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ORIGINAL ARTICLE

Spectrophotometric determination of nifedipine in pharmaceutical formulations, serum and urine samples via oxidative coupling reaction

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Urine

Abstract Two rapid, simple, sensitive and selective spectrophotometric methods have been developed for the quantitative estimation of nifedipine in pharmaceutical formulations and different human body fluids (serum and urine). The proposed methods are based on the reduction of the nitro group to amino group of the drug. The resulting amine was then subjected to proposed methods. Method A is based on the oxidation followed by coupling of nifedipine with 3- methyl-2-benzothiazolinone hydrazone (MBTH) in presence of ferric chloride ($FeCl_3$) to form green colored chromogen at 685 nm. Method B is based on the formation of oxidative coupling reaction between the corresponding drug and brucine – $NaIO_4$ to form violet colored chromogen at 546 nm. The procedures described were applied successfully to the determination of the compound in their dosage forms and body fluids. The results showed that the proposed procedures compared favorably with reference method are satisfactory sensitive, accurate and precise. The optical characteristics such as Beer's law limits, molar absorptivity, Sandell's sensitivity and various statistical data are reported. The results of the analysis for the two methods have been validated statistically and by recovery studies.

1. Introduction

Nifedipine is chemically known as dimethyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl) pyridine-3,5 dicarboxylate. (Fig. 1 A) It is pharmacologically a selective L-type calcium channel antagonist (Matrindale the Extra Pharmacopoeia, 2002). 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, various other cardiovascular disorders and Reynaud's phenomenon (Sorkin et al., 1985; Kahan et al., 1981; Hardman et al., 1996). It is mainly used in the treatment of diuretics and ACE (Tripathi K.D.) inhibitors although calcium channels antagonists are still favored as primary treatment for older black patients – sub lingual. Nifedipine has previously been used in hypertensive emergencies. It

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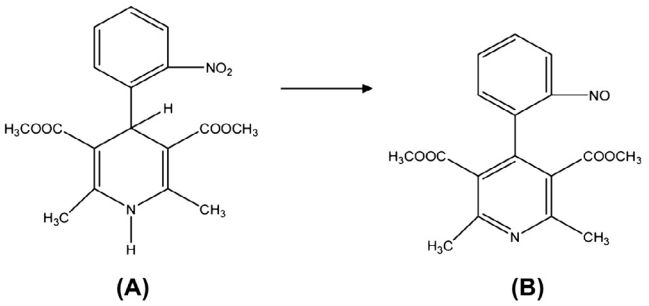

Figure 1 Chemical structures of (A) Nifedipine (B) Nitro phenyl pyridine.

has a very low bioavailability, and it is photosensitive and thermally unstable. This compound, when exposed to daylight and certain wavelengths or artificial light readily converts to a nitro phenyl pyridine derivative (NFPD) (Fig. 1B) (Henry, 1980; Miller, 1987; Stone et al., 1980). Blood pressure diabetes and LDL cholesterol are casual risk factors for cardiovascular diseases (CVD) and their combined effects make this disease common. A pill containing different active ingredients (Polly Pill) to overcome these factors is more beneficial than the common pills with only one, interns of cost and patient compliance. Nifedipine is a common prescribed active ingredient for CVD. Nifedipine, a highly non polar compound, is absorbed completely from the gastrointestinal tract, predominately from the Jejunum, but has a very low bioavailability mainly due to presystemic metabolism. Following absorption, nifedipine is further metabolized in the small intestine and liver to more polar compounds which are primarily eliminated by the kidney (Dokladaiova et al., 1982; Schellens et al., 1991; Kleinbloesem et al., 1984). Nifedipine is a photolabile compound, undergoing oxidative biotransformation in human body into pharmacologically inactive metabolites (Dokladaiova et al., 1982; Suzuki et al., 1985; Ohkubo et al., 1992).

The literature has reported some methods for nifedipine determination in biological fluids, which included gas chromatography, high performance liquid chromatography with either UV detection or electrochemical detection, fluorescence procedures, first derivative spectroscopy, Voltammetric method and LC–MS combining a simple liquid–liquid extraction (Kondo et al., 1980; Sheridan et al., 1989; Jankowski et al., 1994; Suzuki et al., 1985; Ozattin et al., 2002). Additionally, the methods reported to quantify nifedipine in bulk and in pharmaceuticals formulation involved a variety of analytical techniques such as high performance thin layer chromatographic, liquid chromatographic, gas chromatographic, polarographic, micellar electro kinetic chromatography, electro analytical and spectrophotometric methods (Patravale et al., 2000; Rahman et al., 2004; Rahman and Hoda, 2002; Karadi et al., 2000; Tu et al., 1995; Dumitrescu et al., 2001; Richter et al.,1997; Beaulieu et al., 1991; Kalieswari et al., 2002; Kasture and Ramteke, 2005; Milenovic et al., 2008; Rodríguez et al., 2008; Hemmateenejad et al., 2009).

Surprisingly, according to the best of our knowledge few spectrophotometric methods for the determination of nifedipine in pharmaceutical formulations, body fluids and other additives were reported. The present study documents an accurate, sensitive, rapid, selective and reproducible visible spectrophotometric assay which meets an accepted analytical validation. Spectrophotometry is the technique of choice even today in the laboratories of research, hospitals and pharmaceutical industries due to its low cost and inherent simplicity.

2. Experimental

2.1. Apparatus

Shimadzu UV–visible double beam spectrophotometer (model 2450) with 1 cm matched quartz cells was used for all the spectral measurements.

2.2. Materials and reagents

All chemicals and reagents were of analytical grade and water was always double distilled water.

(1) Nifedipine was kindly supplied by Novartis Pharmaceuticals Ltd. Mumbai, India its Purity was found to be 100.024 ± 0.84.
(2) Brucine solution (Loba, 0.2%; 5.067×10^{-3} M): prepared by dissolving 200 mg of Brucine in 100 ml distilled water.
(3) $NaIO_4$ solution (BDH, 0.2%; 9.35×10^{-3} M): prepared by dissolving 200 mg of sodium metaperiodate in 100 ml distilled water and standardized iodometrically.
(4) H_2SO_4 solution (Qualigens, 2.3 M): Prepared by diluting 6.38 ml of 18 M H_2SO_4 to 100 ml with distilled water.
(5) MBTH (Merck, 0.2%): Prepared by dissolving 200 mg in 100 ml distilled water.
(6) $FeCl_3$ (Merck, 0.5%): Prepared by dissolving 500 mg in 100 ml distilled water.
(7) Pharmaceutical formulations: Nicardia retard – 10 mg nifedipine per tablet (Local market Tirupati).

Calciguard – 10 mg and Adalat retard – 10 mg nifedipine per tablet (Novartis Pharmaceuticals Ltd., Mumbai, India).

2.3. Reduction of nitro group in Nifedipine (Olajire Aremu and Offiong Edet, 2009; Tulasamma and Venkateswarlu, 2009)

100 mg of nifedipine pure or equivalent tablet powder was accurately weighed and dissolved in 20 ml of methanol. This solution was treated with 10 ml of 5 N HCl and 0.5 g of Zinc powder was added in the portions, while shaking and refluxed at 80 °C for 10 min. The solution was filtered using a Whattman filter paper 41 to remove the insoluble matter and the volume was made up to 100 ml with methanol to get the concentration 1 mg/ml.

2.4. Preparation working standard solution

The resulting amine from the above solution 10 ml was taken into 100 ml volumetric flask and made up to the mark with methanol to get the concentration 100 μg ml^{-1} and dilution was carried out to the further working standards.

2.5. General procedure

2.5.1. Method A

Aliquots of nifedipine ranging from 1–19 μg ml^{-1} were transferred into a series of 10 ml volumetric flasks. To each flask 1 ml of aqueous solution of ferric chloride (0.5% w/v), 1.0 ml aqueous solution of MBTH (0.2% w/v) were added followed by dil HCl. The final volume was made up to 10 ml with distilled water. The absorbance of the green colored species formed was measured at 685 nm against reagent blank and beer's law was obeyed in concentration range of 1–19 μg ml^{-1}. The amount of nifedipine present in the sample was computed from calibration curve (Fig. 2).

2.5.2. Method B

Aliquots of nifedipine solution 4–18 μg ml^{-1} were transferred into 10 ml volumetric flasks. To, this 3 ml of Brucine solution 1.5 ml sodium meta periodate solution and 2 ml of (1.2 M) sulphuric acid were added to each flask. The flasks were shaken thoroughly and placed in a boiling water bath for about 15 min. The reaction mixture was then cooled to room temperature and total volume was adjusted to 10 ml with distilled water. The absorbance of each solution was measured at 546 nm against a reagent blank. The amount of nifedipine present in the sample was computed from calibration curve (Fig. 3).

3. Results and discussion

3.1. Method A

3.1.1. Absorption spectra (David et al., 2009)

The formation of green colored complex was employed in the quantitative detection of nifedipine with MBTH in presence of ferric chloride. However when MBTH was initially mixed with nifedipine and then with oxidizing agent, a green colored compound was produced with maximum absorbance in the visible range at 685 nm and shown in Fig. 4. MBTH loses two electrons and one proton due to oxidation with Fe (II), forming an electrophilic intermediate, which is the active coupling species. The electrophilic intermediate and the analyte under goes electrophilic reaction with the formation of colored product and the elimination of one molecule of water.

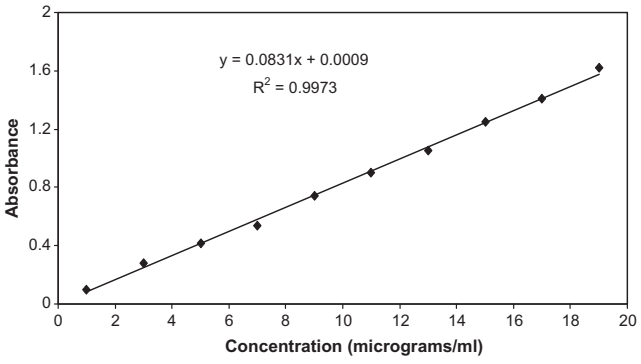

Figure 2 Calibration plot of Nifedipine (Method A).

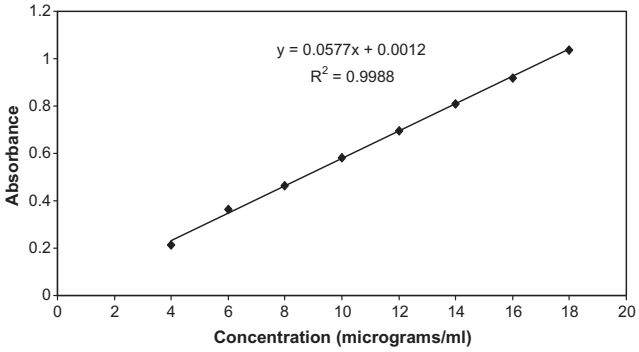

Figure 3 Calibration plot of Nifedipine (Method B).

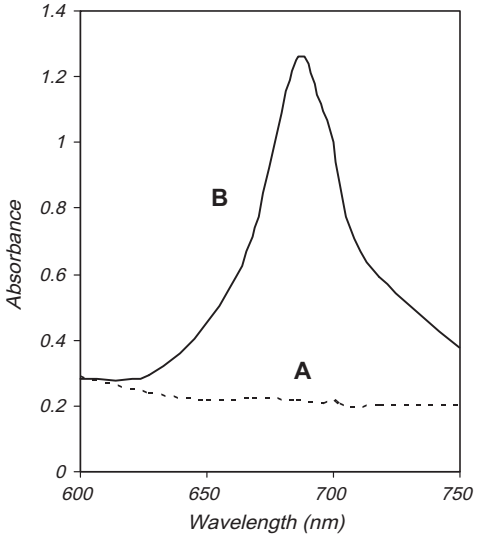

Figure 4 Absorption spectrum of (A) blank (B) MBTH with Nifedipine.

3.1.2. Optimum reaction conditions for complex formation

The optimizations of the methods were carefully studied to achieve complete reaction formation, highest sensitivity and a maximum absorbance. Reaction conditions of oxidative coupling complex were found by studying with preliminary experiments.

3.1.3. Effect of reagent

The addition of 1.0 ml of Fe^{3+}-MBTH solution was sufficient to obtain the maximum and reproducible absorbance for 6.0 μg ml^{-1} nifedipine. Smaller amounts resulted in complete complex formation. Increased concentration had no effect on complex formation, although absorbance increased slightly owing to the reagent background used.

3.1.4. Effect of reaction time and stability of colored species

The optimum reaction time was investigated from 0.5 to 4.0 min by following the color development at ambient temperature (25 ± 2 °C). Complete color intensity was attained after 2.0 min of mixing for complex. Rising the temperature up to 30 °C has no effect on the absorbance of the formed complex, where as above 30 °C, the absorbance start to decay. The absorbance remains stable for at least 3 h.

3.1.5. Effect of nature of acid

The reaction product, green color was found to flocculate with in 20–30 min of color development. To delay the flocculation, acid was added before dilution. Hydrochloric acid was found to give more stable color and reproducible results.

3.1.6. Effect of order in which reagents were added

After fixing all other parameters, a few of the experiments were performed to ascertain the influence of the order in which reagents were added. The following order: Drug-reagent -acid gave maximum absorbance and stability.

3.1.7. Effects of interference

In pharmaceutical analysis, it is important to test the selectivity towards the excipients and fillers added to the pharmaceutical preparations. Several species which can occur in the real samples together with drug were investigated. The level of interference was considered acceptable. Commonly encountered excipients such as talc, starch, glucose etc. did not interfere in the determination.

In order to apply the proposed method to the analysis of pharmaceutical formulations, the influence of commonly used excipients starch, lactose, glucose, sugar,talc, sodium chloride, titanium dioxide and magnesium sterate and additives was studied by preparing solutions containing 2.0×10^{-3} M Nifedepine and increasing concentration of the potential interference up to 1.0×10^{-3} M. The tolerance (Table 2) of each foreign compound was taken as the largest amount yielding an error of less than $\pm 2\%$ in the analytical signal of nifedipine.

3.2. Method B

3.2.1. Absorption spectra (Murali et al., 1984)

Brucine (2,3-dimethoxystrychnidin – 10 one) under acidic conditions has been reported to be an effective reagent for the spectrophotometric determination of NO_3^-, NO_2^- and Ce^{4+}. Sodium periodate (IO_4^-) is an efficient oxidant and is also color stabilizer. This investigation was concerned with the development of a spectrophotometric method for the routine determination of nifedipine using brucine – IO_4^-. The violet color λ_{max} of 546 nm (Fig. 5) developed was stable for 10 h and hence was suitable for routine work with good precision and accuracy.

Table 1 Optical and regression characteristics of the proposed methods.

Parameteras	Method A	Method B
λ_{max} (nm)	685	546
Beer's law limit (μg ml^{-1})	1–19	4–18
Molar absorptivity (L mol^{-1} cm^{-1})	2.77×10^4	1.59×10^4
Sandell's sensitivity (μg cm^{-2}/0.001 A.U)	0.13	0.20
Regression equation ($Y = mX + C$)		
Slope (m)	0.0831	0.0577
Intercept (C)	0.0009	0.0012
Correlation coefficient (r^2)	0.9973	0.9988
% Relative Standard deviation	0.42	0.19
Color	Green	Violet
LOD (μg ml^{-1})	0.21	0.23
LOQ (μg ml^{-1})	0.70	0.53

Table 2 Tolerance of different species in the determination of Nifedipine.

Species added	Maximum tolerable mole ratio
Lactose, glucose, sugar	200
	20
Starch, talc	200
	20
Sodium chloride, titanium dioxide	200
	20
Magnesium sterate	200
	20

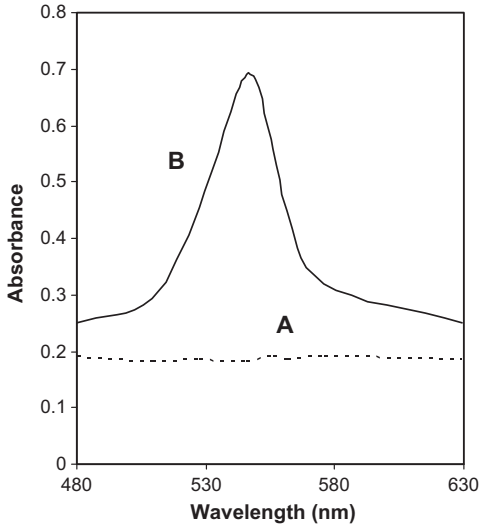

Figure 5 Absorption spectrum of (A) blank (B) brucine with Nifedipin.

3.2.2. Optimum reaction conditions

The optimum conditions for the method was established by varying one parameter at a time and keeping the others fixed and observing the effect produced on the absorbance of colored species and incorporated in the procedure.

3.2.3. Effect of reagent concentration

The effect of reagent was studied by measuring the absorbance of solution containing a fixed concentration of nifedipine (10 μg ml^{-1}) and varied amount of the respective reagent. Maximum color intensity of the complex was achieved with 3 ml and 1.5 ml of 0.2% w/v Brucine and $NaIO_4$ reagent solutions respectively for nifedipine. Although a larger volume of the reagent had no pronounced effect on the absorbance of the formed complex.

3.2.4. Effect of time and temperature

The reaction of nifedipine depends on time, maximum absorbance intensity was observed after 8.0 min for nifedipine at room temperature. The reaction of nifedipine was studied at different temperatures (25–100 °C) the values of maximum absorbance of the oxidative coupling product was almost constant at 25–80 °C for nifedipine, further temperature decreases the absorbance.

3.2.5. Effect of acid

The optimum sulphuric acid strength of the 25 ml of diluted reaction mixture for maximum color development with minimum blank color was 0.1–0.15 M.

3.2.6. Sequence of reagents were added

The following order gives the maximum absorbance and stability i.e. Drug-reagent -acid.

3.3. Method validation

3.3.1. Linearity

Under the optimum conditions described, Beer's law holds over the concentration ranges 1–19 and 4–18 μg ml^{-1} respectively for Methods A and B, calibrations were constructed by plotting absorbance versus concentrations. We calculated the apparent molarabsorptivity, Sandell's sensitivity, relative standard deviation and the regression equations (Table 1).

3.3.2. Sensitivity

The detection limit LOD for the proposed methods were calculated using the following equations (Miller and Miller, 1993)

$$\mathrm{LOD} = 3S/K$$

where S is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of analyte and K is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits were found to be 0.21 and 0.23 μg/ml for MBTH, brucine respectively.

The limits of Quantitations, LOQ defined as (Miller and Miller, 1993):

$$\mathrm{LOQ} = 10S/k$$

According to this equation, the limits of quantitation were found to be 0.70 and 0.53 μg/ml for MBTH, Brucine respectively.

3.3.3. Accuracy and precision (Ayman and Hassan, 2008)

In order to determine the accuracy and precision of the proposed methods, solutions containing four different concentration of nifedipine were prepared and analyzed in six replicates. The relative standard deviation as precision and percentage relative error (Err%) as accuracy of the suggested methods were calculated at 95% confidence levels, and can be considered satisfactory. Precision was carried out by six determinations at four different concentrations in these spectrophotometric methods. The percentage relative error was calculated according to the following equation.

$$\mathrm{Err}\% = [(\mathrm{Found} - \mathrm{Added})/\mathrm{Added}] \times 100$$

The inter-day and intra-day precision and accuracy results are shown in (Table 3). The analytical results for accuracy and precision show that the methods proposed have good repeatability and reproducibility.

3.3.4. Robustness and ruggedness

For the evaluation of the method robustness, some parameters were interchanged, reagent concentration, wavelength range and shaking time. The capacity remains unaffected by small deliberate variations. Method ruggedness was expressed as R.S.D% of the same procedure applied by two analysts and in two different instruments of different days. The results showed no statistical difference between different analysts and instruments suggesting that the developed methods were robust and rugged.

3.3.5. Reference method

An accurately weighed amount of tablet powder equivalent to 100 mg of nifedipine was transferred into 100 ml volumetric flasks. To this 75 ml of methanol was added and shaken well for about 15 min. The contents were diluted with methanol up to the mark and mixed thoroughly. The solution was filtered, then 2 ml of filtrate was pipette out into a 100 ml volumetric flask and made up to the mark with methanol for obtaining a concentration of 20 μg ml^{-1}. Into a series of 5 ml graduated tubes, aliquots of drug solution ranging from 0.5–0.3 μg ml^{-1}were taken and diluted to mark with methanol. Read the absorbance at 266 nm against a solvent blank. The drug was read from its calibration graph.

3.4. Applications

3.4.1. Analysis of pharmaceutical preparations

The proposed methods have been successfully applied to the determination of nifedipine in commercial tablet. The results obtained are shown in Table 4. The two suggested methods were applied successfully to the determination of nifedipine in commercial tablet. Six replicate determinations were obtained and the assay results were in a good agreement with percentage recovery. Moreover, to check the validity of the proposed methods, dosage forms were tested for possible interference with standard addition method. There was no significant difference between slopes of calibration curves and standard addition methods at two methods. To evaluate the validity and reproducibility of the method, known amounts of pure drug were added to previously analyze pharmaceutical

Table 3 The Intra day and inter day precision and accuracy data to nifedipine obtained by the proposed methods.

Method	Nifedipine taken (μg m^{-1})	Intra-day			Inter-day		
		Found (μg/ml)	Recovery[a] ± RSD%	RE%	Found μg ml^{-1}	Recovery[a] ± RSD%	RE%
A	1	1.002	100.20 ± 1.09	0.20	1.003	100.30 ± 1.20	0.30
	3	3.002	100.06 ± 0.36	0.06	3.003	100.06 ± 0.32	0.10
B	4	3.990	99.79 ± 0.32	−0.20	3.999	99.99 ± 0.37	−0.01
	8	8.001	100.01 ± 0.14	0.01	8.002	100.02 ± 0.16	0.02

[a] Average of five determinations.

Table 4 Assay of nifedipine in pharmaceutical formulations.

Formulation	Amount taken (μg ml^{-1})	Amount found (μg ml^{-1})		% Recovery[a] ± RSD		Reference method[b]
		Method A	Method B	Method A	Method B	
Calciguard – 10 mg	3	3.033	3.003	101.10 ± 0.67	100.10 ± 0.32	
	9	9.023	9.012	100.25 ± 0.42	100.13 ± 0.27	103.00
Naciarda retard – 10 mg	4	4.018	4.017	100.45 ± 1.05	100.42 ± 0.92	
	8	8.014	8.012	100.17 ± 0.41	100.17 ± 0.22	

[a] Average of five determinations.
[b] Hemmateenejad et al. (2009).

Table 5 The recovery of nifedipine in urine and serum.

Sample	Added μg ml^{-1}	Amount found μg ml^{-1}		% Recovery[a] ± RSD	
		Method A	Method B	Method A	Method B
Serum (2.5%)	4	4.00	4.001	100.0 ± 0.39	100.01 ± 0.70
	8	8.00	8.002	100.0 ± 0.11	100.03 ± 0.13
Urine (5%)	4	4.00	4.003	100.01 ± 0.39	100.09 ± 0.23
	8	8.002	8.002	100.02 ± 0.09	100.03 ± 0.12

[a] Average of five determinations.

preparations and the mixtures were analyzed by the proposed methods. The percent recoveries are also given in Table 4.

3.4.2. Analysis of recovery of nifedipine from serum and urine samples

This proposed method was also applied to the determinations of nifedipine in serum and urine samples.

The serum and urine samples were prepared for the analysis of recovery of nifedipine with these proposed methods. The serum and urine samples were obtained from healthy volunteers. The urine samples were centrifuged for 15 min at 3000 r/min to remove the suspended matter before determination. The serum and urine samples were added respectively according to the proposed procedure. The results are presented in Table 5. High accuracy and good recoveries are obtained, which indicates that the proposed methods can be successfully applied to recover nifedipine in the serum and urine samples.

4. Conclusions

The reagents utilized in the proposed methods are cheaper, readily available and the proposed method does not involve any critical reaction conditions or tedious sample preparation and extraction procedure. The method is unaffected by slight variations in the experimental conditions, such as reagent concentration or temperature. The proposed methods are sufficiently sensitive to permit determinations even down to 12 μg ml^{-1}. The sensitivity in terms of linearity, molarabsorptivity, precession and RSD of the methods are very suitable for the determination nifedipine in tablets and body fluids (serum and urine). Moreover the methods are free from interference by common additives and excipients. The comparative study of the molarabsorptivity indicated good sensitivity of the proposed methods which follow the order of MBTH > brucine–$NaIO_4$. These advantages encourage the application of the proposed methods in routine quality control analysis of nifedipine in pharmaceutical and body fluids (serum and urine).

The proposed methods were compared with reported methods (Rahman and Najmul Hejaz Azmi, 2005); in this study the method B is based on the formation of molybdenum blue complex by reduction of ammonium molybdate with drug. The concentration of nifedipine was determined at 803 nm and linearity range 2.5–45.0 μg ml^{-1} by spectrophotometric method. In this manuscript, the developed methods have small linearity range 1–19 and 4–18 μg ml^{-1} and wavelength of 685 nm and 546 nm. The statistical analyses show that the data from proposed methods are in good agreement with those of reported methods.

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