodium in rat plasma after oral administration: a sensitive HPLC-UV method. Int J Pharm Pharm Sci 2015;7:343-6.

33. Arafat M. Simple HPLC validated method for determination of diltiazem hydrochloride in human. Int J Pharm Pharm Sci 2014;6:213-6.

34. Plumley C, Gorman EM, El-Gendy N, Bybee CR, Munson EJ, Berkland C. Nifedipine nanoparticle agglomeration as a dry powder aerosol formulation strategy. Int J Pharm 2009;369:136-43.

35. Zaater M, Hasan E, Najib N. Trace-level determination of nifedipine in human serum by reversed phase high performance liquid chromatography. Pol J Pharmacol 2000;52:307-12.

36. Jankowski A, Lamparczyk H. Evaluation of chromatographic methods for the determination of nifedipine in human serum. J Chromatogr A 1994;668:469-73.

37. Grundy JS, Kherani R, Foster RT. Sensitive high-performance liquid chromatographic assay for nifedipine in human plasma utilizing ultraviolet detection. J Chromatogr B: Biomed Appl 1994;654:146-51.

38. Thongnopnua P, Viwatwongsa K. Quantitative analysis of nifedipine in plasma by high-performance liquid chromatography. J Pharm Biomed Anal 1994;12:119-25.

39. Soons PA, Schellens JH, Roosemalen MC, Breimer DD. Analysis of nifedipine and its pyridine metabolite dehydronifedipine in blood and plasma: review and improved high-performance liquid chromatographic methodology. J Pharm Biomed Anal 1991;9:475-84.

40. Gurley BJ, Buice RG, Sidhu P. Reversed-phase high performance liquid chromatographic determination of nifedipine in human plasma. Ther Drug Monit 1985;7:321-3.

41. Rashid TJ, Martin U, Clarke H, Waller DG, Renwick AG, George CF. Factors affecting the absolute bioavailability of nifedipine. Br J Clin Pharmacol 1995;40:51-8.

42. Arafat M. Approaches to achieve an oral controlled release drug delivery system using polymers: a recent review. Int J Pharm Pharm Sci 2015;7:16-21.

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