



Quantitative Analysis of Manidipine Dihydrochloride in Bulk and Synthetic Mixtures by Visible Spectrophotometry

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ABSTRACT

The present work describes three visible spectrophotometric methods for the quantitative estimation of Manidipine in bulk and synthetic mixtures. Method A is based on the reaction of the drug with *para*-dimethylaminobenzaldehyde (PDAB) and methanolic sulfuric acid at room temperature to form a green chromogen measured at 436.97 nm. Method B is based on the reaction of the diazotized drug with *N*-naphthylethylenediamine dihydrochloride (NEDD) to form a pink chromogen measured at 550.20 nm. Method C utilizes the reaction of manidipine with 3-methyl-2-benzothiazoniumhydrazone hydrochloride (MBTH) in the presence of ferric chloride to form a product measured at 480.21 nm. Under optimized reaction conditions, the proposed methods were validated as per ICH guidelines. Linearity was obeyed in the concentration ranges of 25–125 µg/ml with the following linear regression equations for methods A, B, and C, respectively: $Y = 0.002829 X + 0.01319$, $Y = 0.002325 X + 0.004275$, $Y = 0.0035 X + 0.001039$. The limits of detection and limits of quantification for methods A, B and C were 1.2024 and 1.212 µg/mL; 0.9798 and 3.6451 µg/mL, and 3.672 and 2.969 µg/mL respectively. Recovery studies were carried out by using the standard addition method and the results were found to be satisfactory. All methods have been applied successfully for the estimation of manidipine dihydrochloride in bulk and synthetic mixtures.

Key words: 3-Methyl-2-benzothiazonium hydrazone hydrochloride, manidipine dihydrochloride, *N*-naphthyl ethylene diamine dihydrochloride, *para*-diaminobenzaldehyde, visible Spectrophotometry

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INTRODUCTION

Manidipine dihydrochloride (1, 4-Dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-3, 5-pyridine dicarboxylic acid-2-[4-(diphenylmethyl)-1-piperazinyl] ethyl methyl ester hydrochloride)^[1] is a dihydropyridine type, long-acting antihypertensive, calcium antagonist vasodilator drug.^[2] A literature survey revealed only a few HPLC methods reported for the determination of manidipine in biological fluids.^[3-6] Efforts were hence made to develop a simple, sensitive, and selective visible spectrophotometric analytical procedure for bulk and synthetic mixtures containing

mandipine dihydrochloride. This paper describes three simple spectrophotometric methods for the estimation of manidipine using *para*-dimethylamino benzaldehyde (PDAB), *N*-naphthylethylenediamine dihydrochloride (NEDD), and 3-methyl-2-benzothiazoniumhydrazone hydrochloride (MBTH) in the presence of ferric chloride.

Three visible spectrophotometric methods have been developed for the estimation of manidipine dihydrochloride in bulk and synthetic mixtures. The first method (A) was based on the formation of a yellow chromogen with 1% *para*-dimethylaminobenzaldehyde

(PDAB) and methanolic sulphuric acid, which showed an absorbance maximum at 436.97 nm. The second method was based on the formation of a pink chromogen with *N*-naphthylethylenediamine dihydrochloride (NEDD) which showed an absorbance maximum at 550.20 nm. Method C utilizes the reaction of manidipine with 3-methyl-2-benzothiazoniumhydrazone hydrochloride (MBTH) in the presence of ferric chloride to form a colored chromogen estimated at 480.21 nm. Beer's law was obeyed in the concentration range of 25-125 µg/mL. The results of analysis were validated statistically for all the methods and by recovery studies.

EXPERIMENTAL

Manidipine dihydrochloride (Torrent Laboratories, Gujarat), *para*-dimethylamino benzaldehyde (PDAB) (S.D. Fine Chem. India, certified to be 98.5%), *N*-(1-naphthyl) ethylenediamine dihydrochloride (NEDD) (Central Drug house LTD, India, certified to be 99.0%), and 3-methylbenzothiazolin-2-one hydrazone (MBTH) (Himedia Laboratories Pvt. Ltd., India, certified to be 99.0%) were used. All other chemicals and solvents used were of analytical reagent grade. Spectral and absorbance measurements were made on PERKIN ELMER's Lamda 25 UV/Vis spectrophotometer with 1 cm matched cuvettes. A standard stock solution was prepared by dissolving 25 mg of manidipine dihydrochloride in 5-6 mL of methanol and diluted to the mark in a 25 mL volumetric flask.

Method A

To a series of 10 mL volumetric flasks, aliquots of a standard drug solution ranging from 0.25 to 1.25 mL (25-125 µg/mL) were added. This was followed by the addition of 1 mL of PDAB (1%) and 0.5 mL of methanolic sulphuric acid (0.1 M). The solutions were kept aside for three minutes and the volume was made up to 10 mL with methanol. The resulting yellow chromogen was measured at 436.97 nm against the reagent blank [Figure 1].

Method B

Aliquots of a standard drug solution ranging from 0.25 to 1.25 mL (25-125 µg/mL) were taken in a series of 10 mL volumetric flasks. To each volumetric flask were added 1 mL of Hydrochloric acid, 1 mL of an aqueous solution of NaNO₂, and 0.5 mL of an aqueous solution of ammonium sulfamate. The mixture was stirred for five min and the volume made up to the mark after adding 1 mL of NEDD solution. Solutions were then mixed thoroughly and the absorbance was measured at 550.90 nm against the reagent blank [Figure 1].

Method C

Aliquots of the standard drug solution ranging from 0.25 to 1.25 mL (25-125 µg/mL) were transferred into different 10 mL volumetric flasks. To each, an aqueous solution of FeCl₃ (1 mL, 0.1%) and an aqueous solution of MBTH

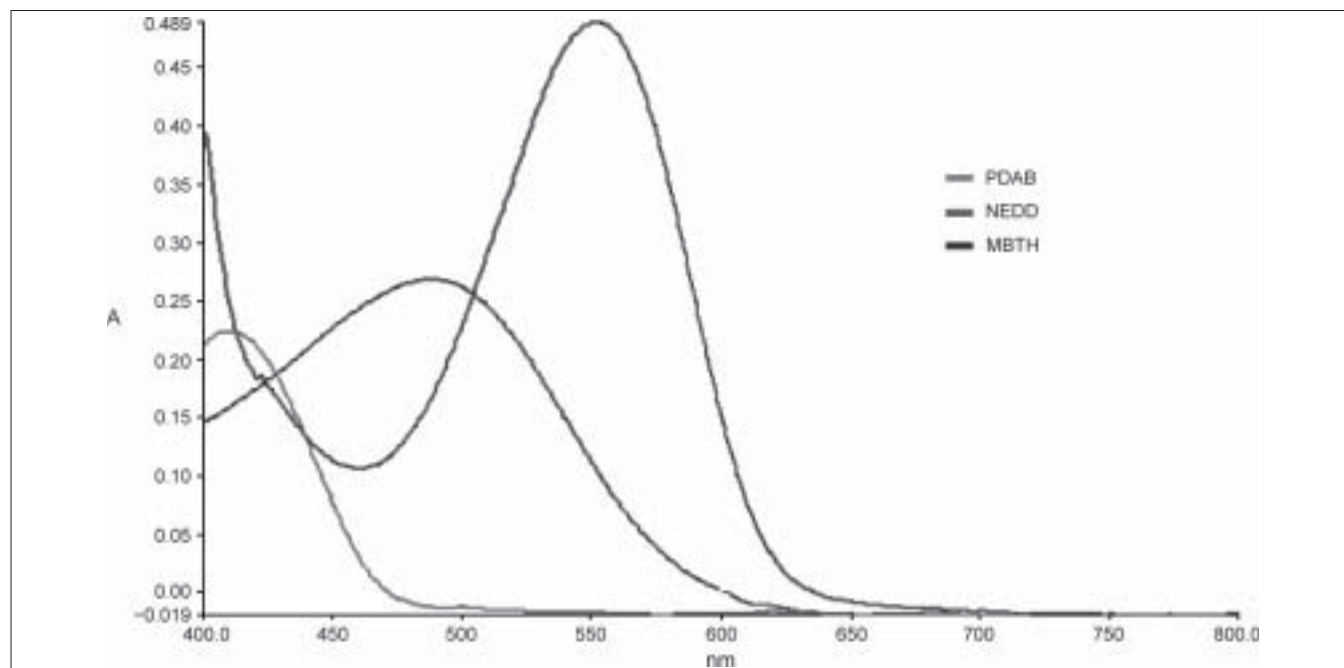


Figure 1: Overlaid Spectrum of Manidipine showing absorption maxima of all three methods

(1 mL, 0.2%) were added. The solutions were swirled and allowed to stand for five minutes and the final volume made up to the mark with the diluent, methanol: water (1:1). The absorbance was measured at 480.21 nm against the corresponding reagent blank [Figure 1].

RESULTS AND DISCUSSION

The primary nitro group of the drug, manidipine dihydrochloride, was subjected to reduction using zinc dust and 0.1 M hydrochloric acid. Reduction of the nitro group to an amino group was achieved after heating the solution at 70°C for ten minutes. The solution was then filtered through Whatman filter paper No. 41 for further analysis.

In method A, the primary amino group reacts with *para*-dimethylaminobenzaldehyde and methanolic sulphuric acid to yield a yellow chromogen showing maximum absorption at 436.97 nm. The second method includes diazotization of the primary amino group to form a diazonium salt. The resultant product was then coupled with NEDD to yield a purple azo dye. In the third method, the drug reacts with MBTH in the presence of FeCl₃ to give a blue product. This is an iron-catalyzed, oxidative coupling reaction of MBTH with the drug. Under the oxidative reaction conditions, MBTH loses two electrons and one proton, forming an electrophilic intermediate that is the active coupling species. This intermediate undergoes electrophilic substitution with the drug to form the colored product. The reaction mechanisms for all methods are shown in Figure 2.

Optimization of reaction condition

Effect of reaction time

The influence of the reaction time on the absorbance of the product was studied on 75 µg/mL of manidipine dihydrochloride with different chromogenic reagents as mentioned under methods A, B and C. The optimum reaction time was found to be 15, 10, and 5 minutes for methods A, B and C respectively [Figure 3].

Effect of the concentration of reaction mixture

The influence of the reaction mixture on the absorbance of the colored product was investigated with 75 µg/mL of manidipine dihydrochloride for all the three methods. The optimum concentration of the reaction mixture was found to be 1% of PDAB and NEDD and 0.2% of MBTH for methods A, B and C respectively [Figure 4].

Validation of the proposed methods

Specificity

The specificity of the proposed methods was ascertained by the analysis of synthetic mixtures that were prepared with excipients such as lactose (100 mg), starch (40 mg), talc (240 mg), and magnesium stearate (120 µg).

Linearity

For evaluation of linearity, the contents of Manidipine dihydrochloride were determined at five concentration levels: 25, 50, 75, 100, and 125 µg/ml for methods A, B and C. Each concentration was independently analyzed (five times). The instrumental absorbance was plotted against each concentration of Manidipine dihydrochloride and the linear regression equation was evaluated by statistical treatment of calibration data. The other regression characteristics were calculated using an Excel worksheet. The limits of detection and quantitation were calculated using the relations:

$$\text{LOD} = 3.3 \times S_0/b$$

$$\text{LOQ} = 10 \times S_0/b$$

Where S_0 is the standard deviation of the calibration line and b is the slope.

Precision

Three concentration levels of a reference Manidipine dihydrochloride solution were selected within the linearity range of methods A, B and C: 50, 75, and 100 µg/mL. Five independent analyses at each concentration level were performed within one day (intraday precision). This analysis was repeated for five consecutive days (interday precision).

Accuracy

The accuracy of the method was evaluated by the standard addition technique. A known amount of standard was added to the preanalyzed sample and the mixture was reanalyzed by all the proposed methods.

CONCLUSION

Optical characteristics such as Beer's law limits, Sandell's sensitivity, percent relative standard deviation, and percentage range of error were calculated for all the methods and results summarized in Table 1. Satisfactory results were obtained by the proposed methods. Hence, the proposed methods are economical, simple, sensitive, and accurate enough for the routine estimation of the drug manidipine dihydrochloride in bulk and pharmaceutical preparations.

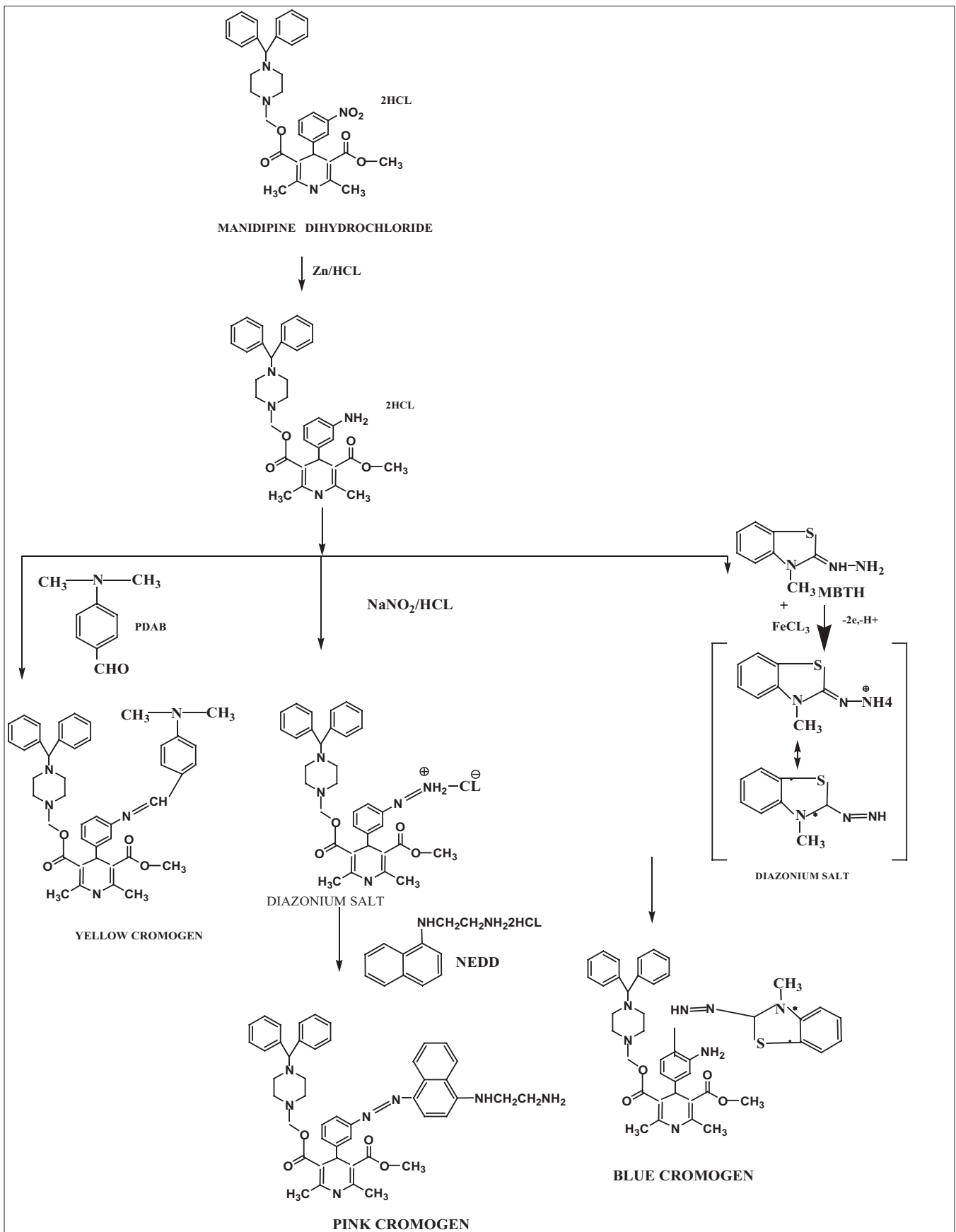


Figure 2: Reaction mechanism for methods A, B and C chromogen

Analysis of manidipine dihydrochloride and synthetic mixtures

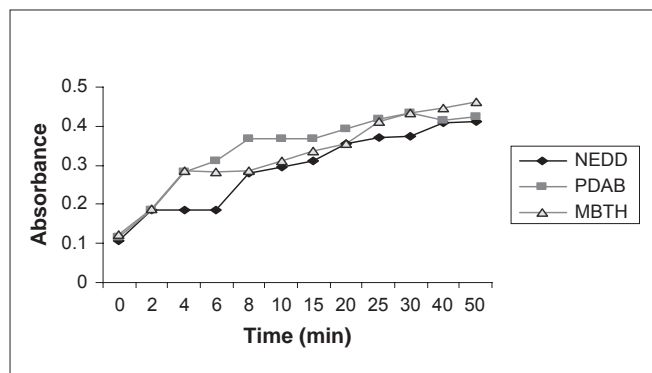


Figure 3: Effect of time on absorbance for methods A, B and C

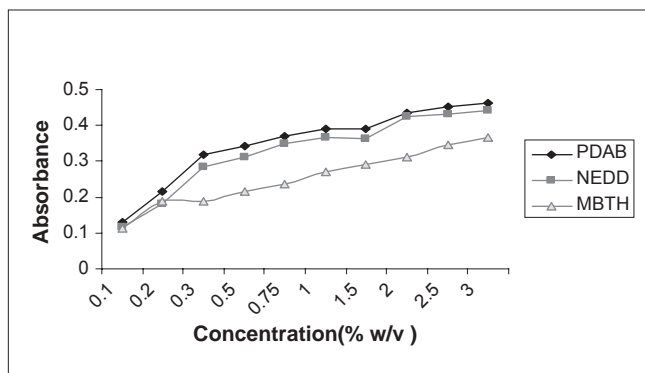


Figure 4: Effect of concentration on absorbance for methods A, B and C

Table 1: Optical characteristics and other parameters

Parameter	Method A	Method B	Method C
λ_{max} (nm)	436.97	550.20	480.21
Beer's law limit ($\mu\text{g/mL}$)	25-125	25-125	25-125
Molar absorptivity ($\text{L/mol}\cdot\text{cm}$)	$2.782 \times 10^{(4)}$	$1.73 \times 10^{(4)}$	$1.79 \times 10^{(4)}$
Correlation coefficient (r)	0.9975	0.9990	0.9982
Sandell's sensitivity ($\mu\text{g/cm}^2$ absorbance unit/0.01 nm)	0.0321	0.0381	0.0357
Regression equation ($Y = mx + c$)*			
Slope (m)	0.0028	0.0023	0.0035
Intercept (c)	0.0131	0.00427	0.00103
Limit of detection ($\mu\text{g/mL}$)	1.2024	1.2121	0.9798
Limit of quantitation ($\mu\text{g/mL}$)	3.6451	3.6727	2.9692
Relative standard deviation (%)	0.3869	0.9370	0.7203
Standard error			
95% Confidence limit	1.96 ± 0.33	1.96 ± 0.40	1.96 ± 0.29
99% Confidence limit	2.575 ± 0.33	2.575 ± 0.40	2.575 ± 0.29
Recovery (%) ^a	99.4 ± 0.38	99.6 ± 0.93	99.3 ± 0.29

* With respect to $Y = mx + c$, where 'c' is intercept, 'x' is the concentration in $\mu\text{g/mL}$ and 'm' is the Slope ^a With respect to Mean \pm SD ($n = 3$)

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