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Spectrophotometric Determination of Hydralazine Using *p*-Dimethylaminobenzaldehyde

O.A. Adegoke* and C.E. Nwoke
Department of Pharmaceutical Chemistry, Faculty of Pharmacy University of Ibadan, Ibadan, Nigeria

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A new spectrophotometric method for the assay of hydralazine hydrochloride in bulk and dosage forms has been developed. The procedure is based on a condensation reaction between alcoholic solution of hydralazine and acidic solution of *p*-dimethylaminobenzaldehyde to generate an instant greenish-yellow coloured product. The hydrazone formed absorbed visible light strongly and optimal detector response was obtained at a wavelength of 470 nm with a molar absorptivity of 3.652×10^3 l mol^{-1} cm^{-1}. Optimization of temperature and time revealed 30 ºC and 10 min as the best conditions for optimal color formation, while methanol was found to be the most suitable alcohol as diluting solvent. The reaction product is stable for at least 24 h. Beer's law was obeyed in the concentration range of 2-10 μg ml^{-1}. Recovery studies over three days gave a mean recovery of 101.87% with a relative standard deviation of 1.29%. There was no interference from commonly used tablet excipients. There was no significant difference between the results obtained with the new method and the BP iodimetric method ($p > 0.05$). The proposed method is simple, sensitive, rapid and economical, and could find application as an in-process quality control method for hydralazine.

Keywords: Hydralazine hydrochloride, *p*-Dimethylaminobenzaldehyde, Condensation, Alcohols, Spectrophotometry

INTRODUCTION

Hydralazine (1-hydrazinophthalate) is a vasodilator which reduces high blood pressure and peripheral resistance. It is used in the management of hypertension and particularly in the treatment of hypertensive emergencies [1]. The official methods proposed by BP [2] and USP [3] for the assay of hydralazine.HCl utilize redox titration involving potassium iodate. End-points are detected potentimetrically [2] or by the use of discharged iodine in chloroform [3].

Various other methods have been described for the assay of hydralazine HCl in dosage forms. Majority of UV-Vis spectrophotometric and chromatographic methods are based on derivatization procedures with some aldehydes. Some of the UV spectroscopic methods include the use of ninhydrin [4], *o*-methoxyacridine [5], 2-hydroxy-1-naphthaldehyde [6], vanillin [7] and nitrite [8]. Other methods include the use of tetracyanomethylene [9], derivative spectrophotometry [10], fluorescence in H_2SO_4 [11] and pulse polarography [12]. Some of the reported chromatographic techniques include the use of 2-nitrocinnamaldehyde [13] and 2-hydroxy-1-naphthaldehyde [14] as pre-column derivatizing reagents. Majority of these methods are time-consuming, require the use of expensive reagents, utilize high calibration range and involve extensive extraction procedures.

This paper reports the colorimetric determination of hydralazine using *p*-dimethylaminobenzaldehyde following the formation of a hydrazone.

*Corresponding author. E-mail: jireade@yahoo.com

EXPERIMENTAL

Chemicals and Reagents

Apresoline® tablets, 25 mg, and Injection, 20 mg, were from Novartis Pharma AG (Switzerland). The hydralazine hydrochloride secondary reference substance was prepared in our laboratory and its purity was assessed by TLC, melting point and iodometric titration. Reagent grade *p*-dimethylaminobenzaldehyde (DMAB), methanol, ethanol, 2-propanol, concentrated sulphuric acid were purchased from BDH (Poole, England). Precoated thin layer plates GF_{254} 0.2 mm (Merck, Germany) were used.

Apparatus

The apparatus used were a UV-Vis spectrophotometer (Unicam Aurora, Helios Scan Software v 1.1, Pye Unicam, England), an analytical balance H80 (Mettler, UK), an ultrasonic bath (Langford Electronics, UK), a vortex mixer (Griffins and George ltd., UK) and a digital colorimeter (Jenway, UK).

Preparation of Stock Solutions

A 0.3% solution of *p*-dimethylaminobenzaldehyde (DMAB) was made by dissolving 0.3 g in 100 ml of 0.5 M sulfuric acid. Hydralazine hydrochloride (10 mg) was dissolved in 10 ml methanol to give a 1 mg ml^{-1} stock solution. A working solution of 100 µg ml^{-1} was thereafter made from the stock solution.

Selection of Analytical Wavelength

A 0.5 ml quantity of DMAB solution was added into a test tube and 0.5 ml hydralazine stock was added. The immediate greenish-yellow colored complex formed was allowed to stay at room temperature for 10 min. The solution was made up to 5 ml with methanol. The absorption spectrum of the complex was determined against DMAB and hydralazine blanks and the wavelength of maximum absorption (λ_{max}) of the product of the reaction was noted.

Optimization Studies

Temperature and time were optimized using the method of steepest ascent [15]. Aliquots of hydralazine stock solution (0.5 ml) was added to the DMAB solution (0.5 ml) in a test tube and the reaction mixture was mixed in a vortex mixer followed by incubation in turn at 30 ºC and 50 ºC for 5 and 20 min. Similar experiments were carried out at 60 ºC and 80 ºC. Each determination was done in duplicate. The absorbance readings of the complex were taken at 470 nm on the colorimeter after making up to 5 ml with methanol.

The optimal reaction time was determined by repeating the above procedure and the reaction was terminated by making up to 5 ml with methanol at 5, 10, 15, 20 and 30 min. The optimal reaction time was taken as the time corresponding to the maximal absorbance of the samples.

The effect of varying acid concentration used in preparing the DMAB solution was investigated by preparing DMAB in 0.125, 0.25, 0.5, 1 and 2 M H_2SO_4 as solvent. In each case, the absorbance reading of 0.5 ml DMAB and 0.5 ml hydralazine in 5 ml methanol was determined after incubation at 30 ºC for 10 min. Optimal acid concentration was taken as one which gave the highest absorbance reading.

The effect of the diluting solvent after condensation reaction was investigated using acetonitrile, ethanol, methanol and 2-propanol. In each case the reaction mixture was made up to 5 ml with the respective solvent. Absorbance readings were taken at 470 nm.

Effect of Reagent Concentration

The effect of varying the concentration of DMAB was carried out using reagent concentrations of 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5% in 0.5 M H_2SO_4. After mixing 0.5 ml of each reagent concentration with 0.5 ml hydralazine solution and making up to 5 ml with methanol, the absorbance readings of the complex formed were made at 470 nm on the colorimeter.

Stoichiometric Ratio Determination

Equimolar solutions (0.0201 M) of DMAB and hydralazine were prepared in their respective solvents. In five different test tubes, 0, 0.25, 0.33, 0.50, 0.67, 0.75 and 1.0 ml of DMAB was added, respectively. The solutions were made up to 1.0 ml with hydralazine solution. Blank determinations of DMAB and hydralazine were similarly carried out using H_2SO_4 and methanol as diluents, respectively. The mixtures were allowed to stand at room temperature for 10 min. At the end of the time interval, solutions were made up to 5 ml with methanol and the absorbance readings were made at 470 nm

the colorimeter.

Validation Studies

At the optimal analytical conditions described above, the calibration lines were obtained using standard solutions of 0, 2, 4, 6, 8 and 10 µg ml^{-1} hydralazine. Linear regression analysis was used to calculate the slope, intercept and coefficient of determination (r^2) of each calibration line. The precision and accuracy of the method were determined as documented by the USP [16]. The limit of detection was computed from the calibration equation as the analyte concentration giving a signal equal to the blank signal plus three standard deviations of the blank [17].

Assay of Dosage Forms

The amount of powdered tablet equivalent to 10 mg hydralazine hydrochloride was dispersed in 6 ml methanol and mixed in an ultrasonicator for 5 min. The solution was filtered and the filtrate made up to 10 ml with methanol. A 1 ml quantity of this solution was further diluted to 10 ml to give a 100 µg ml^{-1} stock solution. Aliquots (0.3 ml) of this solution were added into test tubes containing DMAB (0.5 ml) and the mixture was incubated at 30 ºC for 10 min. The solution was made up to 5 ml with methanol. The absorbance was measured at 470 nm on the colorimeter. Contents of hydralazine in the tablets were then determined by interpolation from calibration lines. Similar procedure was used for the assay of Apresoline® injection.

The analysis of both the tablet and injection samples were also repeated using the BP [2] and USP [3] procedures, respectively. The assay results obtained by the proposed method and the official iodometric titrations were compared using student's t-test and F-ratio test.

Interference Studies

The effect of commonly utilized excipients in drug formulation was studied by spiking solutions of hydralazine stock (0.5 ml) into tablet excipients (*viz*: gelatin, starch, magnesium stearate, lactose and a mixture of the last three substances). In addition, 0.2 ml of hydralazine stock was spiked into tablet and injection solutions containing 0.6 ml hydralazine. The recovery was determined in each case and then compared with the recovery from reference samples alone.

RESULTS AND DISCUSSION

Hydralazine reacted instantly with *p*-dimethylamino-benzaldehyde (DMAB) to give a greenish-yellow color. This color is distinct from the colorless solutions of hydralazine in methanol and DMAB in dilute sulfuric acid. TLC analysis revealed the formation of a new compound. The condensation product exhibited a new λ_{max} at 390 nm and a shoulder at 420 nm, where neither DMAB nor hydralazine has any significant absorption. The UV-Vis spectra of hydralazine, DMAB and hydralazine-DMAB complex are presented in Fig. 1.

As is obvious, the complex exhibited some other peaks at 250 and 300 nm where DMAB has also some significant absorption. However, the only prominent peaks for hydralazine are at 210 and 270 nm. The condensation product therefore has longer chromophores than the starting materials. Optimal difference in absorptivity between the three species was observed at 470 nm on the colorimeter, which was selected as the working wavelength. The use of colorimeter

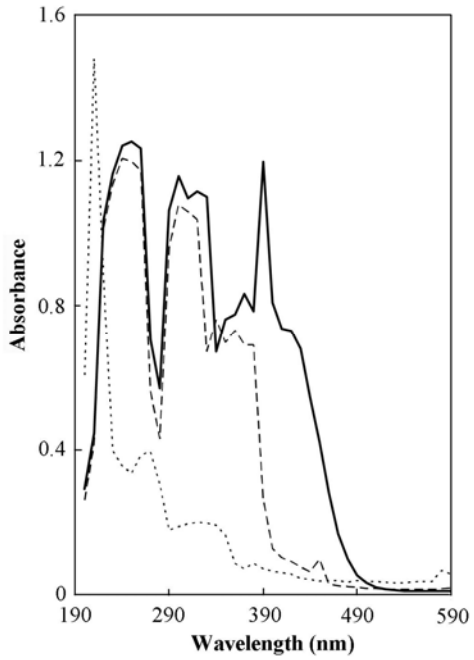
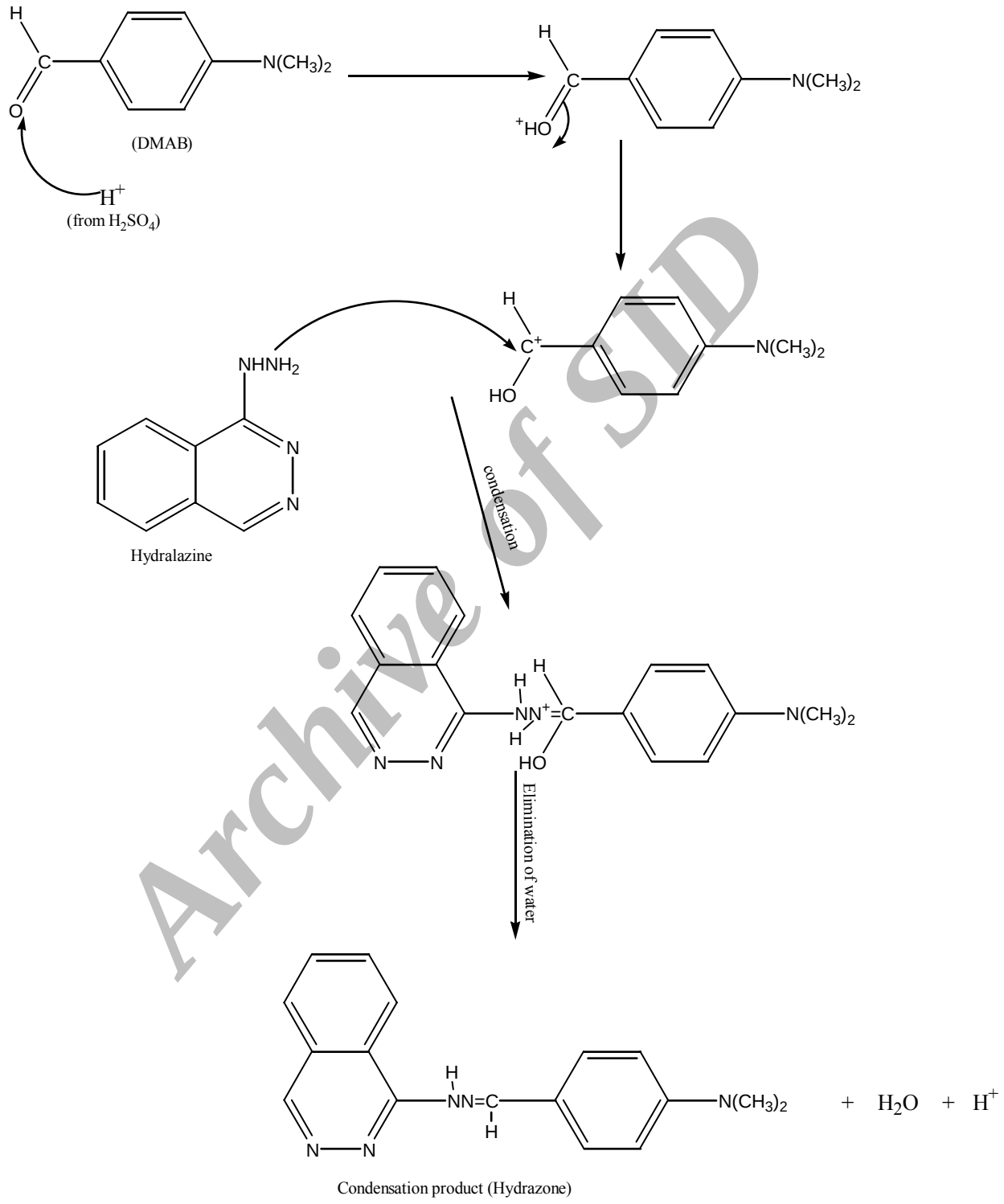


Fig. 1. Absorption spectra of hydralazine (…..), DMAB (– – –) and Hydralazine-DMAB complex (──).



Scheme 1

was preferred as it provides a more readily available instrumentation. Field studies with the reagent will also be possible on the colorimeter.

The reaction between hydralazine and DMAB is presented in Scheme 1. The hydrazone produced is suitable for the detection of primary aromatic amines. In particular, DMAB has already been used for the HPLC determination of phenylpropanolamine [18]. The reagent is also a principal component for detecting microbial indoles (Kovác reagent). The condensation reaction requires the presence of an acid for the protonation of the carbonyl oxygen and thereby leaving the carbonyl carbon fully positively charged. The hydrazine then donates a lone pair of electrons to the carbon. Internal rearrangement thereafter results in the formation of hydrazone and then giving water and proton as by-products. Stability of the hydrazone produced depends on the concentration of the acid and the rate at which the water is removed.

Optimal stability of the condensation product was found to occur when DMAB was prepared in 0.5 M sulfuric acid (Fig. 2). Lower acid concentrations gave low absorbance values while absorbance readings declined with increasing acid concentration beyond 0.5 M. It appears that higher acid concentration catalyses the breakdown of the product. In methanol, ethanol and propanol, as solvent, increasing absorbance values were obtained with each successive chain length increase. It thus implies that higher homologue alcohols are more effective in removing the water formed from the condensation reaction and thereby stabilizing the molecule and promoting the formation of the hydrazone. However, methanol was used as the diluting solvent based on its lower cost and considering the fact that the absorbance of product in the three solvents is not significantly different ($p > 0.05$). A much lower absorbance value was obtained in the presence of acetonitrile, the solvent being incapable of dehydrating the medium and hence producing instability. Optimal reagent concentration was found to be 0.3% (Fig. 3).

The optimization of temperature was investigated in two time levels of 5 and 20 min at 30, 50, 60 and 80 ºC. As presented in Fig. 4, at 5 min, highest absorbance was obtained at 60 ºC and thereafter declined beyond 60 ºC. However, at 20 min, there is no significant difference in the absorbance produced at 30 º and 50 ºC (*i.e.*, 0.75 and 0.77, respectively). Therefore, 30 ºC was selected as being optimum for the hydrazone formation.

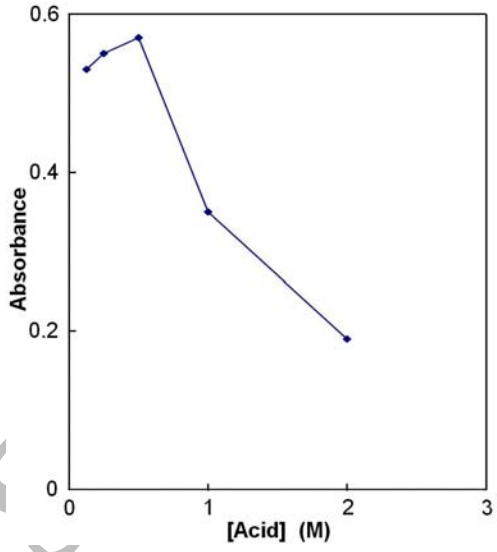


Fig. 2. Effect of acid concentration on hydrazone formation.

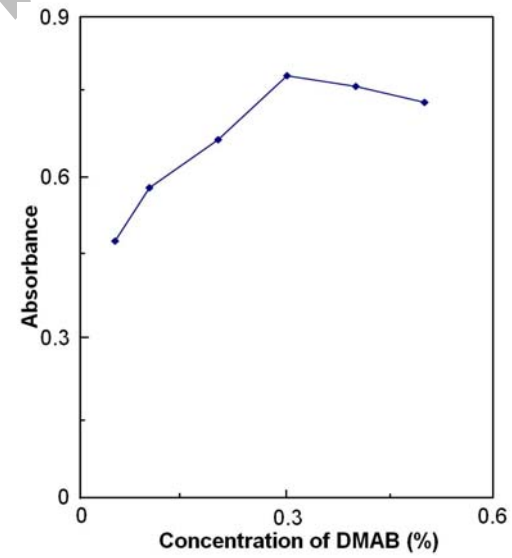


Fig. 3. Effect of reagent concentration on the formation of hydralazine-DMAB complex.

Optimization of reaction time was thereafter carried out at 30 ºC at time levels of 5, 10, 15, 20 and 30 min (Fig. 5). The absorbance value peaked at 10 min and thus optimal conditions for the hydrazone formation are at 30 ºC and 10 min. Higher temperatures resulted in destroying hydrazone,

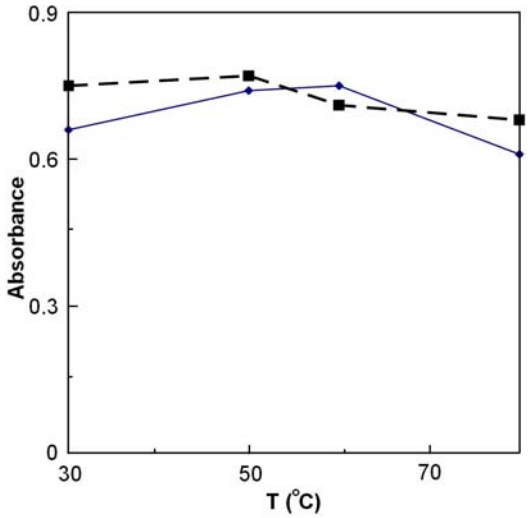


Fig. 4. Optimization of temperature for condensation reaction at 5 min (——) and 20 min (– – –).

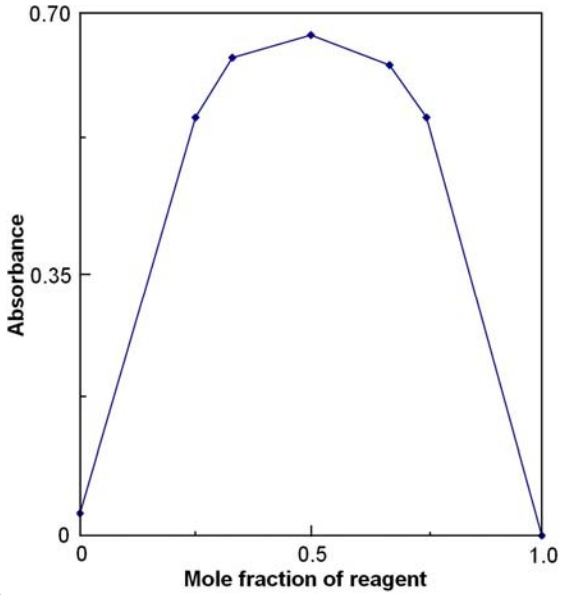


Fig. 6. Stoichiometric ratio determination for the formation of the hydrazone at 30 ºC for 10 min.

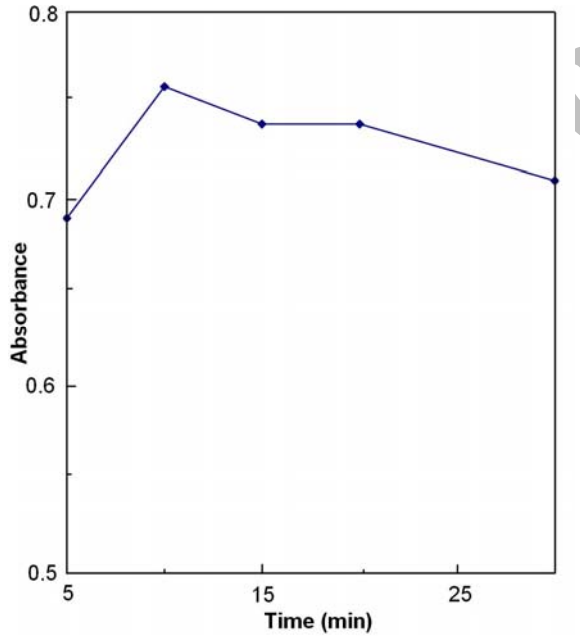


Fig. 5. Optimization of time at 30 ºC for the condensation reaction.

similar to high acid concentrations.

The stoichiometric ratio for the formation of the hydrazone produced the results is shown in Fig. 6. As seen, hydralazine is combined with DMAB at a 1:1 molar ratio. This is anticipated as there is no other envisaged side reactions.

The regression line equation for the analysis of hydralazine using DMAB was found to be $y = 0.0178x + 0.0054$ ($r^2 = 0.9984$), where y is the absorbance and x is the concentration in μg ml^{-1}. The correlation coefficient (r) translates to 0.9992 which represents an excellent curve. The limit of detection obtained is 0.508 μg ml^{-1}. This low limit compares favorably with previously described spectrophotometric methods [4-8]. An advantage of this new method over the other methods is the micro analytical range determinable by the method. Most of the previous methods [4-8] worked in calibration ranges which utilized concentration up to 10 times the range described in this procedure. The 95% confidence limit for the slope and intercept are 0.0178 ± 0.0011 and 0.0054 ± 0.0006, respectively. The molar absorptivity is given as 3.652×10^3 l mol^{-1} cm^{-1}.

The accuracy and precision for the assay of hydralazine was carried out on three successive days using three concentration levels. The results are presented in Table 1. The overall recovery of hydralazine is 101.87 ± 1.31%. This recovery compares favorably with the spectrophotometric methods described by Mari-Buigues *et al*. [6] and by Ahmed *et al*. [7]. The relative standard deviation obtained (1.29%)

Table 1. Accuracy and Repeatability of the Proposed Spectrophotometric Method

Concentration (μg ml^{-1})	Day 1[a]		Day 2[a]		Day 3[a]		Inter-day statistics for each analyte size	
	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)
3	103.1	0.4	102.8	1.0	102.9	0.9	102.9	0.2
7	100.1	0.4	100.1	0.3	100.2	0.2	100.1	0.1
9	102.5	0.8	102.6	0.6	102.6	0.6	102.6	0.1

[a]n = 9, Regression equation: y = 0.0178x + 0.0054 (r^2 = 0.9984); between-day statistics = 101.87 ± 1.31%, RSD (of s.e.m) = 1.29%

Table 2. Recovery of Hydralazine by the Proposed and Official Methods[a]

Drug formulation	Proposed method (%)	Recovery in the presence of additional analyte (%)	95% C.I. (of 6 μg ml^{-1})	Official method (%)	*p*-Value[b] F-test	t-test
Apresoline® tablet	98.8 ± 0.5	99.2 ± 1.0	5.92 ± 0.03	99.1 ± 0.5	0.99	0.40
Apresoline® injection	99.1 ± 0.5	100.9 ± 0.9	5.95 ± 0.03	100.9 ± 1.6	0.13	0.06

[a]Both the BP and USP require the content of hydralazine in tablet and injection to be 95-105%. [b]Statistical analysis done between the results obtained from the proposed method and official iodometric titration method.

is also better than the HPLC method described by Manes *et al*. [14]. Another advantage of the proposed method is the simple methodology involved and the relative stability of the hydrazone produced.

The new method was then applied to the quantitative determination of hydralazine hydrochloride in tablet and injection formulations. The results obtained as well as that of the official methods are presented in Table 2. As is obvious, there is no significant difference in the content of hydralazine obtained by both methods ($p > 0.05$). In the presence of common excipients, the recoveries of hydralazine are as follows: starch, 98.38 ± 0.1%; lactose, 99.38 ± 0.5%; magnesium stearate, 98.38 ± 0.6%; and a mixture of these excipients, 99.68 ± 1.1%. However, the use of gelatin as an excipient was found to seriously interfere in the recovery of hydralazine. This implies that the new method also shares in this disadvantage of the previous methods. Prior quality control of tablet or injection samples for presence of hydrazine will improve the specificity of the procedure.

CONCLUSIONS

The new procedure for the spectrophotometric determination of hydralazine described in this work is simple, rapid and cost-effective with high accuracy and precision when compared with previously reported procedures. It could find application as a convenient technique for the in-process control of hydralazine.

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