

**A RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF DIHYDRALAZINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS**

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**ABSTRACT**

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Dihydralazine in pharmaceutical dosage form. Isocratic elution at a flow rate of 0.8 mL min<sup>-1</sup> was employed on a Hypersil C<sub>18</sub> column at 40°C. The mobile phase consisted of phosphate buffer: acetonitrile 98:2 (v/v) and the detection wavelength was at 305 nm. Linearity was observed in concentration range of 25-150 µg/mL. The retention time for Dihydralazine was 2.75 min. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Dihydralazine in pharmaceutical dosage forms.

**Keywords:** Estimation, Method development, Validation, Dihydralazine, RP-HPLC.

**INTRODUCTION**

Dihydralazine (Figure1) is a drug with antihypertensive properties<sup>[1]</sup>. It belongs to the hydrazinophthalazine chemical class and it has very similar effects to hydralazine. Chemically it is described as 4-hydrazinyl-1-hydrazinylidene-1, 2-dihydrophthalazine<sup>[2]</sup>. Dihydralazine is a direct-acting vasodilator which acts predominantly on the arterioles. It reduces BP and peripheral resistance thus increasing cardiac output. It also tends to improve renal and cerebral blood flow and its effect on diastolic pressure is more marked than on systolic pressure.

Literature survey reveals that more work has not carried out on this particular drug and very few analytical methods<sup>[3-5]</sup> has been reported for the estimation of Dihydralazine. The aim of the present study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of

Dihydralazine in pharmaceutical dosage form as per ICH guidelines.

**MATERIALS AND METHOD**

**Instrumental and analytical conditions :** The HPLC analysis was carried out on Waters HPLC system (2695 module) equipped with PDA detector (2996 module) with auto Sampler and running on Waters Empower software. The column used is Hypersil C<sub>18</sub> (150 × 4.6 mm, packed with 5 µm) and detection was performed at 305 nm. The injection volume of sample was 20 µL and the run time was 7 minutes. An isocratic mobile phase containing 0.025M phosphate buffer and acetonitrile at 98: 2 (v/v) at the pH 3 was carried with the flow rate at 0.8mL min<sup>-1</sup>. The mobile phase was filtered through 0.45µm membrane filter and degassed before use.

**Reagents and chemicals:** Dihydralazine working standard was kindly gifted by Ordain Health Care

Private limited, Chennai. Tablets were purchased from local pharmacy manufactured by Ordain healthcare (Apresol). Ultra pure water was obtained from a millipore system. HPLC grade acetonitrile was obtained from Merck (India) limited. All other chemicals used were AR grade.

**Preparation of mobile phase:** 3.4 gm of Potassium Di hydrogen orthophosphate dissolved in 1000 mL of water and mixed, pH adjusted to 3 with ortho phosphoric acid, sonicated to degas the buffer. Transferred 20 volumes of acetonitrile and 980 volumes of buffer into a 1000 volumes mobile phase bottle and mixed. Then sonicated up to 15 minutes for degas the mobile phase and filtered through 0.45  $\mu\text{m}$  filter under vacuum. Acetonitrile and buffer in the ratio of 50:50 (v/v) used as diluent.

**Preparation of Standard Solution:** Accurately weighed about 10 mg of Dihydralazine and transferred into a 10mL volumetric flask and 7 mL of HPLC grade water was added and sonicate to dissolve it completely and the volume was adjusted with the diluent to get stock solution of 1000  $\mu\text{g}/\text{mL}$ . Then 1 mL of stock solution is transferred into 10 ml volumetric flask and make up to volume with diluent and filter through 0.45  $\mu\text{m}$  filters, which gives a solution of strength 100  $\mu\text{g}/\text{mL}$ .

**Preparation of sample solution:** Weigh 20 Dihydralazine tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 50 mg of Dihydralazine into a 50 ml volumetric flask. Add about 35ml of diluent, sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45  $\mu\text{m}$  filter. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45  $\mu\text{m}$  filter.

## METHOD VALIDATION

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, limit of detection, limit of quantification, robustness and system suitability.

**Linearity:** From the standard stock solution, the various dilutions of Dihydralazine in the concentration of 25, 50, 75, 100, 125 and 150 $\mu\text{g}/\text{mL}$  were prepared. The solutions were injected using 10  $\mu\text{L}$  injection volumes in to the chromatographic system at the flow rate of 0.8 $\text{mLmin}^{-1}$  and the

effluents were monitored at 305 nm, chromatograms were recorded. Calibration curve of Dihydralazine was obtained by plotting the peak area ratio versus the applied concentrations of Dihydralazine, given in table 1. The linear correlation coefficient was found to be 0.999, shown in figure2.

**Precision:** Repeatability of the method was checked by injecting replicate injections of 100  $\mu\text{g}/\text{mL}$  of the solution for six times on the same day as intraday precision study of Dihydralazine and the % RSD was found to be 0.45, given in table 2.

**Accuracy:** Dihydralazine reference standards were accurately weighed and added to a mixture of the tablets excipients, at three different concentration levels (50%, 100% and 150%). At each level, samples were prepared in triplicate and the recovery percentage was determined and presented in table 3

**Specificity:** Spectral purities of Dihydralazine chromatographic peaks were evaluated for the interference of the tablet excipients as per the methodology. In the work, a solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure to evaluate possible interfering peaks and no interference peaks were observed.

**Robustness:** To determine the robustness of the method, two parameters (temperature, composition of mobile phase) from the optimized chromatographic conditions were varied. Statistical analysis showed no significant difference between results obtained employing the analytical conditions established for the method and those obtained in the experiments in which variations of parameters were introduced. Thus the method showed to be robust which is shown in table 4.

**Ruggedness:** Inter day variations were performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. Ruggedness also expressed in terms of percentage relative standard deviation and statistical analysis showed no significant difference between results obtained employing different analyst.

**Detection and quantitation limits:** According to the determined signal-to-noise ratio, Dihydralazine presented limits of detection of 0.4  $\mu\text{g}/\text{mL}$  and limits of quantitation of 1 $\mu\text{g}/\text{mL}$ , where the compounds proportion found in the sample solutions injected on to the chromatograph. However, the objective of the

method is the quantitation of Dihydralazine so that the values obtained should be considered as the limit of method sensitivity.

**System Suitability:** System suitability tests were carried out on freshly prepared standard stock solutions of Dihydralazine and it was calculated by determining the standard deviation by injecting standards in six replicates at 6 minutes interval and the values were recorded and the system suitability parameters are shown in table 5.

**Assay of Dihydralazine tablet:** Three different batches of Apresol were analyzed using the validated method. For the analysis, six replicates of each batch were assayed. Twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 50mg of Dihydralazine was transferred to a 50 ml volumetric flask followed by the addition of 35 ml of diluent. The solution was sonicated for 10 minutes and volume adjusted with the diluents then filtered through 0.45  $\mu\text{m}$  membrane filter. Further dilutions were made to get the final concentration equivalent to 100  $\mu\text{g}/\text{mL}$  of Dihydralazine. The mean peak area of the drug was calculated and the drug content in the tablets was quantified and the results were presented in table 6. All the analyzed batches presented Dihydralazine were very close to the labeled amount. The Dihydralazine content in the tablets samples varied from 98.9 to 99.8%.

## RESULTS AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Dihydralazine was

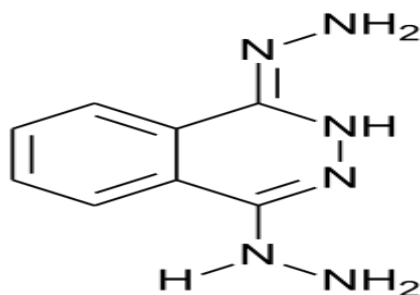
preferably analyzed by reverse phase chromatography and accordingly  $\text{C}_{18}$  column was selected. The elution of the compound from the column was influenced by polar mobile phase. The ratio of the phosphate buffer to acetonitrile was optimized to give symmetric peak with short run time. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase of buffer: acetonitrile at the ratio of 98:2 (v/v). The retention time of Dihydralazine was found to be 2.75 min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The system suitability parameters are given in Table 5. Developed chromatographic method was applied for the determination of Dihydralazine in tablet formulation, given in table 7. A typical chromatogram showing the separation of Dihydralazine is shown in figure 3.

## CONCLUSIONS

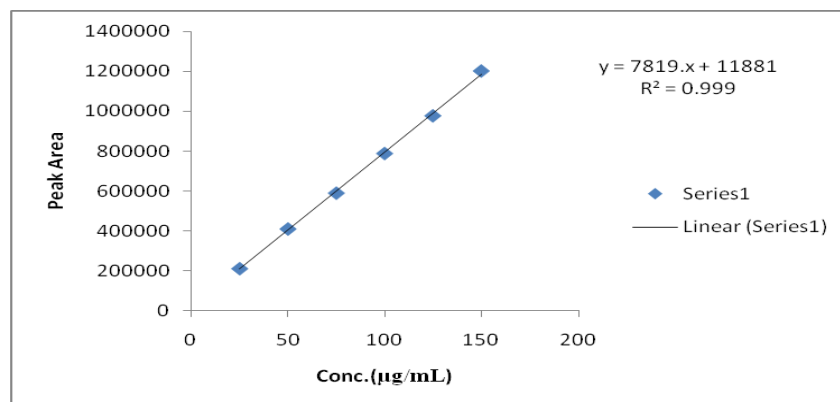
A validated RP-HPLC method has been developed for the determination of Dihydralazine in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Therefore, it is suitable for the routine analysis of Dihydralazine in pharmaceutical dosage form.

## ACKNOWLEDGEMENTS

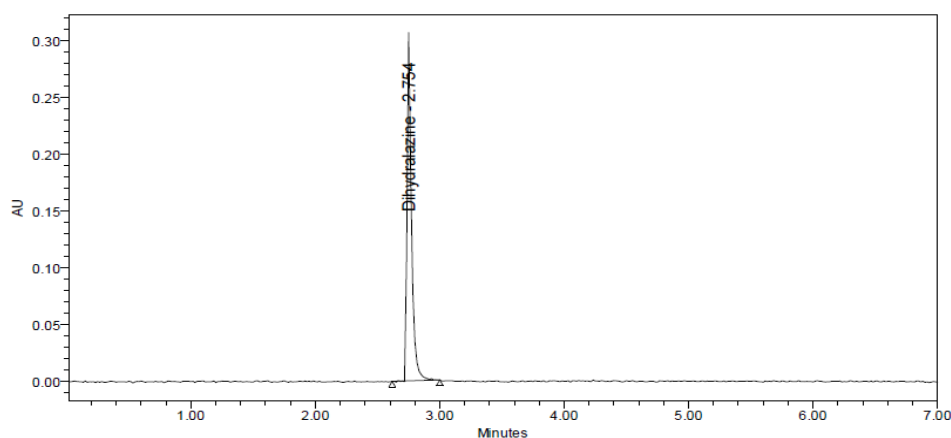
The authors are thankful to Ordain Health Care Private limited, Chennai for providing gift sample of Dihydralazine.



**Figure 1:** Chemical structure of Dihydralazine



**Figure 2:** Linearity curve of Dihydralazine



**Figure 3:** Standard Chromatogram of Dihydralazine

**Table 1:** Linearity of Dihydralazine

Concentration (µg/mL)	Average area
25	211621
50	410260
75	589507
100	787958
125	976311
150	1200636

**Table 2:** Precision of Dihydralazine

Injections	Area
1	740657
2	740758
3	740054
4	743143
5	749222

6	742738
Mean	742762
SD	3395.81
% RSD	0.4571

**Table 3:** Accuracy of Dihydralazine

% Conc	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	5.0	4.94	98.8 %	99.4%
100%	10.0	9.92	99.2%	
150%	15.0	14.89	99.27 %	

**Table 4:** Robustness of Dihydralazine

Parameters	Adjusted to	Average Area	R <sub>t</sub>	SD	% RSD
Temperature as per method 40°C	38 <sup>0C</sup>	776413	2.761	3918.8	0.50
	As it is	750142	2.759	3393.2	0.45
	42 <sup>0C</sup>	710694	2.723	3897.6	0.54
Mobile phase composition Buffer:Acetonitrile (98:2)	Buffer:Acetonitrile (99:1)	762132	2.762	4172.5	0.55
	As it is	748975	2.757	3187.8	0.43
	Buffer:Acetonitrile (97:3)	713882	2.718	5525.8	0.77

**Table 5:** System Suitability of Dihydralazine

Concentration	Injection	Area	R <sub>t</sub>
100 µg/mL	Inj-1	751446	2.757
	Inj-2	749045	2.767
	Inj-3	747306	2.754
	Inj-4	743278	2.767
	Inj-5	744816	2.757
	Inj-6	743422	2.755
Statistical Analysis	Mean	746552.2	2.7595
	SD	3294.373	0.005925
	% RSD	0.44	0.21
	Tailing Factor	1.49	
	Plate Count	26750	

**Table 6:** Contents of Dihydralazine in tablets (n=6)

Sample tablet	Batch	Label Claim(mg)	Amount found(mg) $\pm$ SD	%Amount found
Apresol (25mg)	1	10	9.96 $\pm$ 0.12	99.6
	2	10	9.89 $\pm$ 0.08	98.9
	3	10	9.98 $\pm$ 0.14	99.8

S.D=Standard Deviation

**Table7:** Developed Chromatographic Conditions

Parameters	Method
Stationary phase (column)	HypersilC <sub>18</sub> (150 $\times$ 4.6 mm, packed with 5 $\mu$ m)
Mobile Phase	98:2 (Phosphate buffer : Acetonitrile)
pH	3 $\pm$ 0.02
Flow rate (ml/min)	0.8
Run time (minutes)	7.0
Column temperature ( $^{\circ}$ C)	40
Volume of injection loop ( $\mu$ l)	20
Detection wavelength (nm)	305
Drugs Rt (min)	2.75

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