Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 12, pp.62453-62457, December, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

STABILITY INDICATING RP- HPLC METHOD FOR ESTIMATION OF NISOLDIPINE IN BULK AND TABLET DOSAGE FORM

[1,*]Vishnu Vardhan, D., [2]Venkateshwar Reddy, K. and [3]Srinivas Nayak, A.

[1]Department of Pharmaceutical Analysis, Vaagdevi Pharmacy College Warangal, Telangana, India
[2]CMR college of Technology, Hyderabad
[3]Department of Pharmaceutical Analysis, Satavahana University, Karimnagar, TS, India

ARTICLE INFO

Article History:
Received 06th September, 2017
Received in revised form
13th October, 2017
Accepted 10th November, 2017
Published online 27th December, 2017

Key words:
Nisoldipine, RP HPLC,
Stability Indicating Assay,
Validation.

ABSTRACT

A simple reverse phase HPLC method was developed for the estimation of Nisoldipine in bulk and tablet dosage form. Chromatography was performed by isocratic elution on a Stainless steel Thermo scientific C18 column with dimensions 4.6 x 250mm, packed with octadecylsilane bonded to porous silica (C18) with particle size 5 micron acetonitrile and water in the ratio of 80:20 % v/v is used as mobile phase. The flow rate is 1.0 ml/ min and effluent is monitored at 240 nm. Nisoldipine was eluted at a retention time of 4.4 minutes. The standard curve of Nisoldipine was linear over a working range of 0.3– 8 µg/ml and gave an average correlation coefficient of 0.999. The limit of quantitation (LOQ) of the drug is 0.1 µg/ ml. Recovery studies were carried out by standard addition method and the recoveries are found satisfactory within the range of 99.24 to 99.6 %.The method is precise with % RSD below 2. The method is validated in terms of robustness and forced degradation studies were carried out.

Citation: Vishnu Vardhan, D., Venkateshwar Reddy, K. and Srinivas Nayak, A. 2017. Stability indicating rp- hplc method for estimation of nisoldipine in bulk and tablet dosage form", *International Journal of Current Research*, 9, (12), 62453-62457.

INTRODUCTION

Nisoldipine (ND), is a 1, 4-dihydropyridine derivative calcium channel blocker, used as antihypertensive drug. It has a very poor bioavailability (< 5%), undergoes extensive first-pass metabolism in gut wall and is known to be a CYP3A substrate (Hitomi *et al*., 2000). The drug is not official in any of the pharmacopoeia. Nisoldipine inhibit the transmembrane influx of calcium into vascular smooth muscle and cardiac muscle (Sweetman, 2007). Nisoldipine is most potent and an effective antihypertensive agent. Like other dihydropyridines, it exhibits a mild diuretic effect and vasodilating effect occurs as doses lower than those that affect cardiac contractility (Figure 1). Literature survey revealed that only one RP-HPLC method was reported (Swetha, 2012), but till date there was no stability indicating RP-HPLC method for Nisoldipine. The present study is undertaken in order to develop a new, simple, precise, accurate and specific stability indicating RP-HPLC method in tablet dosage form through stress studies.

MATERIALS AND METHODS

Reagents and chemicals

Nisoldipine bulk drug and tablets were procured as gift sample form MSN MSN laboratories, Hyderabad, A.P, India.

Corresponding author: **Vishnu vardhan, D.,**
Department of Pharmaceutical Analysis, Vaagdevi Pharmacy College Warangal, Telangana, India.

Methanol, Acetonitrile, Hydrochloric Acid, Sodium Hydroxide are purchased from MERCK.

Stock solutions and standards

Standard solution of Nisoldipine (1000µg/ml) was prepared by dissolving accurately weighed amount of Nisoldipine (25 mg) in sufficient quantity of diluent in a 25ml volumetric flask. Then the volume of the flask was made up to the mark with the same. Working standard solution of Nisoldipine were prepared by pipetting 0.9 ml of the stock solution into a 10ml of volumetric flask and the volume was made up to the mark with the diluent.

Apparatus and chromatographic conditions

Quantitative HPLC was performed on Waters HPLC system equipped with waters 515 pump and Waters 2489 dual wavelength UV detector. Empower2 software is used for data acquisition.A Stainless steel Thermo Scientific column with dimensions 4.6 x 250mm, packed with Octadecylsilane bonded to porous silica (C18) having particle size 5 micron.

Method development and optimization:

To develop a suiTable HPLC method for the determination of Nisoldipine, trials were made with different mobile phases, using methanol, water, buffer (0.5% w/v potassium dihydrogen phosphate in water) in different pH with different compositions of mobile phases (80: 20, 75:25).

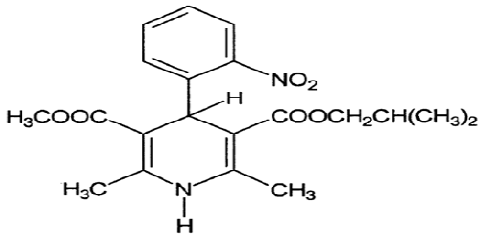

Figure1. Structure of Nisoldipine

The method was optimized finally using combination of Methanol and water in the ratio of 80/20 % v/v with a flow rate of 1.0 ml/ min. The drug was eluted at retention time around 4.4 min with symmetric peak shape. The run time was set for 8 minutes. The detection is performed at wavelength 240 nm.

System suitability

For performing system suitability studies, 100% test concentration under degradation conditions was selected. System suitability test was performed by injecting blank solution once and standard solution of 100% test concentration six times in to stabilized HPLC system. The system suitability was established by evaluating the system suitability parameters from the last peak obtained. System suitability parameters include retention factor (k'), repeatability, resolution (R), tailing factor (T) and theoretical plates (N). It was performed by using the concentration of 90µg/ml. The system suitability data was given in the Table 1.

Table 1. Observation of System Suitability Parameters

S. No	Parameter	Nisoldipine
1	Retention time	4.4
2	Theoretical plates	4625
3	Tailing factor	1.63
4	Area	146.76

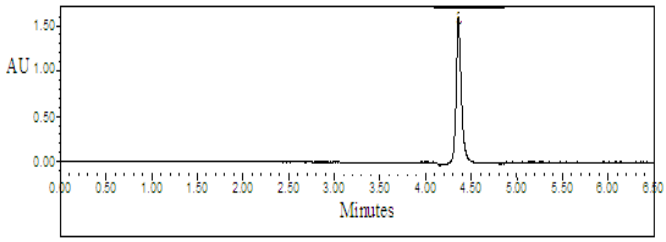

Fig. 2. Representative Chromatogram of Nisoldipine standard sample

Assay of Nisoldipine marketed formulation

Twenty tablets of Nisoldipine were weighed, ground in to a fine powder and mixed thoroughly. A quantity of powder equivalent to 25 mg of Nisoldipine was weighed and transferred in to a 25ml volumetric flask and was dissolved in the diluent. The volume was made up to the mark with the same and the resulting solution was labelled as sample stock solution. The solution obtained was diluted with mobile phase so as to obtain a required concentration. The solution thus prepared was filtered through 0.45µ membrane filter and the filtrate was sonicated for 5min. 20µl of working standard solution and test sample solution were injected six times at the optimized method conditions and the chromatograms were recorded and peak areas were calculated, and the % Assay was calculated by using the following formula.

$$\%ASSAY = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AT - Average area counts of test sample preparation
AS - Average area counts of working standard preparation
WS - Weight of working standard taken in mg
DS - Dilution of standard preparation
DT - Dilution of sample preparation
WT - Weight of sample taken in mg
P - Percentage purity of working standard.
LC - Label claim
AW - Average weight of tablets

***Validation of the assay method* (ICH, 2005; Guidance for Industry, 2001; Lopez *et al*., 2000; BraithWait, 1996; Meyer, 2004; ICH, 1999; Moffat, 2004):**

Linearity

Linearity solutions for assay method were prepared from stock solution at levels from 0.01 to 200µg/ml of analyte concentration. The graph of peak area versus concentration was plotted by least-squares linear regression analysis. The linear fit of the system was illustrated graphically. The linearity range was found to be 0.01 - 200 µg/ml. The standard calibration curve for Nisoldipine was constructed using the average peak-area versus the nominal concentrations of the analyte. Linear least-squares regression analysis was performed to assess the linearity.

Recovery and accuracy

To evaluate the accuracy of the proposed method, recovery studies were carried out by standard addition method, where a known amount of different concentrations of pure drug at five levels of 50%, 75%, 100%, 125% and 150% were spiked with solution of pre analyzed formulation of concentration 100 µg/ ml. At each level recovery studies were carried out in triplicate and expressed as % recoveries. The percentages of recoveries were calculated from the slope and *Y*-intercept of the calibration curve obtained. Accuracy/recovery experiments were performed in triplicate.

Precision

The precision was carried out at three levels, intra assay precision of injection, intermediate precision and reproducibility. Intra assay precision was assessed using 9 determinations covering the range of 50,100 and 150% concentration levels of drug solution. Intermediate precision (inter day precision) was assessed by inducing typical variations like different days and different columns.Reproducibility was assessed by different analysts.

Robustness

Robustness of the method was studied under degradation conditions to study the effects of degradants on Nisoldipine in changes method conditions. It was carried out by considering deliberate changes in detection wavelength, flow rate, mobile phase ratio. Robustness was carried out by changing detection wavelength by ±3 nm. Robustness was checked by changing the proportion of organic solvent in the mobile phase by ±4%. It was also checked for robustness by change in flow rate by ±0.2 ml/ min.

Forced degradation studies

To study the specificity of the method, pure drug was stressed under different degradation conditions. Degradation studies were carried out by exposing drug for acid hydrolysis, alkali hydrolysis, oxidative degradation, thermal degradation and photolytic degradation. Mobile phase is used as solvent for all degradation studies. All the solutions for degradation studies were prepared by dissolving Nisoldipine drug in little amount of mobile phase and the volume was made up to the mark with 0.1N HCl, 0.1N NaOH, 1% H_2O_2. Acid hydrolysis is carried out by exposing the drug to 0.1N HCl. Alkali hydrolysis is carried out by exposing the bulk drug and powdered sample to 0.1N NaOH. Oxidative degradation is carried out by exposing the bulk drug to 1% H_2O_2.Thermal degradation is carried out by exposing the bulk drug in Hot air oven at 50 ^{0}C. Photolytic degradation is carried out by exposing the bulk drug to sun light. The degradation studies were carried at a time interval of 15 minutes. The drug solution was prepared at a concentration of 100µg/ ml.

Acid degradation

10 mg of drug was dissolved in a few ml of mobile phase in a 10ml volumetric flask. The volume was made up to the mark with 0.1N HCl, mixed thoroughly and kept aside. After 15, 30 minutes, solution was mixed and 1ml of this solution was pipetted into another 10ml volumetric flask. To this 1ml solution, 1ml of 0.1N NaOH was added to neutralize the acid and final volume was made upto the mark with mobile phase and its peak area was observed by injecting into HPLC.

Alkali degradation

10mg of drug was dissolved in a few ml of mobile phase in a 10ml volumetric flask. The volume was made up to the mark with 0.1N NaOH, mixed thoroughly and kept aside. After 15, 30 minutes, solution was mixed and 1ml of this solution was pipetted into another 10ml volumetric flask. To this 1ml solution, 1ml of 0.1N HCl was added to neutralize the alkali and volume was made up to the mark with mobile phase and its peak area was observed by injecting into HPLC.

Photo degradation

Drug powder was exposed to sunlight. After 15, 30 minutes, 10mg of the exposed powder was dissolved in mobile phase in a 10ml volumetric flask. From this solution, 1ml was pippeted into another 10ml volumetric flask and its volume was made upto the mark with mobile phase. The peak area of this solution was observed.

Thermal degradation

Drug powder was exposed to 50^{0}C in a hot air oven. After 15, 30 minutes, 10mg of the exposed powder was dissolved in mobile phase in a 10ml volumetric flask. From this solution, 1ml was pippeted into another 10ml volumetric flask and its volume was made up to the mark with mobile phase. The peak area of this solution was observed.

RESULTS

System suitability

A system suitability evaluation usually contains its own set of parameters. For chromatographic assays, these may include tailing factor, resolution, precision, capacity factor, retention time and theoretical plates.

The System suitability Parameters were found to be within the specified limits for the proposed method. The results were given in Table 1.

Assay of Nisoldipine marketed formulation

The representative chromatograms are shown in Figure 1 and 2. The peak areas were mentioned in the Table 2. The assay limits for Nisoldipine was 90-110 % and the results obtained for Nisoldipine was found to be 99.05. Hence the results were within the limits.

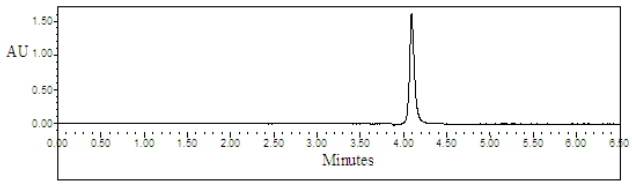

Fig. 3. Representative Chromatogram of Nisoldipine test sample

PARAMETER	NISOLDIPINE
Avg area of sample preparation	153.77
Avg area of standard preparation	152.82
Weight of standard taken	10 mg
Percentage purity	99.1
Avg weight of sample	10.23 mg
Avg weight of standard	10.18 mg
Label claim	10 mg
% Assay	99.05%

Validation of the assay method (Guidance for Industry, 2001; Lopez *et al*., 2000; BraithWait, 1996)

Linearity

The linearity range was found to be 0.3-8 µg/ml for Nisoldipine. Calibration curve was plotted and correlation co-efficient for both the drugs found to be 0.999. Hence the results obtained were within the limits. The linearity curves were shown in Figure 3**.** The linearity results were reported in Table 3.

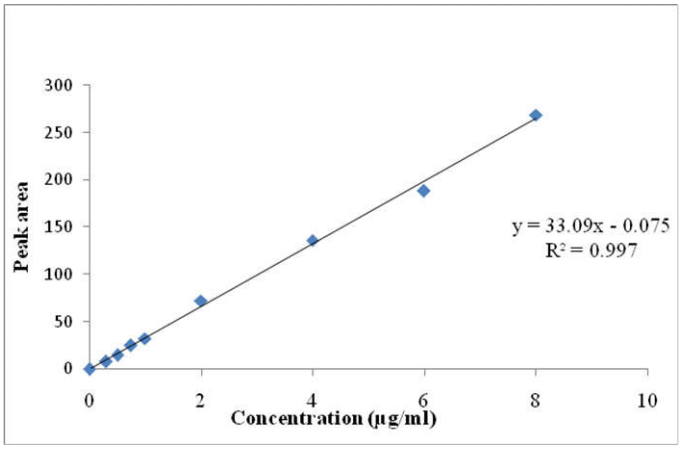

Fig. 3. Linearity curve

Recovery and accuracy

The accuracy studies were shown as % recovery for Nisoldipine at 50 %, 100 % and 150 %, the limits of % recovered should be in range of 98-102 % the results obtained for Nisoldipine were found to be within the limits. Hence the method was found to be accurate. The accuracy studies showed % recovery of the Nisoldipine 99.973%.

Table 3. Results of Accuracy

NISOLDIPINE						
Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean
50%	5.74	149.75	24.775	24.76	99.94	
50%	5.74	148.64	24.775	24.61	99.73	
50%	5.74	149.11	24.775	24.74	99.88	
50%	5.74	148.23	24.775	24.70	99.75	99.67
50%	5.74	149.34	24.775	24.67	99.90	
50%	5.74	148.81	24.775	24.68	99.53	
100%	10.23	149.72	49.550	49.61	100.01	99.9
100%	10.23	149.91	49.550	49.56	100.02	
100%	10.23	149.36	49.550	49.34	99.66	
150%	15.11	148.94	74.325	74.29	99.74	99.89
150%	15.11	149.07	74.325	74.30	99.52	
150%	15.11	149.53	74.325	74.18	99.92	
150%	15.11	149.76	74.325	74.21	99.91	
150%	15.11	148.83	74.325	74.18	99.99	
150%	15.11	149.54	74.325	74.26	99.38	

Table 4. Results of Precision

S.No	Sample Weight	Sample Area-1	% Assay
1	10.23	148.46	99.54
2	10.23	149.52	98.88
3	10.23	148.99	99.23
4	10.23	151.4	99.13
5	10.23	148.65	99.64
6	10.23	149.06	99.88
Average Assay:			99.43
STD			0.36
%RSD			0.25

Table 5. System suitability Results of Nisoldipine for robustness

Change in Flow Rate (ml/min)		System Suitability Results		
		USP Plate Count	USP Tailing	Retention time(min)
Low	0.8	6088	1.53	8.45
Actual*	1.0	6639	1.37	4.42
High	1.2	6837	1.63	4.4
Change in Temperature		System Suitability Results		
		USP Plate Count	USP Tailing	Retention time(min)
5% more	6678	1.591	3.323	5% more
Actual*	6302	1.24	3.374	Actual*
5% less	6280	1.569	2.805	5% less

Table 6. Stability indicating Parameters

S.No	Substance used for degradation	Sample Weight	Sample Area-1	% Assay	%DEG
1	ACID	10.23	148.88	90.63	7
2	BASE	10.23	149.24	94.72	5
3	PEROXIDE	10.23	149.92	98.66	9
4	LIGHT	10.23	147.93	94.82	2
5	HEAT	10.23	148.63	85.51	13
6	Avarage Assay:		148.92	-	-
7	STD		0.73	-	-
8	%RSD		25.4	-	-

Precision

In the precision study, % RSD was found to be 1.82% which indicates that the system has good reproducibility. % RSD was determined for peak areas of Nisoldipine. The acceptance limit should be not more than 2 % and the results was found to be within the acceptance limits. The Results were reported in Table 4.

Robustness

For robustness studies the chromatograms were recorded for standard solutions of Nisoldipine by changing flow rate ± 0.1 and temperature. Robustness studies reveal that the method was reliable. The results were reported in Tables 5 & 6.

Forced degradation studies

Degradation studies were carried out by exposing drug for acid hydrolysis, alkali hydrolysis, oxidative degradation, thermal degradation and photolytic degradation. The results of forced degradation studies were shown in the Table 8. Representative chromatograms were given in Figure 4

Conclusion

The main objective of the present work is to develop a simple stability indicating RP-HPLC method in bulk and tablet dosage form.

The optimum wavelength for the determination of Nisoldipine was selected at 240 nm. Nisoldipine peak was achieved with good, peak shape and symmetry at R_t - 4.4. The assay was performed on the tablet formulation and the % drug content for Nisoldipine was found to be 98.86% which was within the acceptance limits. The system suitability of the proposed method was accomplished from the resolution, theoretical plate count and asymmetric factor of Nisoldipine at the optimized conditions. The mean theoretical plate count and the mean asymmetric factor was in compliance with the acceptance specifications. The developed analytical method was validated according to ICH guidelines with respect to parameters such as precision, linearity, accuracy, limit of detection, limit of quantitation, robustness and specificity. From this forced degradation study it was found that the degradation products did not interfere with the analyte peak. Hence, the proposed method was found to be specific. The developed RP-HPLC method was used for quantitation of drug in the presence of degraded products.

REFERENCES

BraithWait, A. and Smith, F.J.1996. Chromatographic Methods, 5thedition, Kluwer Academic Publisher, PP 1-2.

Guidance for Industry: Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Rockville, MD, 2001.

Hitomi Takanaga, Ayako Ohnishi, Hideyasu Murakami, Hirotami Matsuo, Shun Higuchi. 2000. Relationship between time after intake of grapefruit juice and the effect on pharmacokinetics and pharmacodynamics of Nisoldipine in healthy subjects. Clin pharmacol ther; 67: 201-214.

ICH Q2A, "validation of analytical methods, definitions and terminology", ICH Harmonized tripartite guideline, (1999).

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline. (2005) Validation of Analytical Procedures: Text and Methodology Q2 (R1). Geneva, 1-13.

Lopez, J.A., Martinez, V., Alonso, R.M., Jiminez, R.M. 2000. High-performance liquid chromatography with amperometric detection applied to the screening of 1,4-dihydropyridines in human plasma, *J. Chromatog,* A. 870 105-114.

Meyer V.R. 2004. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, PP 7-8.

Moffat, A. C., Osselton, M. D., Widdop, B. and Watts. J. 2004. *Clarke's Analysis of Drugs and Poisons*. 3rd Edition. Volume 2: 1538, 1690.

Sweetman, S.C. and Martindale, 2007. The complete drug reference. Pharmaceutical Press, London, (U.K). 1556-1557.

Swetha A. and Buchi NN. 2012. Development and validation of rp-hplc- pda method for the estimation of nisoldipine in bulk and formulations. *Int J Pharm Sci.,* 4(3):88-91

****