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Determination of nifedipine in human plasma by solid-phase extraction and high-performance liquid chromatography: validation and application to pharmacokinetic studies

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Abstract

Nifedipine, a dihydropyridine calcium channel antagonist, is widely used in the treatment of hypertension and other cardiovascular disorders. A simple, rapid, sensitive, precise and accurate HPLC method, using solid-phase extraction, for the quantitation of nifedipine in human plasma was developed and validated. The calibration graphs were linear in the 5–400 ng/ml concentration range ($r > 0.999$). Recovery for nifedipine was greater than 93.9% and for internal standard nitrendipine was 96.1%. Intra-day and inter-day precision ranged from 1.4 to 4.2 and 3.9 to 5.6%, respectively. Intra-day and inter-day accuracy was ranged from 94.5 to 98.0 and 93.1 to 98.0%, respectively. The method was not interfered with by other plasma components and was applied for the determination of nifedipine in pharmacokinetic study after single oral administration of 10 mg nifedipine to 18 healthy male subjects.

Keywords: Nifedipine; Determination; HPLC; Clinical pharmacokinetics

1. Introduction

Nifedipine (dimethyl 1,4-dihydro-2,6-dimethyl-4(2-nitrophenyl)pyridine-3,5-dicarboxylate), is a dihydropyridine calcium channel antagonist widely used in the treatment of angina pectoris, hypertension, and other vascular disorders such as Raynaud's phenomenon [1,2]. Nifedipine is a lipid soluble drug that is rapidly and completely absorbed from the gastrointestinal tract after oral administration. The systematic bioavailability of nifedipine is 50–70% due to extensive first-pass metabolism and may result in substantial intersubject pharmacokinetic variability. The elimination half-life of nifedipine is approximately 2–5 h [3,4]. Nifedipine is a photolabile compound, undergoing quick photodegradation when exposed to daylight [5–8].

Numerous methods have been published for the quantitative determination of nifedipine in plasma, including gas chromatography combined with different detectors [8–11] or high-performance liquid chromatography coupled with UV detection

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[11–21], electrochemical detection [22–25] or mass spectrometry [26,27]. However, some of these methods were not sufficiently specific and sensitive or required expensive instruments, some were not fully validated, some required laborious liquid–liquid extraction techniques and some were poorly reproducible, especially for the lower concentration range and were not directly applicable for the quantitative determination of nifedipine in human plasma.

Therefore, the objective of this investigation was to develop and validate a solid-phase extraction high-performance liquid chromatographic method that could be used for the determination of nifedipine in human plasma. The method was proved to be suitable for pharmacokinetic studies of nifedipine that required high selectivity and sensitivity.

2. Experimental

2.1. Chemicals and reagents

Nifedipine was purchased from Sigma (St. Louis, MO, USA) and internal standard nitrendipine was supplied from Elpen (Athens, Greece). Acetonitrile and methanol, both of HPLC-grade, were obtained from J.T. Baker (Deventer, The Netherlands). Analytical reagent grade 85% phosphoric acid was purchased from Merck (Darmstadt, Germany). Bond Elut cyano cartridges, 100 mg/ml, were obtained from Varian (Palo Alto, CA, USA). Water was Milli-Q grade and all other chemicals and solvents used were of analytical grade.

2.2. Chromatographic conditions

The liquid chromatographic system consisted of a Spectra-Physics SP8810 HPLC pump (Colorado Springs, CO, USA), an ISCO V-4 variable wavelength UV–Vis detector (Lincoln, NE, USA), a Hewlett–Packard HP3394A integrator (Avondale, PA, USA), a Timberline Instruments column heater (Boulder, CO, USA) and a Hitachi 655A-40 autosampler (Tokyo, Japan). Separation was performed on a Partisil ODS-3 analytical column (5 μm particle size, 250 × 4.6 mm I.D.) (Whatman, Kent, UK) proceeded by a guard column (20 × 2 mm I.D.) (Upchurch Scientific, Oak Harbor, CA, USA) dry packed with pellicular ODS material (37–53 μm) (Whatman, Kent, UK).

The mobile phase consisted of acetonitrile–water (60:40, v/v) and was filtered through a 0.45-m pore size nylon filter (Alltech, Deerfield, IL, USA) and degassed by ultrasonic treatment before use. The HPLC system was operated isocratically at a flow rate of 1 ml/min at 25 °C and the detector was set at 238 nm. The integrator attenuation was 8 and the chart speed was 0.2 cm/min. The development and validation work was carried out under sodium lamp illumination to prevent photodecomposition of nifedipine in laboratory or daylight [8].

2.3. Standard solutions

A stock standard solution was prepared daily in acetonitrile/Milli-Q water (1:1) and containing 100 μg/ml of nifedipine. This solution was further diluted with acetonitrile/Milli-Q water (1:1) to prepare the calibration solutions containing 0.1, 0.2, 0.4, 1, 2, 4 and 8 μg/ml nifedipine.

An internal standard stock solution was prepared in acetonitrile/Milli-Q water (1:1) and contained 300 μg/ml. This solution was further diluted with acetonitrile/Milli-Q water (1:3) to prepare the working internal standard solution containing 15 μg/ml nitrendipine.

Calibration standard samples were freshly prepared in 1 ml of human plasma by adding 50 μl of the nifedipine calibration solutions and 20 μl of the internal standard working solution to yield concentrations corresponding to 5, 10, 20, 50, 100, 200 and 400 ng/ml of nifedipine and 300 ng/ml of internal standard, respectively.

2.4. Quality control samples

Volumes of 50 ml of human plasma were spiked with 20, 75 and 200 μl of 50 μg/ml nifedipine solution in acetonitrile to obtain quality control samples containing 20, 75 and 200 ng/ml nifedipine, respectively. These samples were divided into aliquots of 3 ml into one-dram vials capped tightly, and kept at −20 °C until analysis. These samples

were used in the analysis of plasma samples as quality controls for the purpose of checking recovery of analyte in the daily analyses of plasma samples.

2.5. Sample extraction

The extraction cyano cartridges were conditioned successively with 2 ml of methanol, 2 ml of Milli-Q water and 2 ml of 0.01% phosphoric acid. After this, a 1-ml plasma sample, spiked with 20 μl of internal standard working solution containing 15 μg/ml, was applied to the cartridges and allowed to run through. The cartridges were washed successively with 2 ml of Milli-Q water, 2 ml of 0.01% phosphoric acid and 2 ml of 20% methanol. Finally, nifedipine and internal standard were eluted with 1 ml of acetonitrile/Milli-Q water (1:1) and 100 μl was injected onto the chromatographic system.

2.6. Pharmacokinetic study

The HPLC method developed was used to investigate the plasma profile of nifedipine after single oral dose of a 10-mg nifedipine tablet. Eighteen non-smoker healthy male volunteers with mean age: 25.1 ± 1.9 years, mean body weight: 73.2 ± 9.2 kg, and mean height: 174.8 ± 6.9 cm, participated in the study. Following written informed consent, each volunteer received a single 10 mg oral dose of nifedipine under fasting conditions. Blood samples (5 ml) were collected into heparinized test tubes pre-dose (0) and at 10, 20, 30, 45, 60, 80, 100 min and 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 h post-dose. Plasma was immediately separated by centrifugation at 3000 g for 10 min and stored in polypropylene tubes at $-20\,^{\circ}C$ pending analysis. Before analysis, the plasma samples were thawed at 18 °C.

3. Results and discussion

3.1. Chromatographic separation and sample extraction

Different published HPLC methods utilizing liquid–liquid extraction were tested with disappointing results due to many interfering peaks which co-extracted from plasma and which made impossible the accurate measurement of nifedipine at low concentrations. The chromatographic conditions described in this assay were arrived after investigating several mobile phases and solid-phase extraction supports. The better clean-up came from cyano cartridges but the eluate apparently contained traces of some protein thus drastically increasing backpressure, following each injection. The problem was eliminated by washing the cartridges before and after sample addition with 0.01% phosphoric acid.

Typical chromatograms, obtained from extracts of drug-free plasma and plasma sample obtained from a male volunteer 2 h after a single oral administration of a 10 mg nifedipine tablet formulation containing 41.4 ng/ml of nifedipine, are shown in Fig. 1. No endogenous plasma components or nifedipine photodegradation products or metabolites elute at the retention time of nifedipine or internal standard. Nifedipine and internal standard were eluted in 4.6 and 6.8 min, respectively. The overall chromatographic run time for an assay was approximately 8 min.

3.2. Calibration curves

Calibration curves were obtained by plotting the peak height ratios of nifedipine/internal standard (y) versus the nifedipine concentrations (ng/ml) in spiked plasma samples (x). Their equations were calculated by using weighted linear regression analysis with a weighting factor of 1/concentration. The linearity of the method was confirmed over the concentration range of 5–400 ng/ml by assaying 1-ml plasma standards in triplicate at seven separate concentrations on three separate occasions. Calibration curves were established on each day of analysis and a typical calibration curve had the regression equation of $y = -0.02325 + 0.03351x$ ($r = 0.999$).

3.3. System reproducibility

System reproducibility, expressed as %R.S.D. and based on absolute peak height, was 4.0 and 0.8% ($n = 9$) for nifedipine at concentrations of 50

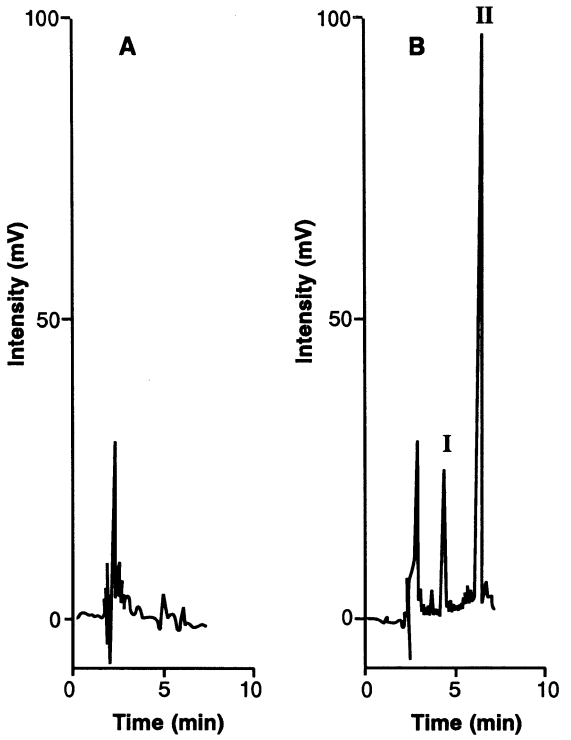

Fig. 1. Examples of chromatograms. (A) extract of 1 ml drug-free plasma; (B) plasma sample obtained from a male volunteer 2 h after a single oral administration of a 10 mg tablet of nifedipine containing 41.4 ng/ml of nifedipine. Peaks: I = nifedipine; II = internal standard, nitrendipine.

and 200 ng/ml, respectively. Mean reproducibility of the internal standard was 0.6% ($n = 18$) at 300 ng/ml concentration in human plasma.

3.4. Recovery from plasma

The absolute recovery of nifedipine and internal standard was determined by direct comparison of peak heights from extracts of plasma samples versus those found by direct injection of standards of the same concentration prepared in acetonitrile/Milli-Q water (1:1). Recovery of internal standard was determined in the same solutions simultaneously. The mean recoveries for nifedipine were 93.9 ± 5.1, 97.6 ± 1.9, and $98.3 \pm 2.3\%$ at the 20, 75, and 200 ng/ml concentrations, respectively ($n = 10$). Mean recovery of internal standard at 300 ng/ml was $96.1 \pm 1.4\%$ ($n = 30$).

3.5. Accuracy and precision

Intra-day precision was determined by calculating the %R.S.D. for five determinations at each concentration of three quality control samples and was found to be less than 4.2%. Intra-day accuracy, assessed by calculating the estimated concentrations as a percent of the nominal concentrations, was better than 94.5% (Table 1).

Inter-day precision and accuracy were assessed by assaying three quality control samples in triplicate on three separate occasions. Inter-day precision for nifedipine was 5.6% based on %R.S.D. values of 5.6, 4.2, and 3.9% for quality control samples containing 20, 75, and 200 ng/ml, respectively. Inter-day accuracy was found to be 98.0, 95.6, and 93.1% for samples containing 20, 75, and 200 ng/ml, respectively (Table 1). Inter-day precision and accuracy for 5 ng/ml nifedipine concentration was found to be 9.2 and 99.2%, respectively.

3.6. Sensitivity

Based on a 1-ml plasma sample the limit of quantification (LOQ), defined as the lowest quantifiable concentration on the calibration curve at which both accuracy and precision should be within the maximum tolerable CV of 20%, was deemed to be 5 ng/ml. This LOQ was sufficient to determine nifedipine concentrations in human plasma obtained in the conduct of pharmacokinetic studies.

3.7. Application to pharmacokinetic study

The present methodology was successfully used to determine plasma concentrations of nifedipine, after single oral administration of 10-mg tablets of nifedipine to 18 healthy non-smoker male volunteers. All activities of the clinical phase were conducted under sodium lamp to prevent photodegradation of nifedipine. Fig. 2 shows mean $\pm$ S.D. plasma concentration–time profile of nifedipine after oral administration of 10 mg nifedipine tablet formulation. Calculation of pharmacokinetic parameters was conducted model-independently. Analysis of nifedipine concentrations in

Table 1
Intra-day and inter-day accuracy and precision for nifedipine in quality control samples in human plasma

Nominal concentration (ng/ml)	Mean found concentration (ng/ml)	Accuracy (%)[a]	Precision[b] (R.S.D.%)
Intra-day			
20	19.6	98.0	4.2
75	73.4	97.9	1.5
200	189.0	94.5	1.4
Inter-day			
20	19.6	98.0	5.6
75	71.7	95.6	4.2
200	186.2	93.1	3.9

[a] Accuracy: found concentration expressed in % of the nominal concentration.
[b] R.S.D.: relative standard deviation.

plasma samples from 18 healthy non-smoker male volunteers following oral administration of 10 mg of nifedipine provided the following pharmacokinetic parameters (mean ± S.D.): C_{max} 46.5 ± 17.8 ng/ml; $AUC_{0-\infty}$ 178.2 ± 64.4 ng h/ml; T_{max} 1.1 ± 0.4 h; elimination half-life, 2.4 ± 0.6 h. The observed values of the pharmacokinetic parameters were comparable to those reported for nifedipine in previous studies [28].

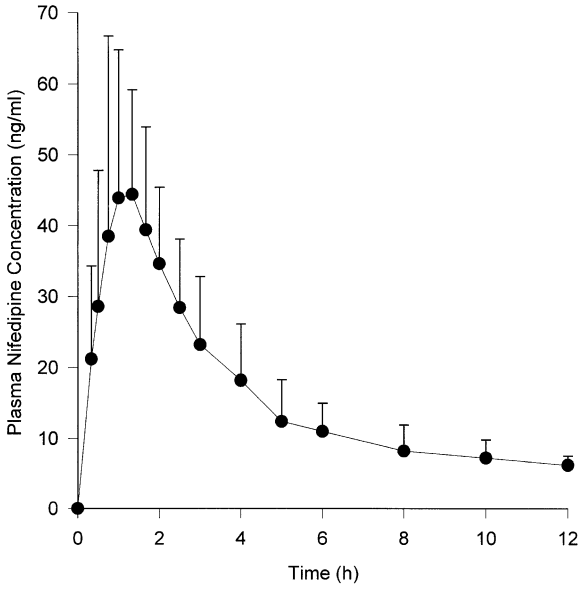

Fig. 2. Mean ± S.D. of plasma nifedipine concentration–time curve following a single oral dose of 10 mg nifedipine in 18 healthy male volunteers.

3.8. Conclusion

In conclusion, a simple, rapid, selective, sensitive, precise and accurate reversed-phase HPLC assay, using solid-phase extraction with cyano cartridges, for the determination of nifedipine in plasma samples was developed and validated. In order to prevent photodegradation of nifedipine in laboratory or daylight all clinical, development and validation activities were carried out under sodium light. The method was found to be suitable for the quantitative determination of nifedipine in plasma samples obtained from 18 healthy male volunteers in the conduct of a pharmacokinetic study, after single oral administration of 10 mg tablets of nifedipine.

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