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Note

High-performance liquid chromatography of nifedipine, its metabolites and photochemical degradation products

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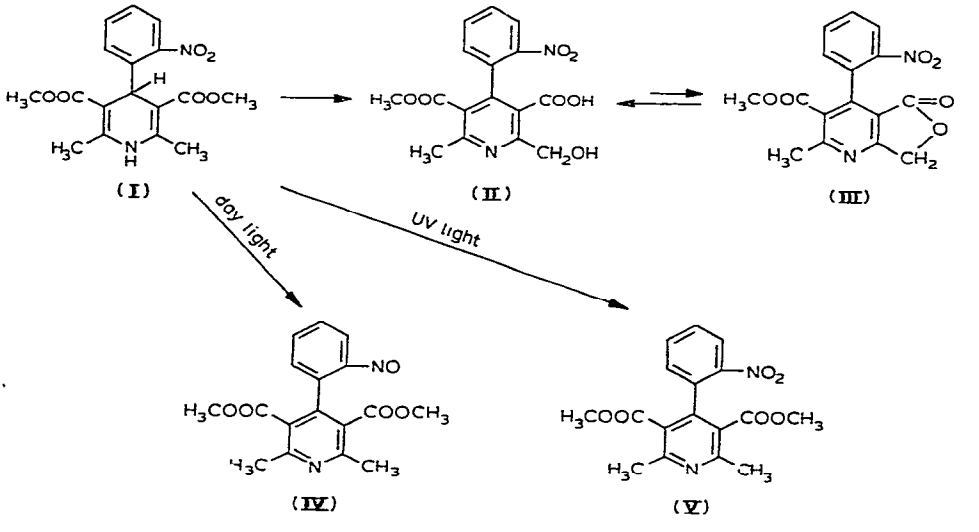
Nifedipine [4-(2′-nitrophenyl)-2,6 dimethyl-3,5-dimethoxycarbonyl-1,4-dihydropyridine] (I) is one of the most useful coronary vasodilators[1–3]. Its metabolism was studied and the metabolites were 4-(2′-nitrophenyl)-2-hydroxymethyl-5-methoxycarbonyl-6-methyl pyridine-3-carboxylic acid (II) and the corresponding lactone (III)[4].

I is a sensitive substance which decomposes in day light to give the 4-(2′-nitrosophenyl)-pyridine homologue (IV), and under UV light to give the oxidation product, 4-(2′-nitrophenyl)-pyridine homologue (V)[5]:



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The quantitative determination of I and its metabolites in body fluids was carried out by tracer studies[6] and fluorimetric methods[7]. However, since the ring structure of I is maintained during biotransformation, the unchanged I, II and III are determined simultaneously.

Recently, gas–liquid chromatography (GLC) and GLC–mass spectrometric methods for determining I in biological samples were reported[5,8,9]; by these techniques I, IV and V can be easily estimated, but polar groups of II and III require a preliminary derivatization step.

In continuing efforts to apply high-performance liquid chromatography (HPLC) in biomedical analysis, an HPLC procedure for the separation and quantification of I, II and III in biological fluids is reported. Furthermore, this procedure has been applied to the simultaneous analysis of I, IV and V in bulk material as well as in pharmaceutical preparations.

EXPERIMENTAL

Materials

I was synthesized in our laboratory according to the literature[1]. II and III were extracted from urine samples and separated by preparative TLC[4].

IV and V were obtained according to the procedure previously described[5]. The structures of II–V were confirmed by mass, infrared and nuclear magnetic resonance spectra.

All the solvents were of HPLC grade (LiChrosolv; Merck, Darmstadt, G.F.R.). Water was distilled in glass and then passed through a 0.45-μm membrane filter (Type FH, Millipore). Other reagents were of analytical grade.

Mobile phase

The mobile phase consisted of 0.01 *M* disodium hydrogen phosphate buffer–methanol (45:55). Before mixing, the buffer was brought to pH 6.1 with 50% phosphoric acid and filtered through a 0.45-μm membrane filter (Type FH, Millipore).

The mobile phase (pH 7.2) was degassed under vacuum for 5 min.

Solutions

Stock solutions. I–V standard stock solutions were prepared with a final concentration of 0.1 mg/ml in methanol. 4-Dimethylaminobenzaldehyde, as internal standard (IS), was dissolved in methanol to give a concentration of 1.5 mg/ml.

Standard solutions. Solutions of I (0.005–0.05 mg/ml) containing 0.015 mg/ml of IS and solutions of II–V (0.001 mg/ml of I.S.) were prepared from the stock solutions. All solutions of I were made in amber glass and used within 24 h.

Sample preparation

Bulk drug substance. To an accurately weighed amount of bulk drug (10 mg) were added 10 ml of the IS solution and the volume made up to 100 ml with methanol.

Capsules. To an accurately weighed amount of capsule contents equivalent to 1 mg of I was added 1 ml of the IS solution and the volume made up to 100 ml with methanol.

Determination of calibration curve for plasma levels

Into individual 15-ml centrifuges tubes were placed accurately pipetted volumes (1.0 ml each) of the standard solutions of I. Then the contents were evaporated to dryness with a stream of nitrogen. Untreated rat plasma (1.0 ml) was added, and the mixture was shaken for 1 min. Ammonium sulfate (0.5 g) was then added to each sample in three divided portions, with shaking and heating after each addition. Ethyl acetate (10 ml) was added, and the mixture was shaken and centrifuged at 1000 *g* for 5 min. The ethylacetate layer was pipetted and filtered through a 0.5-μm Millipore filter into a conical tube and evaporated to dryness under a stream of nitrogen. Methanol (1.0 ml) was added to dissolve the residue, and a 10-μl aliquot was injected into the liquid chromatograph.

Determination of I in plasma

Into 15-ml centrifuge tubes were placed accurately pipetted volumes (1.0 ml) of treated rat plasma. Then 10 μl of the IS solution were added. Each sample was then treated in the same manner as described above. Control blanks with untreated rat plasma also were performed.

Replicate injections of 10 μl were made for each sample.

Recovery

Plasma samples spiked with I in the range 0.005–0.05 mg/ml were subjected to the described procedure and then dissolved in 1 ml of methanol containing 0.015 mg of IS. Peak area ratios were compared to those obtained from the corresponding standard solution of I containing IS.

Chromatographic conditions

The chromatographic system consisted of a Model 6000 A solvent delivery system; a Model U6K universal injector; a Model 440 ultraviolet detector and a Model 730 data module (Waters Assoc., Milford, MA, U.S.A.). Ultraviolet absorption was measured at 254 nm. Samples were chromatographed at room temperature on a 30 cm × 4.0 mm I.D. column packed with μBondapak C_{18} (10 μm) (Waters Assoc.). The flow-rate was 2 ml/min (3200 p.s.i.).

A pre-column of Bondapak C_{18} Corasil was used.

Animal study

Male rats (Wistar Albino, Nossan) weighing 200 g and fasted overnight were used. The animals were treated orally with 50 mg/kg dose of I dissolved in polyethylene glycol 400. Blood samples were collected in 5-ml centrifuge tubes containing 10 mg of potassium oxalate as anticoagulant. The tube contents were centrifuged for 10 min at 1000 *g*, then the separated plasma was pipetted into a glass tube and stored at −4°C until analyzed.

RESULTS AND DISCUSSION

The HPLC analysis of a I–V mixture necessitated (a) the development of a set of HPLC operating parameters that would separate the five components without peak overlapping and (b) the detection and quantification of each compound at low levels.

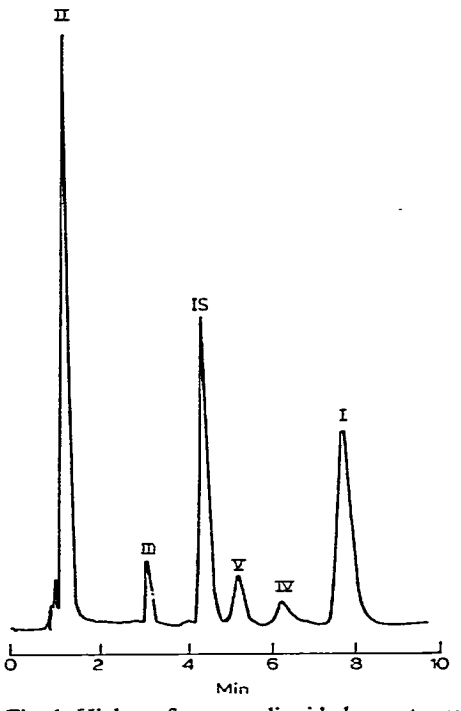

Fig. 1. High-performance liquid chromatogram of nifedipine (I), its metabolites (II, III) and photochemical degradation products (IV, V).

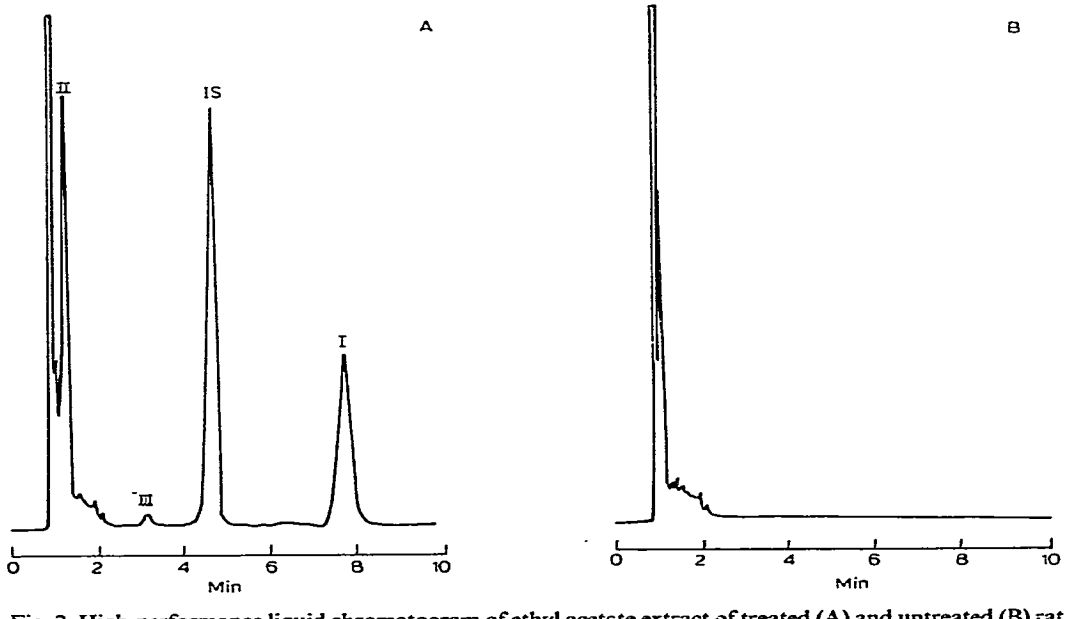

Fig. 2. High-performance liquid chromatogram of ethyl acetate extract of treated (A) and untreated (B) rat plasma.

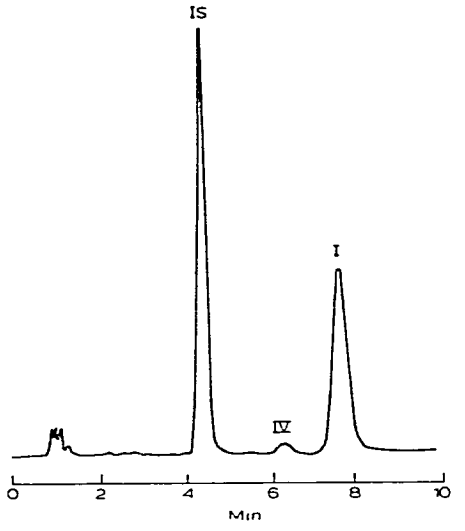

Fig. 3. High-performance liquid chromatogram of drugs.

Reversed-phase HPLC on an octadecyl column was a successful approach to these problems. The compounds were best separated using 0.01 *M* disodium hydrogen phosphate (pH 6.1)–methanol (55:45) as eluent. The IS, I, II, III, IV and V gave capacity factors of 3.58, 6.85, 0.35, 2.29, 5.45 and 4.41, respectively (Fig. 1). This mobile phase allowed the separation of drugs from endogenous plasma substances (Fig. 2A and B) or from the components of the pharmaceutical preparations (Fig. 3) as well as the use of 4-dimethylaminobenzaldehyde as a suitable internal standard.

A flow-rate of 2.0 ml/min (3200 p.s.i. at room temperature) was the most satisfactory since separations could be obtained in less than 10 min.

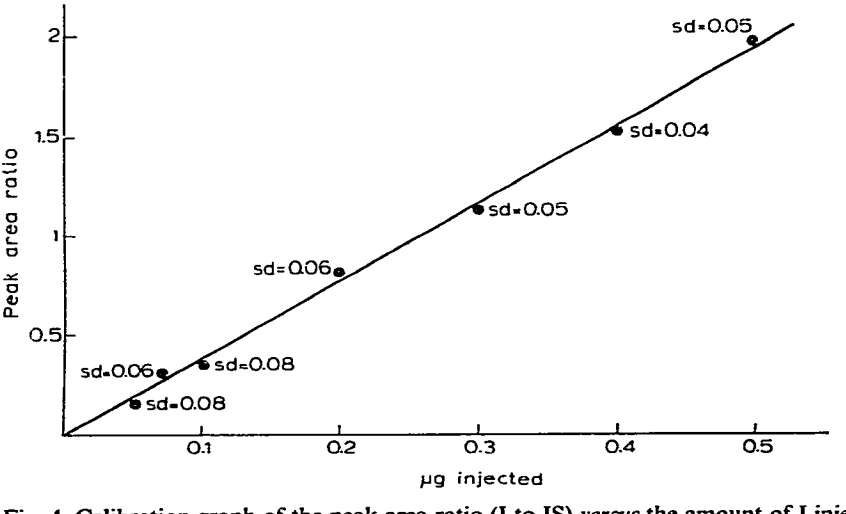

Fig. 4. Calibration graph of the peak area ratio (I to IS) *versus* the amount of I injected. sd = Standard deviation (n = 5).

The recovery for I using the described procedure was 97.5 ± 2.8%. The minimum detectability of all compounds with the described procedure was 10 ng.

Precision over a 30-day period was ±4.1%, using different plasma samples.

A calibration graph of peak area ratio (I to IS) to the amount of injected I in the range 0.05–0.5 μg was prepared from replicate injections (Fig. 4).

In conclusion, the proposed method is suitable for a rapid, sensitive and reproducible quantification of I in biological fluids. Moreover, I as bulk material or as component of pharmaceutical preparations can be rapidly analyzed even in the presence of its photochemical degradation products.

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