



Journal home page:
<http://www.iajpr.com/index.php/en/>

INDO AMERICAN
 JOURNAL OF
 PHARMACEUTICAL
 RESEARCH

SIMPLE SPECTROPHOTOMETRIC METHOD VALIDATION OF NIFEDIPINE SOLID DOSAGE FORM

Patitapabana Parida^{1*}, Subash Chandra Mishra², Subha Prakash Tripathy³

^{1*}Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela, Odisha-769008, India.

²Department of Metallurgical and Materials Engineering, National Institute of Technology, Rourkela, Odisha-769008, India.

³Torrent Pharmaceuticals Limited, Ahmadabad, Gujarat – 380009, India.

| ARTICLE INFO | ABSTRACT |
|--|---|
| <p>Article history Received 20/02/2014 Available online 24/05/2014</p> <p>Keywords Nifedipine, Hypertension, Uv-Visibl Spectrophotometer, Validation.</p> | <p>Simple, sensitive and specific spectrophotometric method was developed for the validation of nifedipine in tablet dosage form in two different brands. Active ingredient showed the absorption maximum in ethanol and chloroform at 235.5 nm and 235 nm respectively. The linearity was established in the concentration range of 2-10 µg/ml for nifedipine in different solvent with correlation coefficient (r^2) of 0.997 - 0.999 respectively. The mean % recoveries were found to be in the range of 99.57 - 99.81 % for nifedipine in different brands. Statistically potency of two marketed brands were determined that there were no significant difference between the two brands where ANOVA at $f(5,3) = 196.0143$ and significant level of p-value observed at 0.004. Hence the proposed method can be applied for the routine analysis of active molecule from the formulations.</p> |

Corresponding author

Patitapabana Parida

Department of Biotechnology and Medical Engineering,
 National Institute of Technology,
 Rourkela, Odisha-769008
 +91-9439781667
 paridap@nitrkl.ac.in

Please cite this article in press as **Patitapabana Parida et al.** Simple Spectrophotometric Method Validation of Nifedipine Solid Dosage Form. *Indo American Journal of Pharm Research.*2014;4(05).

Copy right © 2014 This is an Open Access article distributed under the terms of the Indo American journal of Pharmaceutical Research, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Chemically nifedipine is 3,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-dimethyl ester shown in figure 1. It is used as antianginal agent who lowers blood pressure by vasodilation initiated by calcium antagonistic action [1-3]. It is also used for the treatment of vascular disorders such as Raynaud's phenomenon [4, 5]. The 1,4-dihydropyridine class of calcium channel antagonist inhibits this Ca^{2+} influx through the L-type potential dependent calcium channel which is responsible for the regulation of many physiological functions, including smooth and cardiac muscle contraction [6-8]. Particularly nifedipine is used as a second line treatment in hypertension during pregnancy [9-11]. Aromatization in the dihydropyridine moiety turns it into a pyridine ring and a reduction of nitro group in to nitroso groups at the exposure to ultraviolet-visible irradiation leads to photo-oxidation and produces dehydronifedipine [12, 13]. Nifedipine is freely soluble in acetone [14] and in chloroform, sparingly soluble in ethanol [15] but practically insoluble in water [16].

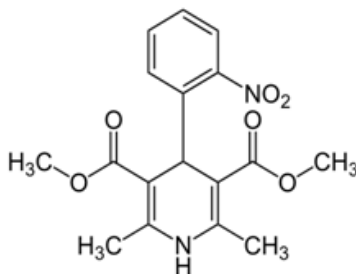


Figure1. Chemical structure for nifedipine

The Uv-visible spectroscopic methods generally simple, sensitive and suitable for the study of chemical concentration in solutions and can be monitored directly [17]. Analytical development for dosage form is necessary to ensure the safety and efficacy throughout the quality associated with accuracy and reproducibility.

MATERIALS AND METHODS

Materials

Nifedipine the active ingredient was received as a gift from J. B Chemicals and Pharmaceuticals Pvt. Ltd, India. Other ingredients such as ethanol and chloroform were provided by Loba Chemie, India. Depin SR tablet 10 mg as Brand-1 (Zydus Cadila) and Nifelat SR tablet 10 mg Brand-2 (Cipla) were purchased from local retailer.

Apparatus

Spectroscopic analysis was carried out using double beam Shimadzu recording UV-Visible spectrophotometer ELICO BL-198 (India) matched quartz cells over the ranges from 200-400 nanometer with wave length interval 0.5 nm in the spectrum mode was used for analytical purpose.

Preparation of stock solution with ethanol

Accurately weighed 2.5 mg of nifedipine was transferred into 100 ml volumetric flask and dissolved in 100 ml of ethanol and diluted up to the mark with water to get a stock solution. The stock solution was sonicated for 2 min and pipette out 10 ml and make up to 100 ml of the same solvent. The concentration of the solution will be 25 $\mu\text{g/ml}$. Later the stock solution was further diluted serially with the respective solvent to get 2, 4, 6, 8, 10 $\mu\text{g/ml}$ solutions and making the final volume up to 10 ml for all the dilutions.

Preparation of stock solution with chloroform

Accurately weighed 2.5 mg of nifedipine was transferred into 100 ml volumetric flask and dissolved in 100 ml of chloroform and diluted up to the mark with water to get a stock solution. The stock solution was sonicated for 2 min and pipette out 10 ml and make up to 100 ml of the same solvent. The concentration of the solution will be 25 $\mu\text{g/ml}$. Later the stock solution was further diluted serially with the respective solvent to get 2, 4, 6, 8, 10 $\mu\text{g/ml}$ solutions and making the final volume up to 10 ml for all the dilutions.

Preparation of calibration curve

Calibration curve was prepared in ethanol and chloroform at λ_{max} 235.5 and 235 nm using UV-Visible spectrophotometer respectively. Serial dilution of 2, 4, 6, 8, and 10 $\mu\text{g/ml}$ were prepared and absorbance was taken. Averages of such 6 sets of values were taken for standard calibration curve and solutions were scanned in the range of 200-400 nm against blank shown in figure 3.

Preparation of working standard solution in ethanol and chloroform

Weigh the powder of 10 tablets of two different brands separately. Weigh accurately a quantity of the powder equivalent to 2.5 mg of nifedipine into a 200 ml volumetric flask. Dissolve with the aid of 100 ml of ethanol. Dilute 10 ml of the filtrate to 100.0 ml with ethanol. Further dilutions were carried out to get 2, 4, 6, 8, and 10 $\mu\text{g/ml}$ for Brand 1 and 2. Measure the absorbance of the resulting solution at the maximum at about 235.5 nm. The standard solution was prepared with chloroform from the above principle.

METHOD VALIDATION

Precision

The Intra-day precision (repeatability) and inter-day precision (intermediate precision) study of nifedipine was carried out by estimating different concentrations of 2-10 µg/ml three times on the same day and on three different days and the results were reported in terms of % RSD. All the experiments were carried in a dark room environment that the raw material is photo sensitive in day light.

Accuracy

Accuracy was assessed by determination of the recovery study. Addition of standard drug to the pre quantified sample preparation at different concentration levels.

Specificity and selectivity

Three different marketed tablets of Nifedipine of concentration 2 mg/ml were prepared in ethanol and chloroform was analyzed by the proposed method. The estimated amounts of marketed formulation were compared with that of pure nifedipine solution of the same strength. Specificity and selectivity of nifedipine solutions prepared in dilution media was checked for change in the absorbance at wavelength 235.5 and 235 nm shown in Figure 2.

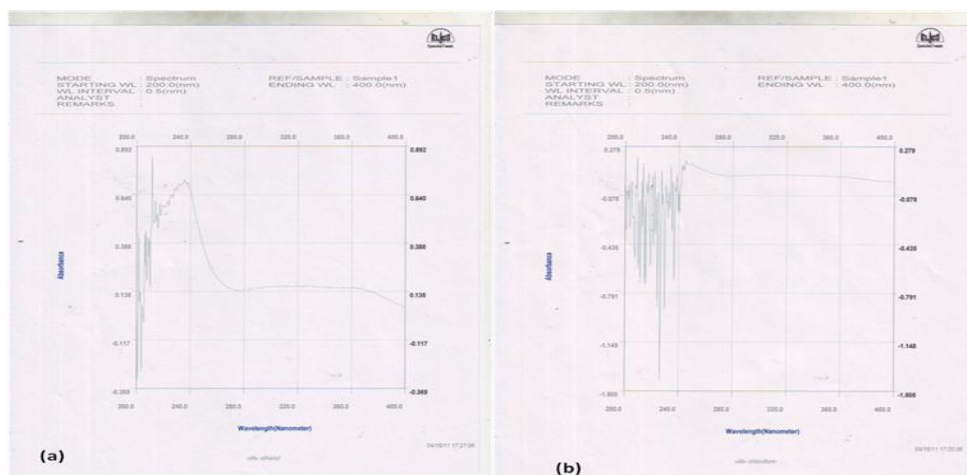


Figure 2. Absorbance in Ethanol (A), Absorbance in chloroform (B)

Linearity

The linearity of the response of the drug was verified at 2 to 10 µg/ml concentrations, but linearity was found to be between 4-10 g/ml concentrations in Table 1. The calibration graphs were obtained by plotting the absorbance versus the concentration data and were treated by linear regression analysis in table 2.

Table 1: Mean absorbance values and statistical data of the calibration curve for the estimation of Nifedipine tablets

| Concentration (µg/ml) | Absorbance for nifedipine from Brand 1 | | Absorbance for nifedipine from Brand 2 | |
|-----------------------|--|--------------------|--|-------------------|
| | measured at 235.5 nm | measured at 235 nm | measured at 235.5 nm | measured at 235nm |
| 0 | 0 | 0 | 0 | 0 |
| 2 | 0.107 | 0.109 | 0.133 | 0.118 |
| 4 | 0.223 | 0.232 | 0.252 | 0.265 |
| 6 | 0.311 | 0.351 | 0.370 | 0.423 |
| 8 | 0.432 | 0.482 | 0.501 | 0.558 |
| 10 | 0.511 | 0.612 | 0.645 | 0.719 |
| Mean | 0.264 | 0.297 | 0.316 | 0.347 |
| RSD | 0.193 | 0.229 | 0.237 | 0.271 |

Limit of Detection

According to ICH guidelines the detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value. Limit of detection can be calculated using following equation as per ICH guidelines, $LOQ = \frac{3.3 \times \text{Standard Deviation}}{\text{Slope}}$.

Limit of Quantification

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Limit of quantification can be calculated using following equation as per ICH guidelines. $LOQ = \frac{10 \times \text{Standard Deviation}}{\text{Slope}}$, shown in table 2.

Table.2 - Validation parameters of proposed method

| Validation | Brand-1 | Brand-2 | Brand-1 | Brand-2 |
|--------------------------------------|-------------------|-------------------|-------------------|-------------------|
| Absorption maxima(nm) | 235.5 nm | 235 nm | 235.5 nm | 235 nm |
| Linearity Range ($\mu\text{g/ml}$) | 2-10 | 2-10 | 2-10 | 2-10 |
| Slope | 0.103 | 0.122 | 0.128 | 0.149 |
| Intercept | 0.097 | 0.132 | 0.131 | 0.031 |
| Correlation Coefficient (r^2) | 0.9975 | 0.9991 | 0.998 | 0.999 |
| Accuracy (Mean \pm SD) | 0.264 \pm 0.193 | 0.297 \pm 0.229 | 0.316 \pm 0.237 | 0.347 \pm 0.271 |
| SE of intercept | 0.0078 | 0.0056 | 0.0063 | 0.0089 |
| SD of Intercept | 0.0175 | 0.0126 | 0.0141 | 0.0199 |
| LOD ($\mu\text{g/mL}$) | 0.5614 | 0.3419 | 0.3656 | 0.4416 |
| LOQ ($\mu\text{g/mL}$) | 1.7014 | 1.0362 | 1.1081 | 1.3382 |

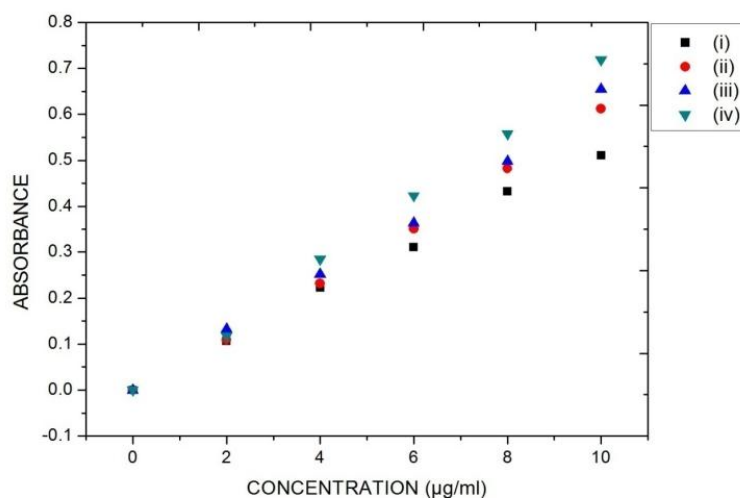


Figure 3. Brand-1 in ethanol at 235.5 nm (i), Brand-2 in ethanol at 235.5 nm (ii), Brand-1 in chloroform at 235 nm (iii), Brand-2 in chloroform at 235 nm (iv)

RUGGEDNESS

Ruggedness of the proposed method was determined by analysis of aliquots from two homogenous slots by analyst using same operational and environmental condition. A linear simple regression by the least squares method was applied. The statistical analysis was calculated by ANOVA the precision was expressed as the percent coefficient of variation of each curve.

RESULTS AND DISCUSSION

Linearity and range

The calibration curve obtained was estimated by its correlation coefficient. The Limit of detection and Limit of Quantification of nifedipine by the proposed method was determined using calibration standards and shown in Table-2. The absorbance of the samples in the range of 2-10 $\mu\text{g/mL}$ was linear with a correlation coefficient (R^2) greater than 0.998. ANOVA analysis results show the significance value of $f(5, 3) = 196.0143$, $p = 0.004$.

Specificity

The specificity of the characterization method was proved by comparing the spectra of raw material and finished marketed products of samples.

Accuracy

Precision of the method was conducted for solutions containing known amounts of pure drug. The analytical results obtained from these investigations for the methods were summarized in Table 2. Results within the range of 99.00–100.07% revealed an precise method as well as indicate non-interference with the excipients of formulation shown in Table 3.

Intra-day and inter-day precision

The intra-day and inter-day precision studies confirmed sufficient sample stability and method reliability where all the RSDs were < 1%.

Table 3. Interday and Intraday results

| Days | Assay of nifedipine as % of labeled amount | | | |
|-------|--|----------|---------------------|----------|
| | Analyst-1 | | Analyst-2 | |
| | Inter -day precision | | Intra-day precision | |
| | Brand-I | Brand-II | Brand-I | Brand-II |
| 1 | 99.29 | 99.42 | 100.19 | 99.63 |
| 2 | 99.86 | 99.32 | 99.57 | 100.15 |
| 3 | 99.41 | 99.72 | 99.88 | 99.51 |
| 4 | 100.72 | 99.68 | 99.95 | 99.38 |
| 5 | 99.31 | 100.13 | 99.46 | 99.20 |
| Mean | 99.718 | 99.654 | 99.81 | 99.574 |
| % RSD | 0.605946 | 0.315405 | 0.295381 | 0.359347 |

The ANOVA analysis result from table 2 showed there is no significant difference at $p = 0.004$, among the assay results obtained in five different days at different times. No interfering intensity was found in the UV spectra due to the tablet excipients. Nifedipine was shown to be stable during all procedure.

CONCLUSIONS

The developed and validated UV estimation method reported here is rapid, simple, accurate, sensitive and specific. The method was also successfully used for quantitative estimation and analysis of nifedipine in dosage form. The developed method was found to be precise, reproducible, and can be used for routine quality control analysis of nifedipine in bulk and pharmaceutical formulation. The procedures can be widely accepted for routine quality control was well established by the assay of nifedipine in pure form and dosage form without interference with commonly used excipients and related substances.

Authors' Statements

Competing Interests

The authors declare no conflict of interest.

REFERENCES

1. Revathi, R., et al., New spectroscopic determination of nifedipine using hydrotropic solubilization. International Journal of Pharmacy and Pharmaceutical Sciences, 2010, 2, 74-6.
2. Boecker, R.H. and F.P. Guengerich, Oxidation of 4-aryl-and 4-alkyl-substituted 2, 6-dimethyl-3, 5-bis (alkoxycarbonyl)-1, 4-dihydropyridines by human liver microsomes and immunochemical evidence for the involvement of a form of cytochrome P-450. Journal of medicinal chemistry, 1986, 29(9), 1596-1603.
3. Muto, K., et al., Synthesis and pharmacological activity of stereoisomers of 1, 4-dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-3, 5-pyridine-dicarboxylic acid methyl 1-(phenylmethyl)-3-piperidinyl ester. Arzneimittel-Forschung, 1988, 38(11A), 1662.
4. Coleiro, B., et al., Treatment of Raynaud's phenomenon with the selective serotonin reuptake inhibitor fluoxetine. Rheumatology, 2001, 40(9), 1038-1043.
5. Askal, H., et al., Spectrophotometric and Spectrofluorimetric Determination of 1, 4-Dihydropyridine Drugs Using Potassium Permanganate and Cerium (iv) Ammonium Sulphate. Bulletin of Pharmaceutical Sciences (Assiut University), 2010, 33, 201-215.
6. Shamsipur, M., et al., A study of the photo-degradation kinetics of nifedipine by multivariate curve resolution analysis. Journal of pharmaceutical and biomedical analysis, 2003, 31(5), 1013-1019.
7. Xiao, R.-P., et al., Dual regulation of Ca^{2+} /calmodulin-dependent kinase II activity by membrane voltage and by calcium influx. Proceedings of the National Academy of Sciences, 1994, 91(20), 9659-9663.
8. Chen, X.-L. and C.M. Rembold, Cyclic nucleotide-dependent regulation of Mn^{2+} influx, $[Ca^{2+}]_i$, and arterial smooth muscle relaxation. American Journal of Physiology-Cell Physiology, 1992, 263(2), C468-C473.
9. De Souza, A.R., M.R. Amorim, and A.R. Costa, Nifedipine antihypertensive treatment effects in pre-eclampsia. Acta medica portuguesa, 2008, 21(4), 351.
10. Walters, B. and C. Redman, Treatment of severe pregnancy-associated hypertension with the calcium antagonist nifedipine. BJOG: An International Journal of Obstetrics & Gynaecology, 1984, 91(4), 330-336.

11. Wood, A.J. and B.M. Sibai, Treatment of hypertension in pregnant women. New England Journal of Medicine, 1996, 335(4), 257-265.
12. Dinarvand, R., et al., Effect of Microencapsulation on Photo-Stability of Nifedipine. Iranian Journal of Pharmaceutical Research, 2010, 5(4), 239-244.
13. Latosińska, J., et al., An innovative method for the non-destructive identification of photodegradation products in solid state: ^1H - ^{14}N NMR-NQR and DFT/QTAIM study of photodegradation of nifedipine (anti-hypertensive) to nitrosonifedipine (potential anti-oxidative). European Journal of Pharmaceutical Sciences, 2012, 47(1), 97-107.
14. Godfraind, T., Actions of nifedipine on calcium fluxes and contraction in isolated rat arteries. Journal of Pharmacology and Experimental Therapeutics, 1983, 224(2), 443-450.
15. Bauer, K.H., Nifedipine concentrate stabilized against the influence of light and a process for its preparation. 1990, Google Patents.
16. Hecq, J. Tejaswi Annapureddy, J. Padma preetha1, et al., Enhancement of solubility of nifedipine by liquisolid compacts technique. Indo American Journal of Pharmaceutical Research, 2013, 2005, 4.4, 3433.
17. Sai Thanuja, V., et al. "Spectrophotometric determination of levetiracetam using 2, 4-dnp in pharmaceutical dosage form., Indo American Journal of Pharmaceutical Research, 2014, 4,1, 5030-5034.



54878478451014243



Submit your next manuscript to **IAJPR** and take advantage of:

- Access Online first
- Double blind peer review policy
- No space constraints
- Rapid publication
- International recognition

Submit your manuscript at: editorinchief@iajpr.com

