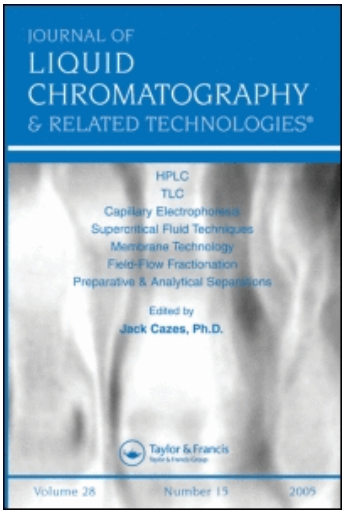

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Microemulsion Liquid Chromatographic Determination of Nicardipine Hydrochloride in Pharmaceutical Preparations and Biological Fluids. Application to Stability Studies

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Abstract: A simple, stability indicating, reversed phase high performance liquid chromatographic method has been developed for the determination of nicardipine hydrochloride (NC) in the presence of its degradation products. Reversed phase chromatography was conducted using a Hibar-C_{18} (150 × 4.6 mm i.d.) stainless steel column at ambient temperature with UV-detection at 238 nm. Microemulsion mobile phase consisting of 0.175 M sodium dodecyl sulphate, 10% n-propanol, 0.3% triethylamine in 0.02 M phosphoric acid of pH 6.5, has been used for the separation of nicardipine hydrochloride and its two degradation products at a flow rate of 1 mL min^{-1}. The calibration curve was rectilinear over the concentration range 1-40 $\mu g\ mL^{-1}$ with a detection limit of 0.024 $\mu g\ mL^{-1}$ (4.65×10^{-8} mol/L) and quantification limit of 0.08 $\mu g\ mL^{-1}$ (1.55×10^{-7} mol/L). The proposed method was successfully applied for the analysis of nicardipine in pure form and commercial capsules, with the mean % recoveries of 100.12 ± 0.28 and 100.87 ± 0.41, respectively. The results obtained were favorably compared to those obtained by a reference method. The method was extended to the *in-vitro* determination of NC in spiked human plasma samples with the mean % recovery of 100.33 ± 3.06 (n = 3). Moreover, the method was utilized to investigate the kinetics of both alkaline induced degradation

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and photo degradation of the drug. The apparent first-order rate constant, half-life time, and activation energy of the degradation product were calculated.

Keywords: Nicardipine hydrochloride, Microemulsion liquid chromatography, Pharmaceutical preparations, Biological fluids, Stability studies

INTRODUCTION

Nicardipine hydrochloride (Figure 1) is 2-[Benzyl(methyl)amino]ethylmethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate hydrochloride.[1] It is a calcium antagonist of the dihydropyridine class. It is currently used for the management of angina pectoris and also used in the treatment of hypertension.[2] Few methods have been reported for determination of nicardipine hydrochloride (NC), these include spectrophotometry,[3,4] voltammetry,[5,6] high performance liquid chromatography,[7–9] liquid chromatography–mass spectroscopy,[10] and capillary electrophoresis.[11] Most of the previous studies focused on the determination of NC in biological fluids.

Microemulsions are clear, thermodynamically stable isotropic mixtures containing oil, water, surfactant, and, also most often, a medium chain alcohol acting as a co-surfactant. They can be considered as two-phase solvents consisting of a micellar phase surrounded by either an aqueous or an organic phase. The micellar phase may contain either an organic solvent or an aqueous phase (reversed micelles). Thus, the microemulsions may either be oil in water (o/w) or water in oil (w/o) mixtures, where the o/w microemulsions are the preferred ones for HPLC.[12] The partitioning and the interfacial adsorption of the analytes in the microheterogenous systems are responsible for the separation obtained. In previous reports, the potential of application of microemulsions as mobile phases in LC analysis was prove.[13–17]

The aim of the present work was to develop, validate, and apply an efficient novel liquid chromatographic method using microemulsion as a mobile phase for rapid and simultaneous determination of NC and its alkaline and photo degradation products. Furthermore, the developed

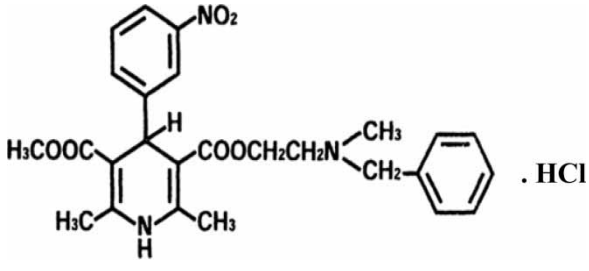


Figure 1. Structural formula of nicardipine hydrochloride.

method was used to investigate the kinetics of the drug degradation in different media at different temperatures.

EXPERIMENTAL

Materials and Reagents

The pure sample of nicardipine hydrochloride was purchased from Sigma (St. Louis, Mo, USA). Capsules containing the drug (Pelcard capsules Batch # 35801, labeled to contain 50 mg of nicardipine HCl/capsule, product of Global Napi, Cairo, Egypt) were obtained from commercial sources in the local market. Sodium dodecyl sulphate (SDS), 99% purity, was obtained from Park Scientific Limited, Northampton, UK. 1-Propanol, methanol, and diisopropyl ether (all of HPLC grade), as well as triethylamine (TEA), were obtained from Riedel-deHäen (Seelze, Germany). 1-Butanol and tetrahydrofuran (HPLC grade) were obtained from Merck (Darmstadt, Germany). 1-Octanol (HPLC grade) was obtained from Aldrich (Gillingham, UK). 1-Butyl acetate was obtained from Fluka (Buchs, Switzerland). Orthophosphoric acid (85 % W/V) was obtained from Prolabo (Paris, France).

Apparatus

Separation was performed with a Perkin Elmer tm Series 200 Chromatograph equipped with a Rheodyne injector valve with a 20 μL loop and a UV/VIS detector. A total Chrom workstation was applied for data collecting and processing (MA, USA). A Shimadzu UV 1601 PC Spectrophotometer equipped with a pair of 1 cm matched cells, recording range: 0–2; wavelength: 238 nm; factor: 1; number of cells: 1; cycle time: 0.1 min was used.

Columns and Mobile Phases

Separation was achieved on a Hibar® C_{18}, prepacked column RT (150 mm × 4.6 mm ID, Lichrosorb® RP-18 (5 μm) combined with a guard column (Merck, Darmstadt, Germany).

The columns were operated at ambient temperature. The analytical system was washed daily with 60 mL of 1 : 1 mixture of water and methanol to eliminate the mobile phase; this did not cause any change in the column performance. The components of the microemulsion were 0.175 M SDS, 10% 1-propanol, 1% 1-octanol, and 0.3% TEA in 0.02 M phosphoric acid.

All the microemulsion components were mixed together and the pH was adjusted at 6.5, or above, using TEA. Other pHs were adjusted using either

orthophosphoric acid or TEA. The mixture was then shaken in an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45 μm membrane filter (Millipore, Ireland). The microemulsion was stable for at least two months when kept in the refrigerator. The column hold up value was the first deviation of the base line obtained.

Standard Solutions

A stock solution containing 1.0 mg mL^{-1} of NC was prepared in methanol and further diluted with 0.5 M NaOH to obtain the working concentration range (1–40 μg mL^{-1}) for the spectrophotometric measurements, and diluted with the mobile phase for the HPLC measurements. This solution was found to be stable for at least two weeks without alteration when kept in the refrigerator.

Preparation of Alkaline Degradation Products

For spectrophotometric measurements, aliquot volumes of methanolic NC were transferred into a series of 10 mL volumetric flasks and diluted with 0.5 M NaOH to the mark. These solutions were heated in a boiling water bath for one hour before measuring the absorbance at 238 nm.

For HPLC measurements, the above solution was neutralized with 0.5 M HCl and completed to the volume with the mobile phase.

Calibration Curve

Aliquots of the standard solution covering the working concentration range of NC were transferred into a series of 10 mL volumetric flasks and diluted with the mobile phase to the mark. Twenty microliter aliquots were injected (in triplicate) and eluted with the mobile phase under the reported chromatographic conditions. The calibration curve was constructed by plotting the peak area ratio against the final concentration of the drug (μg mL^{-1}). Alternatively, the corresponding regression equation was derived.

Analysis of Capsules

The contents of ten capsules were emptied, as completely as possible, and mixed well. An accurately weighed quantity of the powder, equivalent to 10 mg of NC HCl, was transferred into a small conical flask, extracted with 50 mL of methanol, and sonicated for 30 min. The extract was filtered into a 100 mL volumetric flask and completed to volume with methanol. All samples were filtered through 0.45 μm sample filters (RC 25, Sartorius AG, Goettingen, Germany) prior to injection into the HPLC system. The

procedure was followed as described under "Calibration Curve". The nominal contents of the capsules were calculated using either the calibration graph or the corresponding regression equation.

Stability Study

The effect of extreme pH conditions on the stability of NC was investigated by using 1 M HCl and 0.5 M NaOH at different temperature settings (60–100°C) in artificial and diffused day light. Samples were taken for analysis at increasing time intervals.

Determination of Nicardipine in Spiked Human Plasma

The spiked plasma (1 mL) was added into a 10 mL volumetric flask and diluted to volume with the mobile phase, then vortex mixed. This mixture was filtered through a Millipore filter. Twenty microliter of this solution was injected under the above chromatographic conditions.

RESULTS AND DISCUSSION

A microemulsion mobile phase has been utilized in this study for the separation of NC and its two degradation products. Alkaline degradation of NC was prepared according to previous reports.[18,19] Figure 2 shows the chromatogram obtained for a mixture of standard solution of NC and its two degradation products (A and B), obtained under the described chromatographic conditions. The chromatogram revealed that NC was well separated from its degradation product. The mobile phase was chosen after several trials with various proportions of sodium dodecyl sulphate and propanol at different pH values. The chromatographic system described above, allows complete base line separation with good resolution factor (1.90) between each two adjacent peaks. The proposed method was assessed for specificity, linearity, precision, accuracy, stability, and recovery.

The different experimental parameters affecting the separation selectivity of the microemulsion liquid chromatographic system have been investigated and optimized. Hence, the method was applied to the determination of NC in its capsules and spiked human plasma samples.

Method Development

A microemulsion, consisting of 0.175 M SDS, 10% 1-propanol, 1% 1-octanol, and 0.3 %TEA in 0.02 M phosphoric acid of pH 6.5 achieved an optimum separation of the drug from its degradation products, resolution

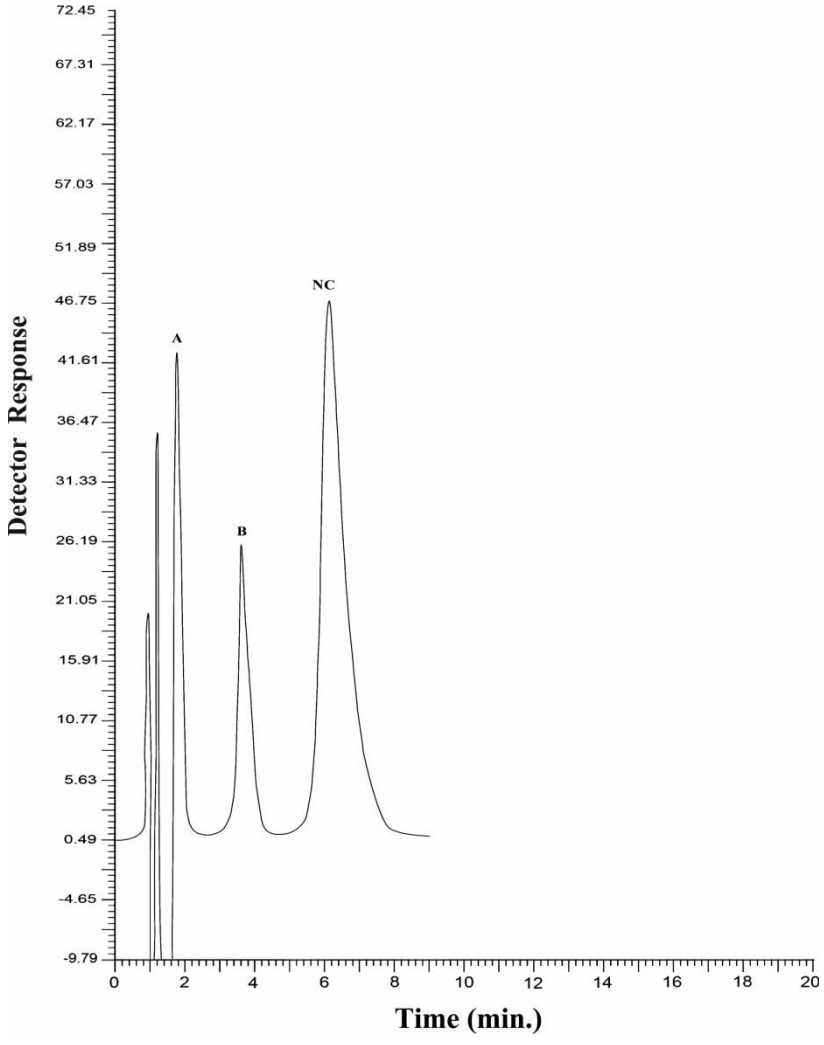


Figure 2. Typical chromatogram of nicardipine hydrochloride (40 μg mL^{-1}) and its degradation products (20 μg mL^{-1}) under the described chromatographic conditions. A: Degradation product A. B: Degradation product B. NC: Nicardipine hydrochloride.

factor of 1.90, in a reasonable time (less than 10 min) with maximum detector response.

The Concentration of the Surfactant

The effect of SDS concentration on retention time and detector response (as peak area ratio) was investigated using microemulsions containing SDS

concentrations ranging from 0.075 to 0.2 M. It was found, that an increase in the concentration of SDS decreased the retention time of both the drug and its degradation products continuously all over the investigated range, due to their distribution into the increased volume of the microemulsion droplets, or to the surface of the droplets, which run with the speed of the mobile phase. Meanwhile, increasing SDS concentration increased the peak height of both the drug and its degradation products up to 0.175 M; further increase in SDS concentration up to 0.2 M did not affect the peak height. Figure 3 illustrates the results obtained. A concentration of 0.175 M was found to be suitable for routine use, as it provided reasonable elution time and selectivity.

Effect of Cosurfactant

Propanol (10%) was replaced with either tetrahydrofuran, 1-butanol, acetonitrile, or methanol, in an attempt to study the effect of the nature of the cosurfactant on the selectivity. The four cosurfactants were found to be equally useful. The resolution factors of the two peaks are given in Table 1, as a function of the cosurfactants investigated. Acetonitrile and tetrahydrofuran provided reasonable resolution of the two peaks, while the use of 1-butanol resulted in overlapped peaks. It is interesting to notice that the retention of the drug and its degradation products was greatly affected by the nature of the cosurfactant, which is in accordance with previous reports in evaluating the effect of various cosurfactants on the separation selectivity in microemulsion eluents.[20] Increasing the cosurfactant concentration over the

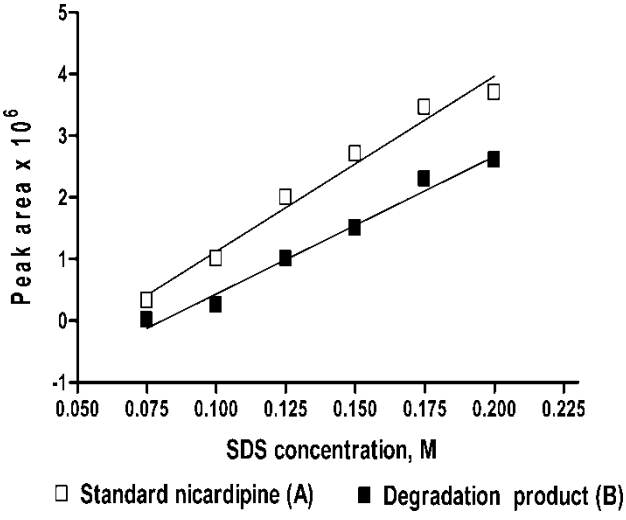


Figure 3. Effect of SDS concentration on the peak area of nicardipine (20 μg mL^{-1}) and its main degradation product (B) using the proposed method.

Table 1. Effect of the type of cosurfactant on the resolution of the proposed HPLC method

	Peak width		
Cosurfactant	NC	Degradation product (B)	Resolution
Propanol	1.50	1.00	1.90
THF	1.90	1.60	1.71
Methanol	2.00	1.75	1.60
Acetonitrile	2.20	1.80	1.25
Butanol	1.40	1.60	1.07

Where: Resolution factor $= 2\Delta t_{R/W1+\ W2.}$

range 6–12%, resulted in decreased retention times of the drug and its degradation products. Best resolution concerning peak width and resolution factor was attained upon using 10% propanol.

Effect of pH

The pH of the mobile phase was changed in the range from 3 to 7 using increasing amounts of triethylamine. The resolution factors of the two peaks were plotted against the pH values. As illustrated in Figure 4, it was found that NC and its degradation product B were considerably retained upon increasing the pH value. However, the increase in retention time of NC was more significant than that of degradation product B, while A is not affected.

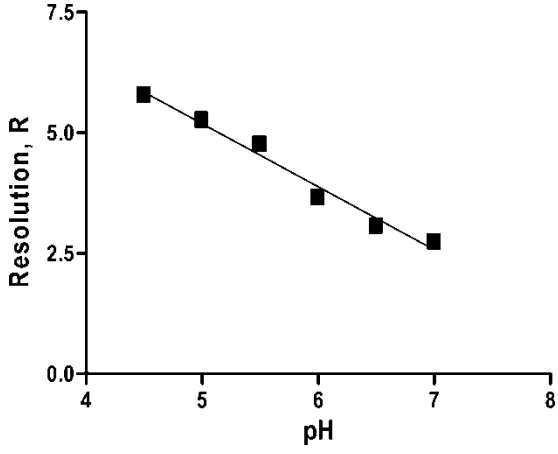


Figure 4. Effect of pH on the resolution of nicardipine (20 μg mL^{-1}) using the proposed HPLC method.

NC differs from its degradation products (A) and (B) in hydrophobicity and dissociation constants as expressed by its Log *P* (octanol/water) and pK_a value, respectively. NC has Log *P* value of 3.70[21] and pK_a value of 7.20,[22] thus, the ionization of NC and its degradation product (B) will decrease with increasing pH and log P values will be expressed. In this study, a pH value of 6.5 seemed to be optimal for the separation and detection of both drug and its degradation product in a short time.

The Internal Organic Phase

Three different organic solvents 1-octanol, butyl acetate, and diisopropylether were tested as internal organic phases (1%) to present a range of polarity. It was found, that the separation could be successfully achieved using each of the three solvents. Butyl acetate and diisopropylether provided poor separation and the longest retention time (8.5, 4.8, and 2.35 min. for NC and its degradation product (B) and (A), respectively) when compared with 1-octanol (6.06, 3.7, and 1.79 for NC and its degradation product (B) and (A), respectively), while the retention time of the degradation product (A) is not affected. 1-octanol seemed to be the optimal organic solvent for separation and detection of both drug and its degradation product.

A micellar mobile phase, identical to the microemulsion system, but without the internal phase 1-octanol, was investigated in our initial attempts for separation achievement. It was found that the two peaks were overlapped and the total run time was reduced. However, the internal organic phase representing the hydrophobic solvent may also be distributed to the hydrophobic stationary phase on the surface of the column packing material, resulting in an increase in the amount of the stationary phase and, thus, changing the selectivity of the system.

Specificity

Attempts were made to degrade NC samples to assess the proposed HPLC method. Solutions of 0.1 mg mL^{-1} NC were prepared in methanol. Separate 4 mL aliquots of this solution were transferred into 10 mL volumetric flasks; the volume was completed with 0.5 M NaOH. These aliquots were then placed in a thermostated water bath at different temperatures (60, 80, 90, 100°C) for different time intervals (10–70 minutes). 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 0.5 M HCl. The volume was completed to the mark with the mobile phase. Triplicate twenty microliter injections were made for each sample.

In alkaline medium, nicardipine gave two degradation products (A and B) with retention times of 3.70 and 1.79 min, respectively (Figure 2). It is postulated that the degradation process involved the ester linkages. Meanwhile, the

nitro group is reduced to be a nitroso derivative with concomitant oxidation of the dihydropyridine ring into pyridine. No significant degradation peaks were observed when using either 0.5 or 1 M HCl.

Analytical Applications

The proposed method was tested for linearity, specificity, accuracy, and precision.

Linearity

The peak area of NC varied linearly with the concentration over the range (1–40 μg mL^{-1}) and (2–20 μg mL^{-1}) for NC and its degradation product B, respectively.
Linear regression analysis of the data gave the following equations:

$$P = -7.189 \times 10^{-4} + 0.0363C \quad (r = 0.9999) \text{ for NC}$$
$$P = 1.520 + 168.13C \quad (r = 0.9998) \text{ for degradation product (B)}$$

Where C is the concentration in μg mL^{-1} and P is the peak area ratio.

While for the degradation product (A), the relationship was not linear, this may be attributed to the reversible oxidation reduction process of the nitro group into nitroso, and subsequent air oxidation to the nitro group.

Limits of Quantitation (LOQ) and Limit of Detection (LOD)

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH Q2B recommendations,[23] below which the calibration graph is non linear and was found to be 0.08 and 0.24 μg mL^{-1} for NC and its degradation product B, respectively. The limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected and was found to be 0.024 and 0.072 μg mL^{-1} for NC and its degradation product B, respectively.

LOQ and LOD were calculated according to the following equations:[23]

$$LOQ = 10\sigma/S$$
$$LOD = 3.3\sigma/S$$

where σ is the standard deviation of the intercept of regression line and S is the slope of the calibration curve.

The linear dependence of the peak area ratios versus the concentration of both analytes was shown for NC and its degradation product (B) by calculation of the regression equations over the ranges given in Table 2.

Table 2. Analytical parameters for the HPLC method of determination of nicardipine and its degradation product (B) adopting the proposed method

Parameters	Standard nicardipine (NC)	Degradation product (B)
Concentration range (μg/mL)	1–40	2–20
Regression equation	$P = -7.189 \times 10^{-4} + 0.0363C$	$P = 9.538 \times 10^{-3} + 0.0692\ C$
Correlation coefficient (r)	0.9999	0.9998
Slope	0.0363	0.0692
Intercept	-7.189×10^{-4}	9.538×10^{-3}
Detection limit (μg/mL)	0.024	0.072
Quantification limit (μg/mL)	0.080	0.240
$S_{y/x}$	7.68×10^{-4}	4.62×10^{-3}
S_a	2.99×10^{-4}	1.69×10^{-3}
S_b	2.10×10^{-5}	2.83×10^{-4}
RSD%	0.28	0.45
%Er	0. 74	0.17

Where:
$S_{y/x}$ = Standard deviation of the residuals.
S_a = Standard deviation of the intercept.
S_b = Standard deviation of the slope.
%RSD = Relative standard deviation.
%Er = Percentage error = %RSD/$\sqrt{n}$.

Accuracy and Precision

The intra-day precision and accuracy of the assays were measured by analyzing 1 μg mL^{-1} NC in spiked human plasma in one day. Also, the inter-day precision and accuracy were determined over three successive days by analyzing the same concentration. The obtained results for both the intra and inter day precision and accuracy are abridged in Table 3.

Degradation Kinetics Study

Figure 5 shows typical chomatograms of NC after being subjected to alkaline degradation. These chromatograms revealed that the peak area of NC decreased over time. The degradation was found to be temperature dependent (Figure 6). The apparent first-order degradation rate constant and the half-life time at each temperature were calculated (Table 4). Plotting log K_{obs} values vs 1/T, the Arrhenius plot was obtained (Figure 7). The activation energy was calculated and found to be 11.06 Kcal $mole^{-1}$. This value is in accordance

Table 3. Accuracy and precision data for the HPLC analysis of (1 μg mL^{-1}) nicardipine in spiked human plasma

		% Recovery		
Regimen	Parameters	Concentration added (μg mL^{-1})	Concentration found (μg mL^{-1})	Recovery (%)
Intra-day		1.00	1.032	103.20
		1.00	1.007	100.70
		1.00	1.046	104.60
	Mean($\bar{x}$)		102.83	
	± S.D.		1.98	
	%RSD		1.93	
	%Er		1.11	
Inter-day	1st day	1.00	0.977	97.70
	2nd day	1.00	1.081	108.10
	3rd day	1.00	1.029	102.90
	Mean($\bar{x}$)		102.90	
	± S.D.		5.20	
	%RSD		5.05	
	%Er		2.92	

Each result is the average of three separate determinations.

with the reported values of activation energy required for the hydrolysis of esters.[24]

Stability

The stability of the methanolic sample solutions at room temperature (25°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 NC in the samples.

Recovery

The recovery of the method was checked by adding known amounts of degraded NC to known amounts of standard NC. The calculated recoveries were satisfactory, indicating that no interference had been observed from the degradation products. The accuracy of the proposed method was evaluated by analyzing standard solutions of the studied drug. The results obtained by the proposed method were compared with those given by the reference method.[25] Statistical analysis of the results using student t test

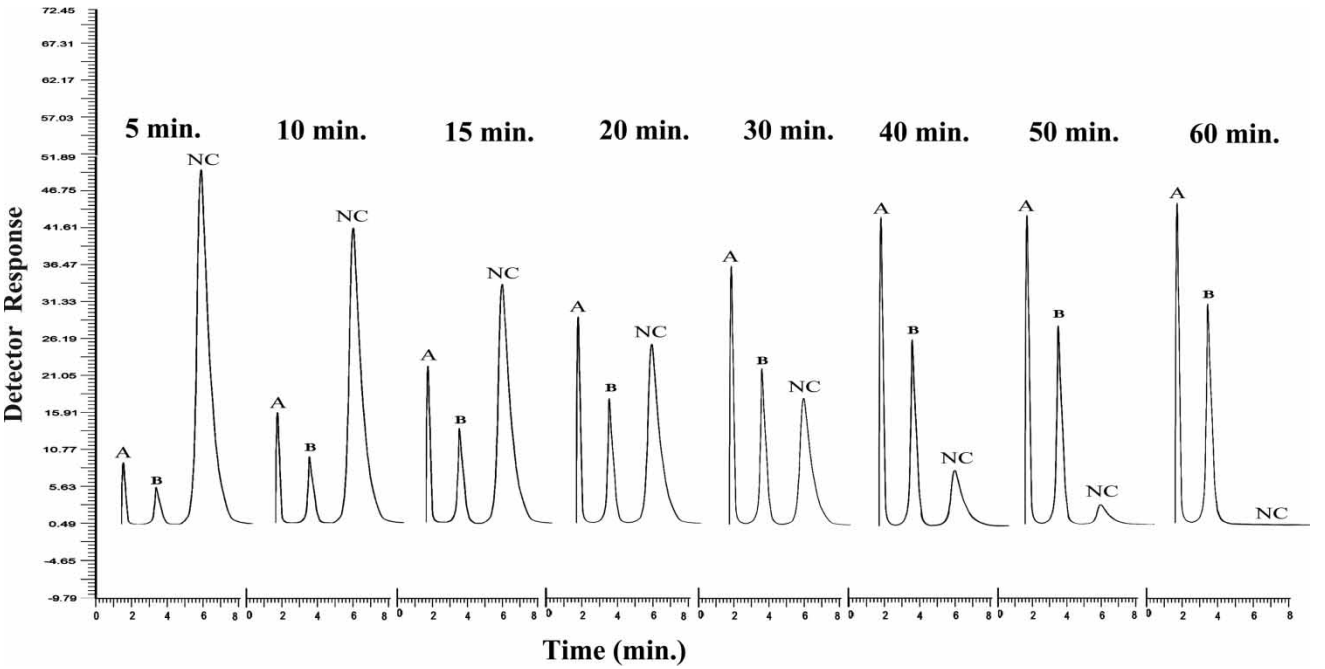


Figure 5. Chromatograms of alkaline degradation of (20 μg mL^{-1}), using 0.5 M NaOH. A: degradation product (A). B: Degradation product (B). NC: Nicardipine hydrochloride.

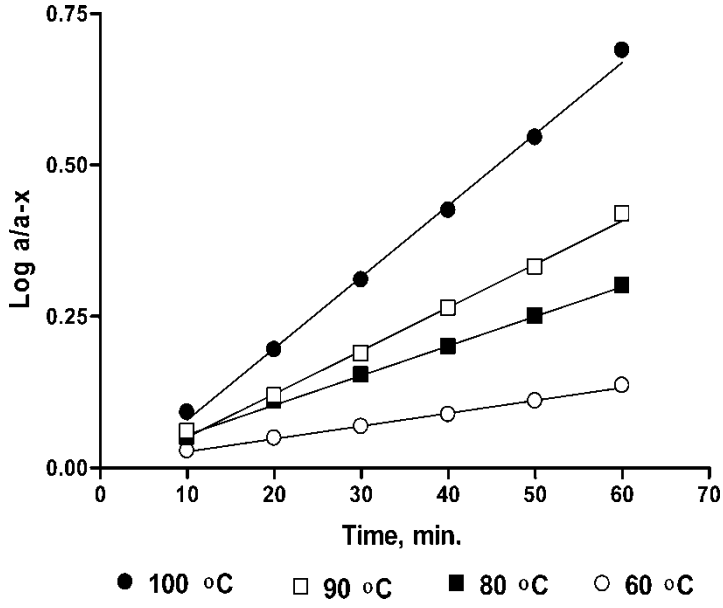


Figure 6. Semilogarithmic plot of nicardipine (30 μg mL^{-1}) versus different heating times with 0.5 M NaOH.

and variance ratio F- test showed no significance difference in the performance of the two methods regarding accuracy and precision, respectively.[26]

Application of the Proposed Method to the Analysis of Nicardipine in its Commercial Capsules

The proposed method was applied to the determination of the drug in capsules. The percentage recoveries are abridged in Table 5. The results shown in (Figure 8) were compared with those obtained using the reference method.[27] Statistical analysis of the results using student t test and variance ratio F- test

Table 4. Effect of temperature on the kinetic parameters of nicardipine hydrochloride (30 μg mL^{-1}) using 0.5 M NaOH

Temperature (°C)	Slope	K(min^{-1})	Log K	$t_{1/2}$(min)	E_a (K· cal · mol^{-1})
60	2.127×10^{-3}	4.90×10^{-3}	−2.309	141	9.38
80	4.87×10^{-3}	0.011	−1.959	63	10.29
90	7.31×10^{-3}	0.0165	−1.783	42	13.50
100	0.0121	0.0273	−1.564	25	$\bar{X} = 11.06$

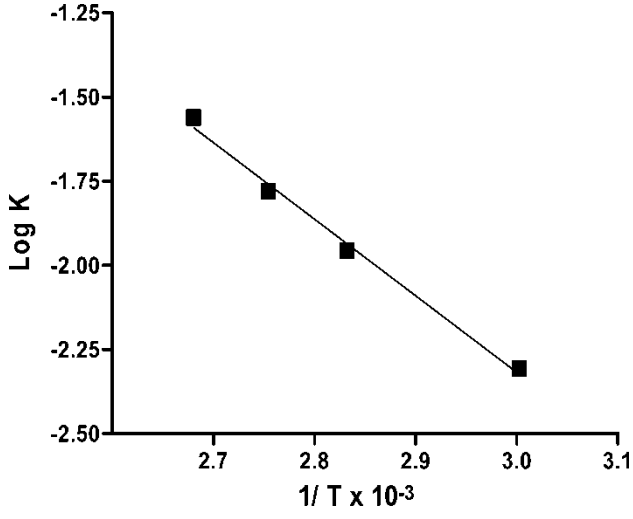


Figure 7. Arrhenius plot for the degradation of nicardipine (30 μg mL^{-1}) in 0.5 M NaOH using peak area measurements.

showed no significant difference in the performance of the two methods regarding accuracy and precision, respectively.[26] Capsule excipients, such as talc (20 mg), lactose (15 mg), magnesium stearate (10 mg), and starch (15 mg) didn't interfere with the assay.

Table 5. Application of the proposed HPLC method to the determination of nicardipine in commercial capsules

	Proposed method			Comparison method (27)
Compound	Concentration taken (μg mL^{-1})	Concentration found (μg mL^{-1})	Recovery (%)	Recovery (%)
1-Pelcard 50 mg capsules[a]	20.0	20.086	100.43	100.00
(NC HCl 50 mg/ capsule)	25.0	25.310	101.24	101.39
	40.0	40.376	100.94	101.45
Mean ± S. D			100.87 ± 0.41	100.95 ± 0.82
Student's t-test			0.150	(2.776)**
F-test			4.00	(19.00)**

Each result is the average of three separate determinations.

[a]Product of Global Napi, Cairo, Egypt, Batch # 35801.

**Figures in parentheses are the tabulated t and F values, respectively at $p = 0.05$ (26).

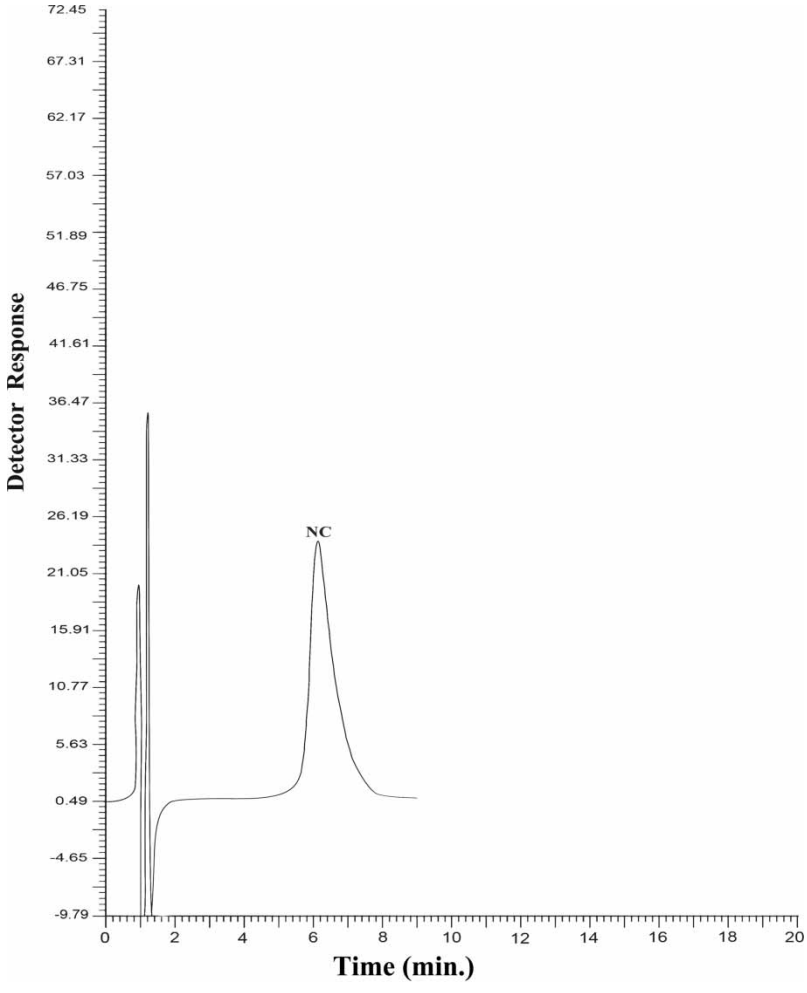


Figure 8. Application of the proposed method for the determination of nicardipine hydrochloride (20 μg mL^{-1}) in capsules. NC: Nicardipine hydrochloride.

Application of the Proposed Method to the Analysis of Nicardipine in Spiked Human Plasma

Nicardipine is rapidly absorbed from the gastro intestinal tract but is subjected to saturable first-pass hepatic metabolism. Bioavailability of about 35% has been reported following a 30 mg dose at steady state. The terminal plasma half life is about 8.6 hours.[2] The method could also be successfully applied to the determination of nicardipine in spiked human plasma (Figure 9). The results are abridged in Table 6.

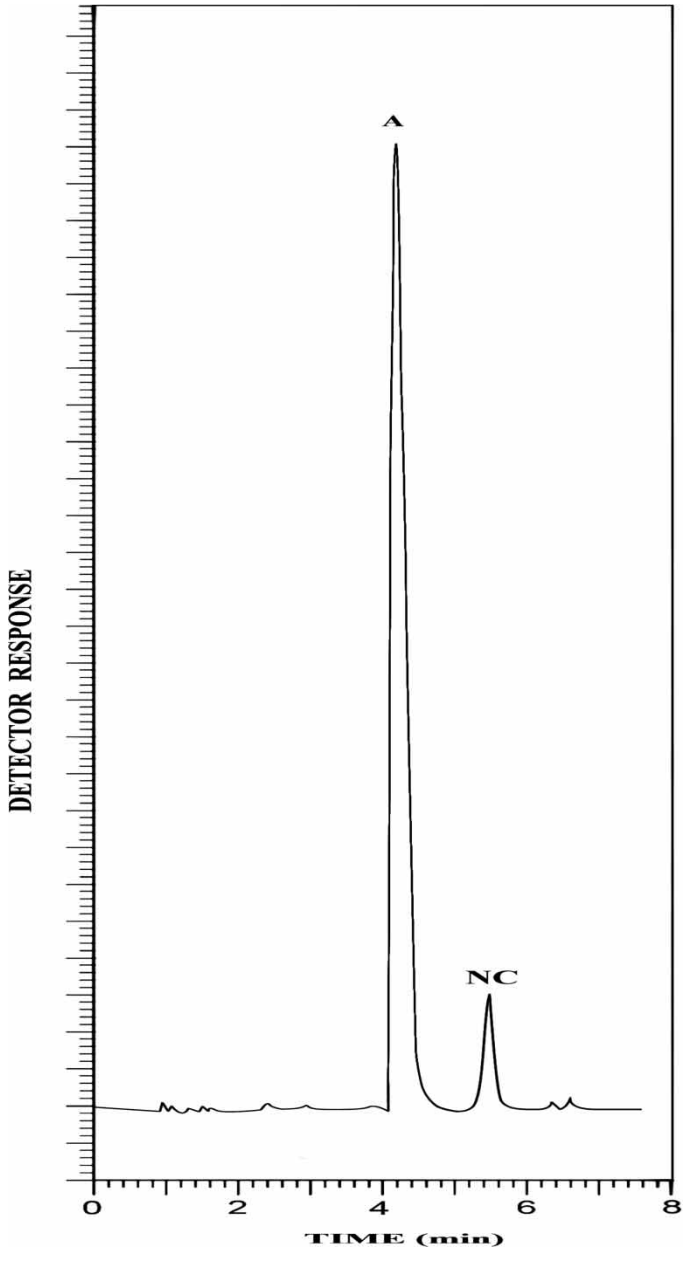


Figure 9. Application of the proposed method for the determination of nicardipine hydrochloride (1 μg mL^{-1}) in plasma. A: Plasma peak. NC: Nicardipine hydrochloride.

Table 6. Application of the proposed method to the determination of NC in spiked human plasma

	Concentration added ($\mu g\ mL^{-1}$)	Concentration found ($\mu g\ mL^{-1}$)	Recovery (%)
	0.5	0.515	103.00
	1.0	0.970	97.00
	1.5	1.515	101.00
Mean found, $\bar{x}$		100.33	
$\pm$ SD		3.06	
% RSD		3.05	
% Error		1.76	

Chromatographic Performance

Three defined symmetrical peaks were obtained upon measuring the UV response of the eluate under the optimum experimental parameters (Figure 2). The nicardipine hydrochloride peak was obtained at retention time of 6.06 with two degradation products, A and B, at t_R 1.79 and 3.70, respectively. It was found that the peak of degradation A was not linear over the concentration range of 2–20 $\mu g\ mL^{-1}$.

Pathway of Degradation

The chromatogram of NC degradation showed 2 peaks in addition to that of nicardipine. It is postulated that the decomposition pathway is as in Scheme 1.

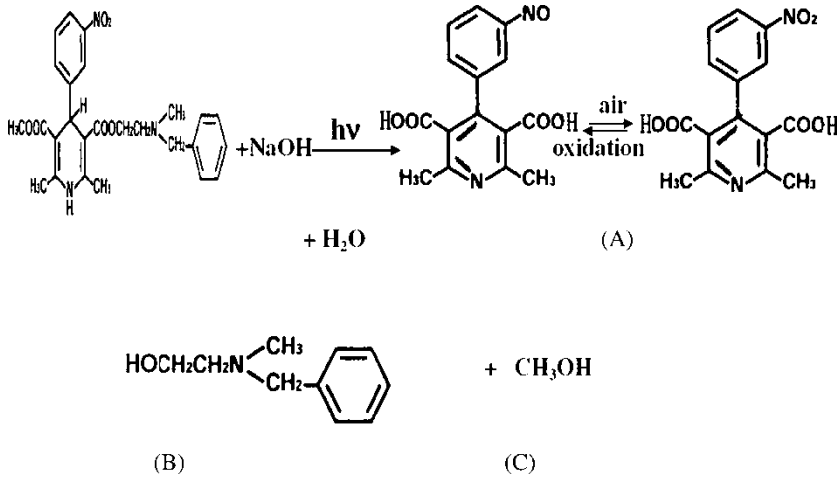
Upon heating nicardipine in alkaline medium, it undergoes the following changes:

The two ester groups attached to the dihydropyridine ring are hydrolysed, resulting in the formation of CH_3OH (C) which is not detectable, and N-methyl-N-(2-hydroxylethyl)benzylamine (B).

At the same time, NC undergoes photochemical decomposition through auto-oxidation reduction resulting in reduction of the nitro group to nitroso and oxidation of the dihydropyridine ring into the pyridine ring (A), as previously reported.[28]

The nitroso group in (A) is reoxidized (atmospheric oxidation) to the nitro group.[29]

TLC of the neutralized hydrolysate using silica gel plates and a mobile phase consisting of chloroform: methanol (9.5 : 0.5) with UV detection[18] gave two spots, one with an Rf value of 0.75, while the other remained on the base line. The first spot is supposed to be the alcohol fraction (B), less polar than the second one (A), which remained on the base line.



Scheme 1.

CONCLUSION

A rapid, precise, and specific HPLC method using a single isocratic system has been developed for the determination of nicardipine, either alone or in presence of its degradation products. The method is considered to be a stability indicating assay for the determination of nicardipine in pharmaceutical preparations and human plasma. A simple sample preparation enables the use of this method for the routine quality control of nicardipine in capsules, with good accuracy. The proposed method is characterized by high sensitivity for the analysis of NC with LOD of 0.024 $\mu g\ mL^{-1}$ (4.65×10^{-8} mol/L) and LOQ of 0.08 $\mu g\ mL^{-1}$ (1.55×10^{-7} mol/L).

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