HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR DETERMINATION OF NIFEDIPINE IN HUMAN PLASMA

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ABSTRACT

A relatively simple normal phase high performance liquid chromatography (HPLC) method was modified for determination of nifedipine in human plasma. The method is based on ultraviolet detection at 235 nm and acidic plasma extraction by a mixture of dichloromethane (30%) and n-hexane (70%) using nimodipine as an internal standard. The system was stabilized with the use of n-hexane (80%), chloroform (17%) and methanol (3%) as mobile phase. The assay was linear up to at least 120 ng/ml of nifedipine in plasma. The limit of reliable determination was at least 3 ng/ml plasma. The reproducibility of the method was satisfactory. The procedure can be used effectively to quantitate nifedipine in the human plasma.

Key Words: HPLC, Nifedipine, Human plasma, UV detection

INTRODUCTION

Nifedipine [dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl-pyridine-3,5-dicarboxylate)] is a calcium channel blocking agent, which selectively dilates arteries with little or no effect on other blood vessels and is used in the treatment of angina pectoris, arterial hypertension and Reynolds phenomenon (1). Pharmaceutical preparations containing nifedipine have been widely used in clinic and the therapeutic effects of nifedipine is related to its concentration in plasma. Since there are reports indicating variability of nifedipine concentrations in blood (2-5), a rapid and sensitive method of assay for comparison of the bioavailabilities of the different preparations and for drug monitoring during therapy is required. Several methods for the analysis of nifedipine in plasma or serum including spectroscopy, gas chromatography and high performance liquid chromatography have been reported (6-9). Most of these methods suffer from time consumption, low sensitivity, derivatization requirement, need to a large volume of plasma or serum, ineffective separation of the drug from its metabolites, expensive equipment and low extraction ratio properties which may makes them inappropriate for the bioavailability studies. Also thermo-oxidation of nifedipine (10), its low plasma levels in man (1-200 ng/ml) and its extensive metabolism (2) have complicated development of the specific analytical methods. In addition most of these methods except one (10) are based on reversed phase HPLC procedures for determination of the drug in dog plasma. The purpose of this study was to modify a normal phase HPLC method for clear separation of the peaks of drug from its metabolite (nitrophenyl-pyridine) in order to determine the exact concentration of nifedipine in bioavailability studies in human.

MATERIALS & METHODS

Chemicals: Methanol, n-hexane, dichloromethane, chloroform, perchloric acid, tangestophosphoric acid and some other chemicals or solvents used in this study were purchased from Merck Chemical Company (Germany). All solvents were HPLC grade and chemicals used as internal standard were analytical grade. Nifedipine and nimodipine powders were supplied by AMSA (Italy) and Bayer (Germany) pharmaceutical companies respectively.

Nitrophenylpyridine and other chemicals used as internal standard were purchased from Merck Chemical Company, (Germany). Plasma was provided by the blood transfer organization in Tehran.

Apparatus: The HPLC system (waters) was equipped with waters 540 pump, a UV tunable absorbance detector model 486 operated at 235 nm, μ-porasil column (30x3.9 mm I.D., particle size of 10 μm), Hamilton syringe (25 and 100 μl), advanced personal computer (NEC), printer P-2500 NEC, and Maxima software (waters).

HPLC operation condition: The mobile phase (n-hexane, chloroform and methanol 80, 17, 3%) was degassed and filtered through a 0.45 μm filter

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(millipore, type HVLP). The flow rate was 1.5 ml/min.

Sample Preparation: To 2 ml of plasma, was added 20 µl of internal standard solution (nimodipine 50 µg/ml) and the solution was treated with 100 µl of 7M perchloric acid and 4 ml of the solvent mixture (dichloromethane 30% and n-hexane 70%). The mixture was shaken for 15 min and then was centrifuged for 10 min at 6000 rpm. Then the upper organic layer containing nifedipine and internal standard was transferred to a clean glass tube using Pasteur disposable pipettes, and evaporated to dryness in a vacuum desiccator. After addition of 100 µl of mobile phase, 25 µl of the solution was injected for analysis. Since nifedipine is highly sensitive to normal laboratory light, all sample handling were performed in a completely darkened room illuminated only with sodium light.

Calibration and validation: For each assay an eight point calibration curve was prepared for concentrations of 5,10,20,40,60,80,100, and 120 ng/ml of nifedipine and equal amounts of nimodipine (1µg) in spiking control samples of plasma (2 ml). Calibration samples were processed identically as described. Ratios of the peak height of standard to the peak height of the internal standard were calculated and calibration curves were conducted by linear regression analysis (fig. 1). The recovery of nifedipine from blood was determined by preparing concentrations of 5, 10, 20, 40, 60, 80, 100, and 120 ng/ml. The within and between run variabilities were determined with the same concentrations used for recovery testing. Results are presented as mean±SD and as coefficient of variation (CV).

RESULTS AND DISCUSSION

Under conditions described in the experimental section, nifedipine, nitrophenylpyridine and nimodipine (internal standard) had retention times of 7.82, 3.8 and 6.38 min, respectively (fig.2). Calibration curve determined in plasma is linear over the range 1-120 ng/ml and the corresponding regression equation was: $y=0.9x + 0.3$ ($r^2 =0.99$), where y is the peak height ratio of nifedipine to internal standard (nimodipine) and x is the nifedipine concentration (ng ml^{-1}) in plasma.

There was not any significant difference between the plasma and the aqueous calibration curves. Variability determined at 5, 10, 20, 40, 60, 80, 100 and 120 ng/ml concentrations are shown in table 1. As it is shown, within run and between run variations determined at these concentrations, was 4.095% and 9.75% respectively.

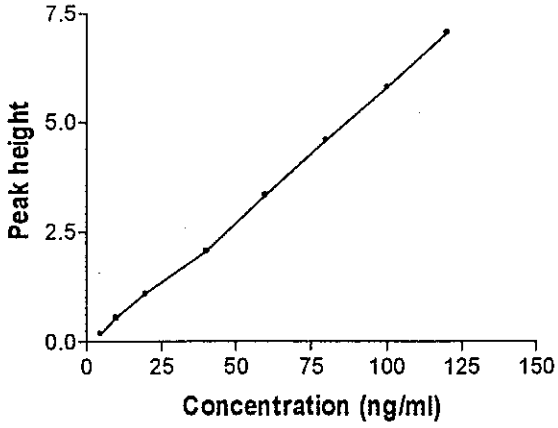

Figure 1: Calibration curve of nifedipine in plasma. $y=0.9x + 0.3$ ($r^2 =0.99$), where y is the peak height ratio of nifedipine to internal standard (nimodipine) and x is the nifedipine concentration (ng/ml) in plasma

Nitrophenylpyridine may cause an error in the analysis of nifedipine (11-13) if it is not isolated clearly during sample preparation and HPLC procedure. In the present method although nifedipine could be detected down to at least 1 ng/ml, the limit of reliable determination was arbitrary set at 3 ng/ml plasma. This value was found from the analysis of different concentration of nifedipine in plasma and detection of reproducible peaks by instrument. Mean recovery based on eight different concentrations and using dichloromethane-hexane (30:70, v/v) as the extraction system was 99.18% (Table 2).

As shown in figure 2, nifedipine and nitrophenylpyridine could be isolated clearly and the peaks were reproducible for many runs. In the present method it is also possible to re-wash the column to eliminate impurities.

In order to find the best solvent for the extraction of the nifedipine and nitrophenylpyridine from human plasma, a number of different solvents were examined. The interfering peaks became negligible and nifedipine or its metabolite nitrophenylpyridine were well separated from endogenous substances when dichloromethane-hexane (30:70, v/v) was used for extraction. This solvent system also provided the best recovery.

The present method has sensitivity to determine nifedipine in low concentrations (1-3 ng/ml). In addition the concentration of nifedipine can be determined in the presence of its metabolite (nitrophenylpyridine). The total HPLC run time for one sample was 9 min allowing 50 to 60 samples to be processed per day.

Table 1. Within and between run variations of nifedipine determined by the present HPLC method

Nifedipine concentration (ng/ml)	Within run (n=4)			Between run (n=4)		
	Mean	SD	CV (%)	Mean	SD	CV (%)
5	7.86	0.43	5.47	5.26	1.25	23.76
10	10.1	0.16	1.58	10.27	1.10	10.71
20	21.1	1.12	5.3	23.26	2.52	10.83
40	39.7	2.79	7.02	39.80	3.87	9.72
60	60.6	2.14	3.53	58.84	7.33	12.45
80	80.02	1.36	1.69	79.85	2.65	3.39
100	99.3	3	3.02	96.70	5.34	5.52
120	120.3	2.15	1.78	122.55	2.25	1.83

Table 2. Recovery of nifedipine from control human plasma assayed by HPLC (n=4)

Nifedipine added to plasma (ng/ml)	Mean±SD of nifedipine determined (ng/ml)	Mean±SD(%) of nifedipine recovery
5	4.8±0.12	96±2.5
10	9.9±0.51	99±5.1
20	19.5±0.95	97.5±4.9
40	37±3.5	92.5±4.2
60	61±4.1	101.6±6.7
80	79±3.9	98.7±4.9
100	99±7.8	99±7.9
120	122±8.5	101.6±6.9

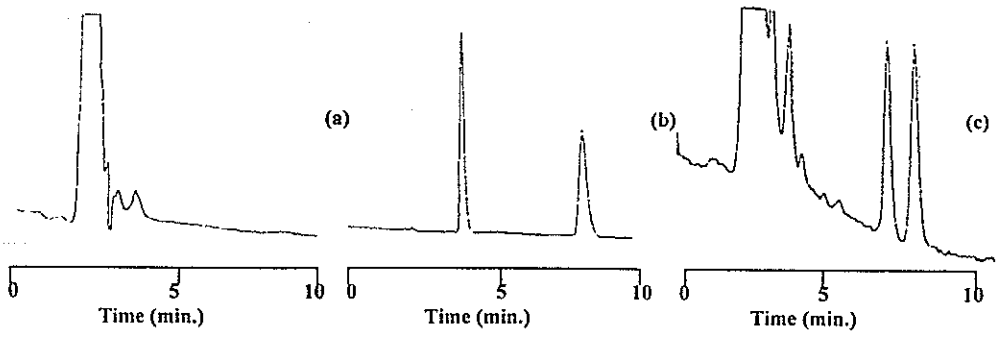

Figure 2: HPLC chromatograms of (a) blank plasma, (b) solution containing nitrophenylpyridine (3.8 min) and nifedipine (7.82 min), (c) plasma with nitophenylpyridine (3.8 min), nimodipine (6.38 min) and nifedipine (7.82 min).

CONCLUSION

This paper describes a simple, robust and easy to automate HPLC method for the analysis of nifedipine based on normal phase chromatography and UV detection. The method is internally calibrated and uses uniform volumes of plasma, so it is suitable for the routine clinical pharmacokinetic studies.

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