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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC- PDA METHOD FOR THE ESTIMATION OF NISOLDIPINE IN BULK AND FORMULATIONS

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ABSTRACT

A simple, precise, rapid and accurate LC-MS compatible RP-HPLC-PDA method has been developed for the estimation of Nisoldipine (NIS) in bulk and tablet formulations. The chromatographic separation was achieved on Phenomenex C_{18} column (250 mm x 4.6 mm, 5.0 µ) using the mobile phase composed of acetonitrile:formic acid (0.02% v/v in water) in the ratio of 70:30 (v/v) at a flow rate of 1.2mL/min and NIS was eluted at 6.2 min. Quantification and linearity was achieved at 234 nm within the concentration range of 5-25µg/mL. The method was validated for specificity, linearity, accuracy, precision, LOD and LOQ as per the ICH guidelines and was found to be suitable to be employed in Quality Control.

Keywords: RP-HPLC, Validation, Nisoldipine

INTRODUCTION

NIS, chemically is 3-isobutyl-5-methyl-1, 4-dihydro-2,6-dimethyl-4-(2-nitro phenyl) Pyridine-3, 5-di carboxylate. It is a calcium channel blocker with vasodilator properties and is used for treatment of hypertension.

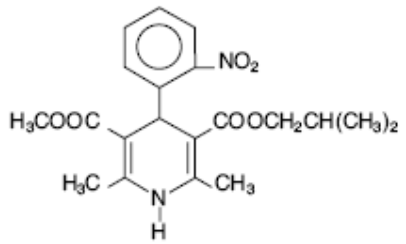

Fig. 1: Nisoldipine (NIS) chemical structure

Literature survey reveals that NIS has been the subject of few analytical chemical investigations including crystal structure elucidations and degradation studies by polarography, voltametry, HPTLC[1-3]. Since NIS is light sensitive, some published papers also dealt mostly with photo stability studies, degradation kinetics, impurity profile and determination of NIS and its photodegradation products by use of different analytical techniques[4]. Methods reported earlier also include LC-MS-MS, HPLC-GC-MS and HPLC for bio-analysis[5-7]. Fully validated HPLC methods for routine analysis were mot published and few reported HPLC methods consisted of phosphate buffer as aqueous phase, which is not compatible with MS detection. The present investigation was aimed at developing a fully validated HPLC - PDA method, which is sensitive, accurate, and compatible with MS detection for the estimation of NIS in bulk and pharmaceutical dosage forms.

MATERIALS AND METHODS:

Chemicals and reagents

SULAR® tablets (Label Claim: 8.5 mg per tablet, Lot # 2022078) were procured from the Shionogi Pharma Inc Atlanta, USA. Formic acid, acetonitrile, and water of HPLC grade were used. All other chemicals and reagents are of HPLC Grade.

Instrument

Chromatographic separation was performed on a Shimadzu Prominence HPLC system equipped with LC 10 AVP binary pumps, DGU 20A Degasser, M20 A PDA detector and LC 10ATVP auto sampler with 200µL loop volume. LC solution software was used to collect and process the data.

Chromatographic Conditions

Mobile phase consisting of 0.02% v/v formic acid: acetonitrile (30:70%) at 1.2mL/min flow rate was used and the mobile phase was filtered through membrane filter (Millipore Nylon disc filter of 0.45µm) and sonicated for 3 min in ultrasonic bath before use. For quantitative analytical purpose, eluents were monitored at 234 nm and a Phenomenex C_{18} column (250mm x4.6mm, 5.0µ) was used and separation was carried out at ambient temperature at an injection volume of 10 µL.

Stock solution and calibration standards

A stock solution was prepared by dissolving accurately weighed 25 mg of NIS in a 25 mL volumetric flask to obtain 1mg/mL solution using HPLC grade methanol. The stock solution was diluted suitably with formic acid (0.02% in water) as diluent in order to obtain calibration standards concentrations in the range of 5-25µg /mL.

Method Validation

Linearity

The linearity of NIS concentrations in the range of 5 to 25µg/mL was determined at an injection volume of 10µL. The data was given in Table 1.

Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the same standard concentration (20µg/mL for standard application). The data was given in Table 2 and shown in Fig 3.

Accuracy

Accuracy (Recovery) of the method was tested by spiking 80, 100 and 120% of NIS working standard (20µg/mL) concentration. The accuracy of the analytical method was established in triplicate across its range and the data was given in Table 3.

LOD and LOQ

LOD and LOQ were determined from standard deviation and slope method as per ICH guidelines[10].

System suitability

The system suitability was assessed using six replicate analytics of drugs at concentrations of 20µg/mL. The acceptance criterion was ± 2% of coefficient of variation (% CV) for retention times and peak areas.

Specificity

Specificity studies were carried for both pure drug and drug product by comparing the 3D plots with blank and placebo. Peak purity tests were also carried out to show that the analyte chromatographic peak is not attributable to more than one component as the impurities are not available by purity index data. The data was shown in Figs 4 & 5.

Application of the method to dosage form

Assay of NIS from SULAR® tablets of 8.5 mg (Lot no 2022078) was carried out by analyzing a powder blend (from a batch of 10 tablets) containing 5 mg of NIS. The weighed samples were placed in 5 mL volumetric flasks and 3 mL methanol was added and sonicated for 2 min to extract the drug form the blend. This solution was filtered using nylon disposable syringe filter (13 mm, 0.45 µm) and the filtrate was diluted with diluent in order to obtain a concentration in the range of 5-25 µg/mL as discussed above. The amount present in the each tablet was calculated by comparing the area of standard NIS and tablet sample. The data was given in Table 5.

RESULTS AND DISCUSSION

Few HPLC, HPTLC, derivative UV spectrophotometry, GC and LC-MS methods were published for the estimation of NIS from biological samples and to check the impurity profiles. No fully validated HPLC - PDA method was published so far for the routine analysis of NIS in bulk and tablet formulations. So, this study was aimed at developing HPLC-PDA method for simple, precise, and accurate analysis of NIS.

Initial trials were made to develop LC conditions for the separation of NIS using 0.02% v/v of formic acid as aqueous mobile phase and acetonitrile as organic modifier. Initial trials were carried out with C_{18} Phenomenex column (250 mm x 4.6mm, 5.0 µ) at 1 mL flow rate and the NIS was eluted at 7.7 min. In another trial flow rate was increased to 1.2 mL and the peak was eluted at 6.2 min. For quantitative analytical purpose wavelength was set at 234 nm, which provided better reproducibility with minimum or no interference. Under the above developed conditions (0.02% v/v formic acid: acetonitrile (30:70%) at 1.2mL/min flow rate) method was validated as per ICH guidelines[8]. A sample chromatogram along with UV spectrum and peak purity profile was shown in Fig 2. The peak purity index was found to be greater then > 0.9999 and indicating no impurities presented in bulk NIS.

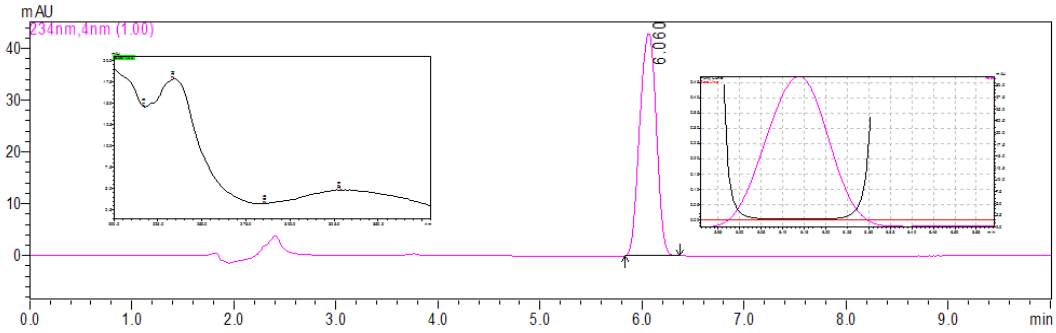

Fig. 2: NIS chromatogram with UV spectrum and peak purity index*

(*Peak Purity Index: 1.00000; Single Point Threshold: 0.998577; Min.peak purity index:1422)

A linear relationship was evaluated across the range (5-25 µg/mL) of the analytical procedure in triplicate. The range of concentrations was selected based on 80-120 % of the test concentration (for assay). Peak area and concentrations were subjected to least square regression analysis to calculate regression equation. The regression coefficient (R^2) was found to be 0.999 and the correlation coefficient (R) was 0.9995 and shows good linearity. The regression data of the calibration curve was given in Table-1.

Precision studies were carried out in terms of repeatability. Six determinations of 100 % concentration at 20µg/mL level was evaluated and the data was given in Table 2 and shown Fig 3.

Table 1: Linearity data for NIS (n=3)

Concentration (µg/mL)	Peak Area (±sd)
5	82524 (1529.351)
10	164532 (13448.8)
15	249743 (8817.289)
20	320587 (15924.49)
25	398779 (1077.913)

$y = 15771 x + 6663$ $R^2 = 0.9991$; $R = 0.9995$

Table 2: Precision data of 20µg/mL

Injection	Peak area
Injection-1	328588
Injection-2	330027
Injection-3	333492
Injection-4	327532
Injection-5	334574
Injection-6	328588
Mean	332463.8
Standard Deviation (SD)	6828.518
% RSD	0.74%

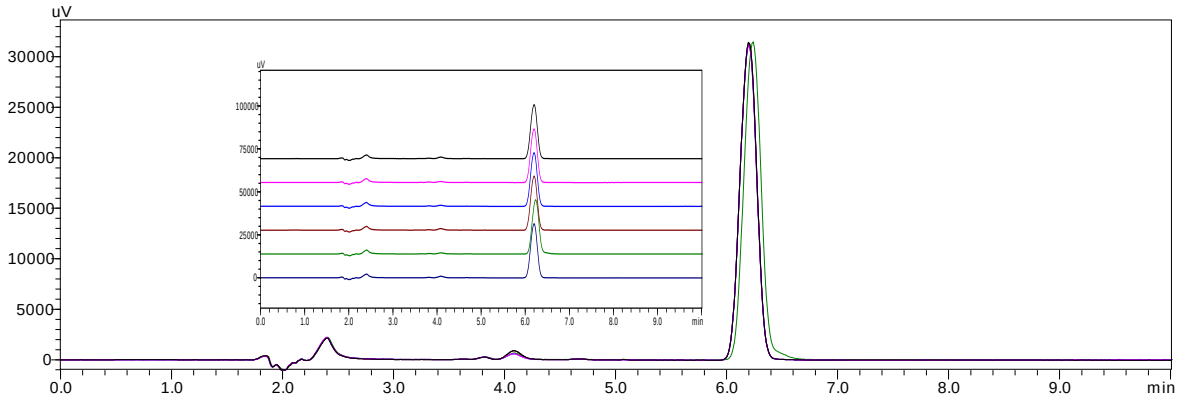

Fig. 3: Overlay chromatograms of NIS 20 µg/mL

Accuracy of the method was examined by performing recovery studies by standard addition method for drug product as the exact components are unknown and for drug substance the analyte peak was evaluated by 3D plot of the chromatogram in order to confirm the existence of one component at the retention time of 6.19 min for NIS (Fig 4) as the impurities are not available. The recovery of the added standard to the drug product sample was calculated and it was found to be 99-102%, with a %RSD less than 2. This indicates a good accuracy in terms of recovery of the method to that of the labelled claim. The obtained recovery results were given in Table 3.

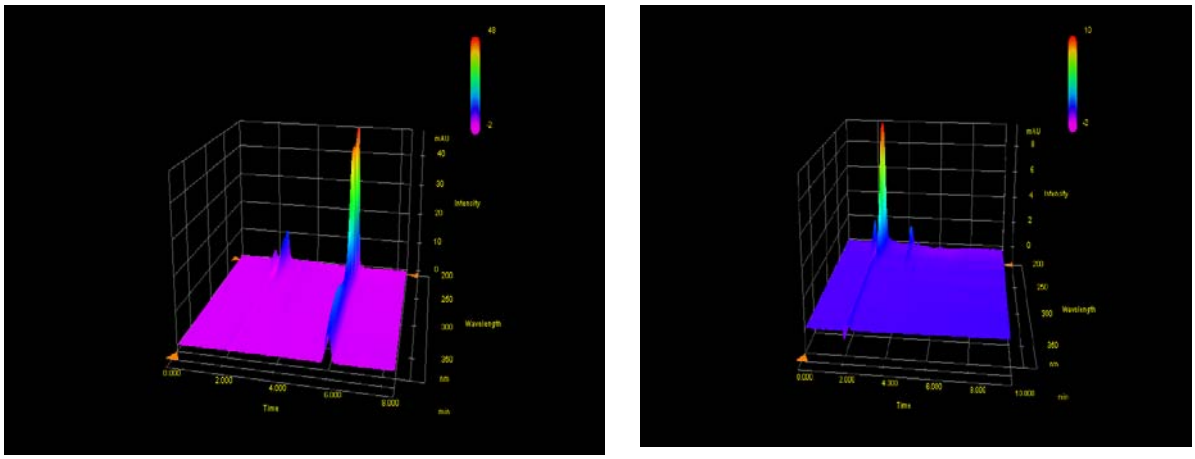

Fig. 4: 3D plots of NIS (left) and diluent (right) chromatograms

Table 3: Accuracy data (n=3)

% Level of Recovery	Amount present (µg/mL)	Amount added (µg/mL)	% Recovery (Mean ± SD)	% RSD
80	20	16	99 ± 1.00	1.10
100	20	20	101.03 ± 0.99	0.99
120	20	24	100.9 ± 0.923	0.91

LOD and LOQ were calculated form the average slope and standard deviation from the calibration curve and were found to be 1.50µg/mL and 4.55 µg/mL respectively, indicating the method was sensitive enough to carryout routine analysis of NIS in bulk and dosage forms.

System suitability was carried out by injecting 20 µg/mL of NIS at different injection volumes. With increment of injection volumes, the RSD for tailing factor was less than 1% and is satisfactory. The data was given in Table 4.

Table 4: System suitability parameters

Injection volume (µL)	Retention time	Tailing factor	Theoretical plates
10	6.083	1.025	7332.840
20	6.060	0.997	6153.687
30	6.050	0.981	5785.782
40	6.031	0.983	5585.994
50	6.017	0.979	5497.863
Mean	6.0482	0.993	6071.233
SD	0.025607	0.019235	749.0576
% RSD	0.42	0.93	-

The specificity of the method was established by spiking diluent solution of commonly used tablet formulation excipients (compressed in the form of a tablet i.e a placebo) and showed no peaks were observed at the retention time of NIS i.e 6.2 min and also over the range of 10.0 min as shown Fig 5. The peak purity index was found to be greater then > 0.9999 and indicating no impurities presented in tablets dosage form[9,10].

The assay of NIS in tablets was calculated by comparing the area of standard NIS and tablet sample along with the consideration of average tablet weight and weight of powder blend taken and dilution factor if any. The assay was found to be within the limits (Table-5) and the present LC conditions can be used for the assay of NIS in different commercially available formulations.

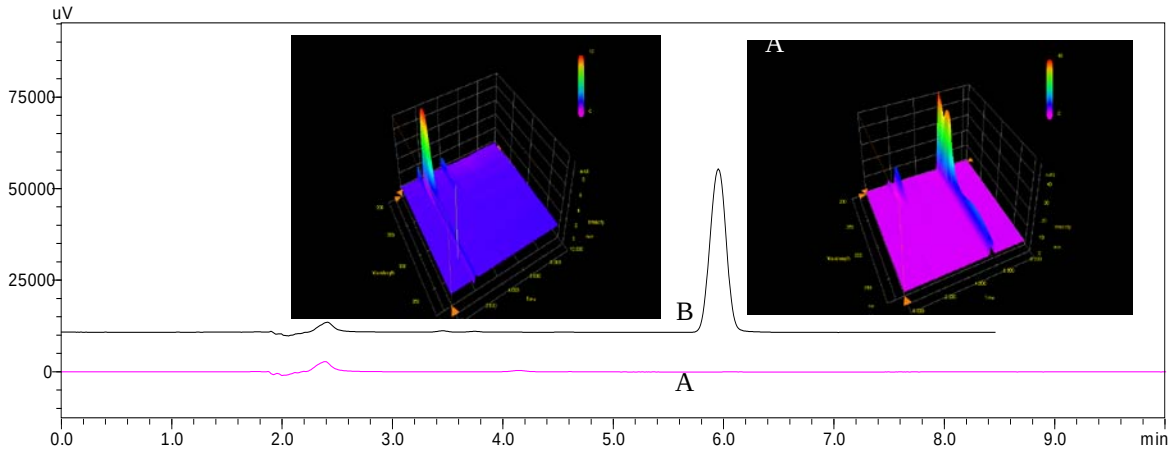

Fig. 5: Overlay of placebo (A) and formulation (B) chromatograms along with in lay of 3 D plots

Table 5: Assay Results (n=3)

Formulation	Labeled Amount (mg)	Amount found (mg) (Mean ±SD)	% Assay (Mean ±SD)	% RSD
SULAR® tab	8.5 mg	7.4 ± 0.3	99.26 ± 0.45	0.4533

CONCLUSION

The proposed RP-HPLC - PDA method was validated fully as per International Conference on Harmonisation (ICH) Guidelines, and found to be applicable for routine quality control analysis for the estimation of NIS in isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be with in the limits. The method provides selective quantification of NIS without interference from blank and placebo. The proposed method is highly sensitive, reproducible, reliable, rapid and specific and also has the unique advantage of LC conditions being compatible with MS detection. Therefore, this method can be employed in quality control to estimate the amount of NIS in bulk and dosage forms.

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