

ORIGINAL RESEARCH ARTICLE

Development and Validation of Stability Indicating RP-HPLC Method for the Determination of Anagrelide HCl in Pharmaceutical Formulation

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Received 24 Dec 2012; Revised 04 Apr 2013; Accepted 18 Apr 2013

ABSTRACT

This study describes the development and validation of stability indicating HPLC method for Anagrelide HCl, an antiplatelet drug. Anagrelide was subjected to stress degradation under different conditions recommended by International Conference on Harmonization. The sample so generated was used to develop a stability-indicating high performance liquid chromatographic method for Anagrelide HCl. The peak for Anagrelide HCl was well resolved from the peaks of degradation products, using a Kromasil C18 (250mm x 4.6 mm, 5 μ m) column and mobile phase comprising of methanol: phosphate buffer (pH-3 adjusted with orthophosphoric acid), using the gradient method at a flow rate of 1 ml/min. Detection was carried out using a UV detector at 258nm. A linear response ($R^2 = 0.9993$) was observed in the range of 10-50 μ g/ml. The method showed good recoveries (99.04 to 99.5%) and relative standard deviation for intra and inter-day were ≤ 1 %. The relative standard deviation for robustness < 1 . The method proved to be simple, accurate, precise, specific and selective. Hence it may be used to assay of the product during stability studies.

Key words: RP-HPLC, stability indicating, validation, Anagrelide HCl

INTRODUCTION

Analytical methods development and validation play a significant role in the discovery, development and manufacture of pharmaceuticals. The official test methods that result from these processes are used by QC laboratories to ensure the identity, purity, potency and performance of drug products. Pharmaceutical drug product development can be a costly and time taking process. Many challenges are faced by pharmaceutical development companies which includes developing an accessible, marketable formulation, ensuring safety and efficacy of the drug product and to receive an approval of the drug product by regulatory agencies. Aimed at receiving the approval by regulatory bodies, the applicant must prove control of the manufacturing process plus validate that the methods used to evaluate drug product performance are accurate and precise. For this reason, the analytical methods developed during development are a key component of the Chemistry, Manufacturing and Controls (CMC) section of a regulatory filing. Conflicts in the analytical method can obstruct

product and process development, thus adjourning submission for regulatory approval and thereby delaying product launch.

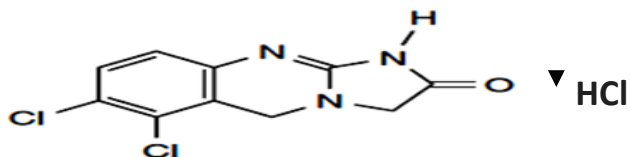
Anagrelide hydrochloride inhibits the activity of cells called megakaryocytes which produce platelets in the bone marrow and hence is used in the treatment of Thrombocythemia. The literature survey reveals that there HPLC method is available for its determination in bulk and GC-MS / LC-MS method for its determination in plasma. The first objective of this study was to report a rapid, sensitive, accurate and precise stability indicating RP-HPLC method for the determination of Anagrelide HCl in bulk drug and in capsule dosage forms.

MATERIALS AND METHODS

Anagrelide HCl as a gift sample was provided by Torrent Pharmaceuticals, Ahmedabad. Anagrelide HCl capsules were procured from a local pharmacy. Perkin Elmer HPLC system comprising of binary LC pump 200 B/200. Separations and quantitation were done using Kromasil C18 (250mm x 4.6mm, 5 μ m) column.

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Figure1: Anagrelide HCl Chemical Structure



Chromatographic Conditions:

The mobile phase was prepared by mixing methanol and phosphate buffer (pH 3, pH adjusted using orthophosphoric acid). The mobile phase was filtered using 0.45 μm filter and degassed by ultrasonic vibrations prior to use. The flow rate was 1 ml/min. All determinations were performed at ambient temperature. An accurately weighed sample (10 mg) of Anagrelide HCl was transferred to a 10 ml volumetric flask and dissolved in acetonitrile first and then volume was made up to the mark using methanol to obtain a solution of strength 1000 $\mu\text{g/ml}$. 0.1ml of this solution was then transferred in 10 ml volumetric flask and made up the volume was made up to the mark with diluent [acetonitrile: methanol (70:30)].

From the solution of Anagrelide HCl (1000 $\mu\text{g/ml}$), appropriate dilutions were prepared using diluent to get final concentrations in the range of 10-50 $\mu\text{g/ml}$. These standard solutions were analyzed in three replicates. The peak areas were plotted against concentration and the data was subjected to linear regression. The standard chromatogram of Anagrelide HCl is shown in (Fig 1).

RP-HPLC Assay procedure:

20 capsules, each containing 0.5mg Anagrelide HCl were weighed. A quantity of powder equivalent to 10 mg was weighed and transferred to 10 ml volumetric flask containing about 7ml acetonitrile, ultrasonicated for 10 min and then the volume was made up to 10 ml with methanol. The solution was filtered using Whatmann filter paper No. 41. From the filtrate appropriate dilutions were made using diluent (acetonitrile: methanol: phosphate buffer) in the ratio 25:25:50 to obtain concentration 30 $\mu\text{g/ml}$. The tablet sample solution was injected and chromatogram was obtained. The peak area of Anagrelide HCl was calculated. Using the regression equations and peak area of the sample, the amount of Anagrelide HCl in the sample was calculated. The amount of Anagrelide HCl per tablet was thus found.

Method Validation:

The linearity of the method was studied by injecting five concentrations of the drug prepared using diluent in the range of 10-50 $\mu\text{g/ml}$ into the

HPLC system and noting the peak areas. The precision of the method was demonstrated by Interday and Intraday variation studies. In the Intraday studies analyses of three different concentrations of the drug were repeated thrice in a day. During the inter day variation study analyses of three different concentrations of the drug was repeated for three consecutive days. Accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 80, 100 and 120% and percent recovery was calculated.

Robustness of the method was determined by carrying out the analysis under conditions during which pH of phosphate buffer (± 0.2 pH), flow rate (± 0.1 ml/min) and wavelength (± 2 nm) were altered and the effect on the area of peak of interest and retention times was noted.

Stress degradation by hydrolysis under acidic conditions:

To 1 ml of stock solution (2000 $\mu\text{g/ml}$) of Anagrelide HCl 1 ml of 0.1 N HCl was added in 20 ml of volumetric flask and made up the volume to the mark with diluent. Volumetric flask was heated at 80°C for 3 hrs. After heating it was cooled to room temperature and was neutralized with 1 ml of same strength of alkali. Volume was made up to mark using diluent. 100 $\mu\text{g/ml}$ solution was prepared and injected in stabilized chromatographic condition. For the blank, 1ml solution of 0.1 N HCl and 1ml solution of 0.1 N NaOH diluted with diluent in 10ml of volumetric flask.

Stress degradation by hydrolysis under alkaline conditions:

To 1ml of stock solution (2000 $\mu\text{g/ml}$) of Anagrelide HCl 1ml of 0.1 N NaOH was added in 20ml of volumetric flask and made up the volume to the mark with diluent. Volumetric flask was refluxed at 80°C for 3 hrs in water bath. After heating it was cooled to at room temperature and was neutralized with 1ml of same strength of acid. Volume was made up to mark using diluent. 100 $\mu\text{g/ml}$ solution was prepared and injected in stabilized chromatographic condition. For the blank, 1ml solution of 0.1 N NaOH and a 1ml solution of 0.1 N HCl diluted with diluent in 10 ml of volumetric flask.

Dry heat-induced degradation:

Anagrelide HCl sample was taken in petriplate and kept in an oven maintained at 80°C temperature for 48 h. 10 mg of the above sample was dissolved and diluted with diluent in

order to make the volume up to 10 ml. From this solution 30µg/ml was prepared using diluent and injected in stabilized chromatographic condition.

Oxidative degradation:

To 1ml of stock solution of Anagrelide HCl, 1 ml of 3% w/v of H₂O₂ was added in 20 ml of volumetric flask and made up the volume up to the mark with diluent. Volumetric flask was refluxed at 80°C for 3 hrs in water bath. After heating it was cooled at room temperature. For the blank, 1 ml of 3% w/v of H₂O₂ was kept at normal conditions for overnight in 10 ml of volumetric flask. Both solutions were heated on a boiling water bath to remove the excess of hydrogen peroxide. Finally made up the volume to 10ml by diluent, and then injected in stabilized chromatographic condition.

Photolytic degradation:

Sample of Anagrelide HCl was exposed to illumination of not less than 1.2 million lux hours. 10mg sample was dissolved in 7ml of acetonitrile and volume made up to 10ml using methanol. From this solution 30µg/ml was made using diluent and injected in stabilized chromatographic condition.

RESULTS AND DISCUSSION

The assay was calculated from the equation of regression line. The percentage of drug found in the formulation was 99.69±0.44%. The results of the analysis show that the amount of the drug was in good agreement with the label claim of the formulation. 30 µg/ml of the tablet sample solution was injected and chromatogram was obtained is shown in (Fig 2).

The data obtained in the calibration experiments when subjected to linear-regression analysis showed a linear relationship between peak areas and concentrations in the range of 10-50 µg/ml for Anagrelide HCl. The equation of the regression line is $y = 102440x + 6368.9$ ($R^2 = 0.9993$).

The developed method was found to be precise as the % RSD values for intra-day and inter-day precision studies were found to be less than 2%. Good recoveries (99.04-99.35%) of the drug were obtained at each added concentration, indicating that the method was accurate. Commonly used tablet excipients were subjected to chromatographic analysis and it was observed that there was no interfering peak at the retention time of Anagrelide HCl. Specificity was also indicated by the resolution of Anagrelide HCl peak from the peaks for degradation product. The peak purity profile by UV detector confirmed the specificity.

The LOD and LOQ were found to be at the submicrogram level for Anagrelide HCl, thus indicating sensitivity of the method. During robustness study, the %RSD was found within < 2%. The method was thus found to be robust since the monitored parameters i.e. the areas of peaks of interest and retention time were not significantly affected when checked by varying the parameters like wavelength, pH and flow rate. Summary of validation parameters of proposed HPLC method is shown in (Table 1).

Anagrelide HCl drug was found to degrade under acidic, alkaline, photolytic and oxidative condition. When Anagrelide HCl was treated with 0.1 N HCl and the sample was withdrawn at an interval of 1, 2 and 3 hrs, and the typical chromatogram obtained after 3 hrs is shown in (Fig 3). Anagrelide HCl was treated with 0.1 N NaOH and the sample was withdrawn at an interval of 1, 2 and 3 hrs and the typical chromatogram obtained after 3hrs is shown in (Fig 4).

When Anagrelide HCl was exposed to heat, peak area for Anagrelide HCl in the degradation sample was found to be less by 0.24% compared to the corresponding peak area for zero time samples. No additional degradation peaks were detected. (Fig 5) shows a chromatogram of Anagrelide HCl exposed to dry heat. When Anagrelide HCl was exposed to the light source as per ICH guidelines, the Anagrelide HCl content exhibited degradation, and additional peaks were detected. (Fig 6) shows chromatograms of Anagrelide HCl samples degraded under Photolytic condition. Upon treatment of Anagrelide HCl with 3% w/v H₂O₂ at 80°C for 3 hrs, additional peaks were detected and peak area for Anagrelide HCl was found to be less by 18.20% compared to the corresponding peak area for zero time samples. (Fig 7) shows the chromatogram of Anagrelide HCl samples degraded under oxidative condition.

Thus the study shows that Anagrelide HCl undergoes degradation in acidic, alkaline, photolytic and oxidative conditions whereas it is relatively stable when exposed to dry heat condition. A stability-indicating method was developed, which resolved all the degradation products formed under a variety of conditions. The method proved to be simple, accurate, precise, specific and selective. Hence it may be

used to assay of the product during stability studies.

Table 1: Summary of validation parameters

Parameters		Results	Acceptance Criteria
Linearity		$R^2 = 0.9993$	NLT 0.995
Accuracy (recovery)		99.3%-99.5%	Within 92% - 102%
Precision (%RSD)	Inter-day Precision	0.53- 0.73 %	NMT 2.0%
	Intra-day Precision	0.28 - 0.87 %	NMT 2.0%
Robustness (%RSD)		0.40 - 0.85 %	NMT 2.0%
Repeatability (%RSD,		0.79 %	NMT 2.0%

Table 2: Stress studies summary

Stress Type	Exposure	Duration	% Degradation
Acid Hydrolysis	80°C	3 hrs.	26.17%
Alkali Hydrolysis	80°C	3 hrs.	10.90%
Peroxide Oxidation	80°C	3 hrs.	18.20%
Photolytic Condition	80°C	3 hrs.	25.89%
Thermal Condition	80°C	48 hrs.	0.24%

Figure 1: Standard peak of Anagrelide HCl (30 µg/ml)

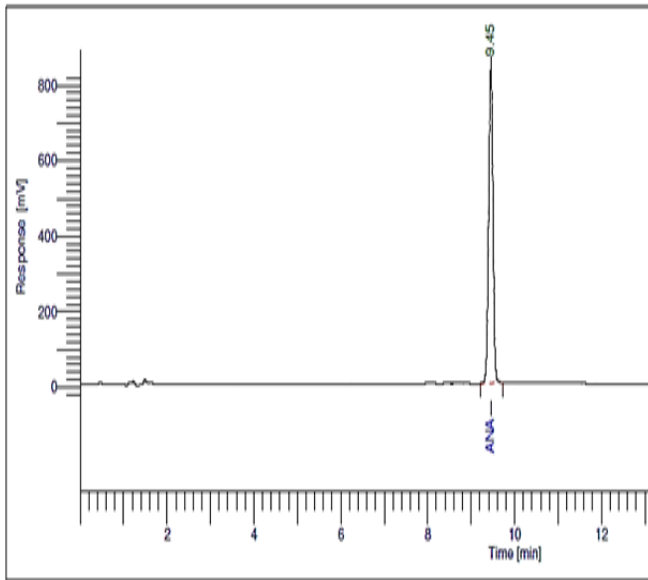


Figure 2: Chromatogram of assay of marketed formulation (30 µg/ml)

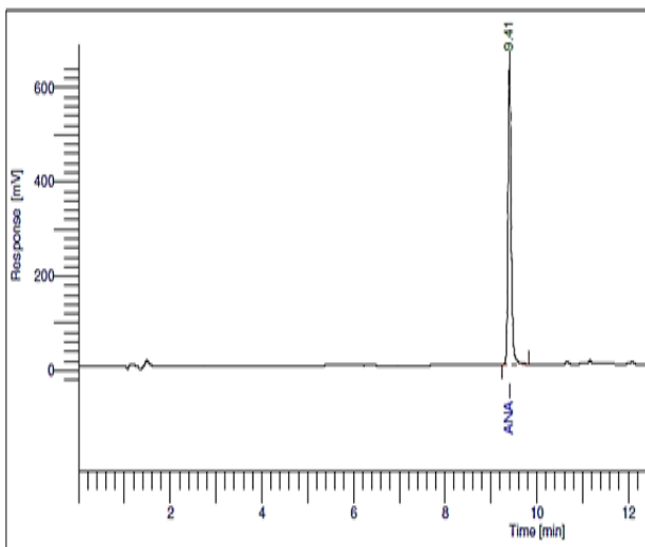


Figure 3: Chromatogram of Acid Hydrolysis (100 µg/ml)

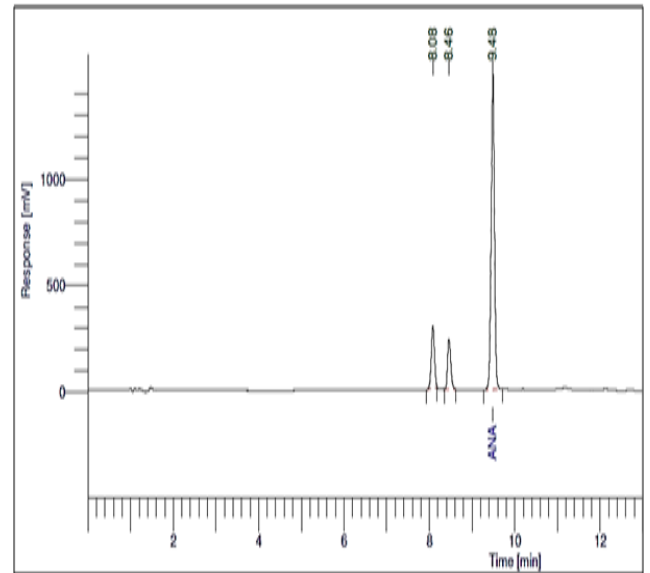


Figure 4: Chromatogram of Alkali Hydrolysis (100 µg/ml)

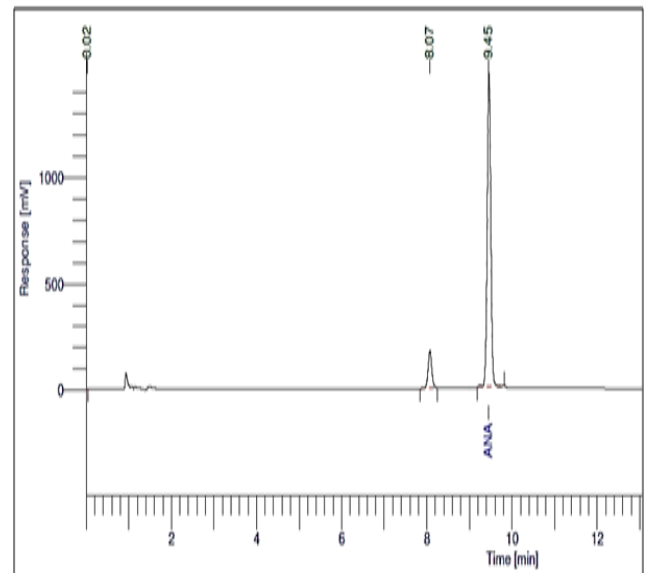


Figure 5: Chromatogram of Thermal Degradation (30 µg/ml)

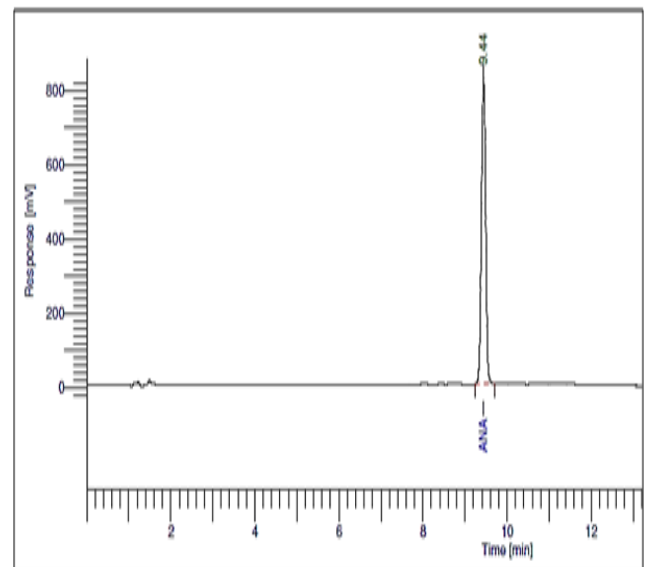


Figure 6: Chromatogram of Photolytic Condition (30 µg/ml)

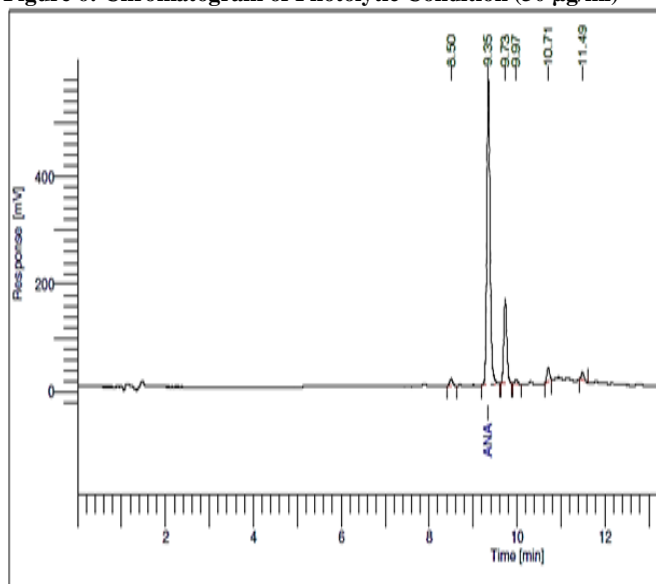
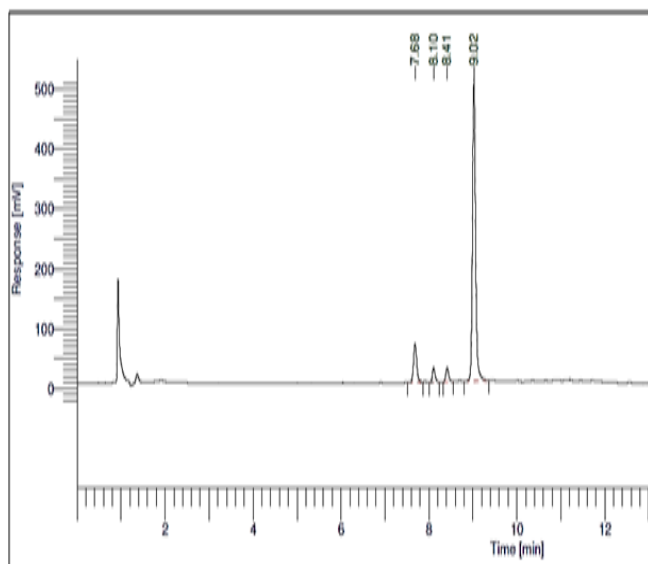


Figure 7: Chromatogram of Peroxide Oxidation (30 µg/ml)



ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Torrent Pharmaceutical Ltd., Ahmedabad, India for providing gift sample of Anagrelide HCl. The authors are also thankful to School of Pharmacy and Technology Management for providing necessary facilities to carry out the research work.

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