VALIDATED HPLC METHOD FOR THE DETERMINATION OF NISOLDIPINE

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ABSTRACT

In this study, novel high performance liquid chromatography method 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 x 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.

Key words: 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 myocardium1. 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 in the management of hypertension and angina pectoris2. Fig. 1 shows the structural formula of Nisoldipine.

Fig. 1: Chemical structure of nislodipine
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\(^13\) 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**

**Instruments and materials**

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.

**Preparation of standard solution**

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 then 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 wavelength used for quantification. Separation was performed on an Agilent ZORBAX Eclipse Plus C18 with particle size of 10 µm (4.6 x 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.

**Validation of the method**

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 and robustness.

**Procedures for tablets**

Twenty tablets of formulation (Sular tablet) were powdered and an amount equivalent to one tablet of Nisoldipine was weighed and then dissolved in the methanol. Then the obtained solution was filtered.
through ordinary filter paper. The desired concentrations for nisoldipine in the range of 5-30 µg/mL were obtained by accurate dilution of this solution. All solutions were sonicated. Finally, all the solutions were filtered through 45 µm Millipore™ filter in order to separate out the insoluble excipients 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 0.1M Hexane sulphonie 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 x 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 (Fig. 2).

![HPLC Chromatogram of nisoldipine using proposed method (15 g/mL)](image)

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

Fig. 2: HPLC Chromatogram of nisoldipine using proposed method (15 g/mL)

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 (peak area) was plotted against concentration to obtain calibration curves. Calibration range of drug concentration of nisoldipine was found
as (5, 10, 15, 20, 25 and 30 μg/mL) in methanol as solvent. The calibration curve was performed in triplicate. Regression analysis was carried out on calibration curve and results are summarized in Table 1. Linearity of the calibration curve shown in Fig. 3 and the adherence to Beer’s law were validated by the high value of the correlation coefficient.

![Calibration curve of nisoldipine by HPLC](image)

**Limit of detection and limit of quantification**

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. The LOD and LOQ were found to be 0.4 and 1.0 μg/mL, respectively (Table 1).

| Concentration (μg/mL) | Peak area | Linear regression for: Y = A + B * X |
|-----------------------|-----------|--------------------------------------|
| 5                     | 80135     | Parameter | Value    | Error     |
| 10                    | 158766    | A         | -856.53333 | 849.32543 |
| 15                    | 239621    | B         | 16050.49714 | 43.61733  |
| 20                    | 319867    |           |           |           |
| 25                    | 401653    | R         | 0.99999   | 912.32186 |
| 30                    | 480121    | SD        | 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 |

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 (intra-day) and for three consecutive days by different analysts. Precision was calculated as intra and interday coefficient of variation (CV) as shown in Table 2.
Table 2: Precision of the intra-day and inter-day assay (n = 5)

| Concentration µg/mL | Intra-day       | Inter-day      |
|---------------------|-----------------|----------------|
| 5                   | 112.2 ± 4.9* (4.4)** | 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)** |

*Accuracy (Mean recovery % ± S.D.)
**CV, coefficient of variation (%)

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 3. These values indicating that the developed method was accurate for the determination of nisoldipine in pharmaceutical formulation.

Table 3: Recovery data obtained for different mixtures of nisoldipine and results of accuracy data using the proposed HPLC method

| Level | Drug added, µg | Drug recovery, µg | Recovery % | Mean ± SD (%) | % RSD |
|-------|----------------|-------------------|------------|----------------|-------|
| 5     | 4.860          | 97.20             |            |                |       |
| 5     | 5.015          | 100.30            |            | 98.64 ± 0.34   | 0.19  |
| 5     | 4.921          | 98.42             |            |                |       |
| 10    | 9.833          | 98.33             |            |                |       |
| 10    | 10.14          | 101.41            |            | 100.02 ± 0.41  | 0.54  |
| 15    | 15.465         | 103.10            |            |                |       |
| 15    | 14.89          | 99.30             |            | 99.20 ± 0.18   | 0.26  |
| 15    | 14.93          | 99.51             |            |                |       |

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). 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. The results obtained were not affected by varying the conditions and were in accordance with the results for original conditions (Table 4). 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 parameters of the used method.

**Table 4: Robustness data for nisoldipine using HPLC system**

| Parameters       | Variations   | Peak area | % RSD | Avg. % RSD | Rt (min.) | Capacity factor | Theoretical plate |
|------------------|--------------|-----------|-------|------------|-----------|-----------------|-------------------|
| Flow rate        | ± 0.1 mL/min | 239521    | 0.21  |            | 7.51      | 1.201           | 3425              |
|                  |              | 235684    | 0.32  | 0.35       | 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              |
| Mobile phase     | ± 2 mL methanol | 224565    | 0.27  | 0.29       | 7.54      | 1.121           | 3386              |

**Determination of nisoldipine in tablets**

The proposed method was successfully applied to determine nisoldipine in Sular tablets without interference from some common excipients used in pharmaceutical preparations such as hydroxypropylcellulose, lactose, starch, microcrystalline cellulose, and magnesium stearate. Replicate analyses have been carried out to obtain the accuracy and precision of the proposed method. The linearity range was in the range of 5-30 µg/mL with mean recovery of 99.95% and mean relative standard deviation of 0.87%.

**System suitability**

The system suitability test was also carried out to evaluate the resolution and reproducibility of the system for the analysis by 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). The experimental results (Table 5) showed that the values were within the acceptable range indicating that the system was suitable for the intended analysis.

**Table 5: System suitability parameters**

| 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
CONCLUSION

The results of the validation studies show that the proposed chromatographic method is specific and accurate. It possesses significant linearity and precision without any interference from the excipients, demonstrating the advantages of the chromatographic technique which established for the quality control of most of the pharmaceuticals due to its simplicity, high resolution, 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.

REFERENCES

1. J. Van Harten, P. Van Brummelen, R. R. Zeegers, M. Danhof, D. D. Breimer, Br. J. Clin. Pharmacol., 25, 709 (1988).
2. S. C. Sweetman, Martindale: The Complete Drug Reference, 36th Ed., Pharmaceutical Press, London (2009) p. 1358
3. A. A. Lueje, J. Sturm, L. J. N. Vergara and J. A. Squella, Electroanalysis, 13, 1485 (2001).
4. A. K. Attia, R. A. Saber and S. A. Abdulla, J. Pharm. Res., 4, 2362 (2011).
5. A. A. Lueji, L. Naranjo, L. J. N. Vergara and J. A. Squella, J. Pharm. Biomed. Anal., 16, 853 (1998).
6. A. K. D. Dogrukol, N. Gokoren and M. Tuncel, Anal. Lett., 31, 105 (1998).
7. W. Likussar, A. Michelitsch and M. S. Zsilavecz, Monatsh. Chem., 125, 509 (1994).
8. Z. J. Zhang, Y. Tian, L. Ni and J. H. Li, J. Chromatogr. Sci., 42, 501 (2004).
9. D. Kong, S. Li, X. Zhang, J. Gu, M. Liu, Y. Meng, Y. Fu, X. La, G. Xue, L. Zhang, Q. Wang, J. Chromatogr. B, 878, 2989 (2010).
10. W. Kang, Anal. Sci., 22, 1597 (2006).
11. D. Agbaba, K. Vucicevic and V. Marinkovic, Chromatographia, 60, 223 (2004).
12. H. R. Wang, L. Zhang, Q. Wang, Z. Yuan, M. Li, J. Chromatogr. B, 835, 71 (2006).
13. V. Marinkovic, D. Agbaba and R. Karljikovic, Il Farmaco, 55, 128 (2000).
14. V. D. Marinkovic, D. Agbaba, K. K. Rajic, S. Vladimirov and J. M. Nedeljkovic, J. Pharm. Biomed. Anal., 32, 929 (2003).
15. M. P. Marques, N. A. G Santos, E. B. Coelho, P. S. Bonato and V. L. Lanchote, J. Chromatogr. B, 762, 87 (2001).
16. J. C. Miller and J. N. Miller, Statistics for Analytical Chemistry, Ellis Horwood Series, Prentice Hall, New York (1993) p. 119.