Journal of Chromatography, 308 (1984) 209—216
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2073

LIQUID CHROMATOGRAPHIC DETERMINATION OF NIFEDIPINE IN PLASMA AND OF ITS MAIN METABOLITE IN URINE

C.H. KLEINBLOESEM* and J. VAN HARTEN

Department of Pharmacology, Subfaculty of Pharmacy, State University of Leiden, Leiden (The Netherlands)

P. VAN BRUMMELEN

Department of Nephrology, University Hospital, Leiden (The Netherlands)

and

D.D. BREIMER

Department of Pharmacology, Subfaculty of Pharmacy, State University of Leiden, Leiden (The Netherlands)

(First received November 29th, 1983; revised manuscript received January 16th, 1984)

SUMMARY

A high-performance liquid chromatographic method was developed for the assay of nifedipine in plasma and its main metabolite (M-I) in urine. After liquid—liquid extraction nifedipine was chromatographed in a reversed-phase system with ultraviolet detection at 238 nm. The method was sensitive to 2 ng nifedipine per ml plasma and the standard curve was linear to at least 400 ng/ml. Standard deviations did not exceed 8.5%. There was no interference with photodecomposition products or metabolites. M-I was determined in urine after liquid—liquid extraction by ion-pair chromatography with ultraviolet detection at 290 nm. The method was sensitive to 0.02 μg M-I per ml urine and the standard curve was linear to at least 5 μg/ml. Standard deviations did not exceed 5.0%. The methods were used to study nifedipine disposition in healthy volunteers.

INTRODUCTION

Nifedipine, dimethyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine carboxylate, is a calcium-channel blocker, which selectively dilates arteries

0378-4347/84/$03.00

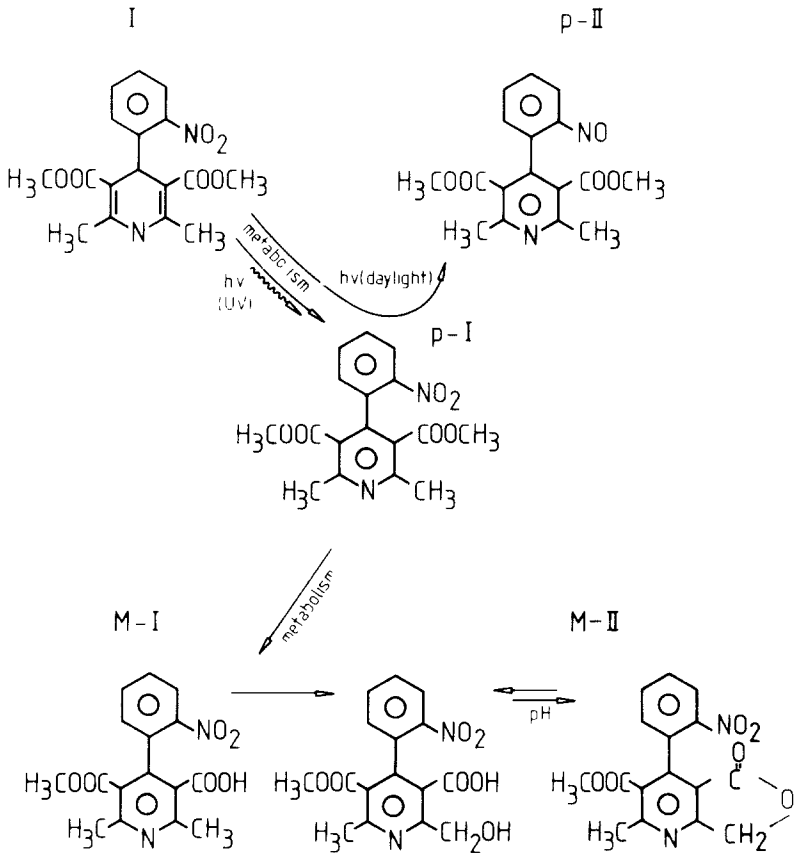

Fig. 1 Photodecomposition and biotransformation scheme. I is nifedipine; p-I and p-II are photodegradation products, and M-I and M-II are metabolites.

with little or no effect on other blood vessels. Therefore nifedipine is used in the treatment of angina pectoris and arterial hypertension [1, 2]. The compound is photolabile; a scheme of its photodecomposition and biotransformation is shown in Fig. 1. In the human body nifedipine is rapidly oxidized enzymatically into its pyridine metabolite (p—I) [3—5]. This product is also formed in ultraviolet (UV) light, whereas the 2-nitroso derivative (p-II) is formed in normal daylight. In the biotransformation process the ester moiety of the side chain can be hydrolysed (M-I) and further oxidation can occur in the 2-methyl position (M-II) [3—5]. M-I is excreted in urine for about 60% of the dose, whereas M-II is excreted for only 3—5% of the dose [6]. It is assumed that these metabolites have no pharmacological activity [3].

For the determination of nifedipine in biological fluids several gas chromatographic methods have been described, with either electron-capture detection or selective ion monitoring, providing a satisfactory detection limit of approximately 1—5 ng/ml [4—10]. However, the thermostability of nifedipine under the chromatographic conditions employed (230—250°C) represents a serious problem, since p-I is formed in non-reproducible amounts. Therefore Kondo et al. [6] and Higuchi and Shiobara [9] oxidized nifedipine prior to

analysis, despite the loss of selectivity. Pietta et al. [11] reported a selective high-performance liquid chromatographic (HPLC) method for nifedipine and metabolites in rat plasma with a detection limit of 10 ng of nifedipine, which is not low enough for pharmacokinetic studies in man. Recently, Bach [12] reported a sensitive liquid chromatographic method for the determination of nifedipine in serum, but some interference with the serum matrix occurred and a lengthy sample clean-up procedure was used.

In the present study a sensitive and selective reversed-phase HPLC method has been developed for the determination of nifedipine in plasma taking photodecomposition and metabolite formation into account. Also an ion-pair HPLC method is reported for the main metabolite of nifedipine in urine (M-I). Preliminary disposition data in healthy volunteers are included in this paper. In addition, the photostability of nifedipine in organic solvents, as well as in plasma, was investigated.

EXPERIMENTAL

Chemicals

Nifedipine, metabolites (M-I, M-II), photodegradation products (p-I, p-II), nitrendipine (internal standard) and Bay a-4160 [2,6-diethyl-4-(2-nitrophenyl)-5-methoxycarbonylpyridine-3-carboxylic acid, internal standard] were kindly supplied by Bayer (Wuppertal, F.R.G.). In all experiments double-distilled water and freshly distilled organic solvents (Baker, Deventer, The Netherlands) were used.

Apparatus and chromatographic system

Nifedipine. The HPLC system consisted of a solvent pump (Waters Assoc., U.S.A., Model M 45), an automatic sampler (Waters Assoc., Model Wisp 710B), a column (100 mm long), constructed from precison bore stainless-steel tubing (I.D. = 2.8 mm, O.D. = 6.35 mm), filled by a slurry technique with MOS-Hypersil® (dimethyloctyl silica), particle size 5 μm (Shandom, Astmoor, U.K.), and a UV detector (Waters Assoc., Model Lambda Max 480) set at 238 nm. Concentrations were calculated by using an integrator (Hewlett-Packard, U.S.A., Model 3390 A). The mobile phase consisted of 0.05 *M* acetate buffer, pH 4.0—acetonitrile (7:5). It was degassed ultrasonically and used at a flow-rate of 1.0 ml/min.

Metabolite I. M-I was chromatographed on a Rad-Pak C_8 cartridge (100 mm long, I.D. 5 mm, particle size 10 μm; Waters Assoc.). The mobile phase consisted of acetonitrile—water + 0.009 *M* cetrimide (1:3) and was degassed ultrasonically before use; the flow-rate was set at 4.0 ml/min. Detection took place at 290 nm.

Preparation of samples

Nifedipine. Plasma (1.0 ml) was transferred to a 15-ml centrifuge tube containing 0.5 ml of 1 *M* sodium hydroxide and 150 ng of nitrendipine (internal standard). The mixture was extracted with 5 ml of dichloromethane—pentane (3:7) on a vortex whirl mixer for 20 sec and centrifuged for 5 min at 2000 *g*. The organic layer was transferred to a 15-ml centrifuge tube using Pasteur dis-

posable pipettes and evaporated to dryness on a vortex vacuum evaporator (Buchler, Fort Lee, U.S.A.) at 40° C. The sample was reconstituted with 200 μl of the mobile phase before injection. All steps were carried out in sodium light, without influence of daylight.

Metabolite I. Urine (1.0 ml) was transferred to a 15-ml centrifuge tube containing 0.5 ml of 1 *M* acetate buffer, pH 4.0 and 3 μg of Bay a-4160 (internal standard). The mixture was extracted with 7 ml of chloroform on a vortex whirl mixer for 30 sec and further processed as described above.

Preparation of calibration graphs

Control plasma samples (1.0 ml) were spiked with 0, 5, 10, 25, 50, 100, 200 and 400 ng nifedipine. Urine samples were spiked with 0, 0.25, 0.5, 1.0, 2.5 and 5 μg/ml M-I. Samples were processed as described and the ratios of peak area of compound to be assayed to peak area of internal standard were calculated. Calibration curves were constructed by linear-regression analysis. Extraction yields were also determined using the same procedure, except that the internal standard was added now after extraction as external standard. The ratios obtained were compared with the ratio of standard amounts of nifedipine and M-I to the internal standard. The average recovery was determined by the slope ratio of the two calibration graphs as obtained after internal and external standardization.

Photostability of nifedipine

To 10 ml-centrifuge tubes 150 ng/ml nifedipine dissolved in dichloromethane—pentane—methanol (3:7:1) (total volume 5 ml) was added. The tubes were exposed to normal laboratory light on a normal summer day for the following periods: 0, 5, 10, 20, 30, 40, 50 and 60 min. After exposure to light, internal standard was added and the samples were analysed after evaporation of the organic solvents. Detection took place at 254 nm. The same procedure was performed after spiking plasma samples with nifedipine and exposing these to light. These samples, however, were also extracted prior to analysis as described above for nifedipine. The identity of the photodegradation product was confirmed by mass spectrometry as the nitroso derivative, by comparing the spectrum obtained with the mass spectrum of the reference compound.

Human experiments

In this preliminary study five healthy volunteers (four males, one female) received 4.5 mg of nifedipine as an intravenous infusion over a period of 50 min. Infusion lines were carefully protected from light by aluminum foil. Blood samples were taken from a forearm vein using a flexible cannula. Samples were drawn in heparinized tubes at 0, 10, 20, 30, and 45 min, and 1, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0 and 8.0 h after starting the infusion. Plasma was separated by centrifugation and samples were stored (protected from light) at −20° C until analysed. Urine samples were collected at regular intervals until 24 h after drug administration and these were stored at −20° C until analysed.

RESULTS AND DISCUSSION

Assay of nifedipine in plasma

In Fig. 2 typical chromatograms are shown which were obtained after extraction of blank plasma to which only internal standard had been added and plasma containing 25 ng of nifedipine and internal standard. No interfering substances seem to be coextracted. There was also a good separation with possible photodegradation products, because the retention times of p-I and p-II were 0.96 and 2.41 min, respectively. These compounds could not be detected in samples of volunteers who received nifedipine, indicating that protection from light had been adequate. Calibration curves were linear with concentration, as shown by the correlation coefficient of 0.999. The method was reproducible and had satisfactory precision; standard deviations did not exceed 8.5%. Extraction yields appeared to be linear in the concentration range 5—400 ng/ml and almost complete (mean 97.6 ± 3.0%; $n = 4$). The lowest measurable concentration was about 2 ng/ml. It can be concluded that the present HPLC method is highly selective, sensitive and rapid. As compared to previously reported GC methods [4—10] thermostability is no problem and the lowest measurable concentration is in the same order of magnitude (1—5 ng/ml); precision, however, is somewhat less: 8.5% vs. 2.8% [6].

Sensitivity is in the same order as that of the HPLC method reported by Bach [12], whereas sample clean-up is less lengthy in the present method.

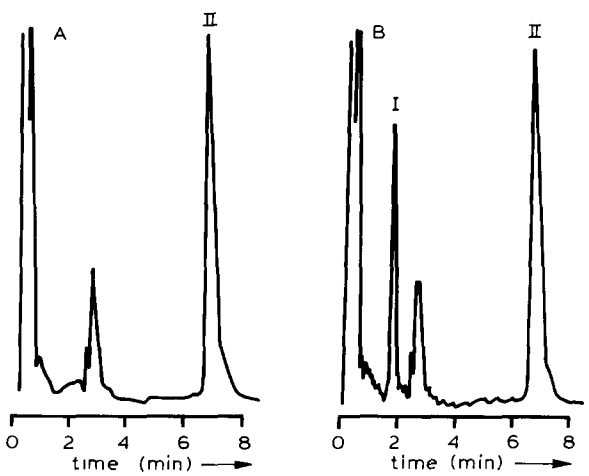

Fig. 2. Chromatograms of (A) control plasma with internal standard (peak II) and (B) plasma spiked with 25 ng/ml nifedipine (peak I) and internal standard (peak II).

Assay of M-I in urine

In Fig. 3 typical chromatograms are shown which were obtained after extraction of blank urine to which only internal standard had been added, and of urine containing 2.5 μg of M-I and internal standard. Interfering peaks were not observed. Calibration curves were linear with concentration, as shown by the correlation coefficient of 0.999. The method was reproducible and standard deviations were below 5%. Extraction yields determined in the same concentration range appeared to be constant and linear with concentration

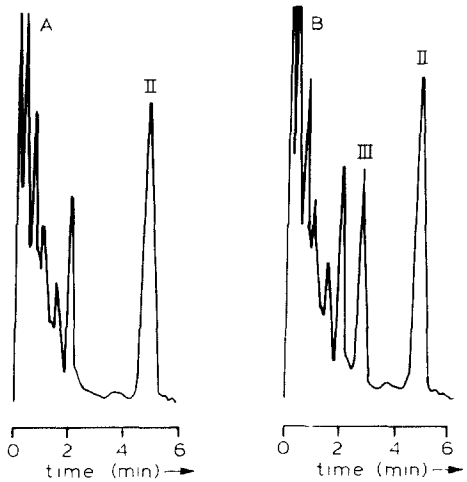

Fig 3. Chromatograms of (A) control urine with internal standard (peak II) and (B) urine spiked with 3 μg/ml M-I (peak III) and internal standard (peak II).

with a mean value of 85.1 ± 4.2% (n = 4). The lowest measurable concentration in urine is 0.02 μg/ml. In comparison to the method of Kondo et al. [6] for this metabolite it can be concluded that the present ion-pair HPLC method is highly selective, and no derivatization step is required. However, the detection limit is somewhat higher (20 ng/ml urine vs. 5 ng/ml) and precision less (5.0% vs. 1.9%).

Photostability of nifedipine

In Fig. 4 the decomposition rate of nifedipine is shown in organic solvents, as well as in plasma. The half-life of nifedipine decompositon was 15 min in organic solvents and 44 min in plasma, which indicates that also in plasma oxidation of nifedipine takes place relatively rapidly in light, so that the whole assay procedure has to be carried out while excluding the light.

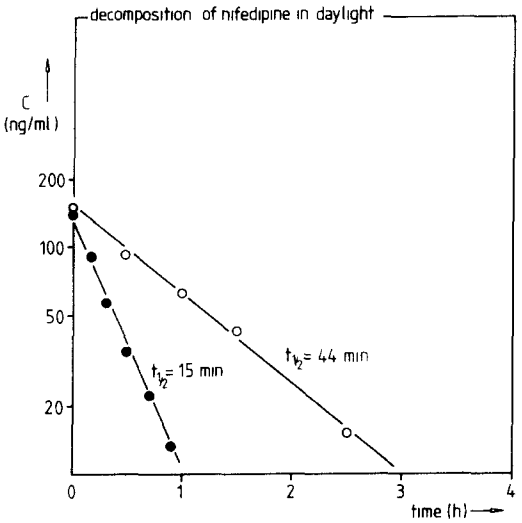

Fig. 4. Decomposition of nifedipine exposed to daylight in organic solvent (•) and plasma (○)

Human experiments

A representative plasma concentration—time curve is shown in Fig. 5. The concentrations of nifedipine could be fitted to an open two-compartment model, using weighted non-linear-regression analysis. Pharmacokinetic data are summarized in Table I. After stopping the infusion a rapid distribution phase ($t_{1/2}$, λ_1 = 10 ± 6 min) was followed by a first-order process of elimination ($t_{1/2}$, λ_2 = 106 ± 24 min). The volume of distribution at steady-state (V_{ss}) was 0.8 ± 0.2 l/kg, which indicates extensive tissue distribution, when considering the high extent of plasma protein binding of about 95—98% [14]. Mean systemic clearance was 0.55 ± 0.12 l/min, which implies that the rate of systemic drug elimination is not only dependent on drug metabolizing enzyme activity, but

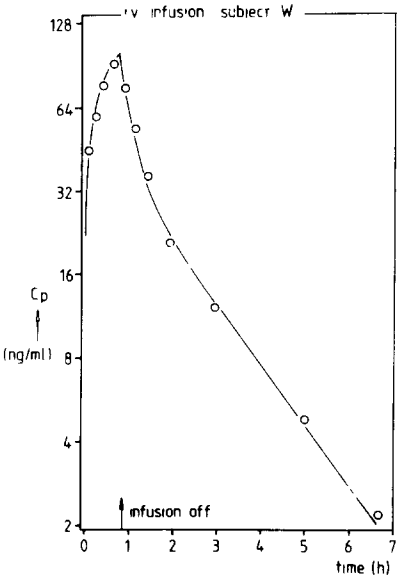

Fig. 5. Plasma concentration versus time profile of nifedipine in a healthy subject (W) following intravenous infusion for 50 min (dose 4.5 mg).

TABLE I

PHARMACOKINETIC DATA* OF NIFEDIPINE FOLLOWING INTRAVENOUS INFUSION

Subject	Age (years)	$t_{1/2,\lambda_1}$ (min)	$t_{1/2,\lambda_2}$ (min)	Cl (l/min)	V_{ss} (l/kg)	M-I (percentage dose per 24 h)
L	27	6	126	0.61	0.99	46.2
S	35	20	120	0.35	0.55	58.3
W	46	11	82	0.54	0.58	79.2
T	50	4	77	0.69	0.75	62.5
B	54	10	125	0.55	1.05	64.3
Mean	42	10	106	0.55	0.78	62.1
S.D.	11	6	24	0.12	0.23	11.9

*$t_{1/2,\lambda_1}$ = half-life of distribution; $t_{1/2,\lambda_2}$ = half-life of elimination; Cl = total plasma cleareance; V_{ss} = volume of distribution at steady-state.

also on hepatic blood flow. These results are in close agreement with the data reported by Rämsch [13].

The 24-h urinary excretion of M-I amounted to approximately 60% of the dose; Horster et al. [3] recovered about 65% of the dose with ^{14}C-labelled nifedipine after a bolus injection; the other part had been excreted in bile. This indicates that relatively small amounts are excreted in urine as other metabolites, since it has been shown by Kondo et al. [6] that unchanged nifedipine and/or p-I are excreted in urine in trace amounts (0.1% of an oral dose). Urinary excretion of M-I is also in agreement with the findings of Kondo et al. [6].

CONCLUSIONS

The phase systems described in this paper for the determination of nifedipine in plasma and its main metabolite (M-I) in urine allows their rapid, sensitive and selective quantitative analysis without interference from endogenous compounds, metabolites or photodegradation products. The described chromatographic procedure can be performed automatically, which permits the relatively rapid assay of many samples of nifedipine. The method is suitable for pharmacokinetic studies in man; drug elimination from plasma can be followed for 3—4 times its half-life following therapeutic dosages of nifedipine. It was also clearly shown in the present study that nifedipine preparations and plasma samples need to be handled without exposure to daylight.

ACKNOWLEDGEMENTS

The authors wish to thank H.C.N. Kappelle-de Vries for typing the manuscript and Drs. W. Onkenhout for producing the mass spectra. The study was supported in part by a grant from Bayer (Wuppertal, F.R.G.).

REFERENCES

1 M. Guazzi, M.T. Olivari, A. Polese, C Fiorentini, F. Magrini and P Moruzzi, Clin. Pharmacol. Ther., 22 (1977) 528.
2 K Hashimoto, T. Kobayashi and E. Kimura (Editors), Nifedipine "Adalat", 1st International Symposium Tokyo, 1973, Bayer Yakuhin Ltd., Osaka, 1975
3 F.A. Horster, B. Duhm, W. Maul, H. Medenwald, K. Patschke and L.A Wegner, Arzneim.-Forsch., 22 (1972) 330
4 R. Testa, E. Dolfini, C. Reschiotto, C Secchi and P Biondi, Farm. Ed. Pract., 34 (1979) 463.
5 H. Medenwald, K. Schlossmann and C. Wünsche, Arzneim.-Forsch., 22 (1972) 242.
6 S. Kondo, A. Kuchiki, K. Yamamoto, K. Akimoto, K Takahashi, N Awata and J. Sugimoto, Chem. Pharm. Bull., 28 (1980) 1.
7 P. Jakobsen, O Lederballe Pedersen and E. Mikkelsen, J Chromatogr , 162 (1979) 81.
8 J. Dokladalova, J.A. Tykal, S.J. Coco, P.E. Durkee, G.T Quercia and J J Korst, J. Chromatogr., 231 (1982) 451
9 S. Higuchi and J. Shiobara, Biomed Mass Spectrom., 5 (1978) 220.
10 K.D. Rämsch, personal communication
11 P. Pietta, A. Rava and P. Biondi, J. Chromatogr., 210 (1981) 516.
12 P R. Bach, Clin. Chem., 29 (1983) 1344.
13 K.D. Rämsch, Schwerpunkt Med., 47 (1981) 55.
14 H. Rosenkranz, K. Schlossmann and W. Scholtan, Arzneim.-Forsch , 24 (1974) 455