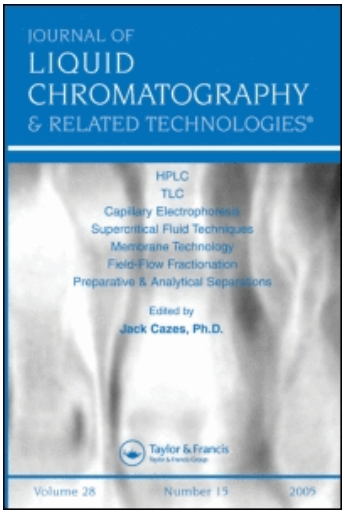

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STABILITY STUDY OF NILVADIPINE IN BULK DRUG AND PHARMACEUTICAL CAPSULES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple, stability-indicating, liquid chromatographic method has been developed for the assay of nilvadipine in the presence of its alkaline-induced degradation products. A μBondapak-C_{18} column was used with a mobile phase consisting of acetonitrile/0.01 M sodium acetate (55:45, v/v) adjusted to pH 3.5 with acetic acid at a flow rate of 1.8 mL min^{-1}. With procaine hydrochloride as an internal standard, quantitation was achieved with UV detection at 254 nm based on the peak areas ratios. Determination of nilvadipine was possible over the concentration range 0.096-0.64 μgmL^{-1} with limit of detection of 0.032 μg/mL (8.3×10^{-8} M). Between-day and within-day relative standard deviations were lower than 2%. The proposed method was successfully applied to the determination of nilvadipine in bulk and capsule form, with a high percentage of recovery. Moreover, the method was utilized to investi-

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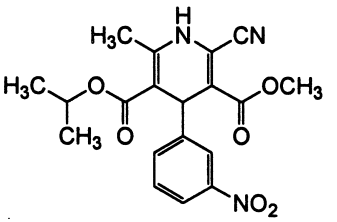
gate the kinetics of the degradation process at different temperatures. The apparent first-order rate constant, half-life and activation energy were calculated. The proposed method was extended to the in vitro detection of nilvadipine in human urine samples.

INTRODUCTION



Nilvadipine (NV), 5-isopropyl-3-methyl-2-cyano-6-methyl-4-(3-nitrophenyl)-1,4-dihydro-3,5-pyridinedicarboxylate, is a calcium antagonist of the dihydropyridine class. It selectively blocks calcium channels in vascular smooth muscles.(1) It is used mainly as an antihypertensive and antianginal drug.(2) Nilvadipine is not yet official in any pharmacopoeia. Few methods have been reported for NV determination, these include GC,(3-6) HPLC,(6-8) and capillary electrophoresis.(9) These previous studies focused on the determination of NV in biological fluids. However, till present, no method has been reported for the assay of NV in commercial preparations or in the presence of its degradation products.

Our goal was to develop a stability-indicating procedure for the determination of NV in the presence of its degradation products and to determine its strength in the bulk substance, marketed capsules in addition to detection in spiked human urine, using a simple reliable liquid chromatographic method. Furthermore, the developed method was used to investigate the kinetics of the drug degradation at different temperatures.

EXPERIMENTAL

Materials and Reagents

Nilvadipine (Batch No. AN 11424) was obtained from Klinge Pharma, Fujisawa Group, Japan. Capsules containing the drug (Escor capsules labeled to contain 8 mg and Escor Forte™ capsules labeled to contain 16 mg of nilvadipine/capsule) were obtained from commercial sources. Urine was obtained from healthy volunteers. All other reagents were of HPLC grade, and were used as received.

Apparatus

Typical Chromatographic Conditions

Column: μBondapak™ C_{18} μm (4.6 mm id × 250 mm).

Eluent: 45% (0.01 M sodium acetate adjusted to pH 3.5) 55% acetonitrile.

Detector: Attn = 8, at 245 nm for potency and 16 at 254 nm in the presence of degradation products.

Flow rate: 1.8 mL min^{-1}

Temperature: Ambient

Injection volume: 20 μL.

Standard Solutions

Stock solution of nilvadipine was prepared to contain 0.16 mg mL^{-1} in methanol: water (8:2). The internal standard procaine HCl solution (IS) was prepared by dissolving 40.0 mg of procaine hydrochloride in 100 mL methanol.

Calibration Curve

Transfer aliquots of the NV stock solution into separate 10 mL volumetric flasks to obtain concentrations of NV ranging between 0.096-0.64 μg mL^{-1}. To each flask add 2.0 mL of IS solution and complete to the mark with the mobile phase. Make triplicate 20 μL injections for each solution. Plot the peak-area ratio of each concentration to the IS against the corresponding standard concentration to obtain the calibration graph.

Analysis of Capsules

Empty the contents of 10 capsules and mix well. Weigh a quantity of the powder equivalent to 16.0 mg of NV and transfer into 100-mL volumetric flask. Add about 40 mL of methanol/water (8:2) and shake for 30 min. Complete to the mark with the same solvent. Centrifuge for 5 min. Transfer aliquots of the supernatant into 10-mL volumetric flask. Complete as under “Calibration Curve”.

Stability Study

The effect of extreme pH conditions on the stability of NV was investigated by using 1 M HCl and 1 M NaOH at different temperatures (20-60°C). Samples were taken for analysis at different time intervals.

Detection of Nilvadipine in Spiked Human Urine

To 1 mL of the spiked urine in a centrifugation tube, add 0.05 mL ethanol, 1 mL of 0.1 M borate buffer (pH 9.0), and 4 mL of n-hexane-ethyl acetate. Shake for 5 min and centrifuge for 2 min. Evaporate 3 mL of the organic phase. Dissolve the residue in 5 mL of mobile phase. Inject 20 µL of this solution under the above chromatographic conditions.

RESULTS AND DISCUSSION

Figure 1 shows the chromatograms obtained for the standard solution of (NV), IS, in the presence of NV degradation products, obtained under the described chromatographic conditions. This chromatogram revealed that NV was well separated from the other compounds. The mobile phase was chosen after several trials with various proportions of acetonitrile-sodium acetate and / at different pH values with different internal standards. The chromatographic system described above, allows complete base line separation with good resolution factor between the adjacent peaks. The proposed method was assessed for specificity, linearity, precision, accuracy, stability, and recovery.

Specificity

Attempts were made to degrade the NV samples to assess the proposed HPLC method. Solutions of 2.0 mg mL^{-1} NV were prepared in methanol. Separate 5-mL aliquots of this solution were transferred into 25-mL volumetric flasks; the volume was completed with either 0.5 or 1 M NaOH. 1 mL aliquots were transferred to separate conical flasks and placed in a thermostated water bath at different temperatures (20, 30, 40, 50, 60°C) for different time intervals (10-150 min). At the specified time interval, the content of each flask was transferred into a 10-mL volumetric flask, then neutralized to pH 7 using predetermined volumes of 1 M HCl. To each volumetric flask, 2.0 mL of IS solution was added, and the volume was completed to the mark using mobile phase. Triplicate 20 µL injections were made for each sample.

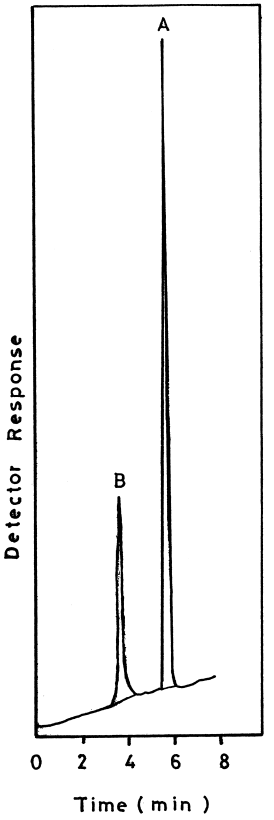
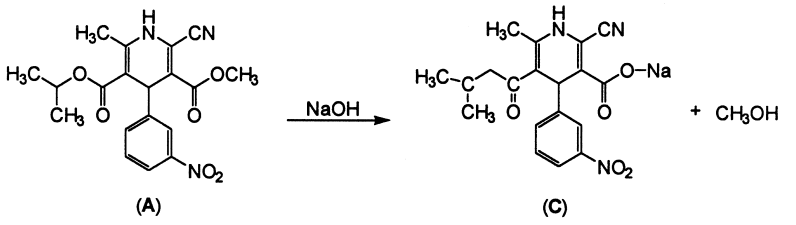
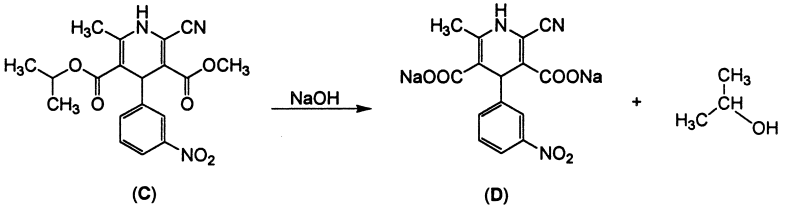


Figure 1. Typical chromatogram of nilvadipine, its degradation products and the internal standard under the described conditions. B: Procaine hydrochloride internal standard (3.63 min). A: Nilvadipine (5.69 min).

In an alkaline medium, nilvadipine gave two degradation products with RT of 1.9 and 2.9 min (Figure 1). It is postulated that the degradation process involved the ester linkages. The reaction is proposed to proceed on two steps. The first involves the methyl ester, resulting in compound C and methanol.



Compound C undergoes further hydrolysis to give compound D and isopropanol.



(C) (D)

Neither methanol nor isopropanol are detected at 254 nm. Therefore, we get two degradation products only, compounds C and D, respectively in Fig. 1. The degradation products – being sodium salts, and consequently more polar than both NV and IS, are eluted first as evident in Fig. 1.

No significant degradation peaks were observed when using either 0.5 or 1 M HCl.

Linearity

The peak area ratio of NV to IS varied linearly with the concentration range used (0.096-0.64 μg mL^{-1}). Linear regression analysis of the data gave the following equation:

$$P = -0.035 + 2.110\ C \qquad (r = 0.9992)$$

where C is the concentration of NV in μg mL^{-1} and P is the peak area ratio. The detection limit (S/N = 2) is 0.02 μg/mL ($\simeq 5 \times 10^{-8}$ M).

Precision and Accuracy

Between-day and within-day precisions were determined for three different concentrations at low, medium, and high levels of the standard curve. The calculated relative standard deviation (RSD) values were within the accepted limits (Table 1). The method proved to be accurate; average accuracy at the three concentrations ranged from 100.45-100.63% of the real concentrations (Table 1).

Degradation Kinetics Study

Figure 2 shows typical chromatograms of (NV) after being subjected to alkaline degradation. These chromatograms revealed that, the peak ratio of (NV)

Table 1. Accuracy and Precision for the HPLC Analysis of Nilvadipine

Added Concentration $\mu g\ mL^{-1}$	Found Concentration (Mean±SD) $\mu g\ mL^{-1}$	Recovery (%)	Within-Day Precision (RSD%)	Between-Day Precision (RSD%)
0.128	0.129±0.0019	100.45	0.74	1.47
0.384	0.387±0.0026	100.63	0.25	0.68
0.512	0.514±0.0022	100.58	0.36	0.43

to the (IS) had decreased by time. The degradation was found to be temperature-dependent (Figure 3). At the selected temperatures (20-60°C), the degradation followed pseudo-first order kinetics (Figure 3). The apparent first-order degradation rate constant and the half-life at each temperature were calculated (Table 2). Plotting log K_{obs} values vs 1/T, the Arrhenius plot was obtained (Fig. 4), The activation energy was calculated and found to be 11.61 Kcal $mole^{-1}$. This value is in accordance with the reported values of activation energy required for the hydrolysis of esters.(15)

Stability

The stability of the methanolic sample solutions at room temperature (20°C) for 24 hours after preparation, was verified by reassaying them (after dilution with the mobile phase). There was no indication of any decomposition of NV in the samples.

Recovery

The recovery of the method was checked by adding known amounts of degraded NV to known amounts of standard NV. The calculated recoveries (Table 3) were satisfactory indicating that no interference had been observed from the degradation products.

Application of the Proposed Method to the Analysis of Nilvadipine in Its Commercial Capsules

The proposed method was applied to the determination of the drug in capsules. The percentage recoveries are abridged in Table 4. The results obtained

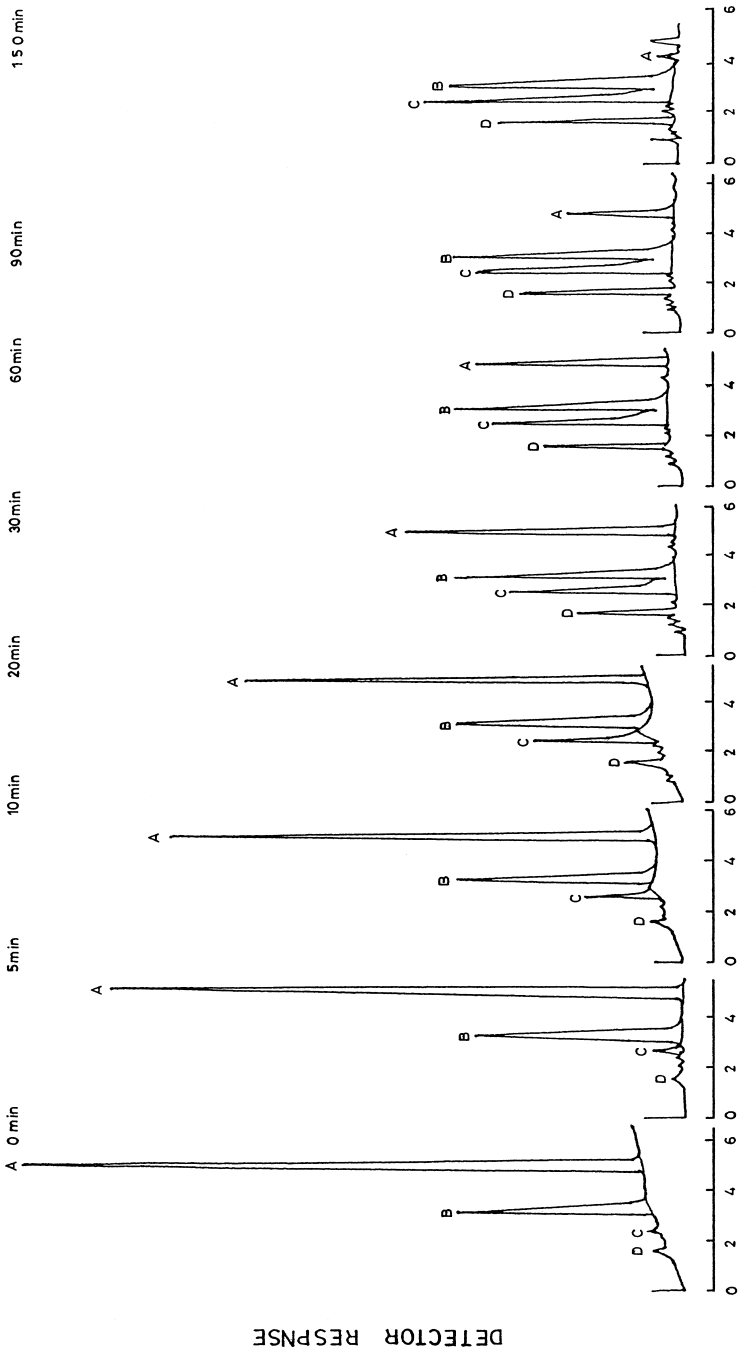


Figure 2. Chromatograms of the alkaline degradation of nilvadipine, using 0.5 M sodium hydroxide. C and D: degradation products of nilvadipine (1.925 and 2.93 min, respectively).

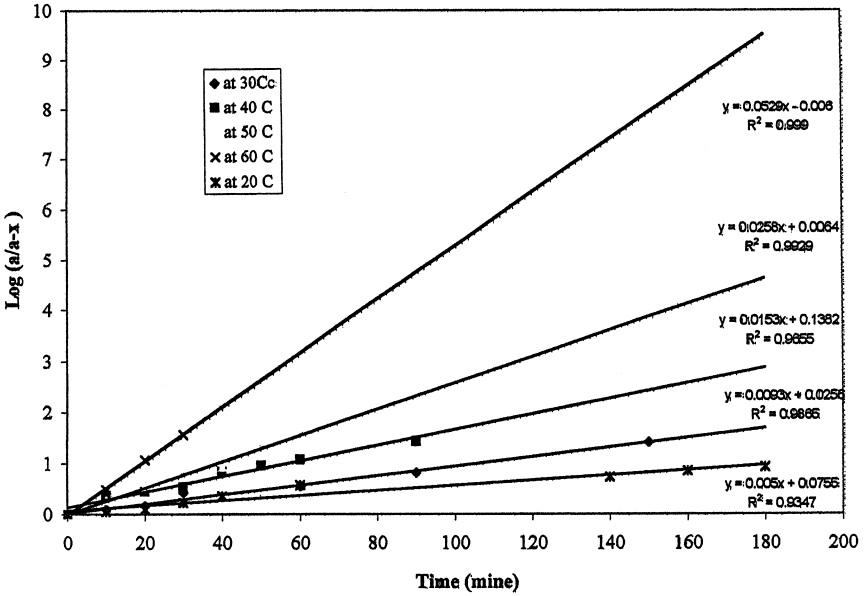


Figure 3. Semilogarithmic plot of nilvadipine (1 μg mL^{-1}) versus different heating times with 0.5 M sodium hydroxide.

were compared with those given upon applying the HPLC method recommended by the manufacturer (Merck, Darmstadt, Germany), and were found to be in good agreement as revealed by statistical analysis of the data. The t-test and F-test reveal no significant difference between the two methods regarding accuracy and precision, respectively(16).

The method could also be successfully applied to the detection of nilvadipine in urine (Figure 5) after extraction, adopting the method of Tokama *et*

Table 2. Degradation Rate Constant (K_{obs}) and Half-Life ($t_{½}$) for Nilvadipine Using 0.5 M NaOH

Temperature (°C)	K_{obs} (min^{-1})	$t_{½}$ (min)
20	0.0058	120.31
30	0.0214	32.38
40	0.0352	19.69
50	0.059	11.75
60	0.1218	5.69

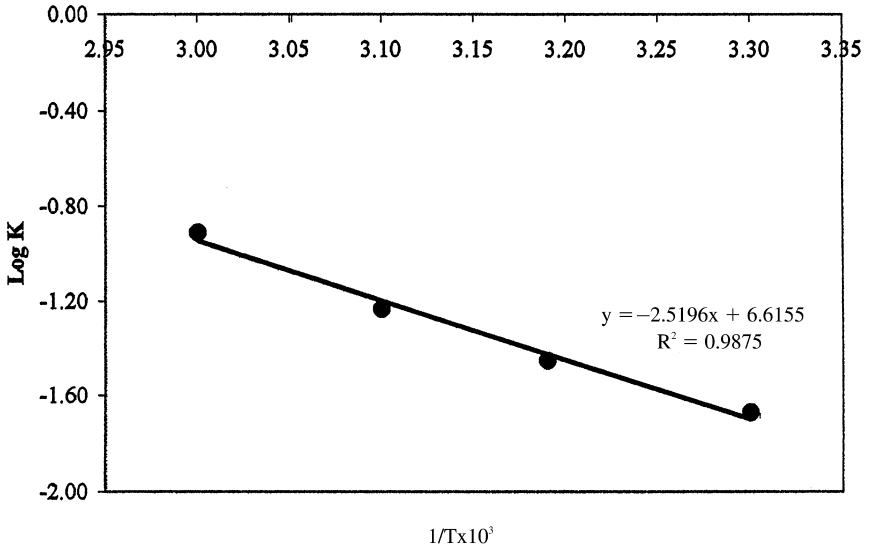


Figure 4. Arrhenius plot for the degradation of nilvadipine in 0.5 M sodium hydroxide using peak area measurements.

Table 3. Determination of Nilvadipine in Presence of Its Alkaline Degradation Products by the Proposed HPLC Method

Added Concentration μg mL^{-1}		Found Nilvadipine μg mL^{-1}	% Recovery
Nilvadipine	Degraded Nilvadipine*		
0.256	0.08	0.252	98.44
0.256	0.20	0.257	100.39
0.256	0.24	0.253	98.83
0.256	0.32	0.255	99.61
Mean			99.32
± SD			0.87

*Alkaline degradation using 0.5 M NaOH.

Table 4. Application of the Proposed Method to the Determination of Nilvadipine in Its Commercial Capsules

	Proposed Method			
Preparation	Amount Taken ($\mu g\ mL^{-1}$)	Amount Found ($\mu g\ mL^{-1}$)	% Recovery	Reference Method † % Recovery
Escor capsules*	0.128	0.128	100.00	
(8 mg nilvadipine/	0.256	0.258	100.78	
capsule)	0.512	0.520	101.56	
	0.640	0.643	100.47	
Mean			100.70	100.00
± S.D.			0.66	0.35
t			1.87 (2.45)	
F			3.56 (6.39)	
Escor Forte	0.128	0.130	101.56	
capsules* (16 mg	0.256	0.258	100.78	
nilvadipine/ capsule)	0.384	0.390	101.56	
	0.64	0.644	100.63	
Mean			101.13	100.63
± S.D.			0.50	0.43
t			1.64 (2.45)	
F			1.35 (6.39)	

The figures in parenthesis are the tabulated values of t and F at p = 0.05.
*Product of E. Merck, Darmstadt, Germany.
†HPLC method recommended by the manufacturer (Merck, Darmstadt, Germany).

al.(7) It is worth mentioning, here, that the internal standard (procaine HCl) did not appear. A peak of unknown source (B), far from that of nilvadipine appeared.

CONCLUSION

A rapid, precise, and selective HPLC method using a single isocratic system has been developed for the determination of bulk nilvadipine. As no interference of the degradation products was observed, the method is suitable as a stability-indicating assay for the measurement of nilvadipine in pharmaceutical preparations. A simple sample preparation enables the use of this method for routine quality control of nilvadipine in capsules, with good accuracy.

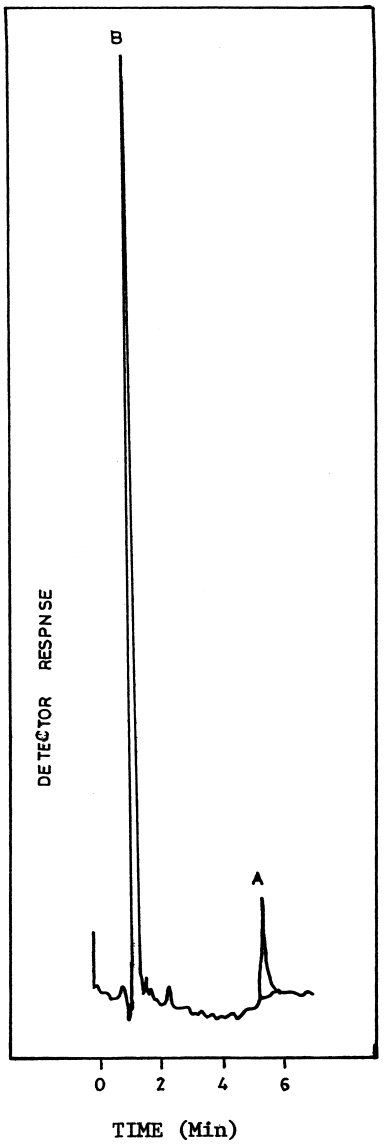


Figure 5. Application of the proposed method to the detection of nilvadipine in urine. A: nilvadipine. B: peak of unknown source.

The proposed method could be successfully applied to the detection of nilvadipine in spiked human urine with a minimum detection limit of 0.032 $\mu g\ mL^{-1}$ (8.3×10^{-8} M).

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