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Short Communication

Evaluation of chromatographic methods for the determination of nifedipine in human serum

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Abstract

Gas–liquid chromatography and high-performance liquid chromatography were compared for the identification and determination of nifedipine in biological samples and the elaboration of the optimum liquid–liquid extraction procedure. The determination limits were 2 and 10 ng/ml, respectively, and the detection limits were 1 and 5 ng/ml, respectively. The calibration graphs were linear in the ranges 2–300 and 10–500 ng/ml, respectively. Recoveries based on three different concentrations were 88.7–95.8% and 93.7–104.2%, respectively. Both methods are sensitive, specific and reproducible enough for pharmocokinetic studies and therapeutic drug monitoring.

1. 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. Therefore, nifedipine is used in the treatment of angina pectoris, arterial hypertension and Reynold's phenomenon [1–3].

Nifedipine exhibits large inter-subject variability in absorption, metabolism and excretion. Hence specific, sensitive and rapid measurements of nifedipine in plasma and serum are required for pharmacokinetic studies and to examine the relationship between blood levels and clinical effects. Several methods for the assay of nifedipine in plasma have been described, including a fluorescence method [4], gas chromatography [5–7] and high-performance liquid chromatography [8–10]. Many of these methods, however, are inappropriate for clinical use because they have low sensitivity, are time consuming, need a large amount of plasma and require expensive equipment, which may not be available in clinical laboratories.

The aim of this study was to evaluate two chromatographic methods, gas–liquid (GLC) and high-performance liquid chromatography (HPLC) for the determination of nifedipine in human serum, for the purposes of pharmacokinetic studies and therapeutic drug monitoring.

2. Experimental

2.1. Materials and reagents

Nifedipine and internal standards (nitrazepam and diazepam) were kindly supplied by Pharmaceutical Enterprise "Polfa". Doubly distilled

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water was used throughout. Other reagents and solvents were of HPLC grade.

2.2. Chromatography

For the HPLC experiments a Kontron Model 400 system consisting of a (Model 420) solvent pump, a (Model 432) UV detector and computer system for acquisition and integration of the data was used. A 250 mm × 4.6 mm I.D. Kontron RP-18 (10-μm) column was used at ambient temperature. The mobile phase was methanol (POCh)–water (70:30, v/v) modified with the addition of 1% glacial acetic acid (POCh). The flow-rate was 1.5 ml/min and the UV detector was operated at 238 nm. Under these conditions the retention time of nifedipine was 4.2 min and that of the internal standard (nitrazepam) was 3.8 min.

Fig. 1 shows typical chromatograms of blank serum and serum spiked with 50 ng/ml of nifedipine and 150 ng/ml of the internal standard.

GLC experiments were performed using a Pye Unicam Model 104 chromatograph with a ^{63}Ni electron-capture detector and a glass column (1.5 m × 4 mm I.D.) packed with 3% OV-17 on Chromosorb W HP (125–150 μm). The operating temperatures were oven 260°C (isothermal), injection port 270°C and detector 300°C. The flow-rate of the carrier gas (argon) was 50 ml/min. Under these conditions, the retention time of nifedipine was 2.7 min and that of diazepam (internal standard) was 4.4 min.

Fig. 2 shows typical chromatograms of blank

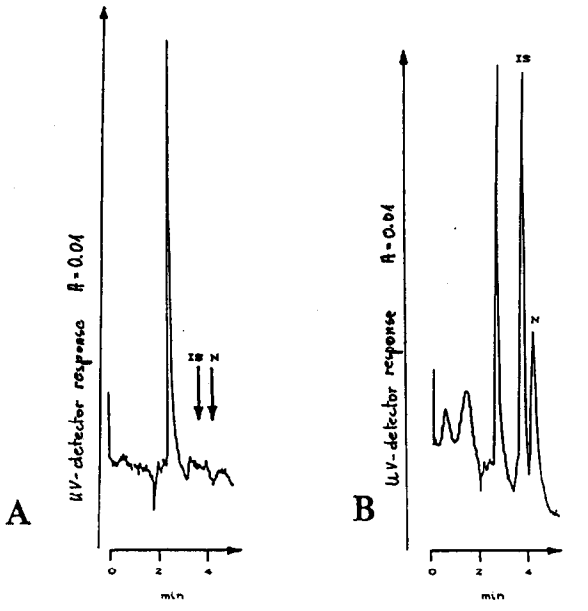

Fig. 1. Typical chromatograms of (A) blank serum and (B) serum spiked with 50 ng/ml of nifedipine (N) and 150 ng/ml of nitrazepam (internal standard, IS). Chromatographic method, HPLC; column, Kontron RP-18 (250 mm × 4.6 mm I.D.); mobile phase, methanol–water (70:30, v/v) modified with the addition of 1% glacial acetic acid.

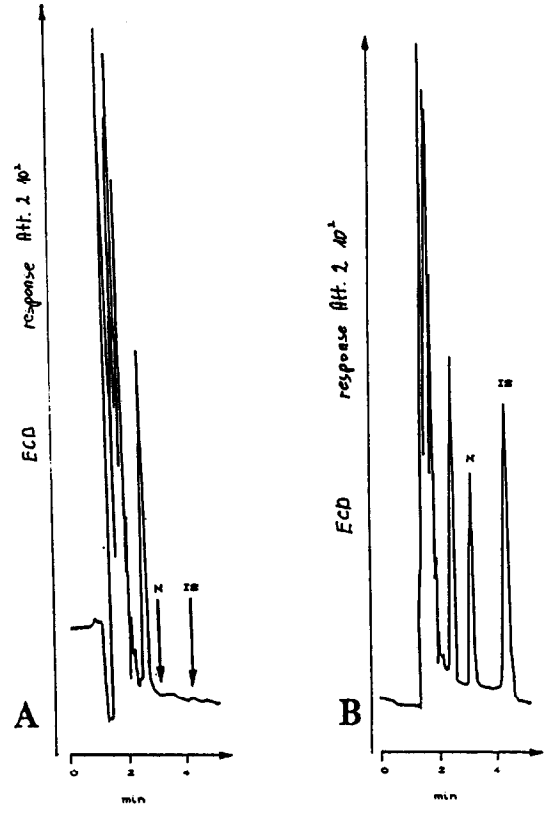

Fig. 2. Typical chromatograms of (A) blank serum and (B) serum spiked with 40 ng/ml of nifedipine (N) and 40 ng/ml of diazepam (IS). Chromatographic method, GLC; column (1.5 m × 4 mm I.D.), packed with 3% OV-17 on Chromosorb W HP (100–120 mesh); column temperature, 260°C; detection, ^{63}Ni electron-capture detector.

serum and serum spiked with 40 ng/ml of nifedipine and 40 ng/ml of the internal standard.

2.3. Extraction procedure

Because nifedipine is very sensitive to light, all samples were stored in complete darkness and analytical operations were done in "gold light". The extraction procedure chosen as the optimum after a number of experiments is described below and was applied for both chromatographic methods considered.

To 1.0 ml of serum sample were added 150 ng of nitrazepam (internal standard for HPLC method) or 40 ng of diazepam (internal standard for GLC method). The sample was then alkalinized with 0.1 ml of 1 *M* NaOH and extracted with 5.0 ml of hexane (POCh)–dichloromethane (Aldrich) (70:30, v/v) by shaking it horizontally for 20 min.

After centrifugation for 10 min, the organic phase was transferred into a conical tube and evaporated to dryness under a stream of nitrogen at 40°C. The residue was dissolved in 120 μl of the mobile phase (for the HPLC method) or 120 μl of benzene (POCh) (for the GLC method). The injection volumes were 20 μl for the HPLC method and 5 μl for the GLC method.

3. Results and discussion

The first stage of the investigation was a search for the optimum pH for nifedipine extraction. The extraction was performed at neutral and alkaline pH, using several organic solvents. It was found that pH does not influence the recovery, but the amount of interfering materials was markedly lower with alkaline extraction.

In order to find the best solvent for extraction of nifedipine from human serum, a number of solvents including chloroform (POCh), dichloromethane and dichloromethane–hexane (30:70, v/v) were tested. Using chloroform and dichloromethane endogenous biological interfering substances were also extracted. The interfering peaks became negligible and nifedipine was well separated from endogenous substances when dichloromethane–hexane (30:70, v/v) was used for extraction. The last solvent system also provided the best recovery, as shown in Table 1. The recovery results were obtained using three different concentrations of nifedipine and HPLC as the detection method.

The determination limits [the lowest concentration that can be determined with a relative standard deviation (R.S.D.) lower than 10%] were 2 and 10 ng/ml for the GLC and HPLC methods, respectively, whereas the detection limits (the lowest measurable concentration that can be distinguished from zero, detected at a signal-to-noise ratio of 3:1) were 1 and 5 ng/ml, respectively.

The calibration graphs were linear in the range of 2–500 ng/ml for the GLC and 10–500 ng/ml for the HPLC method. Recoveries based on three different concentrations and using dichloromethane–hexane as the extraction system were 88.7–95.8% and 93.7–104.2% for the GLC and HPLC methods, respectively.

Table 1
Influence of extraction solvent on recovery of nifedipine from human serum ($n = 6$)

Concentration (ng/ml)	Recovery ± S.D. (%)		
	Chloroform	Dichloromethane	Dichloromethane–hexane (7:3)
25	90.8 ± 8.16	86.4 ± 14.17	104.2 ± 4.13
100	87.6 ± 11.41	81.3 ± 9.21	93.7 ± 4.85
250	84.4 ± 6.12	83.5 ± 8.33	98.1 ± 2.02
150 (IS)	71.4 ± 14.43	68.2 ± 9.13	75.3 ± 7.17

Table 2
Validation of GLC and HPLC methods for nifedipine assay ($n = 6$)

Parameter	Concentration (ng/ml)					
	25		100		250	
	HPLC	GLC	HPLC	GLC	HPLC	GLC
Mean (ng/ml)	26.93	24.43	102.59	94.14	239.24	251.38
S.D.	0.75	2.24	3.09	5.15	2.82	10.97
R.S.D. (%)	2.79	9.20	3.01	5.47	1.18	4.36
Accuracy (%)	7.72	8.66	3.15	4.38	4.30	6.18

For the determination of precision and accuracy, pools of 6 ml of serum were spiked with nifedipine to give concentrations of 25, 100 and 250 ng/ml. Aliquots of 1 ml from each pool were extracted and assayed using the GLC and HPLC methods simultaneously. The results are given in Table 2 and show a slightly better precision and accuracy for the HPLC method. However, the GLC method was slightly more sensitive and the retention time for nifedipine was shorter with the GLC method (2.7 min) than the HPLC method (4.2 min). Nevertheless, the total time of analysis, including elution of the internal standards, was almost the same for both methods. The costs of analysis were also similar for the two methods.

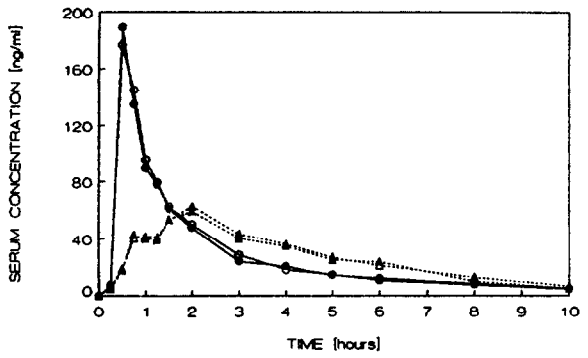

Fig. 3. Concentration *vs.* time profiles for serum from a healthy volunteer after administration of 20 mg of two different forms of nifedipine, determined using GLC and HPLC methods. ● = preparation A (HPLC); ○ = preparation A (GLC); ▲ = preparation B (HPLC); △ = preparation B (GLC).

Hence it can be concluded that both methods are sensitive, specific and reproducible enough for pharmacokinetic studies and therapeutic drug monitoring. In order to confirm these findings, both methods were applied successfully to the determination of nifedipine in human serum. Fig. 3 shows profiles of nifedipine concentration in blood from one healthy volunteer after oral administration of two commercial forms of the drug in a cross-over study, obtained using the two investigated methods. For the same preparation, the curves obtained on the basis of the two methods did not exhibit significant differences. On the other hand, substantial differences were found among two different nifedipine preparations. This finding emphasizes the need for monitoring nifedipine concentrations in blood during therapy.

4. References

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