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# Method Verification and Validation of Hydralazine Hydrochloride: Spectrophotometric Analysis in Pure and Pharmaceutical Formulations

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## Abstract

The new method proposed is based on the formation of hydralazine-Bromophenol blue ion pair simply and without further extraction or heating. The ion pair was prepared in the presence of pH 3 citrate buffer forming a yellow-colored chromogen. A new maximum UV-visible band formed at 416 nm. The color was stable for more than 10 hours and obeyed Beer's Law over the concentration range of 10 - 50 μg/mL. The calculated molar absorptivity and Sandell's sensitivity were $1.01 \times 10^4$ L·mol$^{-1}$·cm$^{-1}$ and 0.0514 μg/mL, respectively. The elements of method validation stipulated by The International Conference on Harmonization [Q2 (R1)] were applied for hydralazine hydrochloride assay in pure and pharmaceutical tablet formulation. The average recoveries of the pure solution and the pharmaceutical formulation were 98.94% and 99.50%, respectively. The results were statistically compared by F-test, which indicates that the method can be precise and repeatable for both pure and pharmaceutical solutions. The method was found to be accurate, reproducible, and cost-effective, and validated for the assay of hydralazine in terms of the routine quality control.

## Keywords

Spectrophotometry, Validation, Hydralazine Hydrochloride, International Conference on Harmonization Q2 (R1)

## 1. Introduction

Hydralazine hydrochloride (HzHCl) has the chemical name 1-hydrazinophthalazine

hydrochloride ($C_8H_8N_4HCl$). It is used as a muscle relaxant to treat hypertension by acting as a vasodilator in arteries and arterioles. The vasodilator reduces the blood pressure and peripheral resistance. HzHCl is administered after heart valve replacement and for treatment of chronic-resistance heart failure [1] [2]. It can be used in combination with $\beta$-blockers to balance the reflex tachycardia and with diuretics to decrease sodium retention for the treatment of hypertension [3] [4]. Unjudicial uptake of HzHCl can increase adverse effects such as postoral hypotension, dizziness, flushing, fluid retention with oedema and weight gain, conjunctivitis, lachrymation, tremor, muscle cramps, and nasal congestion. With excessive or habitual uptake of HzHCl, toxic symptoms may develop, such tachycardia, palpitations, angina pectoris, headache, gastro-intestinal disturbance [5]. Analytical drug quantification requires development of methods that assist researchers in different activities including toxicology, formulation research, clinical research studies, and clinical pharmacology. The development method should be subjected to validation to demonstrate that the quantitative analysis under examined conditions is reliable and reproducible for intended use. The basic objectives of method development and validation are to determine the optimum analytical condition of drugs in the pharmaceutical industry and to measure the appropriate quantity of the commercialized product [6] [7] [8] [9] [10]. HzHCl has been subjected to various analytical methods including chromatographic [11] [12], amperometric [13], atomic spectrophotometry [14] [15], polarographic [16] flourometric [17] [18], and the UV-visible spectrophotometric method [19] [20]. Specifically, the UV-visible spectrophotometric analysis of HzHCl in literature is based on the reaction of HzHCl with a chromophoric agent to produce a new band in the UV-visible range. Different procedures have been achieved by analysts using 9-chloracridine [21], Ninhydrine [22], toluene-p-sulphonic acid [23], tetracyanoethylene [24], nitrite ion [25] [26], 2-hydroxy-1-naphthaldehyde [27], picric acid, 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone, 2,4-dinitrobenzoic acid, bromanil and chloranil [28], and vanillin [29]. A new band was generated for the above-listed methods that range from 390 to 510 nm. The proposed method of reacting HzHCl with the chromogenic agent bromophenol has been developed to be simple, sensitive (with a high detection limit), selective for a specific absorbent specie, and a rapid, one-step method with high accuracy and precision [30] [31]. In addition, the proposed method is conforming to International Conference on Harmonization Q2 (R1) guidelines [32].

## 2. Materials and Methods

### 2.1. Materials

Analytical grade hydralazine hydrochloride (HzHCl) and bromophenol blue indicator (BpB) were supplied from Sigma. Apresoline tablets containing hydralazine hydrochloride (25 mg HzHCl tablet) were purchased from Novartis Pharma AG, Basel, Switzerland.

## 2.2. Instrumentation

A UV-visible double beam spectrophotometer with matched quartz cell (1 cm) (model no. Evolution 201) by Thermo Scientific, 81 Wyman Street Waltham, Massachusetts, US was used to record the absorption spectra.

## 2.3. Preparation of Standard Solution

An aqueous mixture of 10 mL $1 \times 10^{-3}$ M of BpB and 3 mL of pH 3 citrate buffer was mixed with 5, 10, 15, 20, and 25 mL of HzHCl of $1 \times 10^{-3}$ M in 100 mL volumetric flasks.

## 2.4. Preparation of Pharmaceutical Tablet Solution

To assay Apresoline tablets containing HzHCl (25 mg/tablet), ten tablets of Apresoline were grounded and weighed (1.0068 g). Apresoline solution of $1 \times 10^{-3}$ M concentration was prepared by dissolving 0.0792 g of the powder in the double distilled water, 10 mL of $1 \times 10^{-3}$ M BpB, and 3 mL pH 3 citrate buffer and 10 mL of $1 \times 10^{-3}$ M Apresoline solution was diluted in a 100 mL volumetric flask to the mark.

## 2.5. General Procedure

The spectrophotometric method was developed and optimized for present purposes. Different experimental trials of buffer concentration, diluent composition, and other chromophoric conditions were performed to choose the optimum condition for the developed method, which resulted in the optical characteristics and statistical analysis for Hz-BpB ion-pair determination shown in Table 1.

Table 1. Optical characteristic and statistical analysis for calibration graphs for the determination of HzHCl by the proposed method.

| Optical characteristics | Hz-BpB ion-pair |
|---|---|
| $\lambda_{max}$ (nm) | 416 |
| Temp. ˚C | 25 |
| Linear range (μg/mL) | 10 - 50 |
| LOQ (μg/mL) | 0.82 |
| LOD (μg/mL) | 0.27 |
| Molar absorptivity (L $mol^{-1}$ $cm^{-1}$) | $1.01 \times 10^4$ |
| Sandell's sensitivity (μg/$cm^2$/0.001 absorbance unit) | 0.0514 |
| Regression equation (y = bx + a)* | Y = 0.0518x + 0.0221 |
| Slope (b) | 0.0518 |
| Standard deviation on slope ($S_b$) | 0.00006 |
| Intercept (a) | 0.0221 |
| Standard deviation on intercept ($S_a$) | 0.00425 |
| Correlation coefficient ($R^2$) | 0.9937 |

*y = bx + a, where x is the concentration of analyte in μg/mL and y is the absorbance unit.

The method was then validated to confirm that the analytical procedure is suitable for HzHCl quantification in pure and pharmaceutical formulation. The validation characteristics of ICH under consideration in this work are: specificity, linearity, range, limit of quantification (LOQ), limit of detection (LOD), precision, accuracy, solution stability, and robustness.

## 3. Results and Discussion

### 3.1 Method Specification and Verification

Preliminary trials of different compositions and concentrations of the constituents were made to choose the optimum conditions suitable for the method of analysis. According to the findings, specific analytical conditions were derived to prepare the standard and pharmaceutical solutions mentioned in section 2.3 and 2.4.

### 3.2. Selection of Wavelength

The selection of wavelength used to measure the absorbance of standard and pharmaceutical solutions implies a new band for the Hz-BpB ion pair. Both HzHCl and BpB were scanned separately in Figure 1 and Figure 2 showing a $\lambda$max at 328 nm and 590 nm, respectively. Figure 3 showed a new band at $\lambda$max = 416 nm generated. This proves that an ion-pair of Hz-BpB is formed.

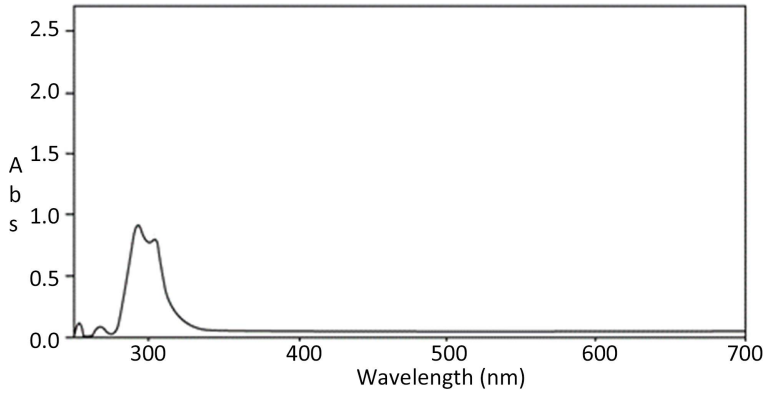


Figure 1. UV-visible spectrum of hydralazine-HCl in pure solution.

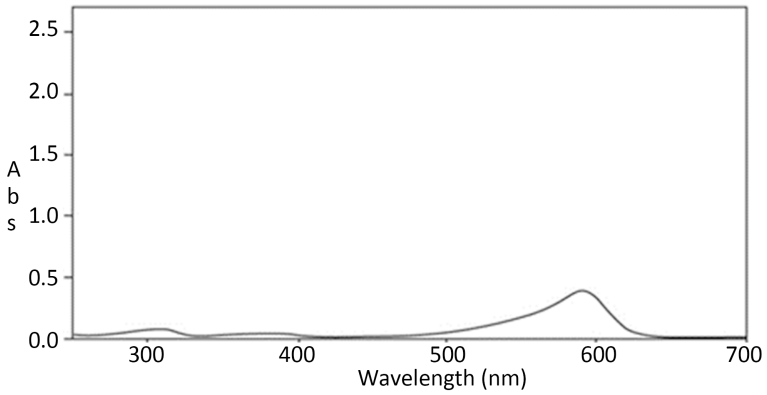


Figure 2. UV-visible spectrum of bromophenol blue and diluent solution.

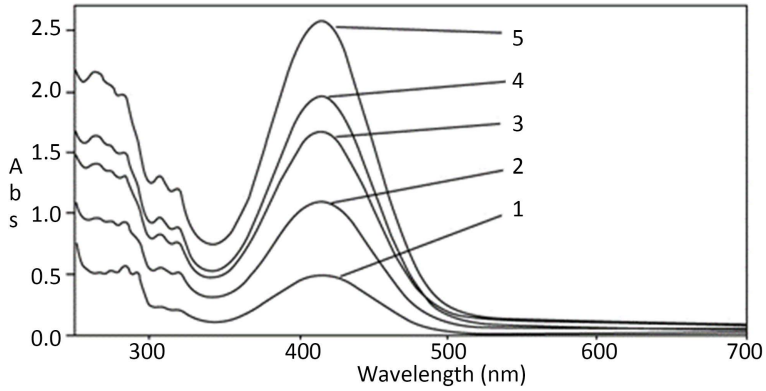


**Figure 3.** UV-visible overlay spectra of Hydralazine-BpB ion-pair solution (1: 9.83 ppm, 2: 19.66 ppm, 3: 29.49 ppm, 4: 39.32 ppm, 5: 49.15 ppm).

## 3.3. International Conference on Harmonization Q2 (R1) Guideline Verification

### 3.3.1. Specificity

The peaks of both the standard solution and the pharmaceutical solution were checked for resolution from the nearest peak by scanning each solution separately (Figure 3).

### 3.3.2. Linearity

A calibration curve of 5 points was created. The curve showed a linear response of a linear equation y = 0.0518x + 0.022 and a correlation coefficient $R^2$ = 0.9937 (Table 2, Figure 4).

### 3.3.3. Precision

Five samples of Apresoline were analyzed as per the procedure for normal weight taken for analysis. Mean, standard deviation, and percentage relative standard deviation (%RSD) were calculated. The method was found to be precise since the %RSD were < 0.5816% and < 1.8568% for the repeatability and intermediate precision shown in Table 3. Analytical application of F-test in case of pure solution with pharmaceutical solution was applied to test the effect of the matrix on the method. The results showed $F_{calc}$ = 11.36, less than that of $F_{Theor}$ = 19.00, at degree of freedom ($v1$ and $v2$) equal to 2 in both solutions. This indicates that the matrix

**Table 2.** Concentration vs. Absorbance table for linearity study.

| Conc. (ppm) | Absorbance |
|---|---|
| 0 | 0.000 |
| 9.83 | 0.490 |
| 19.66 | 1.090 |
| 29.49 | 1.663 |
| 39.32 | 1.953 |
| 49.15 | 2.570 |

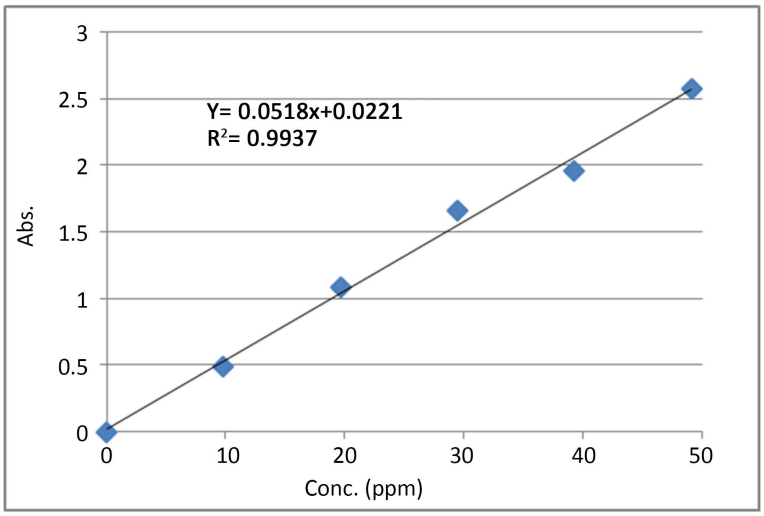


**Figure 4.** Linearity curve for Hydaralzine-BpB ion-pair standard solution.

**Table 3.** Evaluation of data of precision study.

| Sample No. | %Assay | |
|---|---|---|
| Set | Intraday | Interday |
| 1 | 99.8 | 98.8 |
| 2 | 99.2 | 96.5 |
| 3 | 98.8 | 96.2 |
| 4 | 98.6 | 93.8 |
| 5 | 98.3 | 95.8 |
| Mean | 98.9 | 96.2 |
| SD | 0.581 | 1.787 |
| %RSD | 0.588 | 1.857 |

of the pharmaceutical solution has a negligible effect on the magnitude of the random error associated with the proposed method. This means that the proposed method can be precise and repeatable for both pure and pharmaceutical solutions.

### 3.3.4. Accuracy

The standard addition method was used to test the accuracy of the method. Three levels of standard quantity equivalent to 50%, 100%, and 125% were added to the sample. The highest recoveries were (96.90 - 99.77), indicating the high accuracy of the method, as shown in Table 4.

### 3.3.5. Solution Stability Study

The stability study of Apresoline solution was applied. Test preparation Apresoline solution absorbance was measured at different time intervals; results are shown in Table 5 and Table 6. The test preparation Apresoline solution was found to be stable up to 8 h at room temperature. The recoveries did not drop below the minimum as the time increased.

**Table 4.** Evaluation data of accuracy study.

| % Recovery Level | % Recovery | Mean % Recovery | SD | %RSD |
|---|---|---|---|---|
| 50% | 96.8 | | | |
| | 97.1 | 96.9 | 0.173 | 0.179 |
| | 96.8 | | | |
| 100% | 99.8 | | | |
| | 99.2 | 99.5 | 0.300 | 0.302 |
| | 99.5 | | | |
| 125% | 99.8 | | | |
| | 99.8 | 99.77 | 0.058 | 0.058 |
| | 99.7 | | | |

**Table 5.** Evaluation data of solution stability study.

| Time (h) | Abs. Standard | Abs. Pharmaceutical |
|---|---|---|
| 0 | 1.090 | 0.978 |
| 2 | 1.087 | 0.975 |
| 4 | 1.087 | 0.977 |
| 6 | 1.085 | 0.973 |
| 8 | 1.084 | 0.973 |
| 10 | 1.085 | 0.972 |
| Limit at (2 h)* | 0.275 | 0.307 |
| Limit at (4 h)* | 0.275 | 0.102 |
| Limit at (6 h)* | 0.459 | 0.511 |
| Limit at (8 h)* | 0.551 | 0.511 |
| Limit at (10 h)* | 0.459 | 0.613 |

*Limit can be calculated by the following formula:

$$\text{Limit} = \frac{Abs.of\ \ tandard\ \ initial - Abs.of\ \ standard\ \ at\ different\ time}{Abs.of\ \ standard\ \ initial} \times 100 .$$

**Table 6.** Evaluation data of robustness study.

| Sl No. | Abs. at 415 nm | Abs. at 416 nm | Abs. at 417nm |
|---|---|---|---|
| 1 | 0.997 | 0.998 | 0.995 |
| 2 | 0.991 | 0.992 | 0.989 |
| 3 | 0.994 | 0.995 | 0.993 |
| 4 | 0.992 | 0.993 | 0.991 |
| 5 | 0.997 | 0.998 | 0.996 |
| Mean | 0.994 | 0.995 | 0.992 |
| SD | 0.00277 | 0.00277 | 0.00286 |
| %RSD | 0.279 | 0.278 | 0.288 |

### 3.3.6. Robustness

The evaluation of robustness showed the reliability of an analysis with respect to deliberate variations in method parameters. Otherwise, if the measurements are varied in analytical method, a precautionary statement should be included in the procedure. The results in Table 7 showed that, within all variance conditions, the assay of test preparation solution was not affected. Therefore, the analytical method can be considered robust.

Table 7. Evaluation data of system suitability study.

| Sl No. | Absorbance |
|---|---|
| 1 | 1.087 |
| 2 | 1.085 |
| 3 | 1.089 |
| 4 | 1.093 |
| 5 | 1.089 |
| Average | 1.089 |

## 4. Conclusion

A simple and one-step method has been developed for the HzHCl assay in pure and pharmaceutical solutions. The method was characterized and analyzed for optical properties for further specification and robustness. Elements of analytical method validation by the International Conference on Harmonization (ICH) Q2 (R1) were applied and validated to prove that this method is easy, inexpensive, less time-consuming, accurate, and appropriately sensitive for the routine quality control analysis of HzHCl in different solutions.

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## Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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