Validated LC Method for the Estimation of Hydralazine Hydrochloride in Pharmaceutical Dosage Forms

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Abstract: A simple and specific liquid chromatographic method has been developed and validated for the estimation of hydralazine hydrochloride injection using HPLC. All the analytical parameters were determined as per ICH Q2B guidelines. Good chromatographic separation was achieved with Inertsil L10 packed column (4.6 mm x 150 mm, 5 μm particle size) at a wavelength of 230 nm using phosphate buffer and acetonitrile (77: 23) as mobile phase with a flow rate of 1.0 ml/ min. The resolution, between phthalazine and hydralazine hydrochloride peak is not less than 4.0. From the statistical treatment of the linearity data of Hydralazine HCl, it is clear that the response of Hydralazine HCl is linear between 50 % to 150 % level. The correlation coefficient is greater than 0.998. In addition, the analysis of residuals shows that the values are randomly scattered around zero, which fits, and well within the linear model. The developed method showed good linearity, reproducibility, precision and can be suitably applied for the routine quality control analysis in the estimation of commercial formulations.

Keywords: Hydralazine hydrochloride, HPLC, Validation, Estimation.

Introduction

Hydralazine hydrochloride is chemically 1(2H)-phthalazinone hydrazone; 1-hydrazinophthalazine. It acts as a vasodilator and mainly used in the treatment of hypertension and hypertensive emergencies. It reduces high blood pressure and peripheral resistance[1].

For the estimation of Hydralazine hydrochloride official methods are been proposed which involves potentiometric titration and HPLC methods[2,3]. Literature survey reveals that different assay methods like spectrophotometry[4,5], spectrofluorometry[6], oxidimetry[7], and HPLC[8] are available for the estimation of Hydralazine hydrochloride in bulk and in dosage forms. But none of these methods are found suitable for routine quality control studies due to the following reasons like poor sensitivity, suitable at higher concentration only, extraction procedure involved in sample preparation. Based on this, it was felt necessary to develop a validated simple, selective and sensitive HPLC method for the determination of Hydralazine hydrochloride in dosage forms. The proposed method has been demonstrated superior to the existing procedures due to its sensitivity, speed, accuracy and it is suitable for routine quality control analysis.

Materials and Methods

Apparatus

Agilent HPLC system with an isocratic pump (G1310A), a DAD detector system (G1314A) and a thermostatted column (G1316A) compartment was used for the chromatographic analysis. Chemstation software was used for data analysis.

Reagents and solutions

The working standard of hydralazine HCl (100 % potency) was procured as gift sample from Strides Arcolab (Bangalore, India). All the reagents and chemicals used were of analytical grade. High purity millipore water was used.

Standard preparation

40.0 mg of Hydralazine hydrochloride standard was diluted to 100 ml with 0.1 N acetic acid. From this stock solution a known concentration of about 40 μg / ml was prepared.

Sample preparation

Accurately transferred 2 ml of injection volume (equivalent to about 40 mg of Hydralazine hydrochloride) to a 100 ml volumetric flask, diluted with 0.1N acetic acid to volume, and mixed well. 10.0 ml of this solution was further diluted to obtain a concentration of 40 μg/ml.

Assay procedure

The developed HPLC method was successfully applied for the analysis of Hydralazine hydrochloride injection and the active contents in each sample were estimated by comparing with the appropriate standard solution of the drug.

Method Validation

The developed chromatographic method was validated as per ICH Q2B guidelines[9]. The system precision was checked by using standard chemical substance in order to ensure that the analytical system is working properly. The retention time and area of six determinations was measured and calculated the percentage relative standard deviation. In method precision, analysed the sample of hydralazine hydrochloride injection six times of a single batch as per analytical procedure and calculated relative standard deviation which indicates whether the method is giving consistent results for a single batch. For intermediate precision study repeated the method precision set by different analyst on different instrument using different column. Evaluated the stability in analytical solution by injecting the sample and the standard at regular intervals and the area difference for hydralazine hydrochloride in standard and assay preparation was calculated.

The accuracy of the method was determined by performing recovery studies of hydralazine hydrochloride at 50 %, 100 % and 150 % level of the working concentration into the placebo without preservative. The linearity study was performed in the range of 50 % to 150 % of working concentration of hydralazine hydrochloride. Prepared the standard solution in the above range (6 levels) and injected into the chromatograph. The average area for each level, slope, intercept & correlation coefficient was calculated. The intercept was tested for statistical equivalence. Injected the standard solution and resolution solution by altering the flow rate, mobile phase composition, pH of mobile phase and column temperature and calculated system suitability parameters to study the robustness parameter.

Results and Discussion

In order to resolve the active drug different combination of mobile phase at various flow rates were tried. The active compound showed good resolution with a mobile phase composition of acetonitrile - phosphate buffer (pH 3) with isocratic elution at a flow rate of 1 ml/min and the optimum wavelength for detection was set at 230 nm. An Inertsil L10 packed column (4.6 mm x 150 mm, 5 μm particle size), maintained

at ambient temperature (25°C) was found to be suitable for the separation. In the optimized chromatographic conditions, active drug showed a resolution greater than 2.0. System suitability parameters were evaluated and tailing factor for the active compound was observed to be within 1.3.

Range and Linearity

From the statistical treatment of the linearity data of hydralazine hydrochloride, it is clear that the response of hydralazine hydrochloride is linear between 50 % to 150 % level (Table 1). The correlation coefficient is greater than 0.998. In addition, the analysis of residuals shows that the values are randomly scattered around zero, which fits, and well within the linear model (Figure 1). To evaluate, whether the y-intercepts are significantly different from zero, the P value was determined. If P value is > 0.05 then intercept is statistically equal to zero. For hydralazine hydrochloride P value obtained is 0.55 hence it is statistically equal to zero. In addition, the origin is within the lower and the upper limit of the 95 % confidence limit, that gives high degree of confidence to the value obtained for intercept. Moreover, the value of the intercept is less than 5 % of the area response at 100 % level.

Table 1 Summary of linearity results

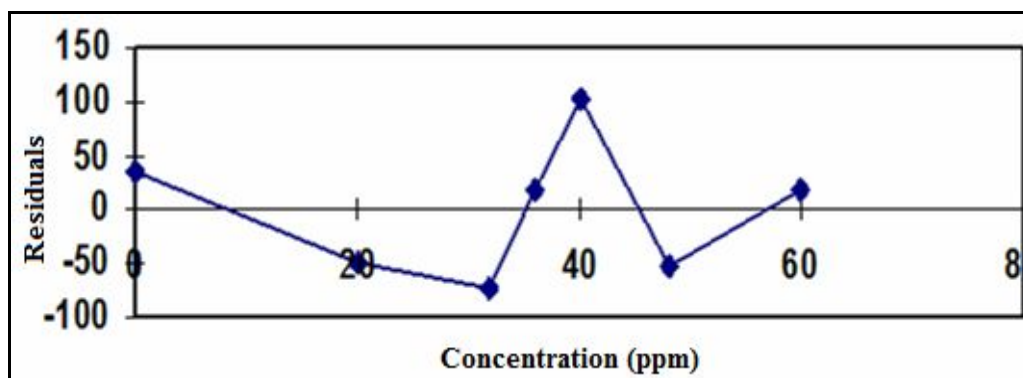| Concentration (µg/ml)	Area response
20.0	1113.00
32.0	1806.28
36.0	2137.03
40.0	2459.46
48.0	2781.08
60.0	3570.74
Correlation coefficient	0.9986
Slope	233.45
Intercept	-34.74
% Intercept	-1.25

Figure 1 Residual Plot: Hydralazine HCl

Accuracy and Precision

Mean percentage recovery for hydralazine hydrochloride at all levels was between 98.0% and 102.0%. In addition, the accuracy results are precise as relative standard deviation calculated on all levels (3 levels x 3) is not more than 2.0 % (table 2). Repeatability was investigated by injecting six replicate of standards, it was observed that the assay values are consistent as evidenced by the value of relative standard deviation. The % R.S.D. values are found to be less than 2%. The % R.S.D. values for intra- and inter-day study was performed in the same laboratory by two analysts did not exceed 3%. Hence, it is concluded that the analytical method is precise for analysis.

Table 2 Accuracy results

Level	Area	Hydralazine HCl added (mg)	Hydralazine HCl recovered (mg)	% Recovery	Mean % Recovery	% RSD
50%	1177.1950	0.1004	0.0986	98.2		
	1175.1650	0.1004	0.0984	98.0		
	1176.7700	0.1004	0.0986	98.2		
100 %	2431.5354	0.2008	0.2036	101.4	99.9	1.5
	2427.3900	0.2008	0.2033	101.2		
	2441.7500	0.2008	0.2045	101.8		
150 %	3597.0950	0.3012	0.3012	100.0		
	3603.7050	0.3012	0.3018	100.2		
	3598.9750	0.3012	0.3014	100.1		

Sensitivity and Selectivity

The sensitivity of the developed method is associated with Limit of detection (LOD) and Limit of quantification (LOQ). The LOQ and LOD were determined based on signal-to-noise ratios and were determined using an analytical responses of 10 and three times the background noise, respectively. The LOQ was found to be 0.3 ppm with a % R.S.D. of 4.15 (n = 6) and the LOD for hydralazine hydrochloride was found to be 0.10 ppm which confirms high sensitivity of the developed method. The chromatogram of standard under the optimized condition gives a symmetrical single peak well separated from the solvent front (Figure 2). Hence, the proposed method is selective and specific.

Figure 2 Standard chromatogram
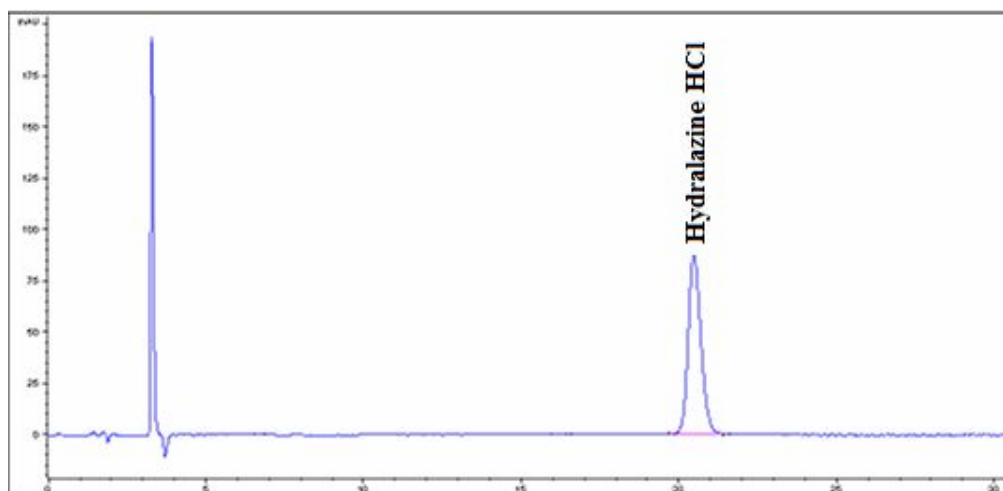
Conclusion

Specific and sensitive HPLC method has been developed for the quantification of hydralazine hydrochloride and validated. All the parameters satisfactorily meet the criteria as per ICH guidelines for method validation. Satisfactory results were obtained for the recovery of the three drugs and were in good agreement with the label claims indicating that both the proposed techniques can be used for the quantification in routine quality control analysis of pharmaceutical dosage forms.

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