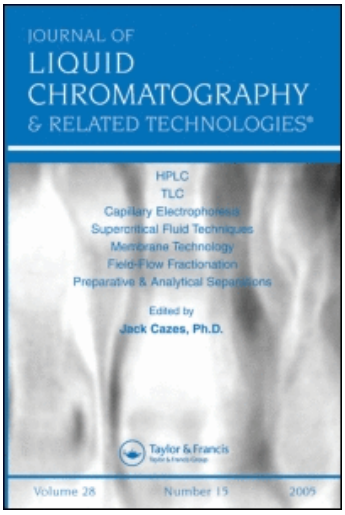

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HIGH PRESSURE LIQUID CHROMATOGRAPHIC DETERMINATION OF NITRENDIPINE IN HUMAN PLASMA AFTER SOLID PHASE EXTRACTION

E. Georgarakis[a]; F. Zougrou[a]
[a] Section of Pharmaceutics and Drug Control, Department of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece

Online publication date: 05 November 1999

To cite this Article Georgarakis, E. and Zougrou, F.(1999) 'HIGH PRESSURE LIQUID CHROMATOGRAPHIC DETERMINATION OF NITRENDIPINE IN HUMAN PLASMA AFTER SOLID PHASE EXTRACTION', Journal of Liquid Chromatography & Related Technologies, 22: 9, 1381 — 1390
To link to this Article: DOI: 10.1081/JLC-100101739
URL: http://dx.doi.org/10.1081/JLC-100101739

J. LIQ. CHROM. & REL. TECHNOL., 22(9), 1381–1390 (1999)

HIGH PRESSURE LIQUID CHROMATOGRAPHIC DETERMINATION OF NITRENDIPINE IN HUMAN PLASMA AFTER SOLID PHASE EXTRACTION

E. Georgarakis, F. Zougrou

Section of Pharmaceutics and Drug Control
Department of Pharmacy
Aristotle University of Thessaloniki
GR-540 06 Thessaloniki, Greece

ABSTRACT

A rapid and sensitive Reversed-Phase High Pressure Liquid Chromatographic (RP-HPLC) method has been developed for the quantitative determination of nitrendipine in human plasma.

A Hypersil C_{18} 150×4.6 mm, 5 μm analytical column was used with a mixture of CH_3CN-H_2O at a volume ratio 50:50 v/v. Detection was performed with a variable wavelength UV-VIS detector at 238 nm. The detection limit was 0.4 ng/mL.

Nimodipine was used as internal standard (IS) at a concentration of 12 ng/mL.

The statistical evaluation of the method was examined performing intra-day and inter-day calibration.

Nitrendipine was extracted from human plasma matrices by Solid Phase Extraction (SPE) technique instead of conventional liquid-liquid extraction methods. Recovery of nitrendipine in spiked samples was 97.75±5.43.

The method was applied to a preliminary pharmacokinetic study in which an oral dose of 20 mg of nitrendipine was given to eight healthy volunteers.

INTRODUCTION

Nitrendipine is a dihydropyridine calcium channel blocker shown to inhibit the movement of calcium through the "slow channel" of cardiac and mascular smooth muscle, thus inducing peripheral vasodilation with consequent reductions in elevated blood pressure.[1]

As evidenced by clinical trials, nitrendipine promptly lowers blood pressure in patients with mild to moderate hypertension, and sustains this effect during long term administration. Combining nitrendipine with other antihypertensive agents such as diuretics or β-blockers often results in successful treatment in patients unresponsive to nitrendipine monotherapy. Headache, oedema, flushing, and palpitations commonly occurring during treatment with nitrendipine are generally mild, usually subsiding with continued therapy.

To support nitrendipine clinical studies (bioequivalence, bioavailability, interaction) a quantitative analytical method with sensitivity, selectivity, and specificity is required to measure low levels of nitrendipine concentration in human plasma.

Chromatographic assays for the determination of nitrendipine in plasma have been limited.[2,3] These methods utilized either Gas Chromatography (GC) with electron-capture detection[3] or large plasma volumes as they use liquid-liquid extraction methods[2] for the isolation of the drug from biological matrices. Nitrendipine has widely been used as an internal standard in the determination of nimodipine.[4-7]

In the present work a solid phase extraction (SPE) procedure is developed for the determination of nitrendipine. The proposed method is a rapid, simple, and reproducible method. It offers an alternative to GC and HPLC procedures available, especially when measurement of nitrendipine is of interest and plasma volume is limited. The proposed method was used to support clinical pharmacokinetic studies.

EXPERIMENTAL

Apparatus

The HPLC system consisted of an SSI 222D HPLC-pump (SSI, State College PA USA), a UV-VIS detector (Avondale PA, USA) at a wavelength of 238 nm and a sensitivity of 0.002 AUFS, a Rheodyne 7010 injection valve (Rheodyne, Cotati California USA) with a 100 μL loop and a Hewlett-Packard (Avondale, PA, USA) HP3396 Series II integrator.

A glass vacuum-filtration apparatus obtained from Alltech (Alltech Associates Inc., Illinois USA) was employed for the filtration of the mobile phase using membrane filters 0.2 μm obtained from Schleicher and Schuell (Dassel, Germany).

A SIGMA centrifuge, model 203 (Osterode, Federal Rep. Germany) was used for the sample pretreatment.

The SPE assay was performed on an Alltech vacuum manifold column processor (Alltech, Illinois USA.).

Materials and Reagents

Nitrendipine and nimodipine were kindly provided by Pharmathen, Pharma Industry (Athens, Greece).

HPLC gradient grade acetonitrile and methanol were obtained from Lab-Scan (Lab-Scan Analytical Sciences, Ltd, Dublin, Ireland). Distilled water was used throughout analysis. Solid phase extraction cartridges were Alltech C_{18} 200 mg (Rigas Labs Thessaloniki, Greece).

The mobile phase was vacuum filtered before use through 0.2 μm membrane filters (Schleicher and Schuell, Dassel, Germany).

Standard Solutions

Stock solutions of nitrendipine and nimodipine (100 μg/mL) were prepared in acetonitrile and stored at 4°C. Working solutions were prepared daily from stock solutions at concentrations: 1,3,5,8,10,20,30, and 40 ng/mL. The solution of internal standard (nimodipine) was added at a concentration of 12 ng/mL.

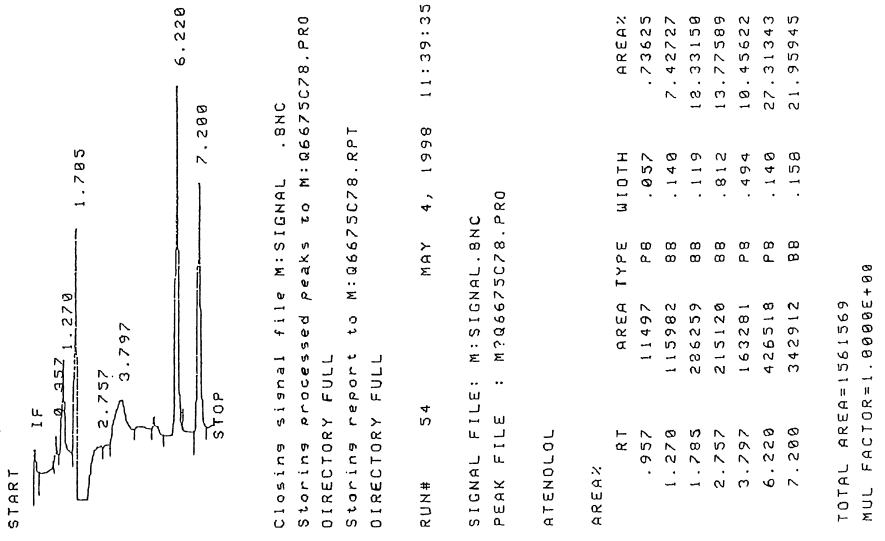

Figure 1. Chromatogram of standard solution of 10 ng/mL Nitrendipine (6.220 min) and 12 ng/mL Nimodipine (7.200 min).

Chromatography

The chromatographic analysis was performed under isocratic conditions and ambient temperature with the detector operating at 238 nm and with a sensitivity setting of 0.002 AUFS. The mobile phase was a mixture of acetonitrile-water (50-50 v/v). The flow rate was 1.3 mL/min leaded to 950 psi inlet pressure. Retention times were 6.220 min for nitrendipine and 7.200 min for nimodipine as shown in chromatogram presented in Figure 1. Peak area ratios of nitrendipine to nimodipine were used to calculate nitrendipine plasma concentrations.

PHARMACOKINETICS OF NITRENDIPINE

In order to determine the pharmacokinetic parameters and bioavailability of nitrendipine, a randomized ascending single-dose two way cross over trial was designed. Eight healthy male volunteers aged 23-43 years body weight 62-102 Kg had each received two treatments of 20 mg of nitrendipine. Blood samples were collected by venipuncture at 0, before administration and at 0.25, 0.5, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, and 8 hours after dosing. Plasma was obtained by centrifugation of blood samples at 3000 rpm and kept frozen (under -25°C) until analyzed.

Table 1

Within-Day Precision and Accuracy for the Analysis of Nitrendipine*

Concentration (ng/mL)	Recovery (%) (x ± SD)	RSD (%)
8	92.57 ± 3.76	4.06
10	96.14 ± 4.00	4.12
20	103.1 ± 5.35	5.19

* (n = 8).

Table 2

Day-to-Day Precision and Accuracy for the Analysis of Nitrendipine Over a Period of 8 Consecutive Days

Concentration (ng/mL)	Recovery (%) (x ± SD)	RSD (%)
3	92.58 ± 4	4.32
5	98.81 ± 5	5.06
10	106.66 ± 1.29	1.21

RESULTS AND DISCUSSION

Performance Characteristics of the Proposed Method

Optimized chromatographic conditions were set and the following analytical characteristics were evaluated:

- Precision and accuracy
- Analysis time
- Calibration data
- Solid phase extraction

Precision and Accuracy

In order to verify the reproducibility, eight replicate injections of each of three standard solutions were made during a day's time and the peak area was

measured in comparison to the peak area of the internal standard. The results were treated statistically to assess the within-day precision and accuracy of the method which is given in Table 1.

The day-to-day precision and accuracy of the method was assessed by the repeated analysis of three standard solutions over eight days. Results are presented in Table 2.

Analysis Time

The analysis time in the proposed method is determined by the retention time of the internal standard, which is 7.200 min.

Calibration Data

Calibration of the method was performed by injection of standards covering the entire working range. Eight concentrations were used in the range between 1-40 ng/mL. Each sample was injected five times.

Linear correlation (r=0.998346) was obtained for nitrendipine using nimodipine as internal standard at a concentration of 12 ng/mL.

Calibration curve for nitrendipine is:

$Y=(0.484377\pm0.033249) + (0.088225\pm0.002272)X$, were X= ng/mL.

Solid Phase Extraction

Aliquots of 200 μL of human plasma were treated with 400 μL of acetonitrile to precipitate proteins. After 2 min vortex mixing, 200 μL of standard solution were added to the sample at concentrations: 1.04, 3.12, 5.20, 10.04, 20.80, 31.20, and 41.60 ng/mL. The sample was subsequently centrifuged at 4000 rpm for 15 min and the supernatant was evaporated at 45°C under nitrogen stream to remove organic solvent. The sample was applied to the cartridges. Just prior to sample application the cartridges were conditioned by passing 3 mL of methanol and then 2×3 mL of deionized water. A washing step with 2×3 mL water followed and then nitrendipine was eluted with 3 mL of methanol. The sample was subsequently evaporated to dryness in a water bath at 45°C, using a stream of nitrogen and the residue was reconstituted with 200 μL of nimodipine. 100 μL of the sample were injected onto the HPLC column.

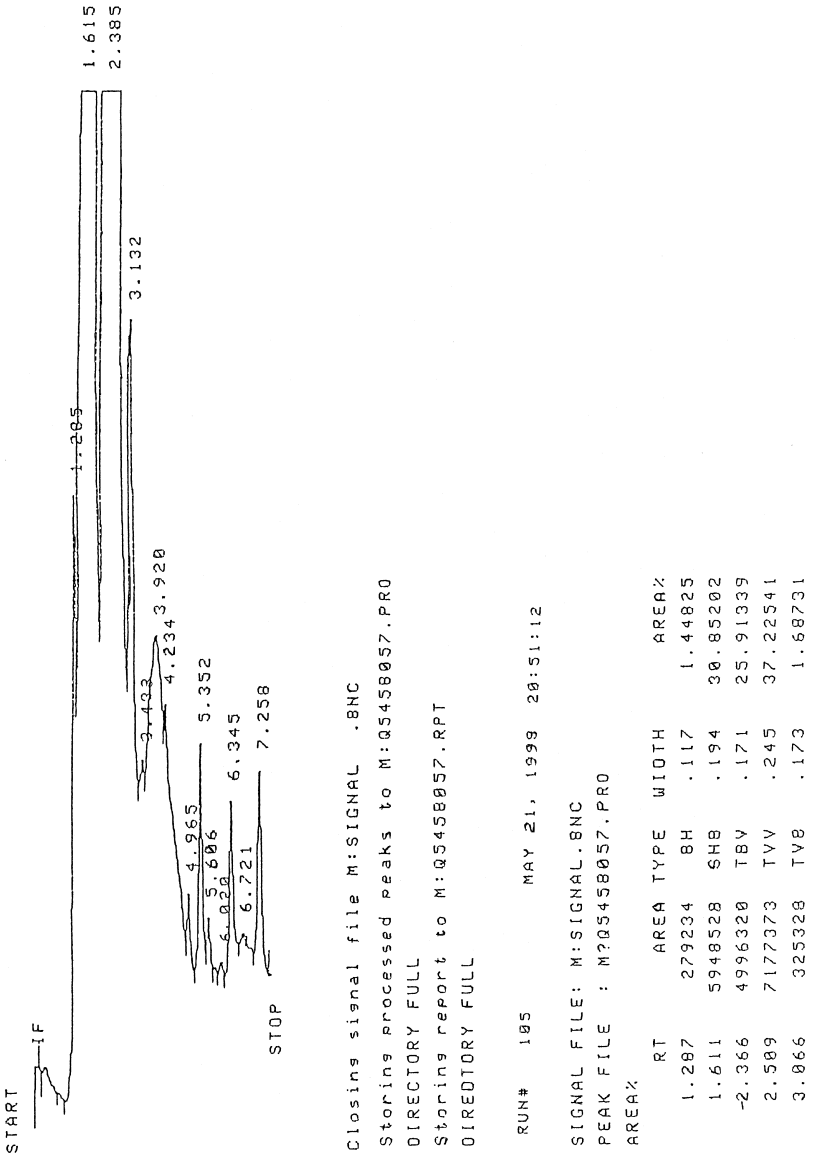

Figure 2. Chromatogram of spiked with Nitrendipine (10.04 ng/mL) human plasma sample after SPE.

Table 3

Precision and Accuracy of Nitrendipine Determination in Spiked Human Plasma Samples*

Concentration (ng/mL)	Added (ng)	Found (ng)	RSD (%)	Recovery (%)
3.12	0.312	0.3	---	96.15
5.20	0.520	0.54 ± 0.01	1.85	103.80
10.04	1.040	0.97 ± 0.04	4.12	93.30

* (n = 5).

At the retention times of nitrendipine and nimodipine no interferences from endogenous compounds were found in the chromatogram of extracted plasma samples as can be seen in Figure 2.

A new calibration curve (r=0.999505) was constructed for the blood plasma:

$Y=(0.436723\pm0.018952) + (0.750092 \pm0.011811)X$, were X=ng/mL.

The plasma samples were spiked with nitrendipine at three different concentration levels. Each sample was injected five times on the HPLC column. The results were treated statistically and are given in Table 3.

PHARMACOKINETICS OF NITRENDIPINE

The proposed method was applied to the measurement of nitrendipine in plasma samples obtained from eight healthy volunteers. These volunteers participated in a bioequivalence study comparing two nitrendipine tablets from two manufacturers [Nelconil (Pharmaten), Bay Press (Bayer)]. The mean nitrendipine plasma levels after the intake of the 20 mg tablets in the eight subjects are shown in Figure 3 while the average pharmacokinetic parameters obtained from the concentration curves are depicted in Table 4.

CONCLUSIONS

A simple, rapid and selective HPLC method for measurement of nitrendipine in plasma was developed.

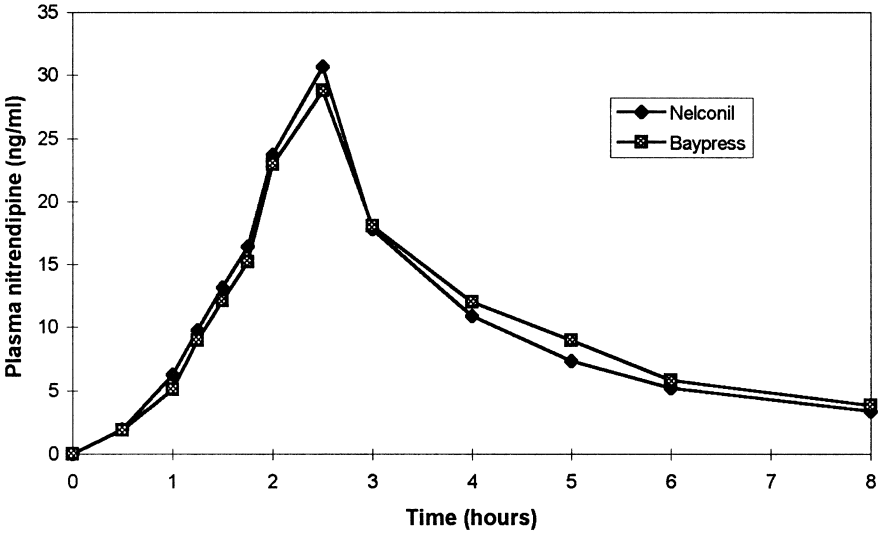

Figure 3. Mean plasma concentration of Nitrendipine after 20 mg oral dose.

Table 4

Pharmacokinetic Parameters of Nitrendipine After 20 mg Dose

Preparations	C_{max} (ng/mL)	T_{max} (h)	Half Life (h)	AUC* (hxng/mL)
Nelconil	31.58 ± 5.42	2.5	1.73 ± 0.47	80.08 ± 14.28
Bay Press	29.83 ± 5.20	2.5	1.97 ± 0.51	82.07 ± 11.60

* AUC = area under the concentration-time curve.

The analysis is completed within approximately 8 minutes which is a reasonably short time for routine analysis. No interferences from endogenous compounds were found in chromatogram from extracted spiked human plasma as can be seen in Figure 2.

The sensitivity, precision, and accuracy of the method are satisfactory. The RSD′s and relative recoveries ranged from 1.21 to 5.06 % and 92.58 to 106.66 % in within day assay and from 4.06 to 5.19 % RSD and 92.57 to 103.1 recovery in day-to-day assay.

The proposed method was applied in human plasma. SPE was used as a clean-up step of the plasma matrices. The extraction method proved to be accurate, sensitive, precise, faster, and with higher recovery than the previously reported methods using liquid-liquid extraction technique, with the advantage of the minimal use of reagents. Recovery of nitrendipine from spiked plasma samples ranged from 93.3 to 103.8 % with RSD′s ranging from 1.85 to 4.12 ng/mL.

The proposed method was applied as a routine method in bioavailability and bioequivalence studies of this drug.

REFERENCES

1. L. K. Goa, E. M. Sorkin, Drugs, **33**, 123-155 (1987).

2. R. A. Janis, G. J. Krol, A. J. Noe, J. Clin. Pharmacol., **23**, 266-273 (1983).

3. J. Kann, G. J. Krol, K. D. Raemsch, J. Cardiov. Pharmacol., **6**, S968-S973 (1984).

4. P. Jakobsen, E. O. Mikkelsen, J. Laursen, F. Jensen, J.Chromatogr., **374**, 385-387 (1986).

5. M. T. Rosseel, M. G. Boguert, L. Hugghens, J.Chromatogr., **533**, 224-228 (1990).

6. Y. B. Liang, X. Y. Ma, G. J. Wu, L. F. Wang, D. K. Liu, Acta Pharmacol. Sinica, **13(2)**, 163-166 (1992).

7. M. Qian, J. M. Gallo, J. Chromatogr., **578**, 316-320, (1992).

Received September 15, 1998
Accepted October 18, 1998
Manuscript 4924

Request Permission or Order Reprints Instantly!