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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY METHOD FOR DETERMINATION OF NIFEDIPINE IN TABLET DOSAGE FORM

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ABSTRACT

A simple and sensitive, HPTLC method has been developed for the quantitative estimation of Nifedipine in it's single component tablet formulation. The separation was carried out on Merck aluminium plates precoated with silica Gel 60 F_{254} using n-hexanel:ethyl acetate in the ratio of 6:4: (v/v) as mobile phase. Nifedipine showed R_f value of 0.59±0.027 and was scanned at 254nm using Camag TLC Scanner3. The linear regression data for the calibration plot showed a good relationship with r=0.9892. The method was validated for precision and recovery. The limits of detection and quantification were 20.60 and 40.42 ng/spot respectively. The developed method was successfully used for the assay of nifedipine tablet formulations. The photo degraded product of Nifedipine formed was investigated by Liquid Chromatography mass spectrometer. The method is simple, sensitive and precise; it can be used for the routine quality control testing of marketed formulations.

KEYWORDS: Pharmaceutical analysis, Thin layer chromatography, Antihypertensive drug, Nifedipine.

INTRODUCTION

Nifedipine a calcium channel blocker is one of the most widely used coronary vasodilators and dihydropyridine derivative that is chemically described as (3, 5-pyridinedicarboxylic acid, 1, 4dihydro-2, 6-dimethyl-4-(2-nitrophenyl)-dimethyl ester. Its molecular formula is $C1_7H_{18}N_2O_6$. Nifedipine is used in the management of hypertension, in the prophylaxis of angina pectoris, and in the treatment of other vascular and non-vascular diseases.[1] Calcium

is necessary for muscle cells to contract. Nifedipine prevents calcium from being released within the muscle cells of the small arteries and thereby causes the muscles to relax and the arteries to dilate or expand. Dilation of arteries reduces blood pressure.[2,6]

There is a need for a simple, rapid, cost effective and reproducible method for assay of NFE in its dosage forms. A literature survey revealed that several HPLC[7,12], SFC[13] and HPTLC[14] in combination reported but no HPTLC method reported for stability study of FEP. Therefore, it was thought of interest to develop simple, rapid, accurate, specific and precise HPTLC method for the analysis of felodipine (FEP) in its tablet formulation. The objective of the current work is, therefore, to develop a simple HPTLC method for analysis of FEP in tablet formulations.

MATERIAL AND METHODS

Nifedipine (FEL) procured from Zidus Cadila Ahemdabad as Gift sample. The pharmaceutical dosage form used in study was a Calcigard tablet labelled to contain 10mg of NIF was obtained from local retail pharmacy. Other solvents are obtained from local market.

Instrumentation

The HPTLC system consisted of a Camag Linomat 5 semi-automatic spotting device (Camag, Muttenz, Switzerland), a Camag twin-trough chamber (10 cm × 10 cm), Camag win CAT Software 1.4.4.6337 and a 100 µl Hamilton syringe. Sample application was done on pre-coated silica gel 60 F254 TLC plates (10 cm × 10 cm). TLC plates were pre-washed with methanol and activated at 80°C for 5 min prior to the sample application. Densitometric analysis was carried out utilizing Camag TLC scanner 3.

Preparation of standard solutions

A stock solution of NIF was prepared by dissolving 10 mg in 10 ml methanol (1000 µg/ml). Further standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration range 10 µg/ml.

Selection of Detection Wavelength

From the standard stock solution further dilutions were done using methanol and scanned over the range of 200- 400 nm. The maximum absorbance showed at 254 nm.

Sample Preparation

Twenty tablets weighed accurately and powered. A quantity of powder equivalent to 10 mg average weight was calculated. The tablets were then powdered and an amount equivalent to one tablet was dissolved in 10 ml methanol. To ensure complete extraction of the drug it was sonicated for 45min. This solution was filtered through a Whatman no. 41 paper.

Selection of Mobile Phase in chromatographic studies

Selection of Mobile Phase in chromatographic studies was carried out on the standard stock solution of NIF. Different mobile phases containing various ratios of Toluene, Methanol, n-Hexane, Ethyl acetate and Isopropyl alcohol were examined. Finally the mobile phase containing n-hexane : Ethyl acetate (6:4 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.

HPTLC method and Chromatographic condition

In the proposed HPTLC method, the samples were streaked on the pre-coated TLC plates in the form of a narrow band 6 mm in length, 10 mm from the bottom and margin and 10 mm a part at a constant flow rate of 150 nl/s by using a nitrogen aspirator. A Camag Twin Trough Chamber was saturated for 20 min at room temperature (25 ± 2°C) with the mobile phase containing a mixture of n-hexane: Ethyl acetate (6:4 v/v). After chamber saturation, the plates were developed to a distance of 80 mm and then dried in hot air. Densitometric analysis was carried out using a Camag TLC Scanner 3 (Camag) in the absorbance mode at 366 nm for all measurements. The slit dimension was kept at 5.0 mm × 0.45 mm and a scanning speed of 20 mm/s was employed. NIF was detected at Rf of 0.59±0.027. The chromatograms were integrated using win CATS evaluation software (Version 1.1.3.0).

Summary of Chromatographic Parameters Selected

a) Solvent used : Methanol
b) Stationary phase : TLC plate precoated with silica gel F_{254}
c) Mobile phase : n-hexane: Ethyl acetate (6:4 v/v/)
d) Chamber saturation time : 15 min
e) Development time : 20 min
f) Detection wavelength : 254 nm

g) Temperature : Ambient

Method validation

Validation of the method done by carried out with respect to the following parameters.

Linearity and range

The standard stock solution of Nifedipine (100 ng/µl) was prepared in Methanol. Different volumes of stock solution (10,20,30,40,50 and 60) were spotted on the TLC plates using semiautomatic spotter under nitrogen stream to obtain concentration of 100-600 ng/spot of Nifedipine. Each concentration was applied six times on the HPTLC plate. Peak area was recorded for each concentration and a calibration plot was obtained by plotting peak area against concentration.

Limit of Detection and Quantification

The limits of detection (LOD) and quantification (LOQ) were calculated from the slope (s) of the calibration plot and the standard deviation of the response (SD).

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (200, 400 600ng/spot) of the drug six times on the same day. The intermediate precision of the method was checked by repeating studies on two different days.

Accuracy

To check the accuracy of the method, recovery studies were carried out by over spotting 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.

Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for NIF in sample was confirmed by comparing the Rf and spectra of the spot with that of standard. The peak purity of NIF was assessed by comparing the spectra at three different levels, i.e. peak start, peak apex and peak end positions of the spot.

Robustness of the method

The robustness of the method was studied, during method development, by small but deliberate variation in mobile phase composition (±2%) chamber saturation period (±10%), development distance (±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 concentration level of 100 ng/band and 200ng/band, to study the effect on peak area of the drug.

Analysis of marketed formulation

Twenty tablets (Calcigard 10mg) were weighted & average weight calculated, tablets finely powered and the powder equivalent to containing 10 mg of NIF from T-1 and T-2 respectively and dissolved in 10 of 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 NIF 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 254 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.

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 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 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 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 volume was applied on TLC plate to obtain the chromatogram.

Photolytic conditions

Photo degradation 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 12 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.

Wet heat studies were also performed by keeping drug solution (1 mg/ml in methanol) in oven (60^0 C) for a period of 12 hr. After exposure 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.

RESULT AND DISCUSSION

It was observed that drug showed considerable absorbance at 254 nm.

The Rf value of FEL was found to be 0.59 ± 0.027. Densitogram of NIF is shown in Figure 1.

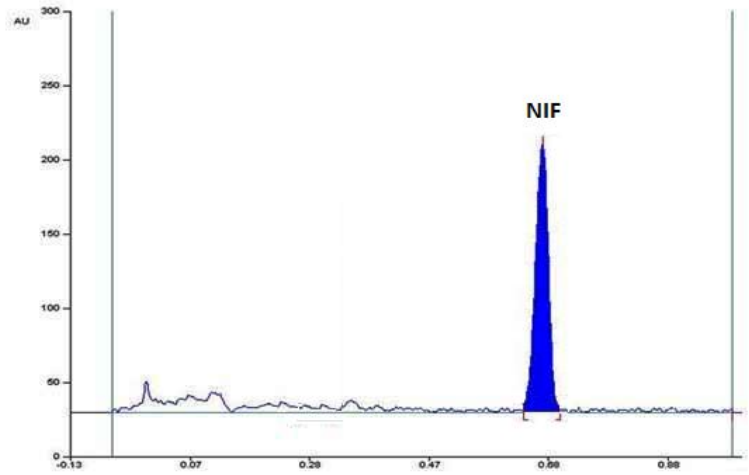

Figure 1: Standard densitogram of NIF (200 ng/band, R_f = 0.59 ± 0.027).

Each standard in five replicates was analyzed and peak areas were recorded. Calibration curves of NIF were plotted of peak area Vs concentration. The result obtained is shown in Table 1. The linearity was established in the range of 100-600 ng/band with regression coefficient equation is y = 3.053x + 668.74 ($R^2$0.989) calibration curve shown in figure 2 and regression analysis of calibration curves shown in table 1.

Table 1: Observation Table for Calibration Curve of NIF (n = 6).

Sr. No.	Concentration (ng/band)	Peak Area*
1	100	800.92
2	200	1283.86
3	300	1788.29
4	400	2235.41
5	500	2762.29
6	600	3343.59

** Avarare of 6 determinations.*

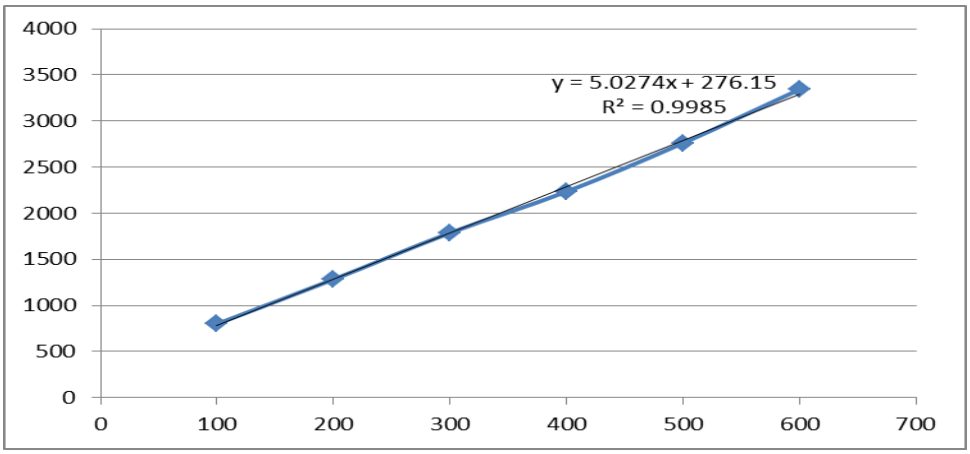

Figure 2: Calibration Curve for NIF.

Table 2: Regression Analysis of Calibration Curves.

Parameter	NIF
Detection Wavelength (nm)	254
Linearity range (ng/band)	100-600
Correlation Coefficient (r)	0.9985
Linear Regression Equation[a] (y = mx + c)	5.0274x + 276.15
Intercept (c)	276.15
Slope (m)	5.027

[a]With respect to y = mx + c, where y is the peak area and x is the concentration (ng/band).

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 Concentration found	SD	% RSD
Intraday (n=3×3)	99.35	0.949	0.956
Interday (n= 3×3)	100.82	1.894	1.879

The results Recovery Studies obtained are shown in Table 4.

Table 4: Recovery Studies of NIF.

Drug	Amount taken (ng per band)	Amount added (ng/band)	Total amount (ng/band)	% Recovery*	% RSD*
NIF	200	100	300	99.35	0.741
	200	200	400	102.42	0.836
	200	300	500	98.83	0.979

**Average of three determinations*

LOD : 20.60 ng/ band

LOQ : 62.42 ng/band

Results of robustness study are presented in Table 5.

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

Sr. No.	Parameters	Variation	% RSD*
1.	Chamber saturation period	± 10%	1.088
2.	Time from application to development	0, 10, 20, 30 min	0.318
3.	Time from development to scanning	0, 30, 60, 90 min	0.770

**Average of three determinations*

The proposed method was evaluated by the assay of commercially available tablet formulation contains 10 mg of NIF. The % drug content (Mean ± S.D.) was found to be 99.796±4.547. The results obtained are shown in table 6.

Table 6: Analysis of Tablet Formulation.

Drug	Label Claim (mg/tablet)	% of Label Claim	SD*	% RSD*
NIF	10	99.796	0.758	0.767

** Average of six determinations*

Degradation Studies

Acidic -Under this condition 1 peak of degradation products were observed.

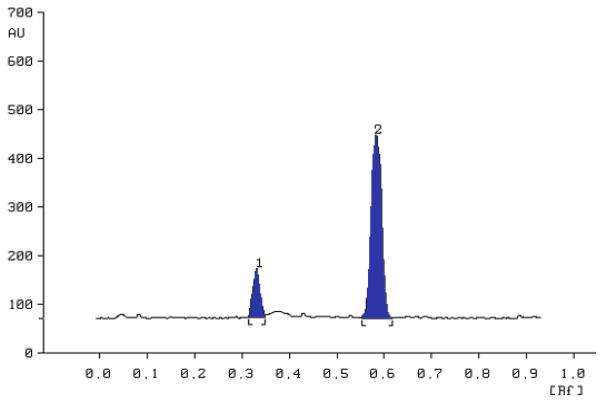

Figure 3: Chromatogram showing the separation of different degradation products of NIF obtained under acidic condition.

Basic - Under this condition 1 peak of degradation product was observed.

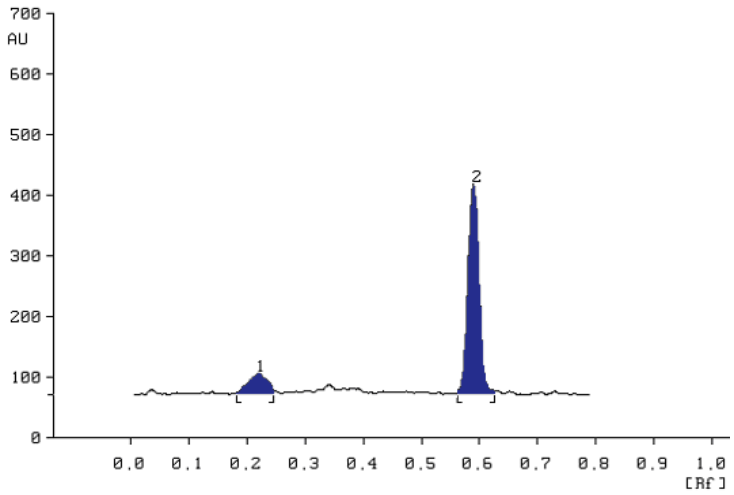

Figure 4: Chromatogram showing the separation of different degradation products of NIF obtained under alkaline condition.

Oxidative condition

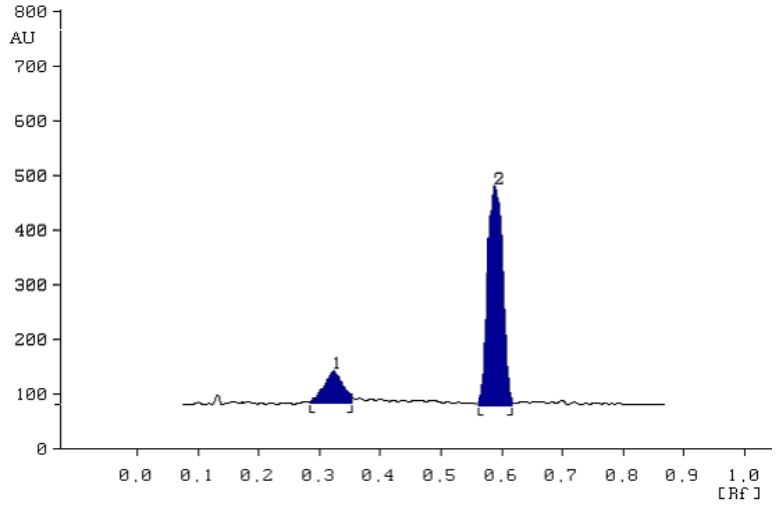

Figure 5: Chromatogram showing the separation of different degradation products of NIF obtained under oxidative condition.

Photolytic condition

For above conditions one additional peak was observed with % of drug 96.46.

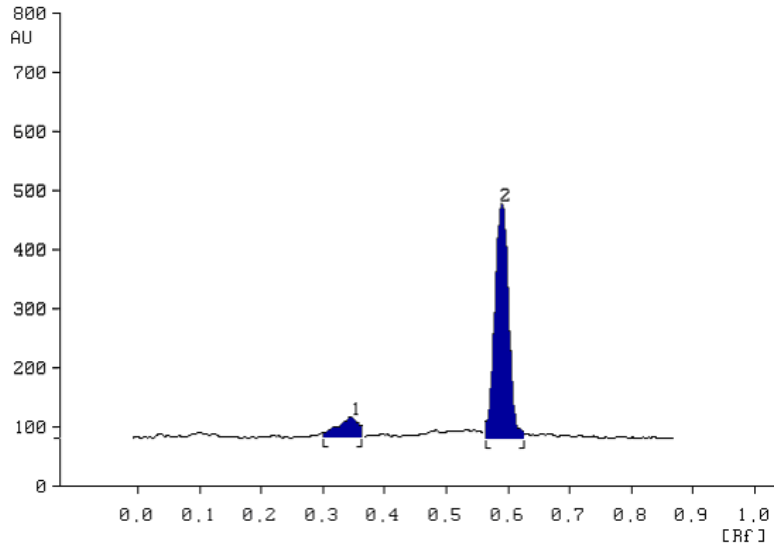

Figure 6: Chromatogram showing the separation of different degradation products of NIF obtained under Photolytic condition.

Table 7: Summary of stress degradation study of NIF.

Sr. No.	Stress Degradation Condition	Percent recovered For NIF (%)
1.	Base (1 N NaOH, kept for 2 Hr.)	80.92
2.	Acid (1 N HCl, kept for 2 Hr.)	85.67
3.	Neutral (kept for 2 Hr.)	76.16
4.	H_2O_2 30% (kept for 2 Hr)	80.12
5.	Photo stability[UV, 200 watt hrs/square meter Florescence, 1.2 million Lux. Hrs]	78.19
6.	Heat dry (60^0C, 12 hrs.)	86.91
7	Wet Heat, (60^0C, 12 hrs.)	65.32

Characterization of Photo degradant

Mass fragmentation

The LC-MS analysis showed the m/z value for following three probable elemental compositions / molecular formula: $C_{17}H_{16}N_2O_5$, $C_{14}H_9N_2O_3$ & $C_{13}H_8NO_2$. This is due to the fact that this drgradents is being derived from Nifedipine molecule. Based on the high resolution mass fragmentation study in comparison to the Std fragmentation pattern of Nifedipin**e.**

Table No 8.

Fragment No.	Mass observed	Theoretical Mass	Molecular Formula
1	329.9	328	$C_{17}H_{16}N_2O_5$
2	253.7	253	$C_{14}H_9N_2O_3$
3	224.7	223	$C_{13}H_8NO_2$

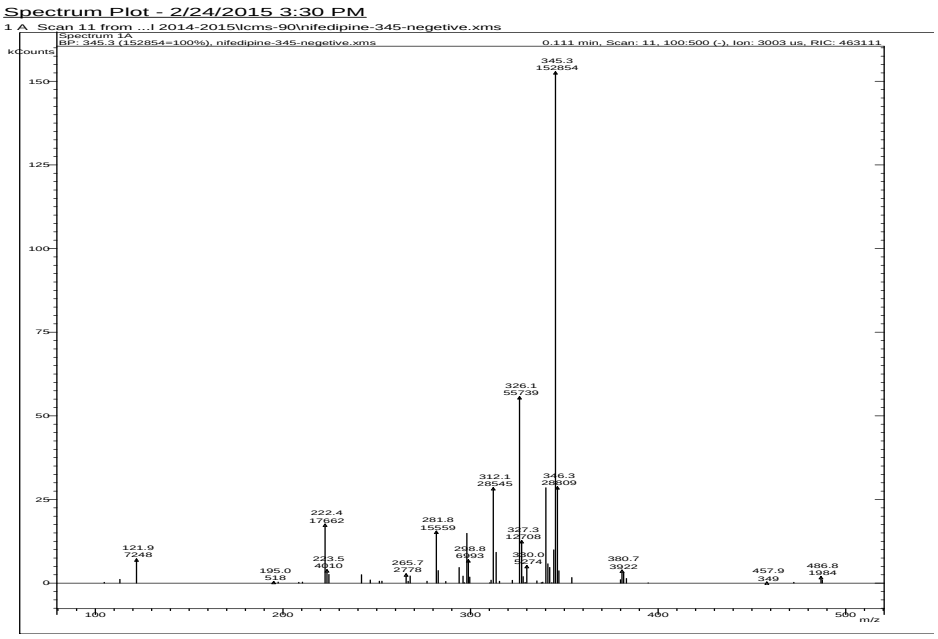

Fig. 7: LS MS Spectrum of Nifedipine STD.

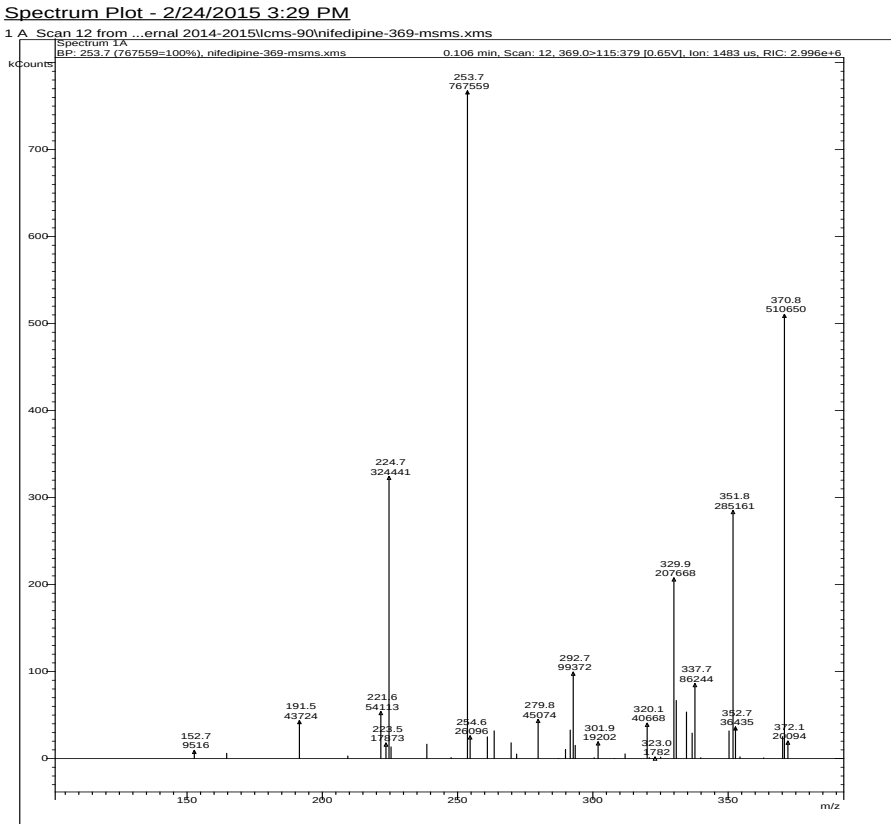

Fig. 8: Spectrum showing degradation of Nifedipine in LSMS plot.

DISCUSSION

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

We identified three probable drgradent of photo degradation. The main changes are from 1-4 dihydro pyridine to stable pyridine ring.

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REFERENCS

1. T.S. Foster, S.R. Hamann, V.R. Richards, P.J. Bryant, D.A. Graves, R.G. McAllister, J. Clin. Pharmacol, 1983; 23: 161–164.

2. B.J. Schmid, H.E. Perry, J R Idle. Determination of nifedipine and its three principal metabolites in plasma and urine by automated electron-capture capillary gas chromatography. J. Chrom. B, 1988; 425: 107-119.
3. Carmen Remuñán, María J.Bretal, AlbertoNúñez, JoséLuisVila Jato. Accelerated stability study of sustained-release nifedipine tablets prepared with Gelucire, Int. J. Pharmacutics, 1992; 80(1-3): 151-159.
4. Y. M. El–Sayed E. M. Niazy S. H. Khidr. High–performance liquid chromatographic method for the determination of nifedipine in plasma and its use in pharmacokinetic studies. Journal of clinical Pharmacy & Theraputic, 1993; 18(5): 325–330.
5. Grundy JS1, Kherani R, Foster RT. Photostability determination of commercially available nifedipine oral dosage formulations. J. Pharm. Biomed. Anal, 1994; 12(12): 1539-35.
6. V B Patravale, V B Nair, S P Gore. HPTLC determination of nifedipine from bulk drug and from pharmaceuticals. J. Pharm. Biomed. Anal, 2000; 23: 6235–627.
7. Zendelovska D, Simeska S, *et l* Development of an HPLC method for the determination of nifedipine in human plasma by solid-phase extraction J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci, 2006; 839(1-2): 85-88.
8. D. Kowalczuka, M. B. Wawrzyckab, Agnieszka Haratym Maj. Application of an HPTLC Densitometric Method for Quantification and Identification of Nifedipine. J. Liq. Chrom. & Related Techn, 2006; 29(19): 2863-2873.
9. Wang XD1, Li JL, Lu Y,et l Rapid and simultaneous determination of nifedipine and dehydronifedipine in human plasma by liquid chromatography-tandem mass spectrometry: Application to a clinical herb-drug interaction study J. Chrom. B., 2007; 852(1-2): 534-544.
10. R. O. C. Filho, P. I. B. M. Franco, E C Conceicao, M I G Leles. Stability studies on Nifedipine using thermogravimetry and differential scanning calorimetry. *J. Therm, Anal. Calorimetry,* 2008; 93(2): 381-385.
11. Meinolf Wonnemann, Barbara Schug, Maria AnschützComparison of Two Marketed Nifedipine Modified-Release Formulations: An Exploratory Clinical Food Interaction Study. Clinical Therapeutics, 2008; 30: 48-58.
12. Milenović D. Milenović Lazić M. Lazić Veljković V. Veljković Todorović Z. Todorović. Validation of an HPLC method for analysis of nifedipine residues on stainless-steel surfaces in the manufacture of pharmaceuticals. Acta chromatographia, 2008; 20(2): 183-194.

13. Y U Awad, S K Patel, Dinesh Kumar, A S Bari Estimation of nifedipine by RP-HPLC tablet dosage form. Int. J Pharm. & Life Sci., 2011; 2(3): 660-612.
14. Krishna S, Rakesh G, Narmada P Padmanabha R A, Prakash V D. Development and validation of RP-HPLC method for nifedipine and its application for a novel proniosomal formulation analysis and dissolution study. Der Pharma Chemica, 2014; 6(1): 279-289.