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A Selective High Performance Liquid Chromatographic Method to Follow the Hydrolytic Degradation of Nicardipine Hydrochloride

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Abstract: A simple, stability indicating, reverse phase high performance liquid chromatographic method was developed and validated for determination of nicardipine hydrochloride (NC) in the presence of its degradation products. The chromatographic separation was performed on Hypersil, BDS-C18, 30 cm x 3.9 mm id, at ambient temperature with UV-detection at 254 nm. A mixture of 20% (v/v) aqueous 0.01 M sodium acetate/acetic acid buffer (pH 4.5) and 80% acetonitrile was used as the mobile phase at a flow rate of 1.5 mL min^{-1}, losartan was used as internal standard. The calibration curve is linear over the concentration range 5-40 µg mL^{-1}, with a regression coefficient of 0.9984 and the % recovery was 99.78±0.17. The method was used to investigate the kinetics of alkaline, acids induced degradation, effect of buffer concentration and temperature. The degradation followed first-order kinetics. The rate constant, half-life time, and activation energy were calculated

Keywords: Nicardipine hydrochloride, HPLC, Hydrolytic Degradation.

Introduction

Nicardipine hydrochloride (Figure 1) is chemically known as 2-(benzyl (methyl) amino) ethyl methyl 1, 4–dihydro-2, 6-dimethyl-4-(3-nitrophenyl) pyridine-3, 5-dicarboxylate hydrochloride[1]. It is a calcium antagonist of the dihydropyridine class and has been widely used for the treatment of hypertension and angina pectoris[2].

Several methods have been reported for determination of nicardipine hydrochloride. These include spectrophotometry[3-5], voltammetry[6-8], high performance liquid chromatography[9-12], liquid chromatography mass spectroscopy[13-15] and capillary electrophoresis[16]. Most of these methods were used for quantitative determination of nicardipine hydrochloride in biological fluids.

Drug degradation is a serious problem which renders the job of quality control analyst very critical and is usually associated with stability of the drug. Investigations on stability during a laboratory process are necessary to establish the precautions required during development and control of dosage form as well as for development and validation of quantitative methods. Generally stability of drugs or pharmaceutical preparations depends on temperature, concentration, pH, oxygen content, light intensity, wave length and other factors. Reviewing the literature, revealed that, only the effect of light on nicardipine HCl has been studied.

The aim of the present work was to develop, validate and apply a stability indicating HPLC method for determination nicardipine HCl and its alkaline and acid hydrolytics products. The developed method was used to investigate the kinetics of the drug degradation in different media at different temperatures.

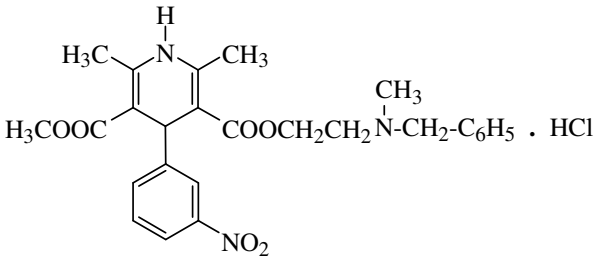


Figure 1. Structural formula of nicardipine hydrochloride.

Experimental

The pure sample of nicardipine hydrochloride was purchased from Sigma (St. Louis, Mo, USA). The commercial capsules (Pelcard 50 mg Batch # 65801) were obtained from commercial sources. Methanol, acetonitrile, (all of HPLC grades), hydrochloric acid (Analar), monobasic sodium phosphate, ammonium acetate, boric acid, sodium acetate and glacial acetic acid (Analar) were obtained from BDH (Poole, UK). Phosphoric acid, ethanol, and potassium hydroxide were obtained from E; Merck (Darmstadt, Germany).Losartan was purchased from Sigma (St. Louis,Mo,USA).

Apparatus

Seperation was performed on Shimadzu class-vp v6.LC-2010A liquid chromatography, equipped with UVB50 varian detecter, rheodyne injector and the wavelength used was 254 nm.

Column and mobile phase

Separation was achieved on Hypersil, BDS- C18, (5 µm), 30 cm X 3.9 mm id. The mobile phase consists of 20 % (v/v) aqueous 0.01 M sodium acetate/acetic acid (pH 4.5) and 80% (v/v) acetonitrile.

Standard solution

A stock solution of 0.04% (w/v) of nicardipine HCl reference material in the mobile phase was prepared and working solutions were prepared by appropriate dilutions.

Internal standard

A stock solution of 0.01% (w/v) of losartan was prepared in the mobile phase and working solutions were prepared accordingly.

preparation of solutions of alkalis and acid degradation

For the effect of alkalis and acids on nicardipine HCl, solutions of the drug with a concentration of 40 µg/mL were treated with solutions of either sodium hydroxide of different molar concentrations (0.1 M, 0.2 M, 0.3 M, 0.4 M, 0.5 M and 1.0 M) of HCl. The experiments were conducted at room temperature. The solutions were then neutralized with 0.1 M, 0.2 M, 0.3 M, 0.4 M, and 0.5 M HCl and 1.0 M NaOH and diluted to volume with mobile phase.

Calibration curve

Aliquots of the standard solution covering the working range (5, 10, 15, 20, 25, 30, 35 and 40 µg/mL^{-1}) of nicardipine were prepared in mobile phase with 20 µgmL^{-1} of the internal standard. Ten 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 of nicardipine HCl to that of losartan (internal standard) versus concentration. The linear regression equation was derived.

Study of the factors affecting the degradation kinetics of nicardipine HCl

Asolution of nicardipine HCl (40 µgmL$^{-1)}$ was used to study the effect of temperature and pH. The solutions were treated with different concentrations of NaOH (0.1 M, 0.2 M, 0.3 M, 0.4 M and 0.5 M) and kept at different temperatures (70 -100 ^{0}C).The solutions were then neuterlized with 0.1 M, 0.2 M, 0.3 M, 0.4 M and 0.5 M HCl and diluted with mobile phase. For the effect of pH four buffer systems (citrate, phosphate, borate and acetate) of different pH were used with the same concentration of nicardipine HCl (40 µgmL^{-1}).

Analysis of capsules

A solution of nicardipine HCl was prepared and injected using the prescribed method and the nominal content of the capsule s were calculated using the calibration graph.

Results and Discussion

Chromatographic separation

Figure 2 is a chromatogram obtained from standard solution of nicardipine and its degradation products (I and II), resulting from alkaline degradation under the prescribed chromatographic conditions. The method allows complete base line separation with a good resolution factor (6.5) between each two adjacent peaks. The proposed method was assessed for specificity, linearity, precision and recovery (Table 1).

Degradation kinetics study

Effect of acids

The result was obtained by treating a solution of 40 µg/mL with 1.0 M HCl. The kinetics parameters for degradation of nicardipine HCl in acid medium were found to be first order. The plotting of log (a/a-x) against time (t) was found to be straight line. The rate constant (k) and the half-life time ($t_{1/2}$) were found to be $1.38x10^{-3}$ min^{-1} and 502 min. respectively.

Effect of alkalis

The effect of alkalis on the degradation of nicardipine HCl was studied by using increasing concentrations of sodium hydroxide: 0.1 M, 0.2 M, 0.3 0.4 M and 0.5 M at room and elevated temperatures. At room temperature, increasing the concentration of sodium hydroxide resulted in increasing the rate of degradation and the value of the reaction rate constant (k) and decreasing in the half-life (t½) (Table 2).

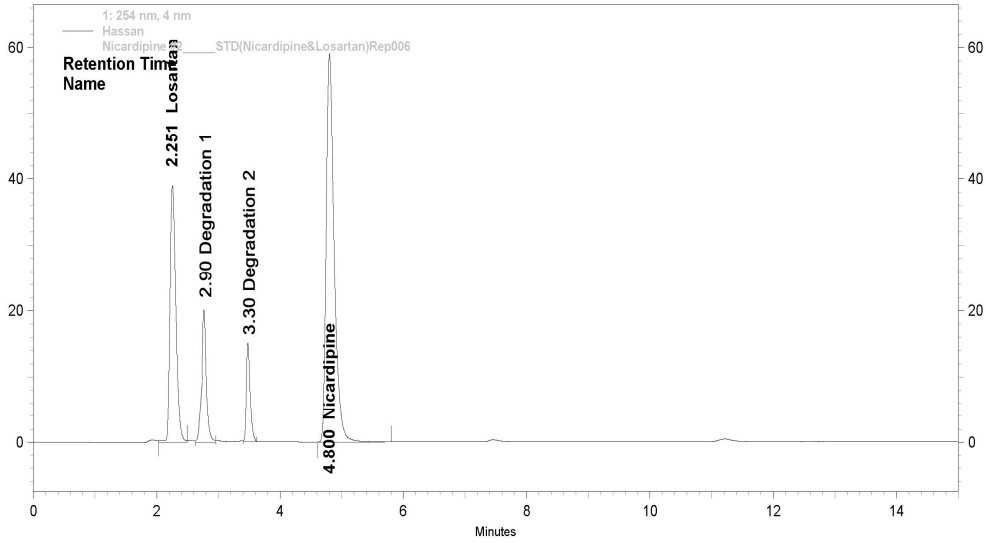


Figure 2. Chromatogram of nicardipine HCl and its degradation products.

Table 1. Analytical parameters for the HPLC method of determination of nicardipine adopting the proposed method.

Parameter	Standard nicardipine
Concentration range, µg/mL	5-40
Correlation coefficient(r)	0.9984
Slope	0.121
Intercept	0.0146
RSD%	0.17
%ER	0.064
% R	99.78+0.17

Table 2. Effect of concentration of sodium hydroxide on the kinetic parameters of nicardipine HCl (40 µg/mL) at room temperature.

Conc. of NaOH, M	K 10^3, min^{-1}	$t_{½}$, min
0.1	1.15	601
0.2	1.84	376
0.3	2.30	300
0.4	2.76	250
0.5	3.68	188

Result of the effect of alkaline degradation at different temperatures (70-100 ^{0}C) are shown in Table 3. The values of the reaction rate constant (k, min^{-1}), half-life times ($t_{1/2}$ min) and activation energy (Ea,kcal.mol^{-1}) are listed. Plotting log K_{obs} values *vs* 1/T, the Arrhenius plot was obtained (Figure 3). From these data, the rate of degradation of nicardipine was found to increase upon increasing both temperature and concentration of sodium hydroxide.

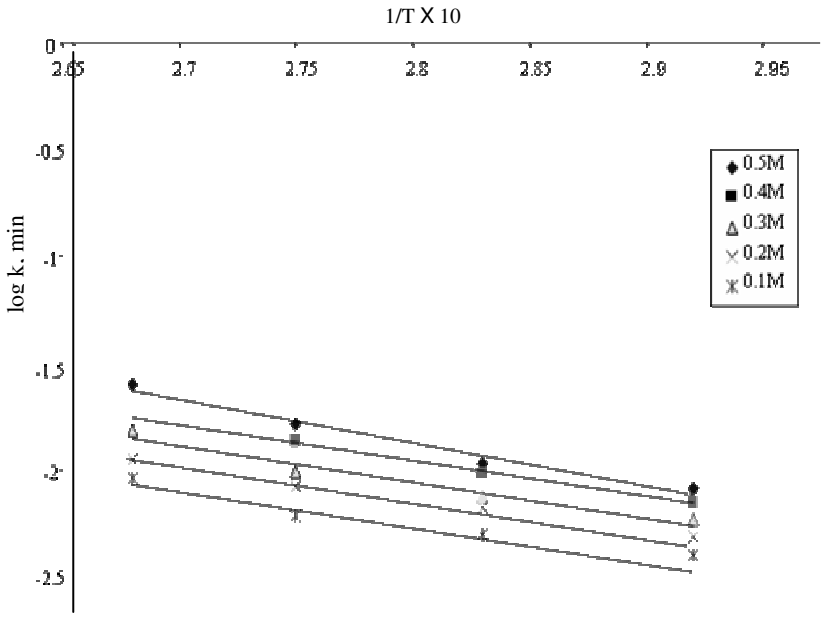


Figure 3. Arrhenius plots for nicardipine HCl at different concentrations of NaOH.

Table 3. Effect of temperature on the kinetic parameters of nicardipine HCl (40 µg/mL) at different concentrations of sodium hydroxide.

Conc.of NaOH, M	Temperature, ^{0}C	K, min^{-1}	t½ min	Ea kcal.mol^{-1}
0.1M	70	$3.91x10^{-3}$	177	X= 7.13
	80	$4.83x10^{-3}$	143	
	90	$5.98x10^{-3}$	115	
	100	$8.98x10^{-3}$	77	
0.2M	70	$4.83x10^{-3}$	143	X =7.20
	80	$6.67x10^{-3}$	103	
	90	$8.29x10^{-3}$	83	
	100	0.01128	61	
0.3M	70	$5.75x10^{-3}$	120	X =8.16
	80	$7.36x10^{-3}$	94	
	90	$9.69x10^{-3}$	71	
	100	0.01496	46	
0.4M	70	$7.13x10^{-3}$	97	X=8.0
	80	$9.44x10^{-3}$	74	
	90	0.01335	52	
	100	0.01893	38	
0.5M	70	$7.83x10^{-3}$	88	X=9.97
	80	0.01036	67	
	90	0.0161	43	
	100	0.0251	28	

Effect of buffer

The result of the effect of type and concentration of buffers on the reaction rate constant at room temperature are shown in Table 4. Based on the effect of different buffers with different pH values on the rate of degradation of nicardipine HCl at 100 ^{0}C (Table 5), the following conclusions are drawn:

- For the same type of buffer: increasing the pH increases the rate of degradation.
- Citrate buffers increase the rate of degradation more than phosphate more than borate and finally acetate.

Table 4. The calculated pseudo-first order rate constant (k_{obs}) and half-lives of degradation of nicardipine HCl at various pH values at room temperature (at 22 ^{o}C).

pH	Buffer composition	K_{obs} day^{-1}	Half life $t_{½}$ (day)	log k_o
1.1	HCl	4×10^{-2}	17.30	– 1.40
2.45	Acetate	1.92×10^{-3}	155.00	– 2.71
3.40	Citrate	1.5×10^{-3}	462.00	– 2.82
4.50	Citrate	8.91×10^{-4}	777.00	– 3.05
5.20	Citrate	1.41×10^{-3}	491.00	– 2.85
6.00	Citrate	0.564×10^{-1}	8.62	– 0.23
7.00	Phosphate	27.7×10^{-1}	17.50	– 0.56
8.30	Borate	0.1×10^{-1}	6.93	– 1
9.00	Borate	0.316×10^{-1}	2.19	– 0.50
10.00	Borate	1.58	0.46	0.19
11.20	NaOH	6.58	0.21	0.81
13.00	NaOH	1.09×10^{-3}	0.44	–2.96

Table 5. Comparison between the different buffer systems on the degradation of nicardipine (40 µg/mL) at 100 ^{0}C.

Type of Buffer	k , min^{-1}	log k	$t_{1/2}$, min
Citrate buffer pH 6	0.0154	- 1.81	44.91
Citrate buffer pH 5.2	0.0108	- 1.96	64.00
Citrate buffer pH 4.5	0.0069	- 2.11	100.00
Phosphate buffer pH 9	0.0159	- 1.79	43.61
Phosphate buffer pH 8	0.013	-1.88	52.79
Phosphate buffer pH 7.5	0.011	-1.95	61.41
Phosphate buffer pH 6.5	0.0092	-2.04	75.22
Borate buffer pH 10	0.014	-1.85	49.33
Borate buffer pH 9	0.011	-1.96	62.69
Borate buffer pH 8	0.0089	-2.04	77.16
Acetate buffer pH 5	0.0076	-2.12	91.00
Acetate buffer pH 4.5	0.0041	-9.38	1.67

Application of the proposed method to analysis of nicardipine in its commercial capsules

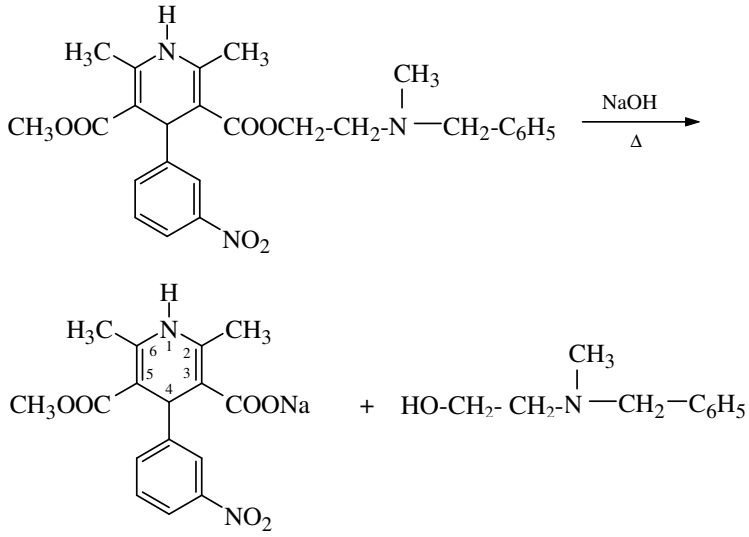
The proposed method was applied to determination of nicardipine in its commercial capsules. The percentage recoveries were obtained Table 6.

Table 6. Application of the proposed HPLC method to the determination of nicardipine in commercial capsules

Compound	Concentration taken, µg/mL^{-1}	Concentration found, µg/mL^{-1}	Recovery, %
Pelcard 50 mg	20	20.04	100.20
capsule	30	30.558	101.86
NC HCl (50 mg/capsule)	40	40.316	100.79
		Mean± S.D =100.95±0.841	

Pathway of degradation

Treatment of nicardipine with sodium hydroxide at elevated temperature resulted in cleavage of the ester linkage and the production of two compounds, I (acid) and II (alcohol) as shown blow:



I II

Compound I is eluted first, as it is more polar than either compound II or nicardipine. It is possible that, the ester group in the side chain at position 5 is also hydrolysed. However, this will not have a pronounced effect the chromatogram developed. Study of kinetic parameters for the degradation of nicardipine involved measuring the peak heights of nicardipine and internal standard only.

Conclusion

A rapid, precise, and specific HPLC method using a single isocratic system has been developed for the determination of nicardipine HCl, either alone or in the presence of its degradation products. The method was used to determine the degradation kinetics parameters of nicardipine and the drug was found to be most stable at pH =4.5.

The energy of activation calculated from Arrhenius plot suggests that a typical hydrolytic reaction is involved. The rate of the decomposition increases considerably in the alkaline medium. This agrees with the fact that ester linkages are more susceptible to cleavage in alkaline medium than in acid medium.

The catalytic effect of four buffers, namely, phosphate, borate, citrate and acetate buffer indicated that the citrate exerts a more catalytic activity than the other three buffers of which acetate apparently has no effect on the rate of degradation of nicadipine hydrochloride.

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