

## Sensitive Spectrophotometric Methods for Quantitative Determination of Hydralazine Hydrochloride in Pure and Pharmaceutical Formulation

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**Abstract:** Two analytical methods for the estimation of hydralazine hydrochloride (HLH) in bulk drug and in their tablet formulations are described. The developed methods are based on the formation of blue colored chromogen due to the reaction of hydralazine hydrochloride with Folin Ciocalteu reagent in presence of alkali, which exhibits  $\lambda_{\max}$  at 640 nm (Method A). The Method B is based on reduction of ferric ions to ferrous ions in presence of drug produces greenish blue colored complex measured at 720 nm against reagent blank. The color was stable for more than 12 hrs for method A and 4 hrs for method B respectively. Beer's law is obeyed over the concentration range of 0.1-1.0 and 0.2-1.8  $\mu\text{g/ml}$  for method A and Method B, respectively. All the variables were studied to optimize the reaction conditions. The calculated molar absorptivity values are  $1.754 \times 10^4$  and  $0.964 \times 10^4 \text{ lit. mol}^{-1} \text{ cm}^{-1}$  for Method A and Method B, respectively. The proposed methods were successfully applied to the determination of HLH in formulations. Good recoveries were obtained and the results were statistically compared with those of a literature method showed good agreement and indicated no significance difference in precision. (The accuracy and validity of the methods were further ascertained by performing recovery experiments via standard addition method). No interference was observed in the presence of common pharmaceutical excipients. The proposed methods does not require any extraction or heating.

**Key words:** Hydralazine hydrochloride • Folin Ciocalteu reagent • Potassium ferricyanide  
• Spectrophotometer • pharmaceutical formulations

### INTRODUCTION

Hydralazine hydrochloride (HLH) is chemically 1(2H)-phthalazinone hydrazone; 1-hydrazinophthalazine [1]. It is a potent vasodilator that has been used for many years, chiefly in the treatment of ambulatory patients with primary hypertension of moderate severity [2]. In therapeutic doses, hydralazine hydrochloride produces little effect on nonvascular smooth muscle or on the heart. Its pharmacological actions are largely confined to vascular smooth muscle and occur predominantly on the arterial side of circulations, many hydralazine derivatives and their formulations are official in World Health Organization (WHO), British Pharmacopoeia (BP) and Indian Pharmacopoeia (IP) [3-6]. The official methods involve potentiometric titration using 0.05 M potassium iodide as a titrant [5] and High performance liquid chromatography (HPLC) [6]. The drug has been assayed by a variety of methods, such as spectrofluorometry [7], oxidimetry [8], polarography [9] and High performance liquid chromatography (HPLC) [10].

Visible spectrophotometry, because of simplicity, cost effectiveness, sensitivity, selectivity, fair accuracy and precision, has remained competitive in an era chromatographic techniques for pharmaceutical analysis. Many visible spectrophotometric methods [11-20] based on different reaction schemes are found in literature for the assay of HLH. But none of these methods are satisfactory for routine quality assurance for one or the other reasons. Some of these methods have low sensitivity [11], work out only at higher concentration of the drug [12] or have less stability [13] or involve extraction [14]. On the basis of this background, it was felt necessary to develop a rapid, low cost, accurate, simple and sensitive spectrophotometric methods, which do not suffer from the above limitations for the determination of HLH in bulk drug and in pharmaceutical formulations. The proposed methods have been demonstrated to be superior to the reported methods with respect to speed, simplicity, sensitivity and cost-effectiveness. Folin Ciocalteu reagent and potassium ferricyanide were previously reported to be a sensitive reagent for

spectrophotometric determination of a considerable number of amine and other functional groups containing medicinal compounds [22-26].

### Experimental

**Apparatus:** All spectral measurements were made on ELICO SL model 164 UV-visible double beam spectrophotometer with 1 cm matched quartz cuvettes. Calibrated glasswares were used throughout the experiment.

**Reagents:** All the chemicals (Folin-Ciocalteu reagent (FCR), sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), potassium ferricyanide, ferric chloride. (S.D Fine Chem. Ltd., Mumbai) used were of analytical reagent grade or chemically pure grade and used without further purification, double distilled water was used for the dilution of reagents and samples. HLH bulk drug was obtained as gift sample from Sequent Scientific Ltd., India. Pharmaceutical formulations of HLH were obtained commercially.

**Standard Drug Solution:** Stock solution of HLH (1000  $\mu\text{g}/\text{ml}$ ) was prepared by dissolving 100 mg of HLH in distilled water and making the volume to 100 ml in a standard volumetric flask. Working solution of lower concentration (100  $\mu\text{g}/\text{ml}$ ) was prepared by further dilution of the above standard stock solution with water. The solution was protected from light before use.

**FC-Reagent (v/v):** 1:4 aqueous solution was prepared by dissolving accurately measured twenty ml of Folin-Ciocalteu reagent in eighty (80) ml distilled water and make up the volume to 100 ml in a standard volumetric flask.

**Sodium Carbonate (w/v):** Twenty five percent (25%) solution was prepared by dissolving 25 g of sodium carbonate in a beaker containing small volume of water and make up the volume to 100 ml in a standard volumetric flask.

**Potassium Ferricyanide (w/v):** First, one (1%) percent solution was prepared by dissolving accurately weighed 1g potassium ferricyanide in distilled water and make up the volume to 100 ml in a standard volumetric flask. The above solution was further diluted to get required concentration (0.2%)

$\text{FeCl}_3$  (w/v). 0.03 M solution was directly prepared by dissolving 0.486 g of ferric chloride in distilled

water and make up the volume to 100 ml in a standard volumetric flask.

### General Procedure for the Determination of Pure Hlh Drug

**Method a:** Different aliquots of working standard (100  $\mu\text{g}/\text{ml}$ ) HLH solution ranging from 0.1-1.0 ml (1 ml =100  $\mu\text{g}$ ) was transferred into a series of 10 ml serially numbered volumetric flasks by means of a micro pipette. To each flask, 3.0 ml of FC Reagent (1:4) and 2.0 ml of sodium carbonate (25%) solution were added with the help of micro burette. The flasks were stoppered, contents were mixed well and kept to room temperature for 10 min. for completion of reaction. The volume was brought up to 10 ml with distilled water to get final concentration of 1-10  $\mu\text{g}/\text{ml}$ . After 5 min., the absorbance of the blue colored solution was measured at 640 nm against reagent blank. The amount of HLH present in the sample was computed from calibration curve.

**Method B:** Different aliquots of working standard (100  $\mu\text{g}/\text{ml}$ ) HLH solution ranging from 0.2-1.8 ml (1 ml = 100  $\mu\text{g}/\text{ml}$ ) was transferred into a series of 10 ml serially numbered volumetric flasks by means of a micro pipette. To each flask, 1.0 ml of ferric chloride solution (0.03M) and 0.5 ml of potassium ferricyanide (0.2%) solution were added with the help of micro burette. The flasks were stoppered, contents were mixed well and kept to room temperature for 10 min. for completion of reaction. The volume was brought up to 10 ml with distilled water to get final concentration of 2-18  $\mu\text{g}/\text{ml}$ . After 5 min, the absorbance of the bluish green colored solution was measured at 720 nm against reagent blank. The amount of HLH present in the sample was computed from calibration curve.

In Methods A and B, a calibration graph was prepared by plotting absorbance versus concentration of drug and the concentration of the unknown was read from the calibration graph or computed from the regression equation derived from the Beer's law data. The calibration graph was then prepared by plotting the absorbance versus concentration of the drug.

**Assay Procedure for Pharmaceutical Tablets:** For analysis of HLH, Two brands of commercially tablets (20) were weighed and ground into a fine powder. An accurately weighed portion of the powder equivalent to 100 mg of HLH was transferred in to a 100 ml beaker containing small volume of water and the solution was shaken thoroughly for about 10-15 min and filtered

through a Whatman filter paper no. 40 to remove the insoluble matter. The filter paper was washed with water and the washings were added to the filtrate, the final volume (100 ml) was made with water. A suitable aliquot of this solution in the working range of HLH (1 ml of the above filtrate was taken in a 10 ml volumetric flask) was treated as per procedure described in the above determination of pure HLH.

## RESULT AND DISCUSSIONS

**Determination of Absorption Maxima ( $\lambda_{\max}$ ):** To determine the  $\lambda_{\max}$ , 10  $\mu\text{g}$  /ml of the HLH was added to 10 ml volumetric flask. Then 2.5 mL of 1:4 solution of FC Reagent and 2.0 ml of 25% solution of sodium carbonate were added for method A and for method B 1.0 ml of 0.03 M solution of ferric chloride followed by 0.5 ml of 0.2% solution of potassium ferricyanide were added. Then solution was made up to the mark with distilled water and mixed well. After 5 min, absorbance were measured (Method A and B) against reagent blank in the range of 400-800 nm.  $\lambda_{\max}$  for HLH were found to be 640 nm for method A and 720 nm for method B. respectively. Absorption spectrum of both the methods are shown in Fig. 1. Under the experimental conditions each reagent blank showed a negligible absorbance at the corresponding  $\lambda_{\max}$ .

**Reaction Sequence:** In the method A, The estimation is based on Folin-Ciocalteu reagents in presence of alkali and produces blue colored complex, with absorption maxima ( $\lambda_{\max}$ ) at 640 nm. In the method B, the drug HLH

reduces ferric chloride to ferrous ions, which in turn couples with reagents having divalent iron like potassium ferricyanide to form bluish green colored potassium ferro ferrous complex and produces bluish green color complex with absorption maxima ( $\lambda_{\max}$ ) at 720 nm. The probable reaction mechanism based on the reported method<sup>25,26</sup> is given in scheme 1.

### Determination of Effective Reagents Concentration:

Optimum reagent concentrations required for the formation of sensitive and quantitative colored products and were determined by varying one reagent concentration and fixing the concentrations of other reagents and its effect on absorbance were measured at 640 and 720 nm respectively.

**Effect of Concentration of FCR and PFCN:** The effect of varying the concentration of complexing agent was studied using the proposed procedure. To a series of solutions containing 10  $\mu\text{g}$  of HLH and fixed concentration of FC Reagent (1:4) for method A and Potassium ferricyanide (0.2%) for method B, in different 10 ml volumetric flask, different volume (1-6 ml) of FCR (Method A) and 0.5-2 ml PFCN (Metho B) were added and solutions were diluted up to the mark with distilled water. After 5 min, absorbance of each solution was measured at 640 and 720 nm for method A and B respectively; it was found that, constant absorbance values were obtained with 3 ml of 1:4 FC reagent or with 1 ml of 0.2% PFCN solution in final volume of 10 ml. Above this range, a decrease in the absorbance was observed.

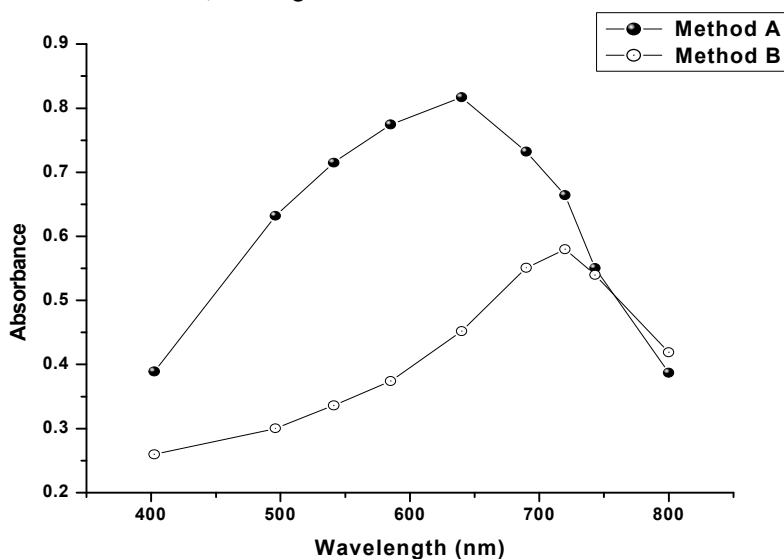
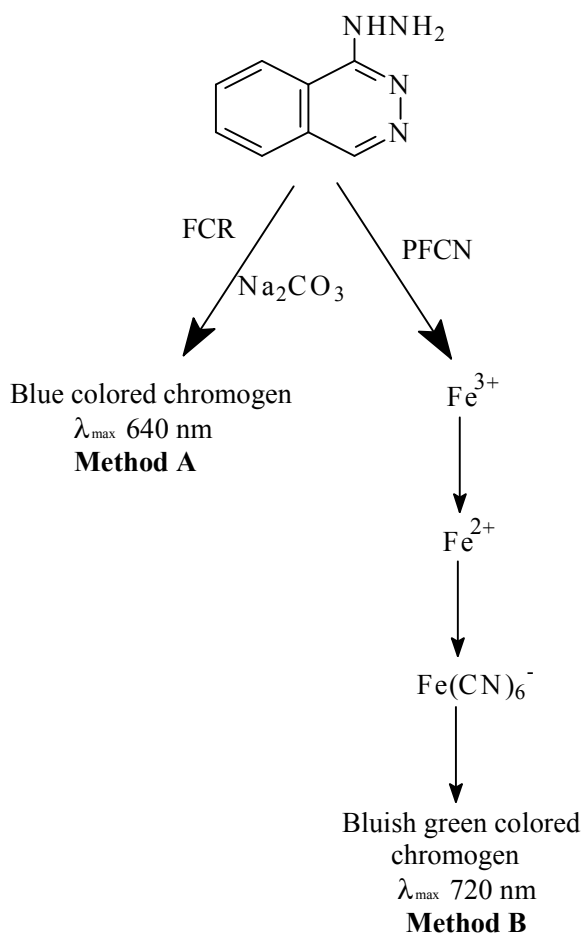


Fig. 1: Absorption spectra of HLH at 10  $\mu\text{g}$  / ml of concentration for Method A and Method B.



Scheme 1:

**Effect of Alkali (Sodium Carbonate) Concentration:**

The effect of sodium carbonate (Alkalinity) concentration on the absorbance was studied; volumes from 0.5-4.0 ml of 25% sodium carbonate solutions were examined. The investigations showed that 1.5-2.5 ml of sodium carbonate gave maximum absorbance and hence 2 ml of 25% sodium carbonate was chosen for all measurements. Other alkaline solutions were tried, but best results were obtained by using sodium carbonate.

**Effect of Ferric Chloride Concentration:** The effect of varying the concentration of oxidation-reduction agent was studied using proposed procedure and adding 0.5-3.0 ml of 0.03 M ferric chloride solution to a series of drug solutions. It was noticed that the maximum color intensity and constant absorbance was formed with 1.0 ml of ferric chloride solution in final volume of 10 ml.

**Effect of Temperature on Colored Product:** The effect of temperature on colored product was studied at different temperatures. It was found that the colored product was stable in the temperature range 0-50°C. At higher temperatures, the blue and bluish green clear solution precipitates out and blanks solution develops a light color and also decrease in the absorbance was observed. However, the color products were stable for more than 12 and 4 hrs for method A and method B respectively, at room temperature.

**Validation of the Method**

**Detection and Quantification Limits:** According to the Analytical Methods Committee<sup>21</sup> the detection limit (LOD) is the concentration of HLH corresponding to a signal equal to the blank mean ( $Y_b$ ) plus three times the standard deviation of the blank ( $S_b$ ). Quantification limit (LOQ) is the concentration of HLH corresponding to the blank mean plus ten times the standard deviation of the blank. The LOD values were found to be 0.089 and 0.096  $\mu\text{g/ml}$  for HLH with FCR and PFCN, respectively. The LOQ values were observed to be 0.296 and 0.320  $\mu\text{g/ml}$  for HLH with FCR and PFCN, respectively.

**Quantification:** The Beer's law limits, molar absorptivity and Sandell's sensitivity values were evaluated and are shown in Table 1. Regression analysis of Beer's law plots at their respective  $\lambda_{\text{max}}$  values revealed a good correlation. Graphs of absorbance versus concentration showed zero intercept and are described by the regression equation,  $Y = bX + c$  (where Y is the absorbance of a 1 cm layer, b is the slope, c is the intercept and X is the concentration of the drug in  $\mu\text{g/ml}$ ) obtained by the least-squares method. The results are summarized in Table 1.

**Accuracy, Precision and Recovery Studies:** The validity of the methods for the assay of HLH was examined by determining precision and accuracy. These were determined by analyzing six replicates of the drug within the Beer's law limits. The low values of relative standard deviation (R. S. D.) indicate good precision of the method. The results of analysis of dosage forms are given in Table 2. The results were reproducible as evident from low R. S. D. values.

To study accuracy of the methods, recovery studies were carried out by the standard addition method. For this, known quantities of pure HLH were mixed with definite amount of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the

Table 1: Analytical parameters of spectrophotometric methods

Parameters	Method A	Method B
$\lambda_{\text{max}}$ (nm)	640	720
Beer's Law limits ( $\mu\text{g}/\text{ml}$ )	1-10	2-18
Molar absorptivity ( $\text{lit. mol}^{-1}\text{cm}^{-1}$ )	$1.754 \times 10^4$	$0.960 \times 10^4$
Sandell's sensitivity ( $\mu\text{g}/\text{ml}$ )	0.0112	0.02048
Regression equation** ( $Y = bx + c$ )		
Slope(b)	0.0691	0.0480
Intercept(c)	0.1153	0.0013
Correlation coefficient (r)	0.9999	0.9998
% Relative Standard Deviation (R.S.D)*	0.1944	1.4622
% Range of error (Confidence)*		
0.05 level	0.0010	0.0055
0.01 level	0.0890	0.0080
Limit of Detection ( $\mu\text{g}/\text{ml}$ )	0.089	0.096
Limit of Quantification ( $\mu\text{g}/\text{ml}$ )	0.2963	0.3203
Stability (h.)	12	4
Color	Blue	Bluish green

\*\* $Y = bX + c$ , where Y is the absorbance and X is the concentration of drug in  $\mu\text{g}/\text{ml}$

\*Average of six determinations.

Table 2: Determination of HLH in pharmaceutical formulations and statistical comparison with the reported method

Tablet brand name	Labelled amount mg	% Found <sup>a</sup> $\pm$ SD	Reported method [20]	Method A	Method B
Nepresol	25	101.03 $\pm$ 0.49		99.73 $\pm$ 0.35	99.44 $\pm$ 0.28
Nepresol	25	101.03 $\pm$ 0.49		100.21 $\pm$ 0.47	99.28 $\pm$ 0.66

<sup>a</sup> Average of six determinations  $\pm$  S.D.

Table 3: Determination of HLH<sup>a</sup> in the presence of excipients

Excipients	Amount taken/mg	% Recovery <sup>b</sup> $\pm$ %RSD <sup>b</sup>
Glucose	20	99.05 $\pm$ 0.36
Sucrose	20	99.27 $\pm$ 0.48
Lactose	25	99.44 $\pm$ 0.36
Dextrose	20	100.22 $\pm$ 0.21
Talc	25	98.15 $\pm$ 0.52
Starch	20	99.36 $\pm$ 0.31
Sodium alginate	15	100.05 $\pm$ 0.34

<sup>a</sup> 10  $\mu\text{g}/\text{ml}$  HLH were used.

<sup>b</sup> mean value of six determinations

drug was then determined and the amount of the added drug was calculated by difference. The average percent recoveries obtained were quantitative (99.28 -99.73 %), indicating good accuracy of the methods.

**Interference Studies:** The effect of the excipients associated with formulations of HLH drug in its pure form and its formulations were investigated using the developed methods. This method does not suffer any interference from commonly associated excipients and additives in the preparation of tablets such as sucrose, lactose, dextrose, starch, talc and sodium alginate. Data of the interference studies are given in Table 3.

**Analysis of Pharmaceutical Formulations and Statistical Comparison of the Results with the proposed Method [20]:** The proposed methods were successfully applied to the analysis of HLH in commercial tablets. Table 2 gives the results of assay and revealed that there is close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically with those obtained by a literature method [20] from which we can conclude that the proposed methods do not differ significantly from reference method.

## CONCLUSIONS

The proposed methods are quite simple and do not require any pretreatment of the drug and tedious extraction procedure. The methods have wider linear range with good accuracy and precision. Hence, the data presented in the manuscript by spectrophotometric methods for the determination of hydralazine hydrochloride in its pure and dosage form demonstrate that the proposed methods are accurate, precise, linear, selective and offer advantages of reagent availability and stability, less time consumption and high sensitivity. Thus it can be extended for routine analysis of hydralazine hydrochloride in pharmaceutical industries, hospitals and research laboratories. Unlike the gas chromatography and high performance liquid chromatography procedures, the UV-visible spectrophotometer instrument is simple and not of high cost, on the other hand in terms of simplicity and expense, the method could be considered superior in comparison with the previously reported methods. Moreover the methods are free from interferences by common additives and excipients.

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