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Note

Rapid high-performance liquid chromatographic determination of nifedipine in plasma with on-line precolumn solid-phase extraction

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Nifedipine, dimethyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarboxylate (Fig. 1), is a calcium antagonist primarily used in treatment of angina pectoris and hypertension [1–3]. Owing to its photolability assays are complicated. For the determination of nifedipine in plasma several gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) methods have been described, which involve carrying out plasma extraction and sample clean-up in a darkroom or under red light [4–12]. However, the known thermoinstability in GC makes it necessary that in some of these methods nifedipine is oxidized prior to analysis to its more stable nitropyridine derivative [8–10]; also, interferences occurred with the serum matrix requiring extensive extraction steps and/or sample clean-up procedures [5,10,11] or the sensitivity of the assay was not low enough for bioavailability studies in humans [7,11].

The method described in this paper is rapid, with no possibility of light degradation, and provides sufficient selectivity and sensitivity for the measurement of low plasma levels.

EXPERIMENTAL

Chemicals

Nifedipine was a gift from Siegfried (Zofingen, Switzerland). Acetonitrile was HPLC grade (Rathburn, Walkerburn, U.K.), and other chemicals were all analytical grade (E. Merck, Darmstadt, F.R.G.). Distilled water was used in all experiments.

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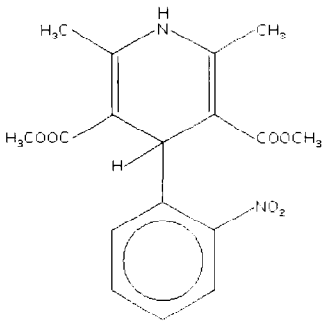

Fig. 1. Structure of nifedipine.

Apparatus and chromatographic system

The chromatographic system consisted of two ConstaMetric III pumps (LDC/Milton Roy, Riviera Beach, FL, U.S.A.) one with a pulse dampener Model 802C (Gilson Medical Electronics, Middleton, WI, U.S.A.), a laboratory-made precolumn (10×4 mm I.D.) dry-filled with LiChroprep RP-2 (25–40 μm), an analytical column (125×4 mm I.D.) filled with Hypersil ODS 5 μm (Forschungszentrum Seibersdorf, Seibersdorf, Austria) and a Spectroflow 773 UV detector (Kratos Analytical Instruments, Westwood, NJ, U.S.A.) set to 355 nm 0.005 a.u.f.s. and connected by an injection valve Model 7125 and a switching valve Model 7010 (Rheodyne, Cotati, CA, U.S.A.), as shown in Fig. 2. The integrator was a Model CI-10 (LDC/Milton Roy).

The mobile phases were 0.02 *M* ammonium carbaminate at a flow-rate of 3 ml/min for pump A and acetonitrile–0.02 *M* acetate buffer pH 4.0 (1:1, v/v) at a flow-rate of 1 ml/min for pump B; both were helium-degassed. Chromatography was carried out at ambient temperature.

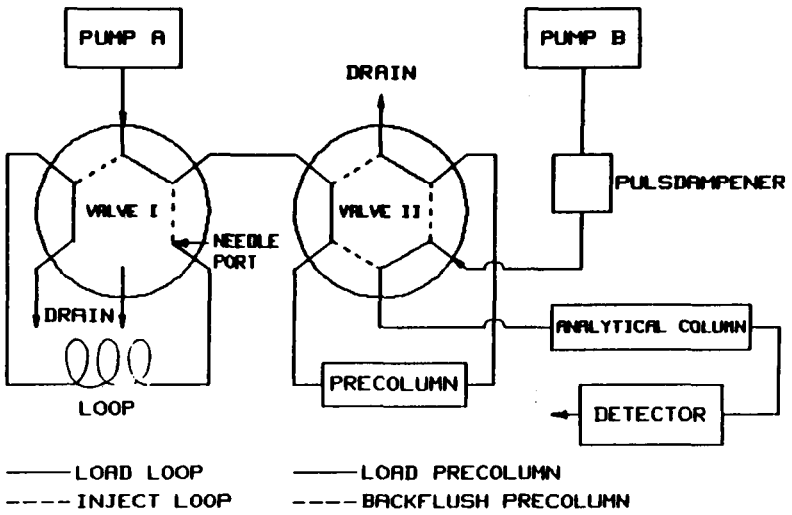

Fig. 2. Schematic configuration of the chromatographic system.

TABLE I

RECOVERY OF NIFEDIPINE AFTER PRECOLUMN WASH

Concentration, 50 ng/ml; $n = 4$.

Methanol (%)	Recovery after wash (%)	Coefficient of variation (%)
20	101.5	2.1
30	100.1	1.1
40	97.5	0.6
50	62.5	1.3

Procedure

To avoid light degradation, plasma samples were collected in glass tubes wrapped with aluminium foil and stored until analysis at −28°C. Thawing and centrifugation were carried out in a darkened room. Freshly centrifuged plasma was injected on a precolumn using a 650-μl loop. After 45 s of rinsing the precolumn, the loop was filled with 20% methanol and injected to remove any less polar compounds retained on the precolumn. After 45 s the rinsed precolumn was switched into the mobile phase stream B, and retained substances were back-flushed on the analytical column. After 1 min the precolumn was switched back and equilibrated with mobile phase A for at least 45 s. So each cycle needs not more than 3.5 min with chromatographic run times of 3 min.

Standard curve preparation

Stock solutions of nifedipine were prepared at concentrations of 10 and 1 ng/μl in distilled water. Appropriate dilution of these solutions with control plasma gave a range of standards corresponding to 0, 2, 4, 10, 50 and 120 ng/ml nifedipine, respectively. The spiked standards were handled like samples.

RESULTS AND DISCUSSION

Photostability of nifedipine

Nifedipine degrades spontaneously to its nitroso or nitropyridine derivative on exposure to light [4,13]. Published decomposition half-lives range from 15 min up to hours, depending on the solvent and spectrum of the light [4,7,14]. Our pilot experiments (not shown) also indicate high degradation of nifedipine in direct light, but in a darkened room samples show no measurable degradation for hours. Nevertheless, in the extraction method reported here, which uses a precolumn, there is practically no light contact with the samples.

Chromatography

Nifedipine can be extracted from plasma on different reversed-phase materials. In pilot experiments RP-2 showed the best results: it retained nifedipine from plasma strongly enough to allow a washing step with up to 30% methanol without loss of nifedipine (Table I). Owing to the irreversible binding of proteins which

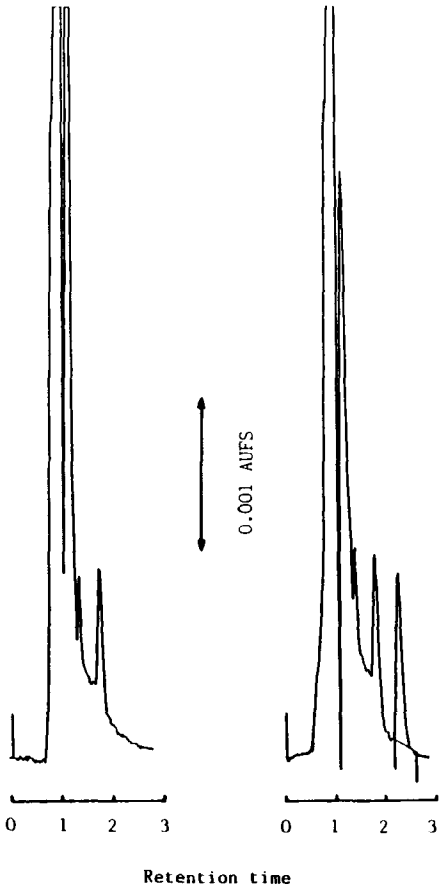

Fig. 3. Chromatograms of control plasma (left) and plasma sample of a healthy volunteer 5 h after oral administration of 20 mg of nifedipine, corresponding to a concentration of 20 ng/ml (right).

results in a poorer separation performance of the entire system, the precolumn had to be changed each 20–25 plasma injections. To enhance selectivity, our choice of detection wavelength was 355 nm because there were often interferences at 280 nm [11] and 238 nm [7].

Typical chromatograms are shown in Fig. 3. Chromatograms of plasma from a healthy volunteer before and 5 h after oral administration of 20 mg of nifedipine are presented. The corresponding nifedipine concentration was ca. 20 ng/ml of plasma. No interfering peaks in the control plasmas at the retention time of nifedipine (2.4 min) were found.

The calibration curve was linear (coefficient of correlation = 0.999, $n = 23$) in the range 0–120 ng/ml of plasma, with a limit of detection of 0.5 ng/ml of plasma (two standard deviations of the blank). The overall recovery (2–120 ng/ml) in relation to water was 99.6%. The intra-assay variability was 0.1–6.7% (median 1.6%, $n = 10$) in the range 12.9–43.9 ng/ml of plasma. The day-to-day accuracy ($n = 5$) was 2.5% at 50 ng/ml plasma.

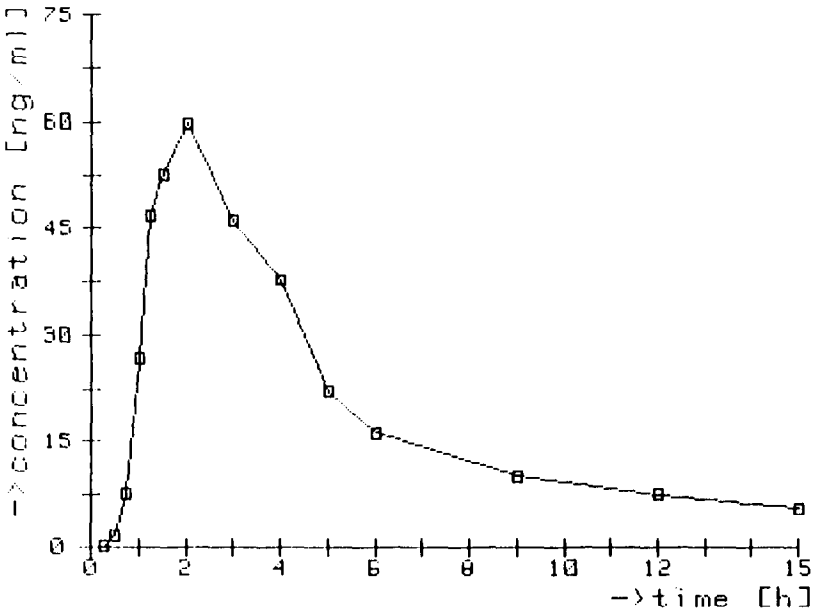

Fig. 4. Plasma concentration-time profile of a healthy volunteer after oral administration of 20 mg of nifedipine.

The method is rapid, allowing ca. 140 samples to be analysed by a single technician within an 8-h working day.

Plasma concentration–time profile

Fig. 4 shows a typical plasma concentration–time curve of a healthy volunteer after oral administration of 20 mg of nifedipine. Importantly for bioavailability studies, it was possible to measure even low concentrations with this method.

REFERENCES

1 M. Guazzi, M.T. Olivari, A. Polese, C. Fiorentini, F. Magrini and P. Moruzzi, Clin. Pharmacol. Ther., 22 (1977) 528.
2 S. Bayley, R.J. Dobbs and B.F. Robinson, Br. J. Clin. Pharmacol., 14 (1982) 509.
3 F. Giontoli, G. Guidi, A. Scalabrino, F. Galeone, E. Pagliai, S. Marini, A. Birindelli and P. Saba, Curr. Ther. Res., 4 (1981) 447.
4 P. Jakobsen, O. Lederballe-Pedersen and E. Mikkelsen, J. Chromatogr., 162 (1979) 81.
5 J. Dokladalova, J.A. Tykal, S.J. Coco, P.E. Durkee, G.R. Querica and J.J. Korst, J. Chromatogr., 231 (1982) 451.
6 M.T. Rosseel and M.G. Bogaert, J. Chromatogr., 279 (1983) 675.
7 C.H. Kleinbloesem, J. van Harten, P. van Brummelen and D.D. Breimer, J. Chromatogr., 308 (1984) 209.
8 S. Higuchi and Y. Shiobara, Biomed. Mass Spectrom., 5 (1978) 220.
9 S. Kondo, A. Kuchiki, K. Yamamoto, A. Akimoto, K. Takahashi, N. Awata and I. Sugimoto, Chem. Pharm. Bull., 28 (1980) 1.
10 E. Zylber-Katz, G. Koren, L. Granit and M. Levy, Biopharm. Drug Dispos., 5 (1984) 109.
11 K. Miyazaky, N. Kohri, T. Arita, H. Shimono, K. Katoh, A. Nomura and H. Yasuda, J. Chromatogr., 310 (1984) 219.
12 H. Suzuki, S. Fujiwara, S. Kondo and I. Sugimoto, J. Chromatogr., 341 (1985) 341.
13 S. Ebel, H. Schütz and A. Hornitschek, Arzneim.-Forsch., 28 (1978) 2188.
14 F.A. Tucker, P.S.B. Minty and G.A. McGregor, J. Chromatogr., 342 (1985) 193.