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Spectrophotometric method for the determination of nifedipine with 4-(methylamino)phenol and potassium dichromate

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Abstract

A new simple, sensitive and reproducible spectrophotometric method for the determination of nifedipine in pure and dosage forms has been proposed. It is based on the reduction of nifedipine with Zn/NH_4Cl, followed by coupling with *N*-methyl-1,4-benzoquinoneimine—the oxidation product of 4-(methylamino)phenol, to give a chromophore which absorbed maximally at 525 nm. The experimental conditions were optimised and Beer's law was obeyed over the concentration range of 5–175 μg ml^{-1}. The molar absorptivity, detection limit, recovery and RSD were found to be 1.9×10^3 l mol^{-1} cm^{-1}, 1.1 μg ml^{-1}, 99.7–100.5% and 0.3–0.8%, respectively. The proposed method was compared favourably with the official B.P. method.

Keywords: Nifedipine; 4-(Methylamino)phenol; *N*-Methyl-1,4-benzoquinoneimine; Potassium dichromate; Visible spectrophotometry

1. Introduction

Nifedipine, chemically dimethyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5-dicarboxylate and pharmacologically a selective L–type slow calcium channel antagonist [1,2], is official in United States Pharmacopeia XXIII and British Pharmacopoeia [3,4]. It is commonly used as an antihypertensive and potent arterial vasodilator in the management of angina and various other cardiovascular disorders [5]. It is also used as a probe drug to assess cytochrome P-450 III A4 enzyme activity in vivo [6]. Nifedipine decreases cyclic guanosine monophosphate in hypoxic lungs like inhaled nitric oxide, exhibits dose dependent depressive effect and causes some common side effects due to excessive vasodilation [7–9].

Several HPLC [6,10–12], reversed phase HPLC [13,14], HPTLC [15], GC [12,16–19] and voltammetric [20] methods have been reported for the assay of nifedipine and its related compounds in pharmaceuticals. A variety of HPLC and GC methods are now widely used for the determination of nifedipine concentration in biological fluids because of their sensitivity and specificity. These methods have adequate sensitivity to assay lower concentrations of the drug and hence use of these methods is justified when the sample matrix is complex and nifedipine concentration is low as in the case with the biological samples. However, the sample matrix is usually less complex and analyte concentration levels are high in case of pharmaceutical analysis, hence it is required to develop a fast, simple and inexpensive method that can be adopted for routine analysis. Therefore, spectrophotometry is still considered as a convenient and low cost technique.

Nifedipine was assayed [21] in pharmaceutical formulations based on the reaction with 4-dimethylaminobenzaldehyde and subsequent determination at 310 nm. Beer–Lambert's law was obeyed over the concentration range 5–60 μg ml^{-1}. Another spectrophotometric method has also been recommended for its determination involving the formation of blue complex with Folin–Ciocalteau reagent [22]. A kinetic spectrophotometric method was described for its determination in

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dosage forms. The method was based on its oxidation by potassium permanganate at neutral pH [23]. In commercial dosage forms, UV-spectrophotometry has also been utilised for its estimation [24,25]. The quantification of nifedipine in combined dosage forms were made using first derivative [12] and second derivative [27] spectra of their solutions in methanol and 0.1 N HCl, respectively.

This work describes a new method for the determination of nifedipine in commercial dosage forms. The method depends on the reduction of nitro group to hydroxylamino group which then reacted with *N*-methyl-1,4-benzoquinoneimine to form coloured product. The reduction of nitro group of nifedipine to hydroxylamino derivative was studied with respect to heating time and concentration of Zn/NH_4Cl. The effects of reagent's concentration, buffer and time on the formation of chromophore were investigated.

2. Experimental

2.1. Apparatus

Spectral runs and absorbances were recorded on a Spectronic $20D^+$ spectrophotometer (Milton Roy, USA). pH-meter model L1-10T (Elico, India) was used to measure pH.

2.2. Materials and reagents

(i) 0.1% ethanolic solution of reduced nifedipine was prepared by heating a mixture of 100 mg pure nifedipine (J.B. Chemicals and Pharmaceuticals Ltd., India) dissolved in 20 ml ethanol, 15 ml of 10% aqueous solution of ammonium chloride (Loba Chemie, India) and 2 g of zinc dust for 8 min on a water bath at 100 ± 1 °C. The content was cooled at room temperature and diluted by adding 30 ml of ethanol. It was filtered on Whatman no. 42 filter paper and washed with ethanol. The filtrate and washings were diluted to volume in a 100 ml volumetric flask. The whole experiment was performed in dark and in amber-coloured glasswares. This solution was stable at room temperature for about 4 days.

(ii) A buffer solution was prepared by mixing appropriate volumes of 1 M hydrochloric acid (E. Merck, India) and 1 M sodium acetate trihydrate (Ranbaxy Chemicals, India).

(iii) 0.2% aqueous solution of 4-(methylamino)phenol (Loba Chemie) was prepared in doubly distilled water. It was always freshly prepared after every 5 h.

(iv) 0.01 M potassium dichromate (Loba Chemie) was prepared by dissolving 0.7355 g in doubly distilled water and made up to 250 ml.

2.3. Recommended procedure

Aliquots of 0.05–1.75 ml of reduced nifedipine (1 mg ml^{-1}) were transferred into a series of 10 ml standard volumetric flasks and then 2.5 ml of buffer solution (pH 2.9), 1.45 ml of 0.2% 4-(methylamino)phenol and 1.2 ml of 0.01 M potassium dichromate were added to each flask successively. The solutions were allowed to stand at room temperature for 18 min and then made up to the mark with doubly distilled water. The absorbance values of the final coloured solutions were measured at 525 nm against a reagent blank. The amount of the drug was computed from a Beer–Lambert's plot.

2.4. Analysis of pharmaceutical formulations

Ten tablets of nifedipine (each claiming 10 mg) were finely powdered and thoroughly mixed. The powdered mixture was transferred in a conical flask. 20 ml of ethanol was added and gently shaken for 2–3 min. Then 15 ml of 10% ammonium chloride solution and 2 g of zinc dust were added and heated on a water bath for 8 min. After cooling at room temperature, the mixture was added with 30 ml of ethanol and filtered on Whatman filter paper no. 42 in a 100 ml standard volumetric flask. The residue was washed with enough ethanol and finally made up to the mark. Nifedipine content was determined using the recommended procedure.

3. Investigation of stability

The stability of nifedipine under the experimental conditions was investigated by incubating 120 μg ml^{-1} in distilled water or ethanol containing common excipients at 30 °C for 3 h in the absence of all lights. At regular time interval the concentration of nifedipine was determined by the proposed and reference methods [23].

4. Results and discussion

The nitro compounds undergo reduction by catalytic hydrogenation in the presence of metals (Zn, Fe, Sn) and other suitable reagents like, HCl, NH_4Cl, NaOH or KOH. Under the proposed condition, nifedipine is reduced to hydroxylamino derivative by Zn/NH_4Cl.

The primary aromatic amines react with 4-(methylamino)phenol and an oxidising agent such as dichromate [28], *N*-bromosuccinimide [29], peroxydisulfate [30] or iodylbenzoate [31] to form a purple red product. It is believed that on oxidation 4-(methylamino)phenol produces *N*-methyl-1,4-benzoquinoneimine. The primary aromatic amines react with *N*-methyl-1,4-benzo-

quinoneimine to form chromophore which absorbed maximally at 520–530 nm. In this study the reaction of hydroxylamino derivative of nifedipine with 4-(methylamino)phenol and potassium dichromate may be assumed to proceed in an analogous manner as the product absorbed maximally at 525 nm [28]. The stoichiometric ratio of hydroxylamino derivative to *N*-methyl-1,4-benzoquinoneimine was determined by Job's method [32] and was found to be 2:1 (Fig. 1). The chromophore formed was found to be positively charged at pH 2.9 as it was adsorbed on cation-exchange resin beads. The possible reaction sequence is presented in Scheme 1.

The optimum conditions for reduction of nifedipine to hydroxylamino derivative were established via a number of preliminary experiments. The effect of variables on the reduction of the drug was studied by taking a separate 10 ml aliquot of 0.1% nifedipine solution.

4.1. Effect of heating time

The aliquot of nifedipine was mixed with 200 mg of zinc dust and 1.5 ml of 10% ammonium chloride solution and the content was heated on a water bath at 100 ± 1 °C. One millilitre of aliquot of this solution was subjected to colour development. The results showed that the intensity of the colour reached its maximum at 7 min of heating and remained unchanged even after 10 min. Therefore, a heating time of 8 min was recommended for reduction.

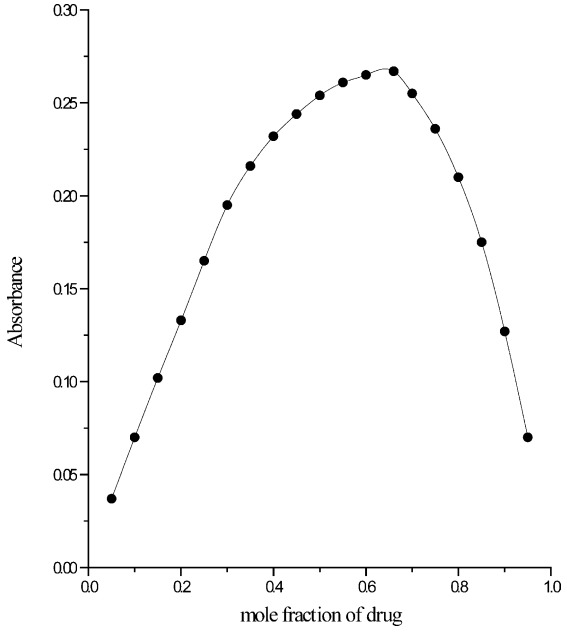


Fig. 1. Job's plot for hydroxylamino derivative of nifedipine and N-methyl-1,4-benzoquinoneimine (each 2.89×10^{-3} M).

4.2. Effect of zinc dust

The effect of the amount of zinc dust on the reduction of 10 mg nifedipine in the presence of 1.5 ml of 10% ammonium chloride solution was studied. It was observed that the absorbance of the coloured solution increased up to 150 mg of zinc dust and then remained constant at higher amounts. Hence, 200 mg of zinc dust was taken as optimum value for reduction.

4.3. Effect of ammonium chloride solution

To study the effect of the concentration of ammonium chloride solution on the reduction of nifedipine, 10 ml of 0.1% nifedipine was mixed with 200 mg of zinc dust and varying volumes of 10% ammonium chloride solution. The content was heated on a water bath at 100 ± 1 °C for 8 min. A plot of absorbance versus volume of ammonium chloride solution showed that the highest absorbance was obtained with 1.25 ml and remained constant beyond this volume. Therefore, 1.5 ml of 10% ammonium chloride solution was taken to reduce the drug for further studies.

The optimum conditions for the development of the proposed method were established by varying the parameters one at a time and observing the effects produced.

4.4. Effect of buffer solution

A constant absorbance was observed in the pH range 2.75–3.1. In this study, therefore, 2.5 ml of pH 2.9 buffer solution was used throughout the experimental investigations.

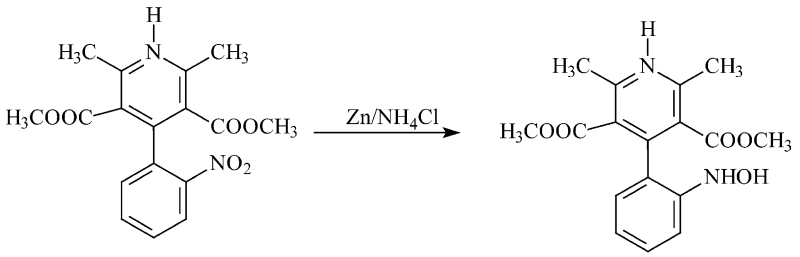
4.5. Effect of time

To 1 ml of 0.1% reduced drug 2.5 ml of buffer solution (pH 2.9), 1.45 ml of 0.2% 4-(methylamino)phenol solution and 1.2 ml of 0.01 M potassium dichromate were added and kept at room temperature (25 °C) for colour development. The intensity of the colour was reached to maximum after 15 min and remained constant for 1 h. The coloured product was diluted to 10 ml with distilled water and absorbance was measured at 525 nm against a reagent blank after 18 min.

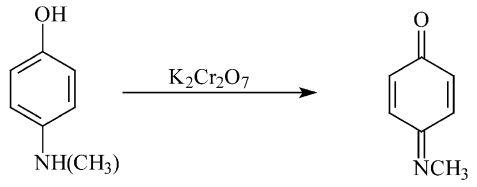
4.6. Effect of 4-(methylamino)phenol solution

1 ml of 0.1% reduced drug, was mixed with 2.5 ml of buffer solution (pH 2.9), varying volumes of 0.2% 4-(methylamino)phenol and 1.2 ml of 0.01 M potassium dichromate and the contents were allowed to stand at room temperature for 18 min. The results showed that a constant absorbance was found in the range of 1.4–

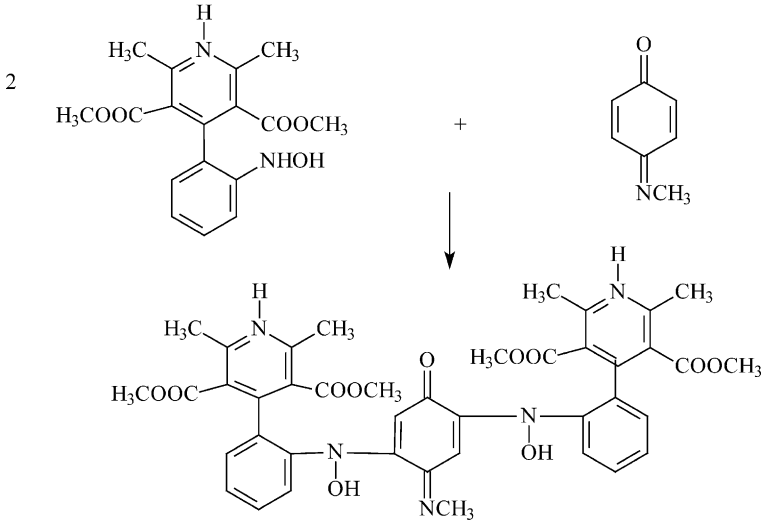
(a) Reduction of nifedipine to hydroxylamino derivative



(b) Formation of 4-N-methylbenzoquinoneimine:



(c) Coupling with hydroxylamino derivative of drug: -



Scheme 1.

1.5 ml. Therefore 1.45 ml of 0.2% 4-(methylamino)phenol was used in all the subsequent works.

4.7. Effect of potassium dichromate solution

In order to study the effect of potassium dichromate concentration, the reaction was carried out in a series of 10 ml volumetric flasks containing 100 μg ml^{-1} reduced drug, 2.5 ml of buffer solution (pH 2.9), 1.45 ml of 0.2% 4-(methylamino)phenol solution. This was followed by different volumes of 0.01 M potassium dichromate ranging from 0.3 to 1.5 ml. The results indicate that the highest intensity and reproducible results are obtained on using 1 ml of 0.01 M potassium dichromate. Therefore, 1.2 ml of this reagent was used throughout this work.

4.8. Analytical data

Under the optimum experimental conditions, a calibration curve was constructed by plotting absorbance at 525 nm versus concentration. Beer's law was obeyed within a concentration range of 5–175 μg ml^{-1}. Regression analysis using the method of least squares was

made to evaluate the slope, intercept and correlation coefficient. The linear regression equation and correlation coefficient are $A = 1.3 \times 10^{-3} + 5.3 \times 10^{-3}C$ (A, absorbance at 525 nm; C, concentration in μg ml^{-1}) and $r = 0.9999$ which indicates an excellent linearity. The molar absorptivity, detection limit and variance [33], standard deviations of intercept and slope [34] were found to be 1.9×10^3 l mol^{-1} cm^{-1}, 1.1 μg ml^{-1} and 1.2×10^{-5}, 1.9×10^{-3} and 1.6×10^{-5}, respectively. The small value of variance marks the negligible scattering of the experimental data points from the line of regression.

The error, S_c, in the determination of a given concentration of nifedipine was calculated by statistical analysis of calibration data using the relation [35].

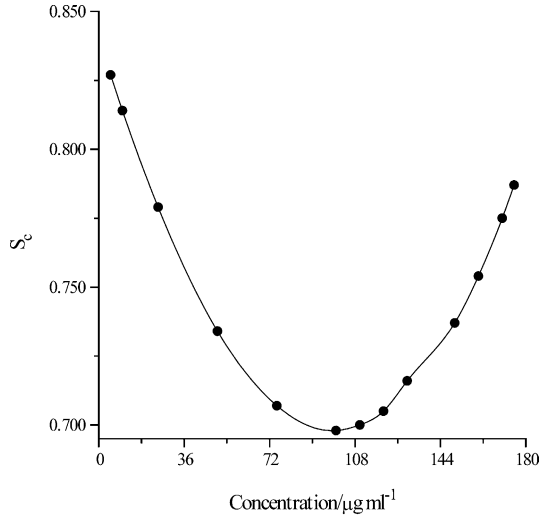


Fig. 2. Errors (S_c) in the determination of the concentration of nifedipine.

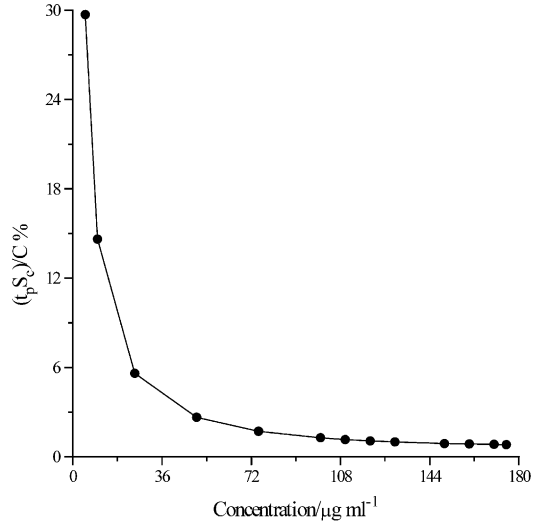


Fig. 3. Variation of confidence limit at 95% confidence level in the form of percent uncertainty on the concentration.

$$S_c = \frac{S_o}{b}\left(1 + \frac{1}{n} + \frac{(A - A')^2}{b^2(\Sigma C^2 - nC'^2)}\right)$$

where C' and A' are average concentration and average absorbance values, respectively, for n standard solutions. Fig. 2 shows the graph of S_c against the concentration of nifedipine. The value of S_c reached a minimum when the actual absorbance was equal to the average absorbance in the calibration graph. Thus the minimum error was found in the determination of about 100 μg ml^{-1} nifedipine. The value of S_c also allows us to establish the confidence limits at a selected level of significance [35]. The results are shown in Fig. 3 in the form of percent uncertainty on the concentration at 95% confidence level. It is apparent from the figure that the relative uncertainty on the concentration can be calculated directly over the full range of the concentration tested and hence, confidence limit can be established.

The stability experiment conducted in the presence of commonly encountered excipients such as starch, talc, lactose and magnesium stearate revealed the fact that under the conditions no degradation of nifedipine was detected. The drug and its photodegradation product, 4-(2-nitrosophenyl)-pyridine homologue may undergo reduction with Zn/NH_4Cl to yield hydroxylamino derivative. However, the determination was done under a condition where contact with light was completely avoided.

The reproducibility of the method was checked by ten replicate determinations at the concentration levels of 60, 100, 120 and 150 μg ml^{-1}. The percent relative standard deviations were found to vary between 0.3 and 0.8.

The accuracy of the method was demonstrated by recovery experiments which were carried out by adding a fixed amount of pure drug to the preanalysed formulations. The analytical results obtained from these investigations are summarised in Table 1 which indicates that common additives and excipients did not interfere with the determination. The percent relative standard deviations can be considered to be very satisfactory.

The proposed method for assay of nifedipine in dosage forms was compared favourably with other existing UV–visible spectrophotometric methods (Table 2). It is evident from the table that the method has advantages of wider linear dynamic range and high precision (%RSD = 0.3–0.8 and 0.4–0.7 for pure and dosage forms, respectively).

Some commercial dosage forms were successfully analysed by the proposed method and official B.P. method [4]. The results (Table 3) were compared statistically by Student's t-test and variance ratio F-test which indicates that there is no significant difference between the methods compared.

Table 1
Spectrophotometric determination of nifedipine in pharmaceutical formulations by standard addition method

Preparations	Amount taken (μg ml^{-1})	Amount added (μg ml^{-1})	Amount found (μg ml^{-1})	Recovery (%)	RSD (%)
Adalat Retard-10	30	30	60.1	100.1	0.8
	40	60	99.5	99.5	0.6
	80	40	120.4	100.3	0.5
Calcigard-10	30	30	59.5	99.2	1.0
	40	60	99.8	99.8	0.8
	80	40	120.7	100.6	0.5
Nicardia Retard-10	30	30	59.5	99.2	0.7
	40	60	100.4	100.4	0.4
	80	40	120.5	100.4	0.5

Table 2
Comparison of the proposed method with existing spectrophotometric methods for assay of nifedipine in pharmaceutical formulations

Reagents	λ_{max} (nm)	Linear dynamic range (μg ml^{-1})	Recovery (%)	RSD (%)	References
Potassium permanganate	530	18–44	99.5–101.3	1.5	[23]
3,4,5-Trimethoxybenzaldehyde	365	10–70	100.2–102.9	1.5	[26]
4-Dimethylaminobezaldehyde	310	5–60	97.8–98.5		[21]
Extractive U.V. [a]	237	0–10	97.8–98.9		[25]
Ethanol and phosphate buffer saline	340		99.7–99.9		[24]
Derivative U.V.	400	4–12	98.5–101.3	1.4	[12]
4-*N*-Methylaminophenol and dichromate	525	5–175	99.7–100.5	0.6	This work

[a] Extracted into chloroform and the solvent was evaporated to dryness. Finally, the residue was dissolved in distilled water.

Table 3
Spectrophotometric determination of nifedipine in pharmaceutical formulations by the proposed method and B.P. method

Preparations	Nominal composition (mg)	Proposed method			Reference method			F-value[c]
		Recovery[a] (%)	RSD[a] (%)	t[b]	Recovery[a] (%)	RSD[a] (%)	t[b]	
Adalat Retard-10	10	99.9	0.6	0.3923	100.1	0.4	1.3975	2.0306
Calcigard-10	10	100.2	0.7	0.6675	100.2	0.5	0.8856	2.2168
Nicardia Retard-10	10	99.9	0.4	0.520	100.4	0.5	1.9876	1.0952

[a] Five independent analyses.
[b] t, the value at 95% confidence level is 2.132 [36].
[c] F, value at 95% confidence level is 6.39 [36].

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