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Oxidative degradation study of nitrendipine using stability indicating, HPLC, HPTLC and spectrophotometric method

D.N. Tipre, P.R. Vavia *

Department of Chemical Technology, Pharmaceutical Division, University of Mumbai, Nathalal Parikh Marg, Matunga, Mumbai 400 019, India

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Abstract

The objective of this study is to develop validated stability indicating HPLC (A), HPTLC (B) and spectrophotometric (C) method for the estimation of nitrendipine. The stability indicating capability of the assays is proved using forced degradation, by exposing drug solution to sunlight, acidic and alkaline medium. The chromatogram and UV spectrum showed nitrendipine well resolved from the degradation product. Degradation of drug is found faster in acidic (0.1 N hydrochloric acid) medium as compared to alkaline (0.1 N sodium hydroxide) medium at 100°C. Also, photodegradation is studied, with special emphasis on the effect of solvents like methanol (**1**), chloroform (**2**), dichloromethane (**3**), acetone (**4**) and ethyl acetate (**5**), on the rate of photodegradation. The degradation of title compound followed first order kinetics in all cases. Estimation of the drug is carried out by the stability indicating methods mentioned, using one point standardization within the linearity range of interest. The methods are compared in respect of performance precision and accuracy. Major route of degradation in all cases is found to be oxidation and degradation product is confirmed as dehydronitrendipine by the use of relevant UV, IR and ^{1}H NMR spectrometry.

Keywords: Nitrendipine; Photodegradation; Stability indicating methods; Spectrophotometric; RP-HPLC; HPTLC

1. Introduction

Nitrendipine [3-ethyl-5]methyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate, a potent dihydropyridine calcium entry blocker, is a useful agent in the long-term treatment of hypertension. There are few studies reported on the stability of nitrendipine. The photodecomposition product of nitrendipine was detected, isolated and identified as nitropyridine analogue [1,2]. The effect of pH and temperature on the stability of the title compound was investigated [3,4]. Analytical methods for quantification of nitrendipine in biological fluids include gas chromatography with mass spectrometric detection [5,6] and with electron capture detector [7,8], liquid chromatography with UV detection [9], radioreceptor assays [10,11] and radioimmunoassays [12,13] have already been published.

* Corresponding author. Tel.: +91-22-4145616; fax: +91-22-4145614.
E-mail address: prv@pharma.udct.ernet.in (P.R. Vavia).

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In the present analytical work, we have developed and compared new, simple and reproducible stability indicating RP-HPLC, HPTLC and spectrophotometric methods for the estimation of nitrendipine. Degradation kinetics was investigated in acidic, alkaline medium and in sunlight (08:00–17:00 h for 2 days). The extent of the influence of methanol (**1**), chloroform (**2**), dichloromethane (**3**), acetone (**4**) and ethyl acetate (**5**) on the photodegradation rate of nitrendipine was investigated. Stability was studied by quantitation of drug by the methods mentioned. The methods were compared in respect of performance, precision and accuracy.

2. Experimental

2.1. Materials

The gift sample of nitrendipine was obtained from Concept Pharmaceuticals Ltd. (Aurangabad, India). Some 0.1 M sodium hydroxide, 0.1 M hydrochloric acid, 0.15 M ammonium hydroxide and 0.15 M acetic acid were prepared as per Indian Pharmacopoeia, 1996. For HPTLC, spectrophotometric method and photodegradation study chloroform, methanol, acetone, dichloromethane and ethyl acetate used were of analytical grade and purchased from Ranbaxy Laboratory (India). HPLC grade methanol, acetonitrile and water purchased from Ranbaxy Laboratory were used for HPLC method.

Borosilicate volumetric flasks were used for photodegradation study and amber-colored calibrated glass apparatus were used throughout analysis.

2.2. Equipment and conditions of stability indicating methods

2.2.1. HPLC

Analyses were carried out using a Jasco HPLC with Jasco UV/VIS intelligent detector and a

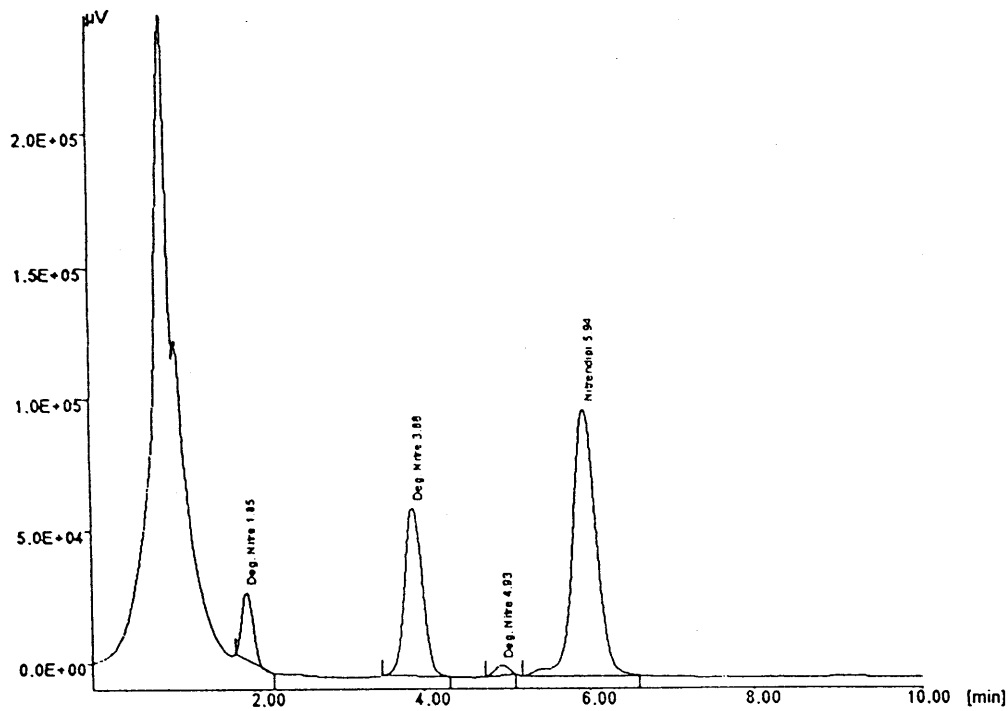

Fig. 1. HPLC chromatogram in acidic and alkaline degradation (major degradation product at $R_t = 3.88$ min, minor degradation product at $R_t = 1.85$, 4.93 min, nitrendipine $R_t = 5.94$ min).

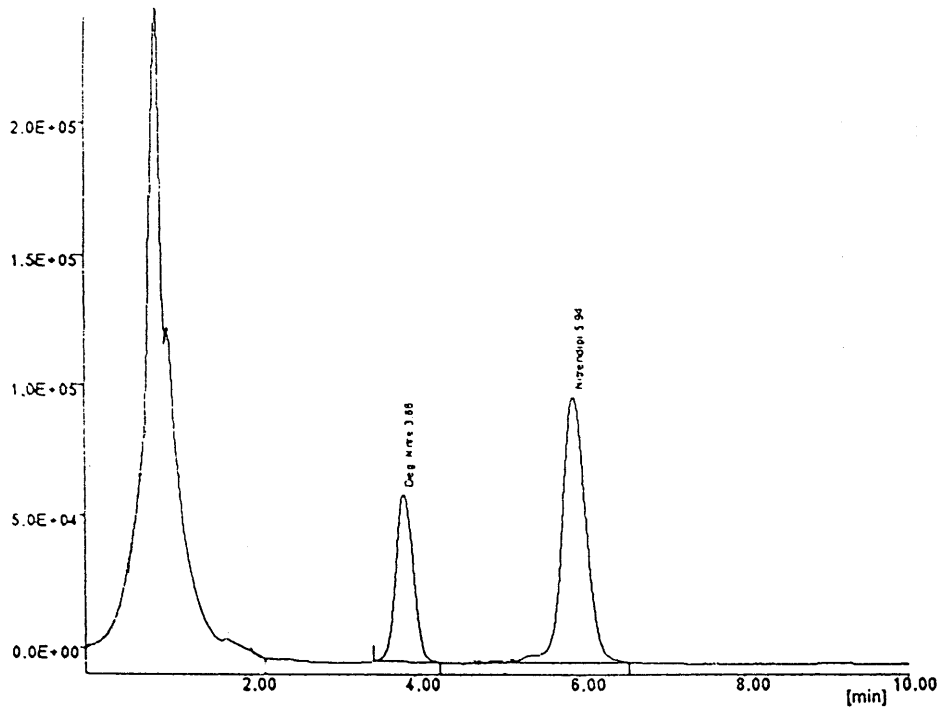

Fig. 2. HPLC chromatogram photodegradation (major degradation product at $R_t = 3.88$ min, nitrendipine $R_t = 5.94$ min).

rheodyne injection valve with a 20 μl-loop. Resolution was achieved on the LiChroCART® (5 μm; 125 × 4). Isocratic elution was employed using a mixture of methanol–water–acetonitrile (45:45:10 v/v/v). Flow rate and detection wavelength was kept at 1.2 ml/min and 235 nm, respectively.

2.2.2. HPTLC

Analyses were carried out using Camag HPTLC system comprising of Camag Linomat IV sample applicator, Hamilton syringe (100 μl), Camag scanner II attached to a Perkin–Elmer LC1-100 integrator. The following standard chromatographic conditions were maintained for analysis.

2.2.3. Test plate

Silica gel $60F_{254}$ aluminum HPTLC precoated plate (Merck, Germany); particle size: 5 μm; format: 10 × 10 cm^2; thickness: 200 μm; bandwidth: 3 mm; separation techniques: ascending; develop-

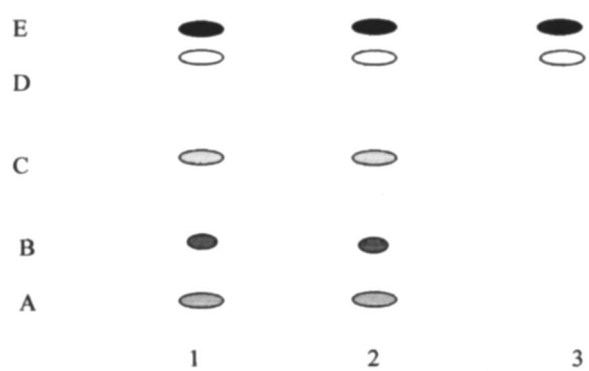

Fig. 3. HPTLC chromatograms. (1–3) acidic, alkaline and photodegradation; (A) Basespot; (B,C) minor degradation product ($R_f = 0.2$, 0.5); (D) Nitrendipine ($R_f = 0.68$); (E) major degradation product ($R_f = 0.78$).

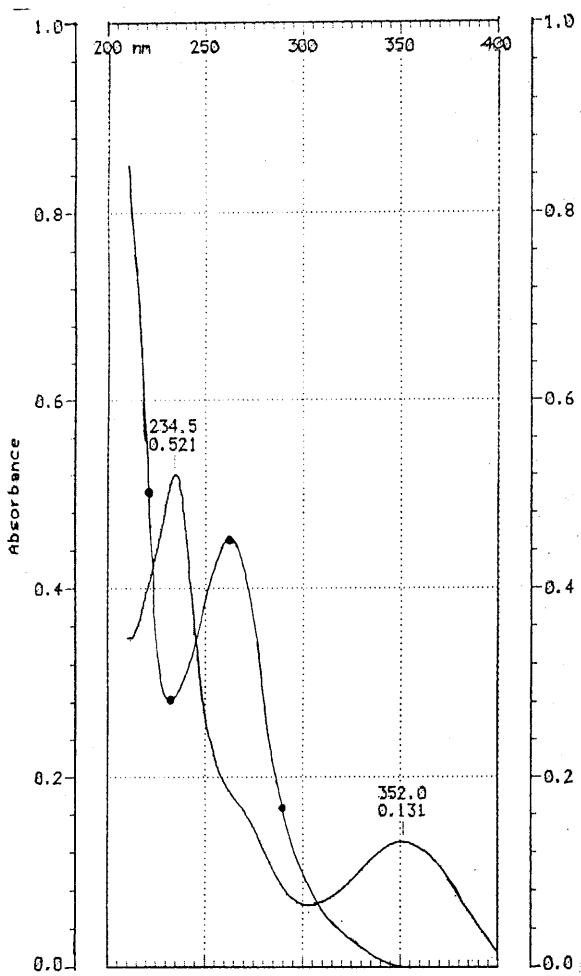

Fig. 4. UV spectrum nitrendipine (——), degraded nitrendipine (—●—).

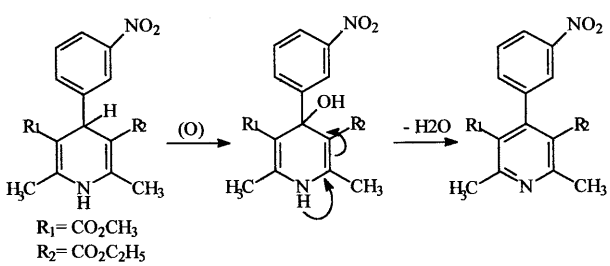

Fig. 5. Plausible mechanism in oxidative degradation of nitrendipine.

ment chamber: Camag twin trough glass chamber $10 \times 12 \times 5$ cm^3; mobile phase: ethyl acetate–chloroform (1:9 v/v); time for chamber presaturation: 30 min; relative humidity (%): 55 ± 5; temperature (°C): 23 ± 3; migration distance: 90 mm; migration time: 15 min; scanning: densitometry; slit dimension: 4×4 mm^2; detection wavelength: 254 nm.

2.2.4. Spectrophotometry

Single beam spectrophotometer (Model: CECIL-CE 2021, Japan) with a quartz cell having a path-length of 10 mm was used for the estimation of the drug. Assay was carried out at 355 nm, where the degraded product did not interfere. Buck Scientific Infrared spectrophotometer M500 (USA) and Brucker FTNMR 500 MHz (Germany) were used to confirm degraded product.

2.3. Sample preparation

Stock solution of the drug (1 mg/ml) was prepared in methanol, acetone, ethyl acetate, chloroform and dichloromethane. These stock solutions were used for the calibration curve in stability indicating methods, in forced degradation study, as external standard in one point analysis and for intra-, inter-day analysis. Drug solution (500 μg/ml) in different solvents was prepared in triplicate for exposure to sunlight.

2.4. Validation of stability indicating methods

The evaluation of intra- and inter-day precision, linearity range and limit of quantification (LOQ) validated the methods.

2.5. Degradation under forced conditions

2.5.1. Degradation in acidic/alkaline medium

Drug stock solution (5 ml) in methanol was taken in two separate 50 ml volumetric flask containing 0.1 M hydrochloric acid and 0.1 M sodium hydroxide (4 ml) and the solutions were kept at 100°C for 72 h. The solutions were then neutralized with 0.2 M ammonium hydroxide for acidic and 0.2 M acetic acid solution.

Table 1
Intra- and interday precision of proposed HPLC method

Intraday precision				Interday precision		
Conc. (μg/ml)	*n*	Found mean conc. ± S.D. (μg/ml)	RSD (%)	*n*	Found mean conc. ± S.D. (μg/ml)	RSD (%)
100	6	98.85 ± 2.45	2.47	3	98.75 ± 2.65	2.68
100	6	97.44 ± 1.95	2.00			
100	6	100.85 ± 2.25	2.23			
50	6	48.65 ± 1.10	2.26	3	48.25 ± 1.15	2.38
50	6	51.78 ± 1.74	3.36			
50	6	49.55 ± 0.80	1.61			
25	6	25.48 ± 0.92	3.61	3	25.15 ± 0.75	2.98
25	6	24.95 ± 0.85	3.40			
25	6	25.75 ± 0.82	3.18			

Table 2
Intra- and interday precision of proposed HPTLC method

Intraday precision				Interday precision		
Conc. (μg/ml)	*n*	Found mean conc. ± S.D. (μg/ml)	RSD (%)	*n*	Found mean conc. ± S.D. (μg/ml)	RSD (%)
100	6	101.11 ± 2.45	2.42	3	100.25 ± 2.60	2.59
100	6	99.45 ± 1.86	1.87			
100	6	100.85 ± 2.40	2.37			
50	6	49.65 ± 1.25	2.26	3	49.25 ± 1.45	2.94
50	6	50.78 ± 1.45	2.51			
50	6	50.55 ± 0.95	1.87			
25	6	25.48 ± 0.98	3.84	3	25.11 ± 0.75	2.98
25	6	26.10 ± 0.75	2.87			
25	6	25.22 ± 0.65	2.57			

Table 3
Intra- and interday precision of proposed specrtophotometric method

Intraday precision				Interday precision		
Conc. (μg/ml)	*n*	Found mean conc. ± S.D. (μg/ml)	RSD (%)	*n*	Found mean conc. ± S.D. (μg/ml)	RSD (%)
100	6	100.45 ± 1.01	1.00	3	100.12 ± 1.10	1.09
100	6	100.45 ± 0.95	0.94			
100	6	99.95 ± 0.95	0.09			
50	6	50.12 ± 0.84	1.67	3	50.48 ± 0.95	1.88
50	6	50.45 ± 1.21	2.39			
50	6	49.47 ± 1.22	2.46			
25	6	25.40 ± 0.45	1.77	3	25.15 ± 0.65	2.58
25	6	25.06 ± 0.45	1.79			
25	6	25.10 ± 0.55	2.19			

Table 4
Degradation study in acidic and alkaline medium[a]

Percent of initial drug content*							
Time (h)	0.1 N HCl			Time (day(s))	0.1 N NaOH		
	A	B	C		A	B	C
0	100	100	100	0	100	100	100
6	68.90	72.95	67.15	14	70.90	72.65	67.45
24	37.00	39.65	35.66	28	55.70	52.63	57.84
30	28.90	26.11	27.11	42	45.50	47.89	42.63
48	18.30	19.55	20.35	56	39.10	37.25	40.62
54	13.70	12.44	15.48	70	29.87	32.75	27.59
				84	26.40	25.15	24.22
				96	24.00	21.77	21.62
				112	20.90	15.42	17.95

[a] (A) HPLC; (B) HPTLC; and (C) spectrophotometric method.
* Mean of three results. Insignificant difference in methods (F-test-one way; $P > 0.05$).

2.5.2. Photodegradation

Drug stock solution (25 ml) in methanol taken in a volumetric flask was exposed to sunlight for 8 h. The degraded drug solutions were suitably diluted with methanol and resolution between degraded products and pure drug was achieved using HPLC, HPTLC and spectrophotometer at standard conditions.

2.6. Isolation of the degradation product

Nitrendipine was degraded in 0.1 M hydrochloric acid, refluxing on water bath and extracted in chloroform. The solvent was removed by distillation and pure degradation product obtained was used for UV, IR and ^{1}H-NMR study.

2.7. Degradation kinetic study in acidic and alkaline medium

Stock solution (25 ml) of drug in methanol was mixed with 0.1 M hydrochloric acid and 0.1 M sodium hydroxide (12.5 ml) separately and the solutions were kept at 100°C. The samples (50 μl) were withdrawn at regular time intervals, neutralized and estimated by HPLC and HPTLC methods by one point standardization using external standard.

Extraction of neutralized solution with chloroform and its estimation within linearity range was then carried out on spectrophotometer. The experiment was carried out in triplicate. Degradation kinetics was studied for reaction rate constant and half-life. The methods were then compared for percentage initial drug contains remained with respect to time.

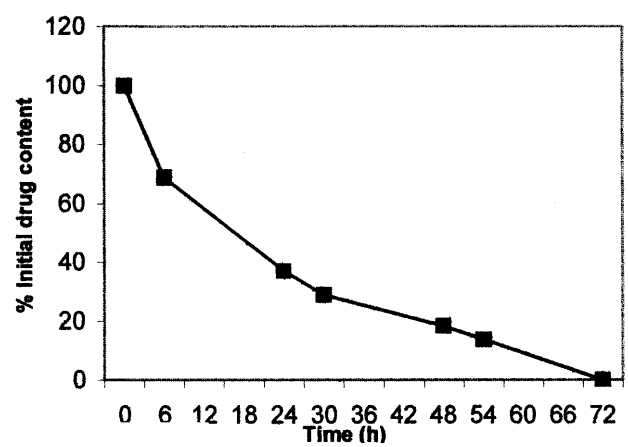

Fig. 6. Degradation in nitrendipine in 0.1 N HCl.

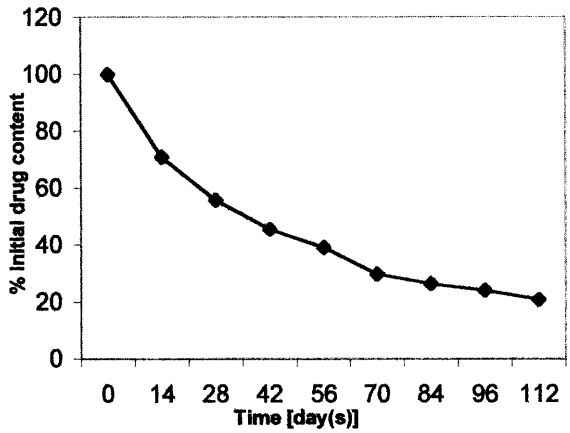

Fig. 7. Degradation in nitrendipine in 0.1 N NaOH.

Table 5
Reaction rate constant (k_{obs}) and half life $t_{1/2}$ in degradation in acidic and alkaline medium[a]

Medium	Acidic[b]			Alkaline[c]		
	A	B	C	A	B	C
k_{obs}	0.034	0.047	0.032	0.013	0.015	0.014
$t_{1/2}$	53.30	46.20	49.50	20.38	14.74	21.65

[a] (A) HPLC; (B) HPTLC; and (C) spectrophotometric method. Insignificant difference in methods (F-test, one way; $P > 0.05$).
[b] k_{obs} (h^{-1}), $t_{1/2}$ (h).
[c] k_{obs} (day(s)$^{-1}$), $t_{1/2}$ (day(s)).

2.8. Photodegradation kinetic study in different solvents

Drug solution in methanol, acetone, ethyl acetate, chloroform and dichloromethane (500 μg/ml) were placed on a wooden plank and kept on a terrace, exposed to sunlight from 08:00 to 17:00 h for 2 days at ≈30°C. Samples (2 ml) were withdrawn at regular time intervals and were suitably diluted with respective solvent and analyzed within the linearity range using external standard by methods mentioned. Degradation kinetics was studied for reaction rate constant, half-life in different solvents and the methods were compared for percentage initial drug contains remained. The experiment was performed in triplicate in each solvent.

3. Results and discussion

Three different analytical assays (HPLC, HPTLC and spectrophotometric) were developed aimed at the selective quantitation of nitrendipine in the presence of its degradation products. The stability indicating capability of the assays was proved using sample solution subjected to forced degradation by exposing them to natural sunlight, alkaline and acidic medium.

The resulting chromatograms and UV spectrum under stressed condition are shown in the figures, where the degradation products are well resolved from nitrendipine. In HPLC, major and minor degradation product in acidic and alkaline degradation was obtained at 3.88, 1.85 and 4.93 min, respectively (Fig. 1). In photodegradation, only major degradation product was found at 3.88 min (Fig. 2). Nitrendipine was observed at 5.94 min.

In HPTLC, major and minor degradation product in acidic and alkaline degradation was obtained at R_f 0.78, 0.2 and 0.5, respectively. In photodegradation, the only major degradation product was found at R_f 0.78. Nitrendipine was observed at R_f 0.68 (Fig. 3).

UV spectrum of nitrendipine in the mentioned solvent showed absorption peaks at 235 and 352 nm, whereas the peak displayed by the degradation product was only at 260 nm (Fig. 4). So diminution in the absorption value at 355 nm was correlated for a decrease in the content of nitrendipine.

In the IR spectrum (KBr) of the degradation product, the strong absorption signal observed at 1735 cm^{-1} corresponded to the presence of the ester carbonyl. In the ^{1}H-NMR spectrum ($CDCl_3$) of the compound, the quartet at δ 4.30 and triplet at δ 1.29 belonged to the protons of the methylene and the methyl group of the ethyl ester, respectively. The protons of the methyl ester displayed a resonance at δ 3.6 as a singlet of three protons. This confirmed that ester group is not hydrolyzed. The hydrogens of the two methyl groups at C2 and C6 on the pyridine ring occurred at δ 1.8 and 1.9 as sharp singlet. Proton signal due to secondary amino group at δ 5.7 and due to proton at C4 at δ 5.1 was not observed in the degraded compound.

Thus, oxidation was observed to be a major route of degradation (Fig. 5) with the UV, IR and ^{1}H-NMR studies. These observations are in agreement with the work of Baloglu et al. [4], who already confirmed dehydronitrendipine as a main degradation product using mass spectrometry.

The methods were validated by the evaluation of intra- and inter-day precision linearity. Drug

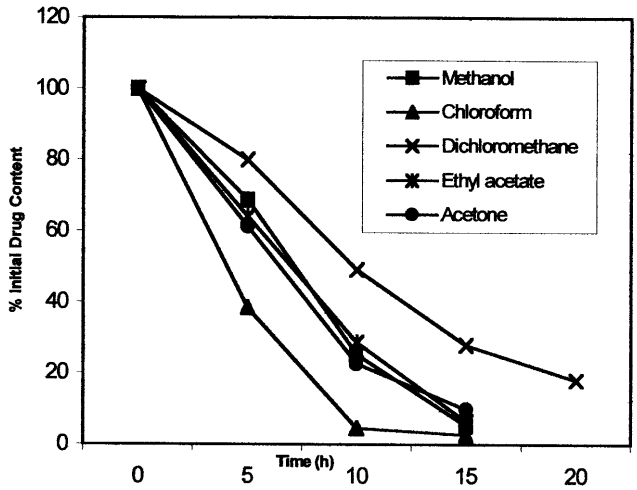

Fig. 8. Photodegradation of nitrendipine in organic solvents.

Table 6
Photodegradation study in sunlight

Time (h)	Method	Percent of initial drug content*				
		Solvents				
		1	2	3	4	5
0	A	100	100	100	100	100
	B	100	100	100	100	100
	C	100	100	100	100	100
5	A	68.74	38.38	80.00	64.19	61.27
	B	67.25	37.12	78.45	66.65	63.55
	C	69.45	39.74	79.85	65.95	64.25
10	A	25.32	4.44	49.10	28.69	22.70
	B	23.74	2.95	50.12	29.84	23.69
	C	26.45	5.45	52.62	30.74	24.96
15	A	5.34	2.64	28.00	6.33	9.71
	B	6.85	2.52	29.64	8.74	10.87
	C	7.85	2.74	31.84	9.74	9.84
20	A	–	–	17.92	–	–
	B	–	–	16.52	–	–
	C	–	–	18.55	–	–

* Mean of three results. Insignificant difference in methods (F-test-one way; $P>0.05$). (A) HPLC; (B) HPTLC; and (C) spectrophotometric method. (1) Methanol; (2) chloroform; (3) dichloromethane; (4) acetone; and (5) ethyl acetate.

Table 7
Reaction rate constant (k_{obs}) and half life $t_{1/2}$ in photodegradation study[a]

Solvent	k_{obs} (h^{-1})			$t_{1/2}$ (h)		
	A	B	C	A	B	C
1	0.19	0.18	0.17	3.53	3.81	4.02
2	0.26	0.27	0.25	2.65	2.55	2.71
3	0.08	0.08	0.07	8.04	8.45	8.99
4	0.18	0.16	0.15	3.81	4.26	4.47
5	0.15	0.15	0.15	4.33	4.53	4.38

[a] Insignificant difference in methods (F-test-one way; $P > 0.05$). (A) HPLC; (B) HPTLC; and (C) Spectrophotometric method. (1) Methanol; (2) chloroform; (3) dichloromethane; (4) acetone; and (5) ethyl acetate.

solution in methanol was used for this study. The relative S.D. (RSD) of the used HPLC method (Table 1) on the basis of peak area for six replicate injections were found to be between 2.00 and 2.47 (100 μg/ml), 1.61 and 3.36 (50 μg/ml), 3.18 and 3.61 (25 μg/ml) in the intra-day assay. The RSD in the interday-assay (3 days, $n = 6$) was 2.68 for 100 μg/ml, 2.38 for 50 μg/ml and 2.98 for 25 μg/ml. The RSD of the HPTLC method (Table 2) on the basis of quantitative results by external standard for six replicate spotting were found to be between 1.87 and 2.42 (100 μg/ml), 1.87 and 2.51 (50 μg/ml), 2.57 and 3.84 (25 μg/ml) in the intra-day assay. The RSD in the interday-assay (3 days, $n = 6$) was 2.59 for 100 μg/ml, 2.94 for 50 μg/ml and 2.98 for 25 μg/ml. The RSD of the spectrophotometric method (Table 3) on the basis of quantitative results by external standard for six replicate absorption value were found to be between 0.09 and 1.00 (100 μg/ml), 1.67 and 2.46 (50 μg/ml), 1.77 and 2.19 (25 μg/ml) in the intra-day assay. The RSD in the interday-assay (3 days, $n = 6$) was 1.09 for 100 μg/ml, 1.88 for 50 μg/ml and 2.58 for 25 μg/ml. Linearity was observed between 5 and 50 μg/ml in HPLC, 50 and 1000 ng in HPTLC and 10 and 50 μg/ml in spectrophotometric method, irrespective of solvents. LOQ was found to be 5.0 μg/ml, 100 ng, 10 μg/ml in HPLC, HPTLC and UV spectrometry, respectively.

In acid and alkaline medium, a decrease of nitrendipine versus time, in 0.1 M hydrochloric acid and 0.1 M sodium hydroxide at 100°C are shown in Table 4. Figs. 6 and 7 show the degradation curve of nitrendipine in acidic and alkaline medium, respectively at 100°C. The decomposition followed first-order kinetics. The reaction rate constants (k_{obs}) and chemical half-life ($t_{1/2}$), calculated by linear regression analysis are shown in Table 5. The reaction rate constant (k_{obs}) and half life ($t_{1/2}$) in acidic degradation determined by HPLC, HPTLC and spectrophotometric methods were found to be 0.034, 0.04, 0.032 $h^{-1}$and 53.30, 46.20 and 49.50 h, respectively. The reaction rate constant (k_{obs}) and half-life ($t_{1/2}$) in alkaline degradation determined by HPLC, HPTLC and spectrophotometric methods were found to be 0.013, 0.015 and 0.014 $(day(s)^{-1})$ and 20.38, 14.74 and 21.65 (day(s)), respectively. The results of the three different analytical methods correspond well statistically (F-test; one way).

In the photodegradation study, a solution of the drug in different solvents was kept in sunlight, as mentioned in Section 2.8. The experiment was performed in triplicate in each solvent. Degradation rate of nitrendipine in solvent was found in the order of chlorofom > ethyl acetate ≅ acetone ≅ methanol > dichloromethane, as shown in Fig. 8. Decrease drug content versus time, degradation rate constant and chemical half-life calculated in different solvents by the method mentioned, is reported in Tables 6 and 7. The results obtained by the methods observed, very close to each other.

4. Conclusion

The three proposed methods for selective quantitation of nitrendipine in the presence of its degradation products proved to be a suitable comparison between those methods applied to the same problem to evaluate their usefulness in degradation kinetic study. HPLC, the leading method having significant official status in drug analysis was found to be simple and efficient compared to other methods. HPTLC method of analysis was found to be sensitive and robust. Spectrophotometric method, though found to be easiest and inexpensive, is less sensitive and hav-

ing insignificant official status in pharmacopoeia compare to HPLC and HPTLC methods.

Major route of degradation was observed to be oxidation and found faster in acidic condition. The photodegradation of nitrendipine was found to be dependant on the solvent system used, which is proved by the quantitative results. Drug was found to be less stable in chloroform and relatively more stable in dichloromethane. Further investigations, like the effect of temperature, ion strength and concentration on stability of nitrendipine will be undertaken.

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