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Validated HPLC Method for the Determination of Nisoldipine

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Abstract

In this study, novel high performance liquid chromatography methods which are described as a simple, selective, sensitive, precise and simultaneous analysis of Nisoldipine in bulk and commercial tablet formulation containing Nisoldipine. Good chromatographic separation was achieved using a specific column Agilent ZORBAX Eclipse Plus C18, 4.6×250 mm and a mobile phase consisting of methanol, 0.01 M potassium dihydrogen phosphate aqueous solution and 0.1 M Hexane sulphonic acid sodium salt (25:65:10, v/v) at pH 4.0 using orthophosphoric acid with a flow rate of 1.0 ml/min. The ultraviolet detector was set at a wavelength of 275 nm. Nisoldipine was eluted at 7.43 min. Due to the high precautions taken during the analysis, no extraneous materials were found to interfere. The linear range for Nisoldipine was 5-30 µg/ml. The linearity, precision, accuracy, robustness, limit of detection and limit of quantification of the proposed method were determined. Regression coefficients ($r^2 \geq 0.999$), recovery (97.2-103.1%), the limit of detection (0.4 µg/ml) and the limit of quantification (1.0 µg/ml) were validated and found to be satisfactory. The proposed method is convenient for quantitative routine analysis and purity control of Nisoldipine in its bulk powder and dosage forms.

Keywords: Nisoldipine; HPLC; Validation; Tablets

Introduction

Nisoldipine, (±)3-isobutyl-5-methyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5-dicarboxylate, 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]. Nisoldipine 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 alone or together with other medicines to treat high blood pressure [2]. Nisoldipine is available as extended release tablets. Figure 1 shows the structural formula of Nisoldipine.

Many analytical chemical investigations have been published for the determination of Nisoldipine, including the determination in formulations by voltammetry [3-6], polarography [7] and HPLC [8-12]. Since Nisoldipine has light sensitivity, its stability, kinetics of degradation and determination of impurities are reported by various techniques including UV [13], polarography [5] and HPLC [14,15].

Therefore, the purpose of this investigation was to develop and validate a method using a simple, rapid, sensitive, precise, accurate and specific reversed phase HPLC-DAD assay. The method uses a simple mobile phase composition and the rapid run time of less than 10 min. Hence, this method can be used for the analysis of large number of samples in quality control laboratories of drugs.

Experimental

Materials and reagents

Nisoldipine was supplied from NODCAR, Egypt, and its pharmaceutical form Sular tablets were manufactured by Rizhao Highrun Biotechnology Co., Ltd, China. All reagents and chemicals of the highest purity and analytical grade were available. Potassium dihydrogen phosphate and HPLC grade methanol were purchased from VWR. Hexane sulphonic acid sodium salt was obtained from Sigma-Aldrich. A standard stock solution 100 µg/ml of Nisoldipine was prepared in 100 ml methanol as solvent. Working solutions were prepared separately by making serial dilutions from the standard solution to obtain calibration graph in the range of 5-30 µg/ml. These solutions were stored at 20°C. Once prepared, analyzed daily for inter and intra-day variations of the method. 20 µl of these solutions were injected into LC system and chromatographic.

Chromatographic conditions

High performance liquid chromatography experiments were carried out using the model of LC system Agilent 1100 Series with degasser, quaternary pump, auto-sampler, column heater, UV-detector and Chemstation-software. The detector was set to scan from 200 to 500 nm and had a discrete channel set at 275 nm, which was the

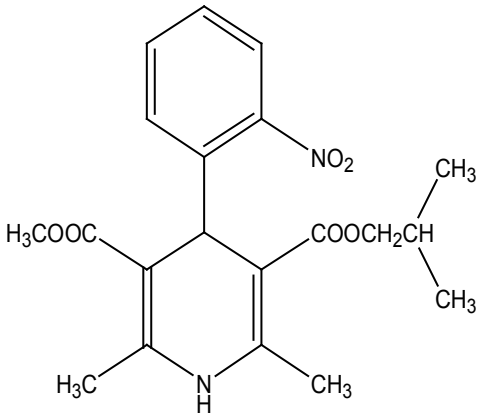

Figure 1: The chemical structure of Nisoldipine.

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wavelength used for quantification. Separation was performed on an Agilent ZORBAX Eclipse Plus C18 with particle size of 10 µm (4.6×250 mm). The mobile phase was consisted of methanol, 0.01 M potassium dihydrogen phosphate aqueous solution and 0.1 M Hexane sulphonic acid sodium salt (25:65:10, v/v) at pH 4.0 using orthophosphoric acid with a flow rate of 1.0 ml/min. Column temperature was set at 25°C and 20 µl of samples was injected to the HPLC system.

Method validation

Validation of an analytical method is the process by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical application of International Conference on Harmonization (ICH) guidelines. Validation is required for any new or amended method to ensure that it is capable of giving reproducible and reliable results, when used by different operators employing the same equipment in the same or different laboratories. The type of validation program required depends entirely on the particular method and its proposed applications. Typical analytical parameters used in assay validation include: linearity range, precision, accuracy, limit of detection, limit of quantification, robustness, selectivity and specificity.

Linearity range: Calibration curve was determined for six different concentrations of standard solution of the drug analyzed in the proposed range of 5-30 µg/ml. Each calibration was injected three times. The calibration curve was performed in triplicate. Calibration curves were calculated applying the least-squares method to concentration versus peak area. Each plot as a linear relationship between concentration and peak area was observed in the range of study.

Precision: Precision, expressed in terms of %RSD, was determined in terms of intraday and interday precisions, analyzing the drug at three different concentrations, determining each concentration thrice, and reflects a high degree of precision. In other words, Intra-day precision was determined by analyzing Nisoldipine (5-30 µg/ml) at three different time points on the same day whereas interday precision on different days and %RSD was calculated.

Accuracy: Accuracy was determined by performing recovery studies by the standard addition method by spiking different concentrations of pure drug in the solution containing Nisoldipine powder for tablet samples within the analytical concentration range of the proposed method at three different set at level of 50%, 100% and 150%. The amount of Nisoldipine was calculated at each level and % recoveries were computed. The results were within the specified limits of ICH guidelines.

Limit of detection (LOD) and limit of quantification (LOQ): The LOD and LOQ were calculated by using the following equations: LOD=3 S.D./m and LOQ=10 S.D./m, where "S.D." is the standard deviation of the intercept of the calibration curve and "m" is the slope of the calibration curve [16].

The LOD and LOQ of Nisoldipine were 0.4 and 1.0 µg/ml, respectively.

Robustness: The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for the routine analysis. Robustness was carried out by varying three parameters (deliberate change) from the optimized chromatographic conditions like mobile phase composition (± 2 ml methanol), flow rate (± 0.1 ml/min.) and pH (± 0.20). No significant change was observed. Also, to assess the stability of sample solutions of Nisoldipine, the samples tested were maintained at 2-8°C for 24 h and also placed into the auto sampler, the stability of these solutions was studied by performing the experiment and observing any change in the chromatographic pattern, compared with freshly prepared solutions.

Selectivity and Specificity of the method were determined by screening five batches of drug. The extraction recovery of Nisoldipine was calculated by comparing the peak area ratio measured for the standard solution with that obtained for drug in dosage form. Results from this testing studies indicated the method was enabled highly selective analysis of the drugs. Because the specificity or selectivity of a developed method is often difficult to ensure, several simple and easy techniques are used in method validation experiments to increase confidence in selectivity (peak purity) [17]. Results from these procedures confirmed the method was selective and specific.

System suitability test: The system suitability test was also carried out to evaluate the resolution and reproducibility of the system for the analysis, using five replicate injections of a reference solution containing 10 µg/ml of Nisoldipine. The parameters measured were peak area, retention time, theoretical plates and tailing factor (peak symmetry).

Procedure for tablets

Twenty tablets of formulation (Sular tablet) were powdered finely and an amount equivalent to one tablet of Nisoldipine was weighed and then dissolved in the methanol. Solutions were then filtered through ordinary filter paper. The desired concentrations in the range of 5-30 µg/ml for Nisoldipine were obtained by accurate dilution and these solutions were then sonicated. Finally, all the solutions were filtered through 45 µm Millipore™ filter, in order to separate out the insoluble excepients before chromatographed.

Results and Discussion

The present work describes development and validation of HPLC method for the determination of Nisoldipine in the bulk powder and in the pharmaceutical tablet formulations.

The mobile phase for the assay of Nisoldipine was optimized and selected by taking different proportions of aqueous and organic phases which gave acceptable asymmetry and theoretical plates with appropriate run time. From the different mobile phases tried mobile phase consisting of methanol, 0.01 M potassium dihydrogen phosphate aqueous solution (25:65, v/v) was found to be satisfactory. However due to amino group present in the structure and the polymeric nature, the chromatogram displayed a tailing peak of Nisoldipine, which was avoided by adding 10% v/v of 0.1 M Hexane sulphonic acid sodium salt. The pH of the mobile phase was also optimized since it is a basic drug, so ionization of the drug was found at pH 4.0 using orthophosphoric acid, where the drug gave symmetric and sharp peak for Nisoldipine at 1.0 ml/min. as flow rate with good theoretical plates and acceptable tailing factor on Agilent ZORBAX Eclipse Plus C18 (4.6×250 mm) with particle size of 10 µm. For quantitative analytical purpose wavelength was set at 275 nm, which provided better reproducibility with minimum interference. Under the chosen experimental conditions, the liquid chromatogram of Nisoldipine showed a single peak of the drug at retention time (RT) 7.43 min with asymmetry of 0.91 (Figure 2).

System suitability

The system suitability was evaluated by calculating the %RSD values of peak area, retention time, asymmetry and theoretical plates of

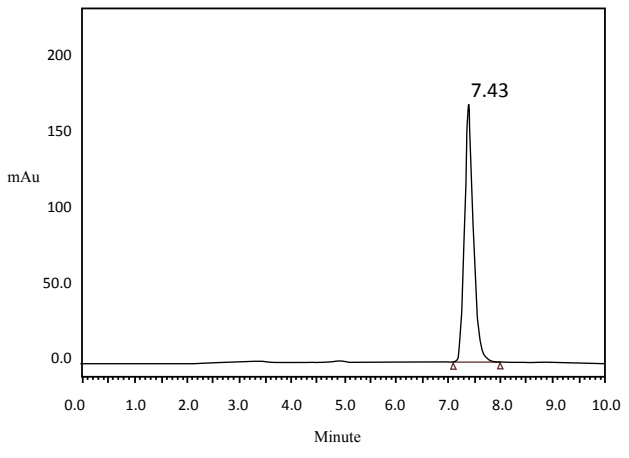
five standard replicates. The experimental results (Table 1) showed that the values were within the acceptable range indicating that the system was suitable for the intended analysis.

Linearity range: The calibration range was established by considering the practical range necessary for assay and to give accurate and precise results with good linearity. Detector response (area of peak) was plotted against concentration to obtain calibration curves. Calibration range of drug concentration of Nisoldipine was as (5, 10, 15, 15, 20, 25 and 30 µg/ml) in methanol as solvent. Regression analysis was carried out on calibration curve and results are summarized in Table 2. Linearity of the calibration curve shown in Figure 3 and the adherence to Beer's law were validated by the high value of the correlation coefficient (Figure 3).

Limit of detection and limit of quantification: The parameters LOD and LOQ were determined on the basis of signal to noise ratio, LOD and LOQ were calculated by the method which was based on the standard deviation (S.D.) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ were found to be 0.4 and 1.0 µg/ml, respectively (Table 2).

Precision: Intra-day and inter-day precision of the assay samples containing Nisoldipine (5, 10, 15, 20 and 25 µg/ml) were analyzed five times in the same day (intraday) and for three consecutive days by different analysts. Precision was calculated as intra and inter-day coefficient of variation (CV) as shown in Table 3.

Accuracy: Accuracy of the method was studied by applying the developed method to prepared synthetic mixtures of tablet excipients to which known amount of Nisoldipine corresponding to 50-150% of the labeled claim had been added. The mean recovery values for Nislodipine were shown in Table 4, indicating that the developed method was accurate for the determination of Nisoldipine in pharmaceutical formulation (Table 4).



Detector A Ch1: Peak Table

Peak	Ret. time	Area	Height	Area %	Height %
	7.43	239621	164.2531	100.00	100.00
Total		239621	164.2531	100.00	100.00

Figure 2: HPLC chromatogram of Nisoldipine using proposed method (15 g/ml).

Parameters	Observed value	Recommended value
Retention Time (Rt)*	7.43 ± 0.09	
Number of theoretical plate*	3124.22 ± 23.25	> 2000
Tailing factor*	0.91 ± 0.03	≤ 2
Capacity factor	6.43	> 2
Resolution	7.56	> 2
%RSD (for Retention time)	0.92	≤ 1

*Each value is the mean recovery ± SD of five determinations

Table 1: System suitability parameters.

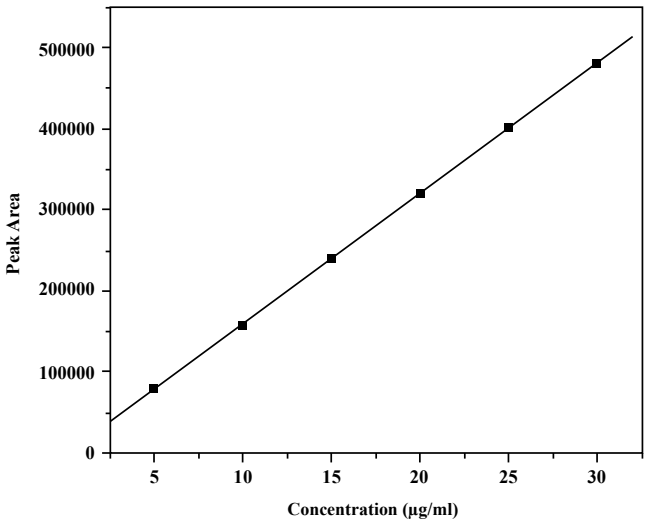


Figure 3: Calibration curve of Nisoldipine by HPLC.

Conc. µg/ml	Peak area	Linear Regression for : Y = A + B* X
5	80135	
10	158766	Parameter Value Error
15	239621	A -856.53333 849.32543
20	319867	B 16050.49714 43.61733
25	401653	R SD N P
30	480121	0.99999 912.32186 6 <0.0001
Limit of detection (LOD)		0.4 µg/ml
Limit of detection (LOQ)		1.0 µg/ml
Analyst to Analyst		Mean ± SD (100.56 ± 0.97) and %RSD 1.13

Table 2: Linearity and regression data for determination of Nisoldipine by HPLC.

Robustness: The robustness of the method was evaluated by assaying the same sample under different analytical conditions deliberately changed from the original analytical condition. The results obtained were not affected by varying the conditions and were in accordance with the results for original conditions (Table 5). The % RSD value (0.51) of assay determined for the same sample under original conditions and with all the conditions of robustness indicates that the developed method was robust and not affected by deliberate changes in the method parameters.

Conclusion

The results of the validation studies show that the LC method is specific, accurate and possesses significant linearity and precision

Concentration µg/ml	Intra-day	Inter-day
5	112.2 ± 4.9[a] (4.4)[b]	85.5 ± 5.4 (6.3)
10	109.5 ± 6.6 (6.0)	98.6 ± 9.6 (9.8)
15	99.9 ± 7.7 (7.7)	104.2 ± 10.0 (9.6)
20	92.7 ± 7.0 (7.1)	101.6 ± 7.9 (7.7)
25	98.0 ± 10.5 (10.7)	99.0 ± 7.3 (7.4)

[a]Accuracy (Mean recovery% ± S.D.)
[b]CV, coefficient of variation (%)

Table 3: Precision of the intra-day and inter-day assay (n=5).

Level	Drug added, µg	Drug recovery, µg	Recovery %	Mean ± SD (%)	% RSD
50%	5	4.860	97.20	98.64 ± 0.34	0.19
	5	5.015	100.30		
	5	4.921	98.42		
100%	10	9.833	98.33	100.02 ± 0.41	0.54
	10	10.14	101.41		
	10	10.03	100.30		
150%	15	15.465	103.10	99.20 ± 018	0.26
	15	14.89	99.30		
	15	14.93	99.51		

Table 4: Recovery data obtained for different mixtures of Nisoldipine and results of accuracy data using the proposed HPLC method.

Parameters	Variations	Peak area	%RSD	Avg. %RSD	Rt (min.)	Capacity factor	Theoretical plate
Flow rate	± 0.1ml/min	239521	0.21	0.35	7.51	1.201	3425
		235684	0.32		7.31	1.425	3375
		228796	0.54		7.19	0.962	3412
pH	± 0.20	245231	0.46	0.48	7.23	1.623	3422
		241124	0.51		7.14	0.711	3463
Mobile phase	± 2 ml methanol	221124	0.31	0.29	7.12	1.322	3433
		224565	0.27		7.54	1.121	3386

Table 5: Robustness data for Nisoldipine using HPLC system.

characteristics without any interference from the excipients, demonstrating the advantages of the LC technique, very well established for the quality control of most of the pharmaceuticals due to its simplicity, high resolution and satisfactory precision and accuracy. Therefore, the proposed method was successfully applied and suggested for the quantitative analysis of Nisoldipine in the bulk powder and in the pharmaceutical dosage forms, contributing to improve the quality control and to assure the therapeutic efficacy.

Future scope for work on Nisoldipine

Simply the current method, the probable degradation pathways of the drug in different stressed conditions can be predicted by analyzing the stressed samples by LC-method, whereby molecular weights and the fragmentation results of the degradation products of Nisoldipine will confirm the most probable degradation pathways of the drug.

References

1. van Harten J, van Brummelen P, Zeegers RR, Danhof M, Breimer DD (1988) The influence of infusion rate on the pharmacokinetics and haemodynamic effects of nisoldipine in man. Br J Clin Pharmacol 25: 709-717.
2. Sweetman SC (2009) Martindale: The Complete Drug Reference, (37thedn), Pharmaceutical Press, London.
3. Lueje AA, Sturm JC, Vergara LJN, Squella JA (2001) A selective voltammetric method to follow the hydrolytic degradation of nitrendipine and nisoldipine. Electroanalysis 13: 1485-1490.
4. Attia AK, Saber RA, Abdulla SA (2011) Electrochemical determination of antihypertensive drug nisoldipine in bulk and tablet dosage form using carbon paste electrode. Journal of Pharmacy Research 4: 2362.
5. Alvarez-Lueje A, Naranjo L, Nunez-Vergara LJ, Squella JA (1998) Electrochemical study of nisoldipine: analytical application in pharmaceutical forms and photodegradation. J Pharm Biomed Anal 16: 853-862.
6. Dogrukol AKD, Gokoren N, Tuncel M (1998) A differential pulse voltammetric determination of nisoldipine using glassy carbon electrode in pharmaceutical preparations. Analytical Letters 31: 105-116.
7. Likussar W, Michelitsch A, Zsilavecz, MS (1994) Determination of nisoldipine in film tablets by differential pulse polarography. Monatshefte für Chemie 125: 509-513.
8. Zhang Z, Tian Y, Ni L, Li J (2004) High performance liquid chromatography-electrospray ionization mass spectrometric determination of nisoldipine in human plasma. J Chromatogr Sci 42: 501-505.
9. Kong D, Li S, Zhang X, Gu J, Liu M, et al. (2010) Simultaneous determination of m-nisoldipine and its three metabolites in rat plasma by liquid chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 878: 2989-2996.
10. Kang W (2006) Simple and sensitive determination of nisoldipine in plasma using liquid chromatography-tandem mass spectrometry. Anal Sci 22: 1597-1599.
11. Agbaba D, Vucicevic K, Marinkovic V (2004) Determination of nisoldipine and its impurities in pharmaceuticals. Chromatographia 60: 223-227.
12. Wang H, Zhang L, Wang Q, Yuan Z, Li M (2006) Validated LC-MS-MS method for determination of m-nisoldipine polymorphs in rat plasma and its application to pharmacokinetic studies. J Chromatogr B Analyt Technol Biomed Life Sci 835: 71-76.
13. Marinkovic V, Agbaba D, Karljikovic-Rajic K, Comor J, Zivanov-Stakic D (2000) UV derivative spectrophotometric study of the photochemical degradation of nisoldipine. Farmaco 55: 128-133.
14. Marinkovic VD, Agbaba D, Karljikovic-Rajic K, Vladimirov S, Nedeljkovic JM (2003) Photochemical degradation of solid state nisoldipine monitored by HPLC. J Pharm Biomed Anal 32: 929-935.
15. Marques MP, Santos NAG, Coelho EB, Bonato PS, Lanchote VL (2001) Enantioselective assay of nisoldipine in human plasma by chiral high-performance liquid chromatography combined with gas chromatography-mass spectrometry: applications to pharmacokinetics. J Chromatogr B Biomed Sci Appl 762: 87-95.
16. Miller JC, Miller JN (1993) Statistics for Analytical Chemistry, Ellis Horwood Series, Prentice Hall, New York, USA.
17. Snyder LR, Barker J, Ando DJ, Kirkland JJ, Glajch JL (1997) Practical HPLC Method Development, (2ndedn), Wiley, New York, USA.

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