International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

A VALIDATED HPLC METHOD FOR THE DETERMINATION OF ANAGRELIDE IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method is developed for the determination of anagrelide in pharmaceutical preparations. Anagrelide was analysed on a reversed-phase column (XTerra symmetry C_{18}, 150×4.6 mm, 5µm) with a mobile phase containing acetonitrile and water (pH 3.0, pH adjusted with ortho phosphoric acid) in a ratio of 40:60 v/v at a flow-rate of 1.2 mL/min and PDA detection was performed at 250 nm. The retention time anagrelide was 2.349 min. The linearity range was found to be 5-30 µg/mL. The method was validated and it was concluded that the developed method was accurate, sensitive, precise, rugged and useful for the quality control of anagrelide in pharmaceutical preparations.

Keywords: Anagrelide, HPLC, XTerra Column

INTRODUCTION

Anagrelide (ANA), 6,7-dichloro-1,5-dihydroimidazo (2,1-b)quinazolin-2(3*H*)-one is inhibiting the maturation of platelets from megakaryocytes.[1] The exact mechanism of action is unclear, although it is known to be a phosphodiesterase inhibitor. [2] It is a potent (IC_{50} = 36nM) inhibitor of phosphodiesterase-II. It inhibits PDE-3 and phospholipase A2. [3] The use of anagrelide was associated with a rapid increase in the degree of reticulin deposition (the mechanism by which fibrosis occurs), when compared to those in whom hydroxyurea was used. Patients with myeloproliferative conditions are known to have a very slow and somewhat variable course of marrow fibrosis increase. This trend may be accelerated by anagrelide. Interestingly, this increase in fibrosis appeared to be linked to a drop in hemoglobin as it progressed. Fortunately, stopping the drug (and switching patients to hydroxyurea) appeared to reverse the degree of marrow fibrosis. Thus, patients on anagrelide may need to be monitored on a periodic basis for marrow reticulin scores, especially if anemia develops, or becomes more pronounced if present initially. Less common side effects include: congestive heart failure, myocardial infarction, cardiomyopathy, cardiomegaly, complete heart block, atrial fibrillation, cerebrovascular accident, pericarditis, pulmonary infiltrates, pulmonary fibrosis, pulmonary hypertension, pancreatitis, gastric/duodenal ulceration, renal impairment/failure and seizure. [3, 4] A literature survey revealed that few analytical methods available for determination anagrelide in plasma by GC-MS [5] and LC-MS [6] and Bulk drugs by HPLC. [7] The objective of this study was to develop a new, simple, economical, selective, accurate and precise reverse phase high-performance liquid chromatographic method with good sensitivity for assay of ANA in tablet dosage form.

MATERIALS AND METHODS

Instrumentation: A Waters HPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2996 PDA

detector was employed throughout the analysis. The data was acquired using Empower 2 software. The column used was XTerra symmetry C_{18} (150×4.6 mm, 5µm). A Bandline sonerex sonicator was used for enhancing dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment.

Chemicals: Anagrelide was generous gift from Dr. Reddy Ltd., Hyderabad (INDIA). HPLC grade acetonitrile and ortho phosphoric acid obtained from Rankem, New Delhi, India. High purity deionized water was obtained from a Millipore, Milli-Q (Bedford) purification system.

Stock and standard solution: A stock solution of ANA (1000 µg/mL) was prepared by accurately weighed 100 mg of ANA reference standard into 100 mL volumetric flask and dissolved in 50 mL diluent (Water: Acetonitrile, 50:50 v/v) and volume was made up to the mark with diluent. The stock solution is protected from light using aluminum foil. Aliquots of the standard stock solutions of ANA ware transferred using A-grade bulb pipettes into 100 mL volumetric flasks and solutions were made up to the mark with mobile phase to give the final concentrations of 5-30 µg/mL.

***Sample Preparation*:** To determine the content of ANA in capsules (label claim: 0.5 mg), 20 capsules were taken and contents were weighed and mixed. An aliquot of powder equivalent to the weight of one capsule was accurately weighed and transferred to 10 mL volumetric flask and was dissolved in 5 mL of diluent and volume was made up to the mark with diluent. The flask was sonicated for 25 min to affect complete dissolution. The solution was filtered through a 0.45 µm micro filter. Suitable aliquot of the filtered solution was transferred into a 100 mL volumetric flask and made up to the volume with mobile phase to yield the concentration of 20µg/mL. The experiments were performed six times under the chromatographic conditions described above. The peak areas were measured at 250 nm and concentration in the sample was determined by comparing the area of sample with that of the standard.

Chromatographic conditions: The chromatographic elution was carried out in isocratic mode using a mobile phase consisting of acetonitrile and water (pH 3.0, pH adjusted with ortho phosphoric acid) in a ratio of 40:60 v/v. The analysis was performed at ambient temperature using a flow rate of 1.2 mL/min with a run time of 5 min. The eluent was monitored using PDA detector at wavelength of 250 nm.

Method validation [8]

Linearity: By appropriate aliquots of the standard ANA solution with mobile phase, six working solutions ranging between 5-30 µg/mL were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of ANA to obtain the calibration curve.

Accuracy: Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of ANA to which known amounts of standard ANA corresponding to 50, 100 and 150% of label claim were added. The accuracy was expressed as the percentage of analyte recovered by the proposed method.

Precision: Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of ANA at concentration of 10, 20 and 30µg/mL. Determinations were performed with three replicates on the same day as well as on three consequent days.

Reproducibility: The reproducibility of the method was checked by determining precision on a same instrument, analysis being performed by another person in same laboratory. It was analyzing the samples of ANA at different concentration (10, 20, 30 µg/mL) were determined in triplicate and calculate the amount of drug present in the sample.

Limit of detection and limit of quantification: Limit of detection (LOD) and limit of quantification (LOD) were calculated based on the ICH guidelines.

Robustness: The robustness of the method was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from1.2 to 1.1 mL/min and 1.3 mL/min. The organic strength of the mobile phase was varied by ±2%.

System suitability tests:To ensure the validity of the analytical procedure, a system suitability test was established. Data from ten injections of 20µL of the working standard solution containing 25µg/mL were used for the evaluation of the system suitability parameters like tailing factor, number of theoretical plates and retention time.

RESULTS AND DISCUSSIONS

A RP-HPLC was proposed as a suitable method for the quantification of ANA in tablet dosage forms. The best chromatographic conditions were adequately selected. The selection of mobile phase and flow rate were made on the basis of peak shape, baseline drift, time required for analysis, economical and the mobile phase consisted of acetonitrile and water (pH 3.0, adjusted pH with ortho phosphoric acid) in the ratio of 40:60 v/v at flow rate of 1.2 mL/min and analyzed at 250 nm. The retention time observed (2.349) allows a rapid determination of the drug. In Figure 1, a typical chromatogram obtained under these conditions is shown.

The calibration plot of peak area against concentration was linear in the range of 5-30 µg/mL. Calibration data, with their % relative standard deviation (%RSD) and linear regression equation are listed in Table 1. The range of reliable quantification was set at 5-30 µg/mL as no significant difference was observed in the slope of the standard curve in this range.

The linear regression data for the calibration curve is indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance. The LOD and LOQ were determined based on a signal-to-noise ratios and were based on analytical responses of 3 and 10 times the background noise, respectively. The LOD was found to be 0.08µg/mL. The LOQ was found to be 0.26µg/mL.

The accuracy was assessed from three replicates containing concentration of 15, 20 and 25µg/mL. The recovery of the method, determined by spiking a previously analyzed test solution with addition of standard ANA solution, was found to be in the range of 99.25-100.63%. The values of % recovery and %RSD are listed in Table 2, indicates that the method is accurate.

Precision of the method was measured in accordance with ICH guidelines. Repeatability of the method was determined as intra-day variation while intermediate precision was determined by measuring inter-day variation for triplicate determination of ANA at three different concentrations.

The results of the determination of repeatability and intermediate precision are listed in Table 3. The low %RSD values indicate that the method is precise. Reproducibility of the method was performed in the same laboratory on a same instrument which was performed by another analyst. The results from determination of reproducibility are listed in Table 4. The assay values and low %RSD values indicate that the method is reproducible.

The robustness was determined by analyzing the same sample under a variety of conditions. The factors consider were: variations in the flow rate (±0.1) and percentage of acetonitrile (±2%). The results and the experimental range of the selected variable are given in Table 5, together with the optimized conditions. There were no significant changes in the chromatographic pattern when the above modifications were made in the experimental conditions, showing that the method is robust.

The system suitability tests were also carried out to evaluate the reproducibility of the system for the analysis to be performed. The results of system suitability tests are given in Table 6, showing that the parameters are within the suitable range.

The proposed method was applied to the analysis of marketed formulations and the results obtained are given in Table 7. The blank solution was prepared containing the components indicated in tablet dosage form except the active ingredient. No interference was observed from the tablet excipients. The low %RSD and assay values indicated that the suitability of this method for routine analysis of ANA in pharmaceutical dosage forms, shown in Table 7.

CONCLUSION

A single reversed-phase gradient HPLC method was developed and validated for the quantitation anagrelide in capsule. The method proved to be sensitive, selective, precise, linear, accurate and stability-indicating. The method was successfully applied to the analysis of anagrelide demonstrating acceptable precision and adequate sensitivity for the detection and quantitation of the impurities. So it may be reasonable to claim that the method can be extended to the analysis of drug formulations and stability samples as well.

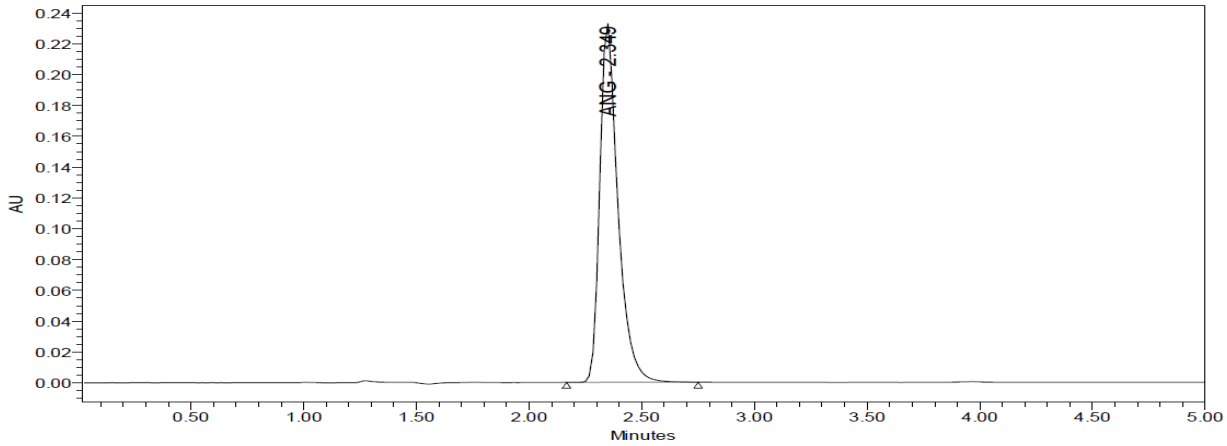

Figure 1: A typical chromatogram of standard anagrelide

Table 1: Linearity regression data for the calibration plot

Analyte	Conc. (µg/mL)	Mean area ±SD (n=3)	RSD (%)	Linear regression equation
ANA	5	355962±3886	1.092	y = 62260x + 27964 r^2=0.9991
	10	620080±4482	0.717	
	15	965737±4999	0.518	
	20	1305098±3791	0.295	
	25	1567446±24923	1.59	
	30	1905775±12939	0.679	

Table 2: Results of recovery studies

Analyte	Amount (%) of drug added to analyte	Theoretical content (µg/mL)	Conc. found (µg/mL) ± SD (n=3)	RSD (%)	Recovery (%)
ANA	50	15	15.06±0.047	0.489	100.42
	100	20	20.13±0.269	1.334	100.63
	150	25	24.81±0.48	1.935	99.25

Table 3: Intra-day and Inter-day precision of the method

Analyte	Conc. (µg/mL)	Intra-day precision		Intermediate precision	
		Mean area ±SD (n=3)	RSD (%)	Mean area ±SD (n=3)	RSD (%)
ANA	10	632352 ±6805	1.076	631155 ±1538	0.244
	20	1303302 ±6994	0.537	1312070 ±11619	0.886
	30	1895372 ±12460	0.657	1902765 ±7859	0.413

Table 4: Reproducibility of the proposed method

Analyte	Conc. (µg/mL)	Conc. found (µg/mL) ± SD (n=3)	RSD (%)	Amount found (%)
ANA	10	10.02±0.155	1.547	100.23
	20	20.18±0.33	1.633	100.92
	30	30.32±0.504	1.662	101.08

Table 5: Results from testing the robustness of the method

Conc. of analyte (µg/mL)	Flow rate		Retention Time	RSD (%)	% Organic		Retention Time	RSD (%)
	Original	used			Original	used		
20	1.2	1.1	2.226±0.024	1.074	40	39.2	2.393±0.022	0.903
		1.2	2.323±0.032	1.364		10	2.338±0.03	1.305
		1.3	2.204±0.018	0.831		40.8	2.255±0.01	0.436

Table 6: Results of system suitability tests

Parameters	Results
Retention time (min)	2.349
Tailing factor	1.41
Theoretical plates (N)	4011

Table 7: Analysis of anagrelide in tablets

Tablet Formulation	Label Claim per Tablet (mg)	% Drug found ± SD (n=6)	RSD (%)	SEM
AGRYLIN	0.5	99.85±1.145	1.147	0.4675

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