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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPTLC METHOD FOR DETERMINATION OF NISOLDIPINE (NISO) IN TABLET DOSAGE FORM

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ABSTRACT
A HPTLC method for the estimation of Nisoldipine (NISO) was developed using pre-coated silica gel F_{254} plates with chloroform: glacial acetic acid: methanol (8.5: 1:0.5 v/v) as mobile phase. The optimum wavelength for detection and quantitation was 227 nm. The retention factor was found to be 0.29 ± 0.008. NISO was subjected to stress test conditions like acid, alkali, neutral, oxidative, photolytic and thermal degradation. Amount of drug remaining after degradation study was observed 92.43, 82.66, 89.62, 86.72, 65.48 and 99.68% in acid, alkali, neutral, oxidative, photolytic and thermal degradation condition respectively. The method was validated for parameter such as linearity, precision, accuracy, range, specificity and robustness. Limit of detection and limit of quantification was found to be 20.60 ng and 62.42 ng respectively. The parentage recovery was found to be 100.56%. The linearity was established in the range of 100-1000 µg/ml with r^2 0.996. This HPTLC method is sensitive, less time consuming than other chromatographic procedures.

KEYWORDS: Nisoldipine, HPTLC, method development, validation, degradation studies.

INTRODUCTION
Nisoldipine (NISO), (±) 3-isobutyl-5-methyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5dicarboxylate, is a second generation of dihydropyridine calcium antagonist which has a selective arteriolar vasodilatation but shows negligible effects on the other vessels and myocardium.[1] NISO is a yellow crystalline substance, practically insoluble in water but soluble in methanol. It has a molecular weight of 388.4 g/mol. It is used in the management of hypertension and angina pectoris.[2] The structural of NISO is show in figure 1.

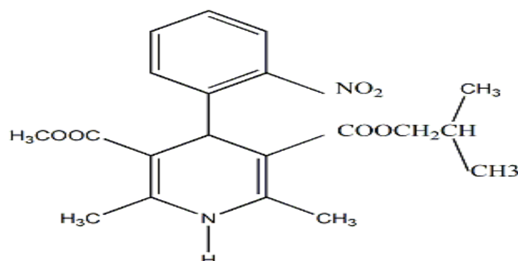

Figure 1: Chemical structure of Nisoldipine.

Many analytical methods have been published for the determination of NISO, including the determination in formulations by voltammetry[3,6], polarography[5,7] and HPLC.[8,12] Since NISO has light sensitivity, its stability, kinetics of degradation and determination of impurities are reported by various techniques including UV[13] and HPLC.[14,15] According to literature survey no stability indicating HPTLC method has yet been reported for determination of Nisoldipine in tablet dosage form by using methanol as solvent and Chloroform: Glacial acetic acid: Methanol (8.5: 1:0.5 v/v) as a mobile phase.

The objective of work is to develop a simple, precise, accurate stability indicating method for the estimation of NISO in formulation by using HPTLC.

MATERIALS AND METHODS
Nisoldipine (NISO) was obtained as a gift samples from Emcure Pharmaceuticals Ltd. Pune, India. Methanol, Toluene, Chloroform, Glacial acetic acid, Distilled Water, HCl, NaOH, 30% H_2O_2 all chemicals are AR Grade was purchased from Merck Specialties Private Limited.

Camag HPTLC system, Camag Linomat V sample applicator, Camag TLC Scanner 3, Win CATS software Version- 1.4.2, Pre-coated silica Gel F254 Merck TLC plates and Hamilton syringe of 100 µl.

HPTLC method and chromatographic conditions
TLC plates were pre-washed with methanol. Activation was done in oven at 105ºC for 20 min. The plates were allowed to cool at room temperature. The chromatographic estimations were performed using following:

Solvent used : Methanol
Stationary phase : TLC plate precoated with silica gel F_{254}
Mobile phase : Chloroform: Glacial acetic acid: Methanol (8.5: 1:0.5 v/v)
Chamber saturation time : 15 min
Development time : 20 min
Detection wavelength : 227 nm

Preparation of Standard Stock Solutions
Standard stock solution of NISO was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1 mg/ml from which 1 ml was further diluted to 10 ml with methanol to get stock solution of 100 ng/µl.

Selection of Mobile Phase and Chromatographic Conditions
Chromatographic studies were carried out on the standard stock solution of NISO. Different mobile phases containing various ratios of Toluene, Methanol, Chloroform and Glacial acetic acid were examined. Finally the mobile phase containing Chloroform: Glacial acetic acid: Methanol (8.5: 1:0.5 v/v) was selected as optimal for obtaining well defined and resolved peaks. Other chromatographic conditions like chamber saturation time, run length, sample application rate and volume, sample application positions, distance between tracks, detection wavelength, were optimized to give reproducible R_f values, better resolution, and symmetrical peak shape for the drug.

Selection of Analytical Wavelength
From the standard stock solution further dilutions were done using methanol and scanned over the range of 200-400 nm and the spectra was obtained.

Analysis of Tablet Formulation
Twenty tablets (Brand Name – Sular. Label Cliam - Each film coated tablet contains 8.5 mg of NISO) were weighed accurately and finely powdered. A quantity of powder equivalent to 10 mg of NISO was weighed and transferred to a 10 ml volumetric flask containing about 5 ml of methanol, shaken for 5 min and volume was made up to the mark with the methanol. The solution was filtered using Whatman paper No. 41. From the filtrate 1 ml was further diluted to 10 ml with methanol to get sample stock solution of NISO 100 ng/µl. From sample stock solution 2 µl volume was applied on HPTLC plate to obtain final concentration of 200 ng/band. After chromatographic development peak areas of the bands were measured at 227 nm and concentration of drug in the sample was estimated from the respective calibration curves. Procedure was repeated six times for the analysis of homogenous sample.

Evaluation of Analytical Method (Method Validation)
Linearity & Range
The standard stock solution of NISO (100 ng/µl) was applied on HPTLC plate in range of 1-10 µl with the help of CAMAG 100 µL sample syringe, using Linomat 5 sample applicator to obtain final concentration 100-1000 ng/band. The plate was developed and scanned under above established chromatographic conditions. Each standard in five replicates was analyzed and peak areas were recorded. A calibration curve of NISO was plotted of peak area Vs concentration.

Intra-day and inter-day precision
The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra day studies, 3 replicates of standard solution (200, 400 and 600 ng/band) were analyzed in a day and percentage RSD was calculated. For the inter day variation studies, 3 replicates of standard solutions (200, 400 and 600 ng/band) were analyzed on 3 consecutive days and percentage RSD were calculated.

Accuracy
To check the accuracy of the method, recovery studies were carried out by overspotting standard drug solution to pre-analyzed sample solution at three different levels 50, 100 and 150%. Basic concentration of sample chosen was 200 ng/band. The areas were noted after development of plate. The drug concentration was calculated by using regression equations.

Detection Limit (DL)
DL was calculated from the formula: -

$$DL = 3.3\ \sigma/S$$

Where
σ = Standard deviation of the response (y- intercept)
S = Slope of the calibration curve

Quantification Limit (QL)
QL was calculated from the formula: -

$$QL = 10\ \sigma/S$$

Where
σ = Standard deviation of the response (y- intercept)
S = Slope of the calibration curve

Specificity
The specificity of the method was ascertained by analyzing standard drug and sample. The spots for both drugs in sample were confirmed by comparing the Rf and spectra of the spots with that of standard drug spots.

Robustness
The robustness of the method was studied, during method development, by small but deliberate variations in chamber saturation period (± 10%), time from

application to development (0, 10, 20, 30 min) and time from development to scanning (0, 30, 60, 90 min). One factor at a time was changed at a concentration level of 200 ng/band to study the effect on the peak area of the drugs.

DEGRADATION STUDIES

Acidic condition

For acid decomposition studies, 1 ml of pure drug solution (concentration 1 mg/ml) was mixed with 1 ml of 1 M Methanolic HCl and volume was made up to 10 ml with methanol and solution was refluxed for 2 hr. 10 µl neutralized volume was applied on TLC plate to obtain the chromatogram.

Alkaline condition

For alkali decomposition studies, 1 ml of pure drug solution (concentration 1 mg/ml) was mixed with 1 ml of 1 M Methanolic NaOH and volume was made up to 10 ml with water and solution was refluxed 2 hr. 10 µl neutralized volume was applied on TLC plate to obtain the chromatogram.

Neutral conditions

For neutral decomposition studies, 1 ml of pure drug solution (concentration 1 mg/ml) was mixed with 9 ml of AR grade water and solution was kept for 2 hr. 10 µl neutralized volume was applied on TLC plate to obtain the chromatogram.

Oxidative conditions

For oxidation decomposition studies, 1 ml of pure drug solution (1 mg/ml) was mixed with 1 ml of 30% H_2O_2, volume was made up to 10 ml with water and solution was kept for 2 hr. 10 µl neutralized volume was applied on TLC plate to obtain the chromatogram.

Photolytic conditions

Photodegradation was performed by spreading the drug substance in petri dish as thin film and kept in photo stability chamber equipped with ultraviolet light with energy of not less than 200 Watt hours/Square meter and fluorescence light illumination not less than 1.2 million lux hours. Suitable controls were kept in dark for comparison for the same period. From drug exposed; solution of 1 mg/ml was prepared in methanol and from resulting stock solution 1 ml was further diluted to 10 ml with methanol. 10 µl volume of this solution was applied on TLC plate to obtain the chromatogram.

Thermal Degradation

Dry heat studies were performed by keeping drug sample in oven (60^0 C) for a period of 2 hr. From drug exposed to dry heat condition; solution of 1 mg/ml was prepared in methanol and from resulting stock solution 1 ml of solution was further diluted to 10 ml with methanol. 10 µl volume of this solution was applied on TLC plate to obtain the chromatogram. Drug was found to be stable for above mentioned condition.

RESULTS AND DISCUSSION

It was observed that drug showed considerable absorbance at 227 nm. The UV spectrum of the drug is shown in figure 2.

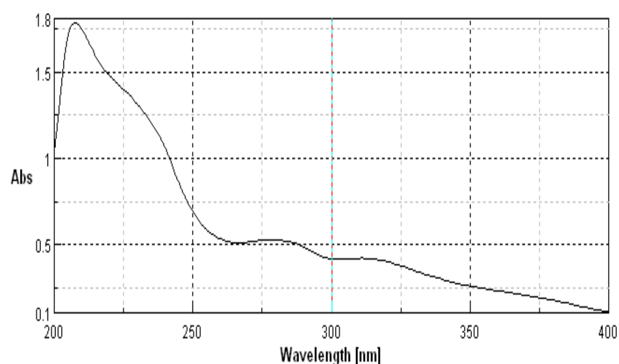

Figure 2: In situ spectrum of NISO measured from 200 to 400 nm.

The retention factor of NISO was found to be 0.29 ± 0.008. Representative densitogram of standard solution of NISO is shown in figure 3.

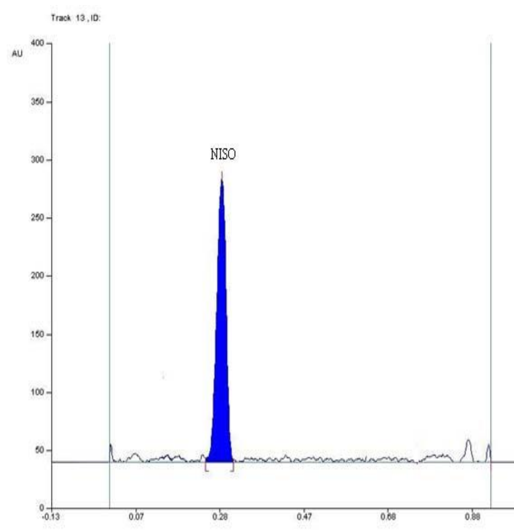

Figure 3: Standard densitogram of NISO (200 ng/band, $R_f = 0.29 \pm 0.008$).

Linear response was observed in the concentration range 100-1000 ng/band with high correlation coefficient. The proposed method was also evaluated by the assay of commercially available tablet formulation contains 8.5 mg of NISO. The % drug content (Mean ± S.D.) was found to be 98.79±0.758. The results obtained are shown in table 1.

Table 1: Analysis of Tablet Formulation.

Drug	Label Claim (mg/tablet)	% of Label Claim	SD*	% RSD*
NISO	8.5	98.796	0.758	0.767

* *Average of six determinations.*

The linearity was established in the range of 100-1000 µg/ml with regression coefficient equation is y=8.498x + 723.8 (r^2 0.996). The result obtained is shown in table 2 and calibration curve shown in figure 4.

Table 2: Observation Table for Calibration Curve of NISO (n = 5).

Sr. No.	Concentration (ng/band)	Peak Area*
1	100	1399.004
2	200	2361.98
3	400	4307.96
4	600	6021.84
5	800	7604.34
6	1000	8993.98

**Avarare of five determinations.*

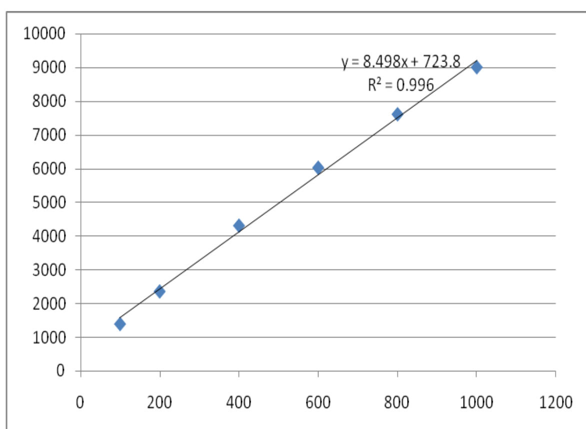

Figure 4: Calibration Curve for NISO.

For Intra-day, the % RSD was found to be 0.956. For Inter-day, the % RSD was found to be in the range of 1.879. The results obtained for Intraday and Inter day variations are shown in table 3.

Table 3: Statistical Validation of Intra-day and Inter-day Precision Studies.

Precision	% of Label Claim	SD	% RSD
Intraday (n=3×3)	99.35	0.949	0.956
Interday (n= 3×3)	100.82	1.894	1.879

The percentage mean recovery was found to be 100.56±0.518. The results obtained are shown in table 4.

Table 4: Recovery Studies of NISO.

Drug	Amount taken (ng/band)	Amount added (ng/band)	Total amount (ng/band)	% Recovery*	% RSD*
CA	200	100	300	99.45	0.541
	200	200	400	100.42	0.536
	200	300	500	101.83	0.479
	200	100	300	99.45	0.541
Mean				100.56	0.518

**Average of three determinations.*

The LOD of NISO was found to be 20.60 ng/band and the LOQ was found to be 62.42 ng/band. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% R.S.D. < 2). The results obtained for robustness of method are shown in table 5.

Table 5: Robustness Data in Terms of % RSD of Retention Factor.

Sr.No.	Parameters	Variation	% RSD*
1.	Chamber saturation period	± 10 %	1.088
2.	Time from application to development	0, 30, 60 min	0.318
3.	Time from development to scanning	0, 30, 60 min	0.770

**Average of three determinations.*

In acid degradation studies drug showed 7.57% of degradation with additional degradation peak (D1) at Rf 0.19 show in figure 5.

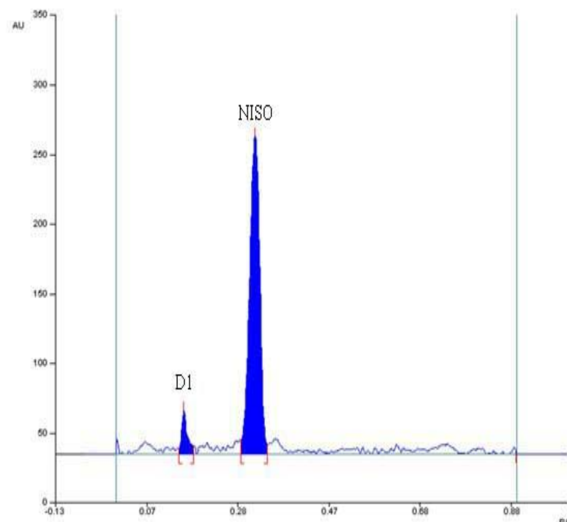

Figure 5: Chromatogram showing the separation of different degradation products of NISO obtained under acidic condition.

In base degradation studies 17.34% of degradation was observed for drug with degradation product (D1) at Rf 0.20 show in figure 6.

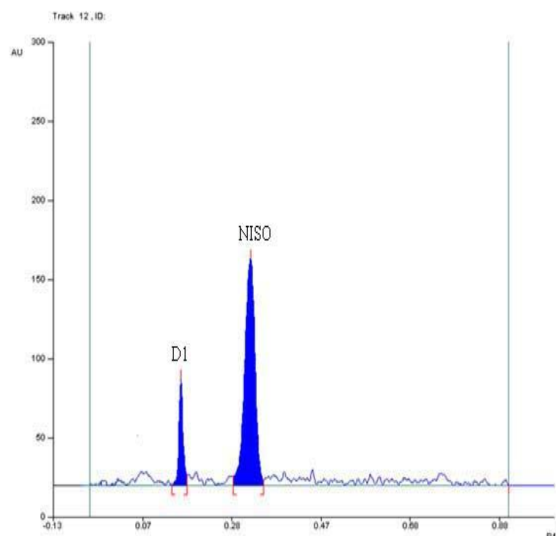

Figure 6: Chromatogram showing the separation of different degradation products of NISO obtained under alkaline condition.

In neutral hydrolysis, 89.62% of NISO was recovered with appearance of degradation product (D2) at Rf 0.63 show in figure 7.

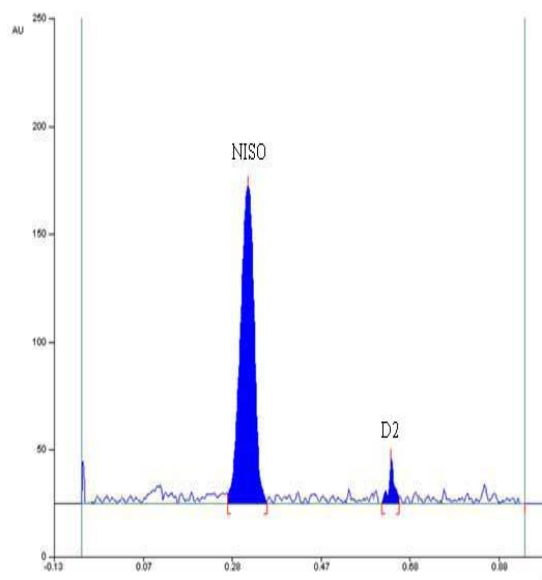

Figure 7: Chromatogram showing the separation of different degradation products of NISO obtained under neutral condition.

In the oxidative condition 13.28% degradation was observed for drug with appearance of degradation product (D3) at Rf 0.89 shown in figure 8.

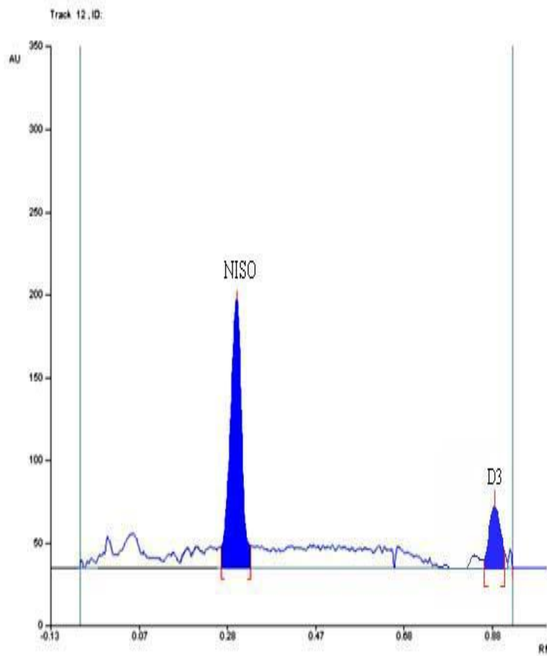

Figure 8: Chromatogram showing the separation of different degradation products of NISO obtained under oxidative condition.

In photo-degradation studies after exposing to UV light, 34.52% of degradation was observed. The peak area was found to be reduced with appearance of degradation product (D4 & D5) at Rf 0.13 and 0.59 respectively shown in figure 9.

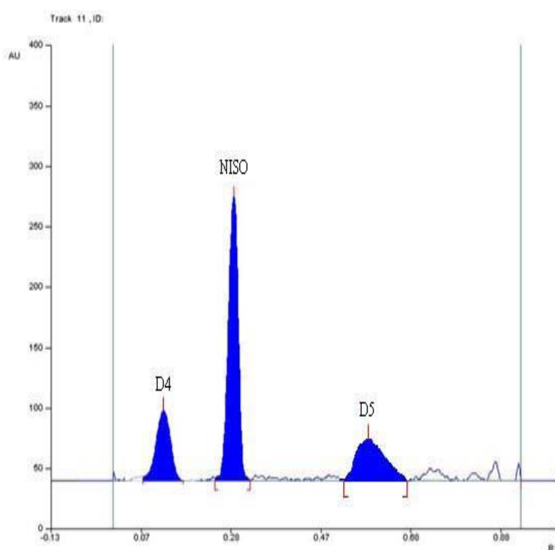

Figure 9: Chromatogram showing the separation of different degradation products of NISO obtained under Photolytic condition.

In dry heat 99.68% of NISO was recovered without appearance of degradation product show in figure 10. In this condition Drug was found to be stable.

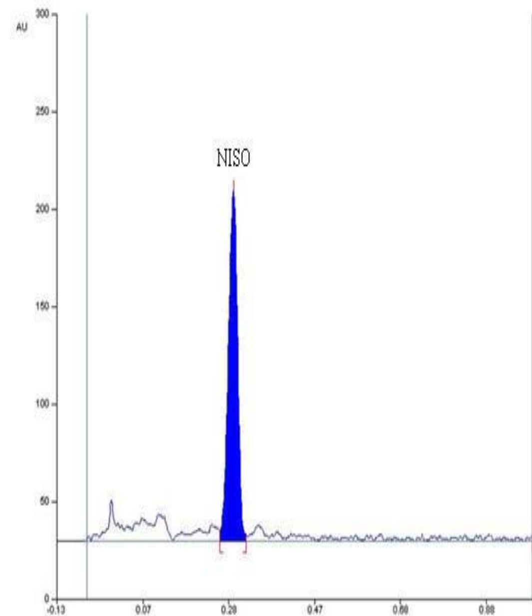

Figure 10: Chromatogram showing degradation of NISO obtained under Dry heat condition.

The summery of forced degradation is shown in table 6.

Table 6: Data of Forced Degradation Studies.

Stress conditions	NISO		
	% Assay of active Substance	% Degradation	Rf values of degraded products
Acid	92.43	7.57	0.19
Alkali	82.66	17.34	0.20
Neutral	89.62	10.38	0.63
Oxidative	86.72	13.28	0.89
Photolysis	65.48	34.52	0.13 & 0.59
Dry heat	99.68	0.32	---

CONCLUSION

The developed HPTLC method is simple, accurate, precise, specific and stability indicating one. The method can be used to determine the purity of NISO drug by detecting the relative impurities. As the method could effectively separate the NISO from the degradation products; therefore, it can be used as stability indicating one.

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