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Research Article

Development and validation of a reversed-phase HPLC method for the determination of Anagrelide in capsule

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Abstract

Development and validation of a simple and sensitive reversed-phase high performance liquid chromatorgaphic (RP-HPLC) procedure for the analysis of Anagrelide in tablet dosage forms is described. The drug was separated on Agilent Eclipse XDB C18, 150 x 4.6 mm, 5m column and UV detection carried out at 250 nm. The elution was achieved isocratically with a mobile phase mixture of acid buffer (pH 4) and acetonitrile in the ratio of 70:30%v/v. The retention time for anagrelide was 5.8 min. The recovery of the drug in HPLC was 99.63 to 100.50 %. The method was validated by doing specificity, precision, linearity, accuracy, LOD, LOQ and range.

Key words: Anagrelide, RP-HPLC, capsule.

1. Introduction

Anagrelide is a drug used for the treatment of essential thrombocytosis or overproduction of blood platelets. It also has been used in the treatment of chronic myeloid leukemia[1]. Anagrelide works by inhibiting the maturation of platelets from megakaryocytes[2]. There are few analytical methods for determination anagrelide in plasma by GC-MS[3], LC-MS[4]. There is HPLC estimation in bulk drugs[5] and in pharmaceutical preparations[6]. The present method development aims to develop a simple, sensitive, accurate RP-HPLC method for the estimation of anagrelide in pure and its dosage form.

2. Experimental

2.1. Reagents and chemicals

Sodium hydroxide, ortho phosphoric acid are analytical grade, methanol, milli-Q water and acetonitrile are HPLC grade. Anagrelide pure sample was obtained from Aizant Drug Research Solutions, Hyderabad, India. Anagrelide formulation is obtained from pharmacy. The mobile phase was a mixture of buffer and acetonitrile in the ratio of 70:30 %v/v, which was set at a flow rate of 1.0 ml/min.

2.2. Preparation of buffer

6.8 gm of Potassium dihydrogen ortho phosphate was accurately weighed and transferred in to a 1000 ml volumetric flask, then 900 ml of milli-Q water was added, sonicated and make up to the final volume with the same. The pH of the solution was adjusted to 4 with ortho phosphoric acid solution.

2.3. Instrumentation

Waters 2695 HPLC system with 2996 PDA detector, A Shimadzu HPLC system equipped with Agilent Eclipse XDB C18, 150 x 4.6 mm, 5m column, Rheodyne injector with 10 µl loop and Empower -2 Software was used.

2.4. Standard preparation

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100 mg of pure anagrelide was weighed accurately and transferred in to a 100 ml volumetric flask. To this 2 to 3 ml of 0.01M sodium hydroxide solution was poured and shaken well to dissolve it completely, after complete dissolution the volume was made up to the mark with methanol. That solution was used as a stock solution having 1000 µg/ml of drug.

2.5. Calibration curve

The stock solution was further diluted with mobile phase to get various concentrations range of 50 to 150 µg/ml of drug. From this solution 10 ml was injected into the HPLC system. The chromatograms for each concentrations were recorded, peak area was taken in Y axis and concentration (µg/ml) is taken in X axis; calibration curve was plotted.

2.6. Sample solution preparation

Twenty capsules containing anagrelide were weighed accurately and emptied. The material equivalent to 5 mg anagrelide was dissolved in 3 mL of 0.01 M sodium hydroxide and shaken for some time, 30 ml of methanol was added, sonicated for 15 minutes, and filtered through 0.2µ filter unit. The residues were washed thoroughly with methanol and further diluted 50 ml with diluent. From this solution 10 ml was injected into the HPLC system. The chromatograms were recorded.

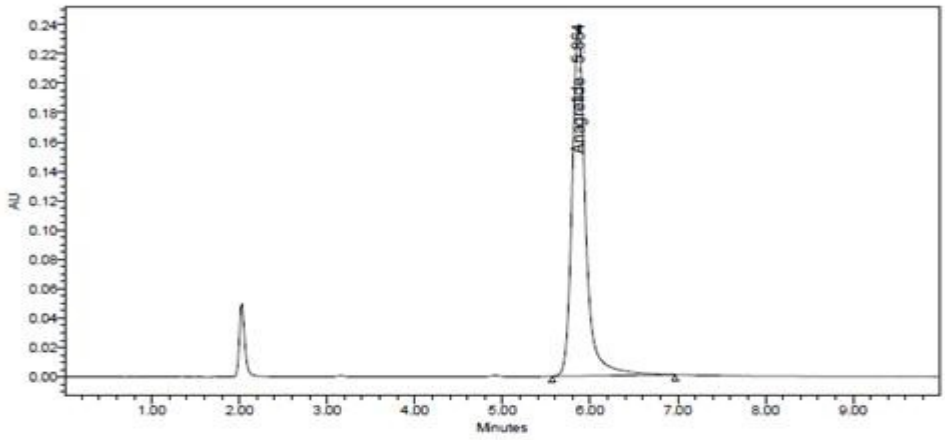
3. Results

In this study, the chromatography of anagrelide was investigated on a Agilent Eclipse XDB C18 column and was separated by using mobile phase of buffer and acetonitrile in the ratio of 70:30%v/v. The linearity was observed in the concentration range of 50-150 µg/ml. The results are shown in Table 1. The retention time is 5.8 min for drug as seen in Fig.1.

Table 1. Linear regression data for calibration curves.

Parameters	Values
Concentration range, µg/ml	50-150
Slope, m	40853
Intercept, b	20101
Correlation coefficient	999

Figure 1. A typical chromatogram of Anagrelide.



3.1. Method validation

3.1.1. Precision

Intra-day precision was calculated from results obtained from six replicate analysis of samples on the same day. Inter-day precision was calculated from results from the same samples analyzed on three consecutive days. The results obtained are listed in table 2.

3.1.2. Linearity

The linearity of the method was tested using the calibration solutions described above. Plot of concentrations

against responses were linear in the range of 50–150 µg/ml. The mean regression equation was $Y = 40853\ X + 20101$. The correlation coefficient was 0.999.

Table 2. Assay results and precision studies

Formulation	Labeled amount (mg/ capsule)	(%) label claim* ± S.D	% Recovery	Precision**	
				Inter-day	Intra-day
Anagrelide Capsules	1	99.14 ± 0.369	99.63 to 100.50	0.3726	0.9673

* Average of six determinations. **%RSD of five determinations.

3.1.3. Limit of detection

The linearity for anagrelide was performed from 50 to 150 µg/mL. Linearity graph was plotted and correlation coefficient determined. Limit of detection (LOD) was predicted for anagrelide from the linearity curve using the formula. LOD = 3.3 × (standard deviation of intercepts)/slope. The limit of detection (LOD) for anagrelide was confirmed to be 0.0087 µg/mL.

3.1.4. Limit of quantitiation

The limit of quantification (LOQ) was predicted from the linearity curve using the formula. LOQ = 10 × (standard deviation of intercepts)/slope. The limit of quantitation (LOQ) for anagrelide was confirmed to be 0.0265 µg/mL.

3.1.5. Accuracy

Accuracy of the method was studied by recovery experiments. Recovery studies were carried out by adding a known quantity of pure drug to a pre-analyzed formulations and the proposed method was followed. From the amount of drug found, percentage recovery was calculated. The results are shown in Table 2.

3.1.6. Application of the HPLC method to the capsules

Twenty capsules containing anagrelide were weighed accurately and emptied. The powder equivalent to 5 mg anagrelide was dissolved in 3 mL of 0.01 M sodium hydroxide and shaken for some time, 30 ml of methanol was added, sonicated for 15 minutes, and filtered through 0.2µ filter unit. The residues were washed thoroughly with methanol and further diluted 50 ml with diluent. From this solution 10 ml was injected into the HPLC system. The chromatograms were recorded.

4. Discussion

The proposed RP-HPLC method was found to be simple, accurate, specific and precise. When compared to other available methods like GC-MS, LC-MS this HPLC method is cost effective and simple solvent system is sufficient. The method can be used successfully for the effective qualitative and quantitative analysis of anagrelide in capsule.

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