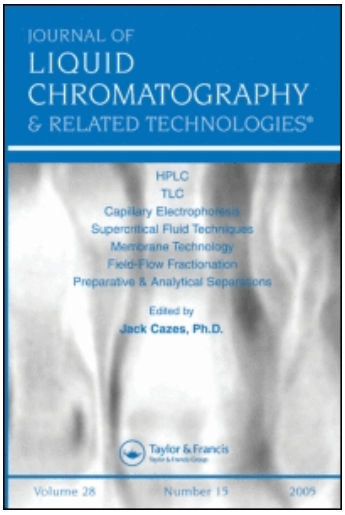

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PREPARATIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE PURIFICATION OF NITRENDIPINE AND ITS DIMETHYL ESTER

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ABSTRACT

Normal-phase preparative HPLC was applied to the purification of nitrendipine and its dimethyl ester from raw materials. For each separation, the chromatographic conditions were optimized by adjusting the sample size to achieve an efficient purification with a yield of 99% in the shortest time. The contaminating impurities were removed in a single step by preparative chromatography. This work demonstrates how gram quantities of analytically pure materials can be conveniently obtained using preparative HPLC.

INTRODUCTION

The calcium antagonists are a heterogeneous group of cardiovascular drugs used to block the entry of calcium ions to the interior of the cell, producing a reduction in peripheral vascular resistance. This class of compounds (dihydropyridines) are 4-aryl-1,4-dihydropyridine 3,5-dicarboxylates. The ester functions in the 3,5-positions may be varied widely without a significant reduction in potency. They proved, however, to be important in respect to vascular selectivity and duration of action, both of which are critical parameters

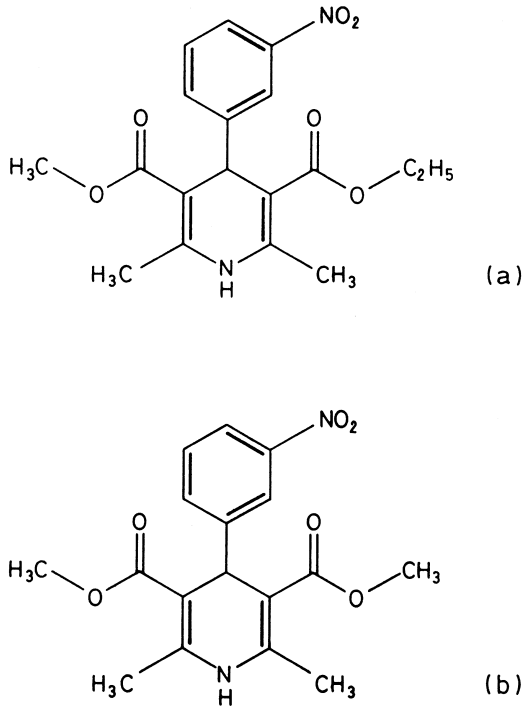

Figure 1. Chemical structures of nitrendipine (a) and its dimethyl ester (b).

for the selection of an antihypertensive agent.[1,2] The calcium antagonist nitrendipine is chemically ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. The chemical synthesis of nitrendipine leads to some pyridine derivatives as well. The most important impurities are: a) ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate, b) dimethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, and c) diethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. The dimethyl ester is an impurity which must be controlled in the industrial process of nitrendipine. It is necessary to have a standard of this compound to check the production of nitrendipine. Chemical structures of nitrendipine and its dimethyl ester are shown in Figure 1.

The present paper describes the optimization of chromatography methods from analytical scale to preparative using silica as stationary phase to isolate gram quantities of analytically pure nitrendipine and its derivative dimethyl ester.

It is necessary to develop both preparative and analytical high-performance liquid chromatographic (HPLC) separations in order to isolate moderate quantities (mg or g) in high purity (≥99.9%) for evaluation of chemical analysis, spectroscopy studies (NMR, MS, X-ray) and for determining physico-chemical properties.[3-5] Analytical high-performance liquid chromatography (HPLC) is a widely applied technique and its obvious extension, preparative HPLC, is now well developed and it may be utilized successfully in the quantitative separation of complex mixtures, purification of compounds with low α values being readily achieved.[6,7]

EXPERIMENTAL

Apparatus

The instrumentation used for analytical chromatography was from Waters (Milford, MA, USA) consisting of a Waters 600E Gradient Module, a Waters 484 tunable absorbance detector and a Waters 745B recorder. The Waters 484 detector is equipped with a 10 mm flow cell path length. For preparative chromatography, the high performance liquid chromatographic apparatus consisted of a Waters Delta Prep 4000 equipped with a Rheodyne sample injection valve (Model 7010) (Rheodyne, Cotati, CA, USA), an automatic sample loader (Waters 170), a Waters 484 tunable absorbance detector, a Waters 745B recorder, and a Waters fraction collector to recover the isolated compounds. The 3 mm path length of the 484 detector's preparative flow cell is ideal when high concentrations of UV absorbing material are present.

Materials

Nitrendipine and its dimethyl ester were supplied by Astur Pharma (Asturias, Spain) and they were obtained by chemical synthesis. Solvents for chromatographic separations (chloroform and hexane) were LiChrosol grade (Merck, Darmstadt, Germany). Mobile phases were filtered through a 0.2 µm filter (Millipore, Bedford, MA, USA) and degassed prior to use.

Columns

For analytical separations a Waters Silica µ-Porasil 125 Å (3.9 x 300 mm, 10 µm) column was used. Preparative separations were conducted on a Waters Silica µ-Porasil 125 Å (19 x 300 mm, 10 µm). Prior to use, the columns were washed with chloroform and hexane.

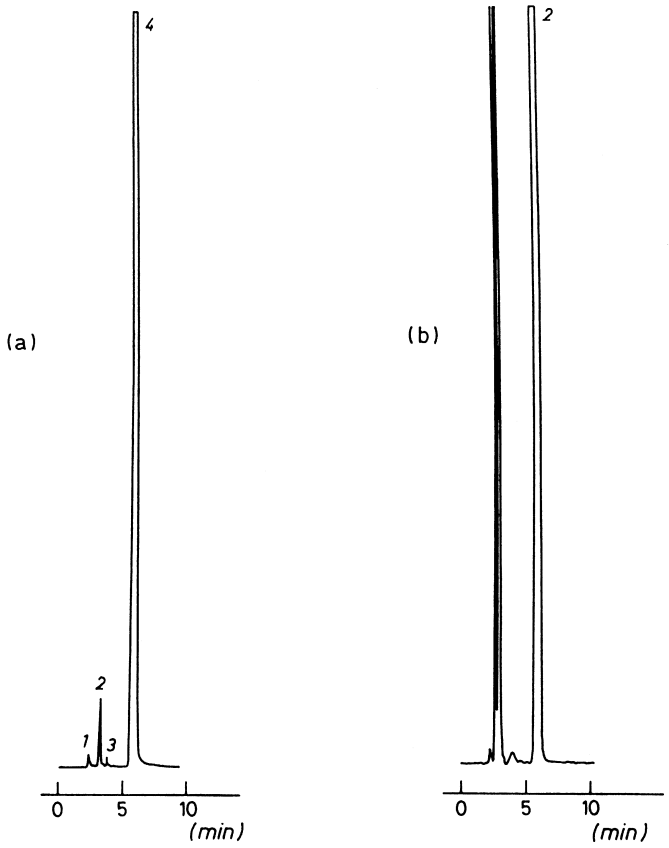

Figure 2. Analytical chromatography on a Silica µ-Porasil column 125 Å (10 µm), 3.9 x 300 mm, at a flow rate of 1 ml/min. a) nitrendipine, in chloroform; and b) dimethyl ester, in chloroform-hexane (75:25). Detection was at 235 nm. Injection: 20 µg. Peak assignement: 1= ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate, 2= dimethyl 2,6-dimethyl-4-(3-nitro-phenyl)-1,4-dihydropyridine-3,5-dicarboxylate, 3= diethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate 4=ethyl methyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (nitrendipine).

RESULTS AND DISCUSSION

Analytical Chromatography

The nitrendipine and its dimethyl ester were chromatographed analytically in order to characterize and separate the impurities and to develop conditions for

use in a wider diameter column of the same packing material. Amounts of 20 µg of the nitrendipine and its dimethyl ester are chromatographed on a 3.9 x 300 mm column of Waters Silica µ-Porasil 125 Å (10 µm). The separation was performed at a flow rate of 1 mL/min and at ambient temperature. The analytical chromatograms are shown in Figure 2. The stationary phase is a valuable column for the resolution of solute and a wide variety of impurities. Nitrendipine was separated using chloroform as mobile phase. The selection of suitable mobile phases is important because in preparative chromatography the solubility of samples in the eluting solvent is crucial. The best separation of the dimethyl ester was using chloroform-hexane (75:25) as mobile phase. Detection at a wavelength of 235 nm was carried out for all separations. Crude nitrendipine appeared to be 98% pure and its dimethyl ester 72% pure. The chromatographic conditions developed in the present study may separate the nitrendipine or its dimethyl ester and their impurities. They were used to determine the operating conditions of the preparative system.

Other mobile phases (acetonitrile-tetrahydrofuran-water) can be used to separate the analytes on a C_{18} column. However, in preparative chromatography an organic mobil phase as chloroform is more suitable when the solvent evaporation is performed. In this work the objective has been to obtain some grams of analytically pure compounds to use these purified materials as standards. This mobile phase should be avoided in an industrial chromatographic process because chloroform has been regarded as a carcinogen.

Preparative Chromatography

Whereas analytical chromatography emphasizes the resolution and identification of each fraction that elutes from the column, preparative chromatography emphasizes the purity and yield of the desired final product. The quantity of sample that can be processed in a given time is referred to as throughput, an important factor in scaling up. If large amounts of purified material are needed the column should be overloaded. Even though peaks are overlapping, a heart-cut will produce a highly purified component with a greater throughput per unit of time than is possible by separation at the loading limit.[8,9]

For preparative chromatography, a solution of nitrendipine (160 mg mL^{-1} chloroform) was filtered through a Nylon 66 membrane filter (0.2 µm). The sample was loaded with a 10 mL syringe. Amounts of 1.6, 3.2, 8, and 16 mg of the nitrendipine are chromatographed on the 3.9 x 300 mm column of Silica µ-Porasil. As sample size is increased, the initially Gaussian peak is distorted into the shape of a right triangle. The critical sample weight for a given preparative separation is of major importance.[10] Injecting a sample of 3.2 mg results in an 100% yield of product in near 100% purity.

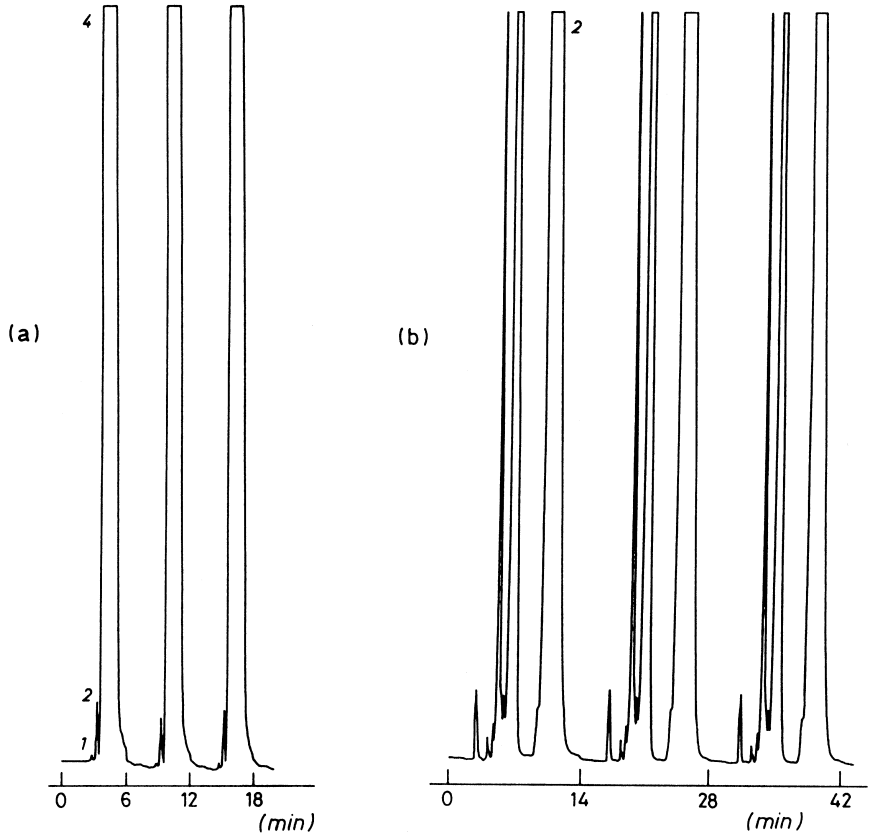

Figure 3. Preparative chromatograms showing several injections on a Silica µ-Porasil column 125 Å (10 µm), 19 x 300 mm, at a flow rate of 25 ml/min. a) nitrendipine in chloroform (injections: 80 mg), b) dimethyl ester in chloroform-hexane (75:25) (injections: 25 mg).

The most obvious way to increase throughput is to increase the column bed volume by making the column wider or taller, or both. Enlarging the column diameter is the most practical means to achieve increased capacity. The separation was next scaled up for use with a 19 mm i.d. column that was otherwise identical with the analytical column. This has permitted injections of 80 mg of nitrendipine per run. The flow rate was increased to 25 mL/min. The optimized mobile phase of the analytical system was transferred without any modification to preparative chromatography. Practically, the same resolution was achieved as in the analytical experiments. The separation is shown in Figure 3.

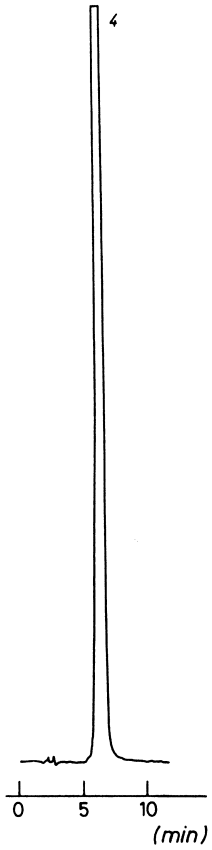

Figure 4. Analytical HPLC of the purified nitrendipine after preparative HPLC. The experimental conditions are shown in Figure 2.

For each experiment, fraction collection began as soon as a slope change was noted in the detector signal. Injections were made with a cycle period of 6 min and 780 mg/h of nitrendipine (100% pure) were finally produced. The solvent consumption (chloroform) was 1.5 L/h. In order to recover the product in the eluent after collection a rotary evaporator at 50°C was used. The purity of the nitrendipine was confirmed by analytical HPLC (Figure 4). The minor impurities that are eluted before the major material were separated in the preparative run.

A similar procedure was applied for the purification of the dimethyl ester. The overload separation was reached injecting a sample of 1 mg. Then, the separation was carried out using the preparative column (Silica µ-Porasil, 19 x

300 mm). Amounts of 25 mg of the dimethyl ester were injected within 14 min and several grams of the dimethyl ester were obtained with a purity of 99.8%. The preparative chromatogram is shown in Figure 3.

A standard of each component was injected as a single-point calibration standard for every analysis. Standard curves were run showing that absorbance was linear for the concentration ranges encountered for the two compounds used in the experiments.

The purpose of this investigation was to develop a small-scale isolation procedure for purification of nitrendipine and its dimethyl ester. The chromatographic conditions developed allow removal of its impurities. These purified materials may be needed as standards.

Due to the different ester moieties in the 3,5-positions of the heterocyclic ring, the nitrendipine molecule becomes chiral. There are two species that differ only in the configuration at carbon atom 4 of the dihydropyridine nucleus. Nitrendipine is a racemic, optically inactive mixture of these two optically active enantiomers. Nitrendipine contains not less than 98.5% and not more than 101.5% of ethyl methyl (4RS)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-pyridine-3,5-dicarboxylate, calculated with reference to the solvent-free substance. The pure isolated nitrendipine in this work could be used as starting material to separate the optical isomers. This separation could offer the provision of sufficient material for pharmacological testing.

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