Validated Spectrophotometric Method for the Assay of Nifedipine in Bulk and Commercial Dosage Forms

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ABSTRACT: A validated spectrophotometric method has been described for the assay of nifedipine in bulk and commercial dosage forms. The method is based on the oxidation of drug with Fe(III) at pH 1.42. The reduced Fe(II) subsequently reacts with 1,10-phenanthroline to produce a red colored tris(1,10-phenanthroline) iron(II) complex, which absorbs maximally at 505 nm. Beer's law is obeyed in the concentration range 0.5 - 14.0 μg mL^{-1} with molar absorptivity and Sandell's sensitivity of 2.805×10^4 L mol^{-1} cm^{-1} and 0.012 μg cm^{-2} per 0.001 absorbance unit, respectively. The various experimental parameters were optimized. The method has been successfully applied for the quantitation of the drug in commercial dosage forms. The results of analysis were statistically compared with those of a reference method showing acceptable recovery and precision. The experimental true bias of all samples based on recovery experiments were within ± 2 %.

KEYWORDS: spectrophotometry, nifedipine, ammonium ferric sulfate, 1,10-phenanthroline, validation.

INTRODUCTION

Nifedipine is chemically known as dimethyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5 dicarboxylate. It is pharmacologically a selective L-type calcium channel antagonist.[1] It causes coronary vasodilation and increases coronary blood flow. It reduces the total peripheral vascular resistance, for which it is widely used in the treatment of hypertension, angina pectoris and various other cardiovascular disorders. The drug is official in The United States Pharmacopoeia[2] and British Pharmacopoeia,[3] which describe high performance liquid chromatographic and non-aqueous titration methods, respectively for its assay in bulk and dosage forms.

Several methods have been reported for the determination of nifedipine in biological fluids and/or pharmaceutical formulations. These include high performance thin layer chromatography,[4] high performance liquid chromatography,[5] gas chromatography coupled with mass spectrometry,[6] micellar electrokinetic chromatography,[7] flow injection analysis,[8] electroanalytical methods[9] and UV spectrophotometry.[10]

The estimation of nifedipine in single and combined dosage forms has been made using second order[11] and first order[12] derivative spectra, respectively. Nifedipine was assayed in dosage forms based on the reaction of the drug with 4-dimethylaminobenzaldehyde in H$_3$PO$_4$ medium resulting in the formation of a yellow coloured product, which can be quantitatively measured at 380 nm.[13] The quantification of the drug was done based on

the reaction of nifedipine with Folin Ciocalteau reagent,[14] and chloranil.[15] Two spectrophotometric methods have been developed based on the reaction of the drug with potassium hydroxide in dimethyl sulphoxide and ammonium molybdate in acidic buffer solution to form a coloured product with absorbance peaking at 430 nm and 830 nm, respectively.[16] A kinetic spectrophotometric method has also been described based on the oxidation of drug with KMnO$_4$ at neutral pH.[17] The other spectrophotometric method was based on the reduction of the nitro group of the nifedipine with Zn/NH$_4$Cl to hydoxylamino derivatives.[18] Extractive spectrophotometric methods have also been reported for the estimation of drug in pharmaceutical preparations, based on the colored complex of the drug with reagents like bromocresol green, bromophenol blue, bromothymol blue and eriochrome black-T.[19]

This paper describes a simple and sensitive validated spectrophotometric method for the determination of nifedipine in pharmaceutical formulations. The method is based on the reaction of drug with ammonium ferric sulfate and 1,10-phenanthroline in acidic pH resulting in the formation of ferroin, which absorbs maximally at 505 nm. The proposed method is validated as per ICH guidelines.[20]

MATERIALS AND METHODS

A Spectronic 20D$^+$ spectrophotometer (Milton Roy, U.S.A.) with matched glass cuvettes was used for all spectral and absorbance measurements. A water bath

shaker (NSW 133, New Delhi, India) was used to control the heating temperature for color development. An Elico model Li-10 pH meter was used to measure the pH of the solutions.

All chemicals used were of analytical or pharmaceutical grade. Solutions of ammonium ferric sulfate (0.01 M) and 1,10-phenanthroline (0.01 M) were prepared in 0.001 M sulfuric acid and ethanol, respectively. Buffer solutions ranging from pH 0.65 - 2.32 were prepared by mixing 50 mL of 1.0 M sodium acetate with different volumes (100 – 51 mL) of 1 M HCl solution, transferring the solutions to a standard flask, and diluting to 250 mL with doubly distilled water.[21]

Reference standard of pure nifedipine and a nifedipine nitrosophenyl pyridine analog were kindly provided by JB Chemicals and Pharmaceuticals Ltd. (Mumbai, India) and Novartis Pharmaceuticals Ltd. (Mumbai, India), respectively. Commercial dosage forms of the drug such as Nicardia Retard (JB Chemicals & Pharmaceuticals Ltd., Mumbai, India), Calciguard (Torrent Pharmaceuticals Ltd., Ahmedabad, India), and Adalat Retard (Bayer, Mumbai, India) were purchased from a local market. The standard solution of 0.01% nifedipine was prepared in methanol and protected from light.

Preparation of Degraded Nifedipine

A standard solution of pure nifedipine (1.0 mg mL^{-1}) was exposed to diffused sunlight (natural) for 2 h. An amount equivalent to 8.0 µl of the degraded nifedipine solution and the nifedipine nitrosophenyl pyridine analog reference standard solution were spotted on a thin layer chromatographic plate of silica gel G (Merck, India) as stationary phase. The chromatograms were developed in a mobile phase of chloroform-ethyl acetate-cyclohexane (19: 2: 2, by vol.). The TLC plates were air-dried and spots were located by placing the plates in iodine chamber. In each case, a single spot having the same R_f value of 0.5 was observed under UV lamp, which indicated that the degraded product of nifedipine is nifedipine nitrosophenyl pyridine.[22]

Recommended Procedure for the Determination of Nifedipine

Into a series of boiling test tubes, different volumes (0.05 - 1.4 mL) of 0.01% of nifedipine solution were pipetted. To each test tube, 1.0 mL of ammonium ferric sulfate and 5.0 mL of pH 1.42 buffer solution were added, mixed well and heated in a water bath at 100 ± 1°C for 5 min. The contents of each test tube were cooled at room temperature (25 ± 1°C) and 2.0 mL of 1,10-phenanthroline was added. Finally, the contents of the tubes were transferred to 10 mL standard volumetric flasks and then diluted to volume with doubly
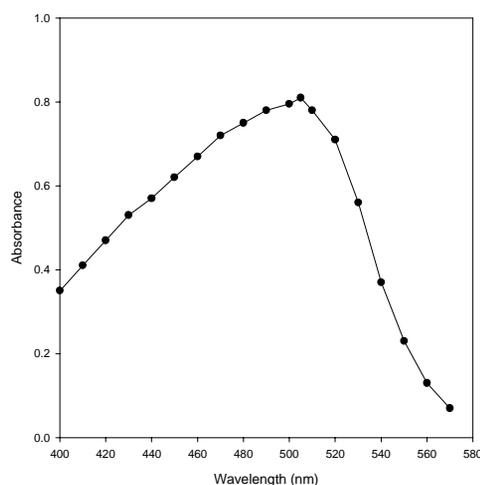
distilled water. The absorbance was measured at 505 nm against the reagent blank prepared similarly, except without the drug. The color intensity of the complex did not change appreciably over a period of 5 h. The concentration of nifedipine was calculated from the corresponding linear regression equation.

Procedure for the Assay of Nifedipine in Pharmaceutical Formulations
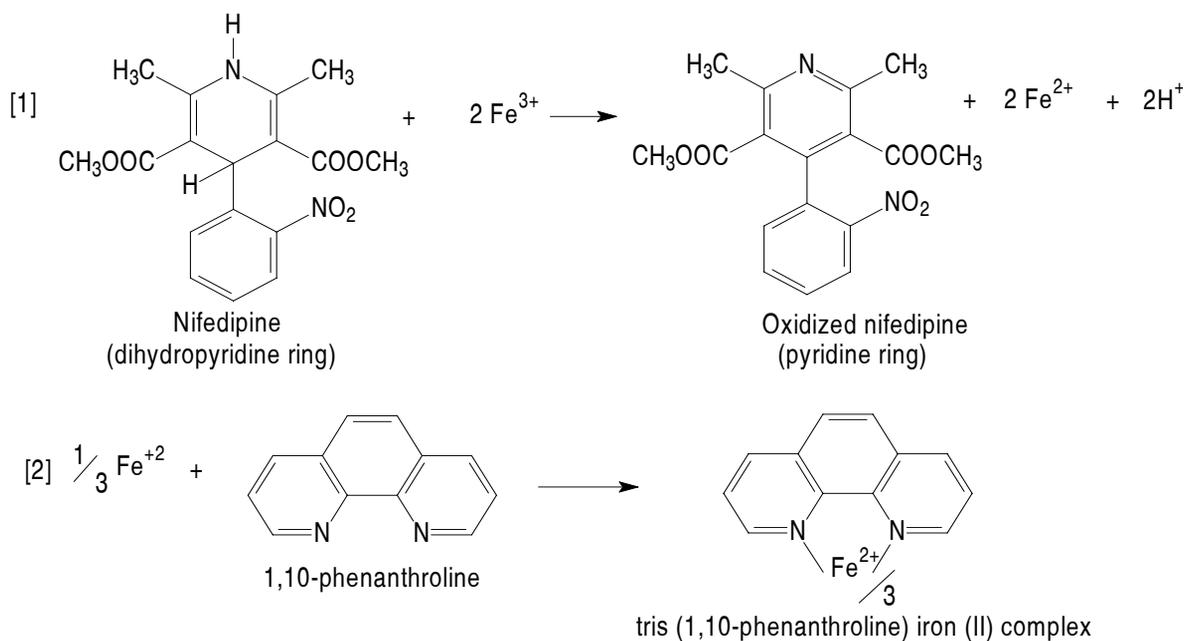
Five tablets of nifedipine (each claiming 10 mg) were finely powdered in a mortar and pestle and a portion equivalent to 20 mg of nifedipine was weighed accurately and stirred well with 3 × 15 mL portions of dichloromethane. The residue was filtered using Whatmann No. 42 filter paper. The filtrate was evaporated to dryness under vacuum and the residual drug was dissolved in methanol and transferred to a 100.0 mL standard volumetric flask, which was then filled to volume with methanol. It was further diluted according to the need and subjected to the recommended procedure for the determination of nifedipine.

RESULTS

Nifedipine can undergo photodegradation accompanied by loss of pharmacological activity. This process involves the reduction of th aromatic nitro group to a nitroso group and/ or the oxidation of the dihydropyridine ring to a pyridine ring.[23] In this study, Fe(III) oxidizes the dihydropyridine ring of nifedipine to a pyridine ring and the reduced Fe(II) forms a red coloured complex with 1,10-phenanthroline of tris(1,10-phenanthroline) Iron(II), ferroin, which exhibits an absorption band peaking at 505 nm (Fig. 1).

Fig 1. Absorption spectrum: 1.0 mL of 0.01% nifedipine + 1.0 mL of 0.01 M ferric ammonium sulfate + 5.0 mL buffer solution of pH 1.42 and 2.0 mL of 0.01 M 1,10-phenanthroline in a 10 mL standard volumetric flask.

Scheme 1

Therefore, based on the literature background and our experimental findings, the reaction sequence is shown in Scheme 1.

Optimization of Variables

The optimum reaction conditions for the quantitative estimation of nifedipine were established via a number of preliminary experiments.

To study the effect of heating time for the development of maximum color, 0.5 mL of 0.01% nifedipine was mixed with 1.0 mL of 0.01 M ammonium ferric sulfate and 5 mL of buffer solution and heated for 7.0 min. in a water bath at $100 \pm 1°C$. After cooling at room temperature, 2.0 mL of 0.01 M 1,10-phenanthroline was added. The intensity of the developed color was measured at room temperature $(25 \pm 1°C)$ after dilution to 10 mL in a standard volumetric flask with doubly distilled water. The maximum intensity of color was obtained after 3.0 min of heating and remained constant up to 7.0 min. Therefore, the optimum heating time was fixed at 5.0 min throughout the experiment.

The influence of pH on the color development was studied using sodium acetate-HCl buffer. The maximum absorbance was obtained in the pH range of 1.09 - 1.85 (Fig. 2). Therefore 5 mL of pH 1.42 buffer solution was used throughout the experiment.

The influence of the volume of 0.01 M ammonium ferric sulfate on the formation of the colored complex was critically examined. The highest absorbance was obtained on addition of 0.3 mL of 0.01 M ferric

ammonium sulfate; above this volume up to 1.3 mL, the absorbance remained constant (Fig. 3a). Therefore, a volume of 1.0 mL of 0.01 M ammonium ferric sulfate was used throughout the determination process.

To study the effect of the volume of 0.01 M 1,10-phenanthroline for maximum color development, different volumes (0.2 - 2.3 mL) were mixed with 0.5 mL of 0.01 % nifedipine and 5 mL of buffer solution (pH 1.42). The results are presented in Fig. 3b. The highest absorbance was obtained by the addition of 1.0 mL of
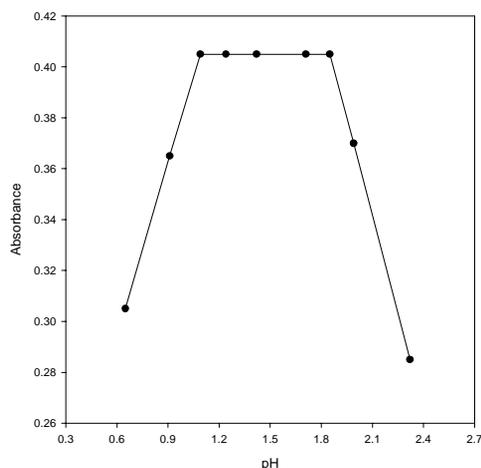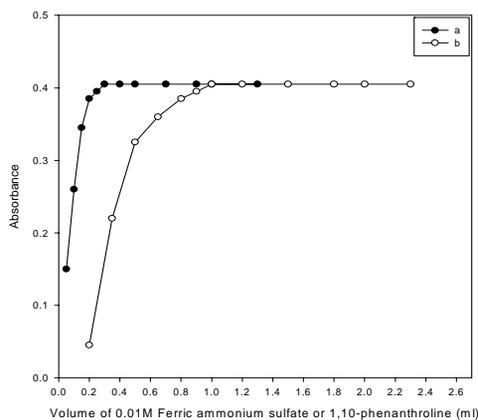

Fig 2. Effect of pH of sodium acetate-HCl buffer solution: 5.0 $\mu g\ mL^{-1}$ nifedipine + 1.0 mL of 0.01 M ammonium ferric sulfate + 5.0 mL buffer solution of pH 1.42 and 2.0 mL of 0.01 M 1,10-phenanthroline. Each set is examined in a 10 mL standard volumetric flask.

Fig 3. Effect of the volume of (a) 0.01 M ammonium ferric sulfate and (b) 0.01 M 1,10-phenanthroline.

0.01 M 1,10-phenanthroline which remained constant up to 2.3 mL. Therefore, 2.0 mL of the reagent was used throughout the experiment.

Study of Interferences

A study of interferences of some common excipients has been made during determination of 5.0 μg mL^{-1} nifedipine. It was observed that starch, glucose and lactose could be tolerated with a maximum amount of 10.0 mg, 18.0 mg and 51.34 mg, respectively. However, the drug content from the powdered tablets was extracted with dichloromethane, which completely eliminates any interference by the common excipients found in drug formulations. Thus, the proposed method is stability indicating assay for the analysis of nifedipine in drug formulations in the presence of various excipients found in tablet formulations.

Solution Stability

The solution stability of nifedipine in bulk and tablet solutions was monitored by keeping the solutions at room temperature (25 ± 1^0 C) under darkness for several days and then recording the absorption spectra of the solutions, and also by performing TLC analysis. The standard drug and quality control sample solutions showed no change in the absorption spectra for at least 7 days. The spots on TLC plates for the drug and quality control sample solutions were monitored each day. It was found that a single spot was observed in each solution with an R_f value of 0.30 on TLC using silica gel G as stationary phase and chloroform-ethyl acetate-cyclohexane (19: 2: 2, by vol.) as mobile phase, confirming the presence of nifedipine and thus indicating that no degradation of the drug takes place for at least seven days.

Robustness

Each operational parameter was closely investigated

and challenged for the robustness of the proposed method. The operational parameters examined are as follows: volume of 0.01 M ammonium ferric sulfate (1.0 ± 0.3 mL); volume of 0.01 M 1,10-phenanthroline (2.0 ± 0.3 mL); buffer solution of sodium acetate-HCl (pH 1.42 ± 0.33); heating time (5 ± 2.0 min) and heating temperature (100 ± 1ºC).

The robustness of the proposed method relative to each operational parameter was judged by analyzing the content of nifedipine in tablet formulations under variable experimental conditions. A sample solution containing 14 mg mL^{-1} of nifedipine (Nicardia retard-10) was assayed five times using the proposed method. The results showed a mean percent recovery and relative standard deviation of 100.14 and 0.42 %, respectively, for the proposed method. Thus, the operational conditions for the proposed method were found to be very robust.

Analytical Data

Under the optimized experimental conditions, a straight-line calibration graph was obtained by plotting the absorbance versus the concentration of nifedipine. Beer's law was obeyed in the concentration range 0.5 - 14.0 μg mL^{-1} with molar absorptivity and Sandell's sensitivity of 2.805×10^4 L mol^{-1} cm^{-1} and 0.012 μg cm^{-2} per 0.001-absorbance unit, respectively. Linear regression analysis using the method of least square treatment of calibration data (n = 9) was made to evaluate the slope, intercept and correlation coefficient. The linear regression equation was A = 8.10×10^{-4} + 8.095×10^{-2} C (where A is the absorbance and C is the concentration of nifedipine, μg mL^{-1}) with a coefficient of correlation of r = 0.9999, which indicates an excellent linearity. The confidence limits for the slope and intercept of the line of regression were computed using the relation, $b \pm tS_b$ and $a \pm tS_a$ at the 95% confidence level and found to be $8.095 \times 10^{-2} \pm 2.602 \times 10^{-4}$ and $8.100 \times 10^{-4} \pm 1.916 \times 10^{-3}$ C, respectively.

The limits of detection (LOD) and quantitation (LOQ) were calculated using the following equation:

$$LOD = 3.3 \times \frac{S_0}{b} \text{ and } LOQ = 10 \times \frac{S_0}{b}$$

and were found to be 0.06 and 0.18 μg mL^{-1}, respectively.

The variance ($S_o^{\,2}$) was calculated using the following relation:

$$S_0^{\,2} = \frac{\sum (A_{expt.} - A_{calc.})^2}{n-2}$$

where $A_{expt.}$ is the absorbance on ordinate and $A_{calc.}$ is the absorbance calculated from the regression equation. The variance was found to be 2.22×10^{-6} μg mL^{-1}, indicating negligible scattering of the experimental

Table 1. Standard addition technique for the determination of nifedipine in pharmaceutical formulations.

| Pharmaceutical formulations | Amount (μg mL^{-1}) | | | Recovery ± RSD [a] (%) | SAE [b] | C.L.[c] |
	Taken	Added	Found ± SD[a]			
Nicardia retard-10	3.0	4.0	6.994 ± 0.054	99.920 ± 0.773	0.024	0.067
	7.0	7.0	13.996 ± 0.049	99.973 ± 0.350	0.022	0.061
Calciguard-10	3.0	4.0	7.007 ± 0.056	100.095 ± 0.798	0.025	0.069
	7.0	7.0	13.994 ± 0.051	99.955 ± 0.367	0.023	0.064
Adalat retard-10	3.0	4.0	7.031 ± 0.050	100.448 ± 0.703	0.022	0.061
	7.0	7.0	13.994 ± 0.051	99.955 ± 0.367	0.023	0.064

[a] Mean for five independent analyses.
[b] SAE, standard analytical error.
[c] C.L., confidence limit at 95% confidence level and four degrees of freedom (t = 2.776).

data points around the line of regression.

The accuracy of the proposed method was checked by performing recovery experiments. For this, a known amount of the pure drug was added to the preanalysed dosage forms and then determined by the recommended procedure. The results obtained in Table 1 showed that the mean recovery and relative standard deviation were in the range of 99.920-100.448 % and 0.350-0.798 %, respectively. These results also suggested that there is no interference from the common excipients present in dosage forms.

The performance of the proposed method with that of other existing UV-visible spectrophotometric methods was compared (Table 2). It is evident from the table that the proposed method is more sensitive and can compete with other existing methods in the determination of nifedipine at lower concentration levels.

The proposed method has been successfully applied for the determination of nifedipine in pharmaceutical

Table 2. Comparison of the proposed method with other existing UV-visible spectrophotometric methods.

Reagents	λ_{max} (nm)	Beer's law limit (μg mL^{-1})	Molar absorptivity (L mol^{-1} cm^{-1})	Recovery (%)	RSD (%)	References
Ethanol and phosphate buffer saline	340.0	-	-	99.70 - 99.90	-	10
4 -Dimethylaminobenzaldehyde	380.0	5.0 - 60.0	-	97.80 - 98.50	-	13
Potassium hydroxide	430.0	5.0 - 50.0	1.108×10^4	100.06 - 100.24	0.78	16
Ammonium molybdate	830.0	2.5 - 45.0	1.455×10^4	99.97 - 100.14	0.52	16
Potassium permanganate	530.0	18.0 - 44.0	-	99.50 -101.30	1.50	17
4-Methylaminophenol and dichromate	525.0	5.0 - 175.0	1.900×10^3	99.70 - 100.50	0.60	18
Bromocresol green	415.0	5.0 - 32.5	6.410×10^3	99.90 - 100.10	0.82	19
Bromophenol blue	415.0	4.0 - 37.5	4.850×10^3	99.90 - 100.10	0.72	19
Bromophenol thymol blue	415.0	6.5 - 33.0	5.260×10^3	99.80 - 100.90	0.66	19
Eriochrome black T	520.0	4.5 - 22.5	7.690×10^3	100.00 - 100.20	0.68	19
Ammonium ferric sulfate and 1,10-phenanthroline	505.0	0.5 - 14.0	2.805×10^4	99.81 - 100.18	0.37	This work

Table 3. Quantitative analysis of nifedipine of the proposed method using point and interval hypothesis tests at 95% confidence level.

| Pharmaceutical Preparations | Proposed Method | | Reference method | | t- & F-values[b] | θ_L & θ_U values[c] |
	Recovery %	RSD[a] %	Recovery %	RSD[a] %		
Nicardia retard-10	99.955	0.367	100.142	0.421	t = 0.534 F= 1.318	θ_L = 0.988 θ_U = 1.008
Calciguard-10	99.972	0.350	100.078	0.457	t = 0.292 F= 1.708	θ_L = 0.989 θ_U = 1.009
Adalat retard-10	100.061	0.401	100.401	0.509	t = 0.835 F= 1.619	θ_L = 0.992 θ_U = 1.008

[a] Mean for five independent analyses.
[b] Theoretical t-value (ν = 8) and F-value (ν = 4, 4) at 95 % confidence level are 2.306 and 6.39, respectively.
[c] In pharmaceutical analysis a bias based on recovery experiments of ±2% (θ_L= 0.98 and θ_U = 1.02) is acceptable.

formulations. The results (Table 3) of the proposed method were compared with those of the reference method[19] using point and interval hypothesis tests.[24] The results showed that the calculated paired t- and F-values are less than the theoretical ones, thus confirming no significant difference between the performance of the proposed method and the reference method at a 95% confidence level. It is also evident from the table that the results of bias (lower and upper limits) based on recovery experiments of the proposed method are acceptable (within the range of ± 2%). The Canadian Health Protection Branch has recommended a bias of ± 2% ($\theta_L = 0.98$ and $\theta_u = 1.02$) in pharmaceutical analysis, which was, based on recovery experiments of the developed method[25].

CONCLUSIONS

The proposed method is found to be more sensitive and it does not require any pretreatment of the drug and tedious extraction procedure prior to its determination. The true bias of all samples is less than ± 2%, confirming acceptable recovery and precision. The method was successfully applied to enable estimation of drug in pharmaceutical dosage forms at a lower concentration level (each tablet claiming 10 mg), and can compete with other existing assay methods for routine quality control analysis of nifedipine in pharmaceutical formulations.

ACKNOWLEDGEMENTS

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