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Research Article

Method Validation and Estimation of Anagrelide Hydrochloride in Pharmaceutical Dosage by RP-HPLC Method

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Abstract

A simple, fast and reproducible reverse phase liquid chromatography (RP-HPLC) method was developed for the determination of Anagrelide Hydrochloride in bulk drug and pharmaceutical dosage form. The method was developed using Inertsil ODS-3V C_{18} (150 × 4.6 mm, 5 µm) column, mobile phase 0.1% triethylamine in Milli-Q-water (pH 3.0 adjusted with orthophosphoric acid) and acetonitrile in the ratio of 70:30% v/v with isocratic elution at a flow rate of 1 mL/min. System Suitability test were performed for the assurance of quality performance of method. The drug was subjected to accelerated degradation for photolytic, hydrolytic, thermal, oxidative conditions. The retention time of Anagrelide Hydrochloride was found to be 6.985 min. The method was validated for accuracy, precision, specificity, linearity, limit of detection, limit of quantitation and robustness as per ICH guidelines. All the parameters were within limits. The mean recovery was 99.9%. Limit of detection was found to be 2.99 µg/ml and limit of quantitation was found to be 9.96 µg/ml. The proposed method gave good resolution of Anagrelide Hydrochloride and its degradation products. The developed method can be used for the routine quality control analysis.

Keywords: Anagrelide hydrochloride; RP-HPLC; ICH guidelines; Validation

Introduction

Anagrelide Hydrochloride [1] Monohydrate 6,7-dichloro-1H,2H,3H,5H-imidazolidino[2,1-b]quinazolin-2-one $C_{10}H_7Cl_2N_3O$. $HCl.H_2O$ Anagrelide HCl [2,3] inhibits phosphodiesterase III which is found in thrombocytes and cause that few analytical methods available for determination of anagrelide [4-8] by RP-HPLC, LC-MS, UV spectrophotometry. The aim of the present work was the development of a RP-HPLC method for the estimation of Anagrelide Hydrochloride in a pharmaceutical dosage for and its validation according to ICH guideline raise in cAMP levels that in turn show the inhibitory effect on platelet aggregation. Two major metabolites, active and inactive, have been identified. The active metabolite, 3-hydroxy Anagrelide, shows similar potency and efficacy as Anagrelide. The inactive metabolite, 5, 6-dichloro-3,4-dihydroquinazolin-2-ylamine, does not participate in the overall effect of Anagrelide. Literature survey revealed.

Materials and Methods

Chromatographic conditions

The present work was aimed at the analytical method development and validation for the estimation of Anagrelide Hydrochloride in capsule dosage form by RP-HPLC method. The chromatographic mode used in this method was RP-HPLC and the detector used in this was PDA detector. Inertsil ODS -3V C_{18} (150 × 4.6 mm, 5 µ) was used as stationary phase and the mobile phase used in this method was 0.1% triethylamine Milli-Q water, pH 3.0 and Acetonitrile. Ratio of mobile phase used in this was (70:30% v/v). The wave length used for detection was 254.0 nm and flow rate was maintained at 1.0 ml/min and the injection volume was 20.0 µL and the temperature of the column was maintained at 25°C.

Preparation of 0.1% v/v triethylamine buffer, pH 3.0

An accurately measured 1000 mL of Milli-Q water, 1.0 mL of tri ethylamine was added to it and mixed well. The pH of the solution was adjusted to 3.00 ± 0.05 with orthophosphoric acid and filtered through 0.45 µ Membrane filter.

Preparation of standard solution

An accurately weighed amount of 25.0 mg of Anagrelide Hydrochloride working standard was transferred into 100 mL volumetric flask, about 70.0 mL of acetonitrile was added and sonicated for 5 minutes to dissolve and 3 drops of 0.1 M hydrochloric acid were added and the volume was made up with acetonitrile and the contents were mixed well. Diluted 2.0 mL of the above solution into 50 mL volumetric flask with diluent and mixed well (8.2 µg/mL).

Preparation of test solution

An accurately weighed 5 capsules of Anagrelide Hydrochloride were directly transferred into 250 mL volumetric flask, 150.0 mL of diluent was added to it. Sonicated for 30 minutes with intermediate shaking. Maintained the sonicate bath temperature below 25°C throughout the sonication. After sonication, the volume was made up to the mark with diluent and centrifuged the solution at 4000 RPM for 10 minutes. Collected the supernatant and injected into HPLC system.

Results and Discussion

The present work was aimed at the analytical method development and validation for the estimation of Anagrelide Hydrochloride in capsule dosage form by RP-HPLC method.

Accuracy

Accuracy of the method was performed with concentration level of 50%, 100% and 150% and the percentage recovery was calculated and found to be between 98.0 to 102.0. % RSD should be not more than 2.0 at each % level. Results are depicted in Table 1.

Precision

The percentage assay of six replicate injections of the drugs were performed and the % RSD of individual drugs were calculated and found to be 0.8 which are found to be within the limits. Results are depicted in Table 2.

Linearity

The linearity of the method was tested for Anagrelide at 5 concentration levels and the correlation coefficient was calculated for both the drugs and found to be within limits i.e., correlation coefficient 0.99. The calibration graph was found to be linear in the range of 4-12 µg/ml of concentrations. Results are given in Table 3. And the graph was given in Figures 1-3.

LOD and LOQ

Limit of detection is the minimum amount of analyte that can be detected but not necessarily quantified. Limit of quantitation can be defined as the lowest amount of analyte that can quantified. LOD and LOQ can be determined by the analysis of samples with known concentrations of analyte. The LOD was found to be 2.99 and LOQ was found to be 9.97 The values of LOD and LOQ were shown in the Table 4.

Specificity

There was no absolute interference from placebo preparations at retention time of Anagrelide Hcl. This shows that the method is specific.

System suitability

The system suitability parameters were evaluated. The %RSD for five replicate injections of Anagrelide Hcl was 0.2 respectively and found to be within the limits. The tailing factors for Anagrelide Hcl was found to be 1.09 respectively. The theoretical plates for Anagrelide Hcl was found to be 5144 and found to be within the limits. The system suitability parameters were evaluated and found to be within the limits. Results are depicted in Tables 5 and 6.

Forced degradation

In acid degradation 0.1 N HCl, heated on Water bath for 60 minutes @ 60°C % net degradation is 2.4 the peak purity is 1.0000. In base degradation 0.2 N NaOH heated on water bath for 60 minutes @ 60°C the % net degradation is 2.7 the peak purity is 1.0000. In water degradation water heated on water bath for 60 min @ 60°C % net degradation is 1.7 and peak purity is 1.0000. In thermal degradation 24 Hours @ 105°C there is no % net degradation and peak purity is 1.0000. In UV light degradation 4.00 Days at 200 watts/m^2/hr there is no % net degradation. In visible light degradation 4.00 days at 1.2 million lux hours there is 0.0% net degradation. In humidity degradation, the % degradation is 0.1 and peak purity is 1.0000.

Conclusion

A new RP-HPLC method was developed for the estimation of Anagrelide Hydrochloride in bulk and pharmaceutical dosage form.

Levels	Response 1	Response 2	Mean response	Amount added	Amount recovered	%Recovery	Mean % recovery	%RSD
80%	865850	866480	866165	4.03	4.03	99.9	100.3	0.4
	866070	864181	865125	4.00	4.02	100.6		
	864145	863678	863911	4.00	4.02	100.5		
100%	1078756	1073554	1076155	5.02	5.00	99.7	99.9	0.3
	1072564	1074562	1073563	5.00	4.99	99.8		
	1075623	1074523	1075073	4.99	5.00	100.3		
120%	1300665	1304405	1302535	6.02	6.06	100.6	101.0	0.3
	1307222	1302634	1304928	6.01	6.07	101.0		
	1310882	1307154	1309018	6.01	6.09	101.3		

Table 1: Accuracy data of Anagrelide.

Sample determination	Assay value %w/w
Preparation 1	99.6
Preparation 2	101.6
Preparation 3	99.4
Preparation 4	99.8
Preparation 5	100.1
Mean	100.0
%RSD	0.8

Table 2: Method precision data.

Level	Concentration (µg/ml)	RT	Response
Level-1	4.1	6.98	461443
Level-2	6.2	6.98	688968
Level-3	8.2	6.98	900077
Level-4	10.3	6.98	1141007
Level-5	12.3	6.98	1391482

Table 3: Linearity of detector response for Anagrelide.

Parameter	Concentration (µg/mL)	RT	Response	S/N Ratio
LOD	0.043	6.97	3350	2.99
LOQ	0.13	6.99	10850	9.97

Table 4: LOD and LOQ Response for Anagrelide.

Injection. No.	Retention time	Standard responses for Anagrelide peak	Acceptance criteria
1	6.98	897553	
2	6.97	898663	
3	6.98	898772	
4	6.98	899138	
5	6.98	901182	%RSD should be NMT 2.0
6	6.99	900670	
Mean		899329.7	
Standard deviation		1354.721	
%RSD		0.2	

System Precision and system suitability.

Table 5: System precision data.

System suitability parameters	Results	Acceptance criteria
The % relative standard deviation for area of Anagrelide for five replicate injections	0.18	NMT 2.0
The theoretical plates of Anagrelide	5144	NLT 2000
The tailing factor for Anagrelide peak	1.09	NMT 2.0
% recovery of Check standard	99.4	98.0-102.0

Table 6: System suitability data.

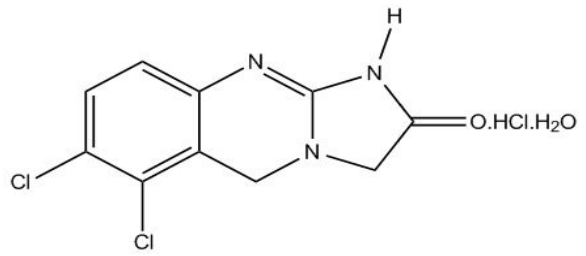

Figure 1: Structure of Anagrelide HCl.

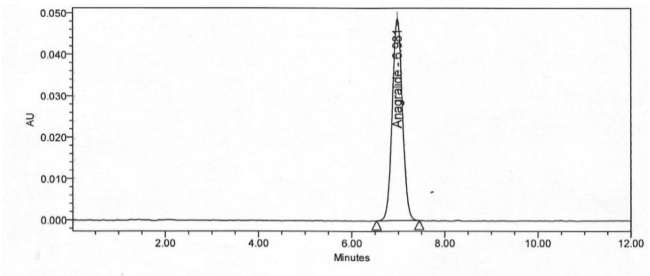

Figure 2: A typical chromatogram for Anagrelide.

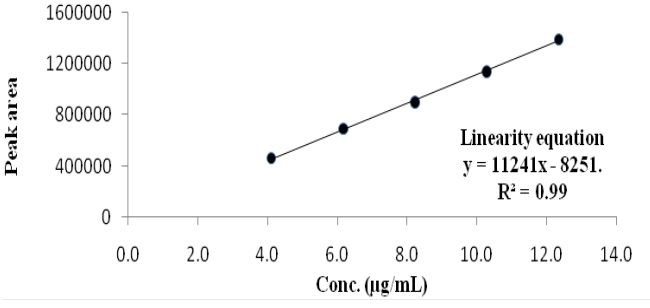

Figure 3: Linearity of detector response for Anagrelide hydrochloride.

the developed method can be used for routine quality control analysis and stability studies of Anagrelide Hydrochloride.

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