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A VALIDATED UV SPECTROSCOPIC METHOD FOR THE DETERMINATION ANAGRELIDE IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT

A simple, reproducible and cost effective spectroscopic method developed and validated for anagrelide in bulk and tablet dosage form. The drug was determined by using dimethyl sulfoxide and 0.1 N NaOH (1:4) as diluent at 300 nm. Validation was performed as ICH guidelines for linearity, accuracy, precision, LOD and LOQ. The %recovery study for the proposed method is 98-108%w/v indicating no interferences of the tablet excipients. Linearity was obtained in concentration range of 2-10µg/ml for anagrelide with correlation coefficient of 0.999. The detection limit and quantitation limit was found to be 0.0419 and 0.127 respectively.

Key words: Anagrelide, UV spectrophotometer, ICH guidelines

INTRODUCTION

Anagrelide is 6,7-dichloro ;1,5;dihydroimidazo[2,1-b]qinazolin;2(3H)-one and is used for the treatment of essential thrombocytosis or overproduction of blood platelets. It also has been used in treatment of chronic myeloid leukemia. Anagrelide (Figure 1) works by inhibiting the maturation of platelets from megakaryocytes[1-4]. It has structural formula $C_{10}H_7C_{l2}N_3O$ and molecular weight is 310.55. Each anagrelide hydrochloride capsule, for oral administration, contains either 0.5mg or 1mg of anagrelide. Sudhakar S.Pujeri .et al has reported a RP-HPLC method for quantitative analysis of Anagrelide by using C18 column and the retention time was found to be 8.378 min. Venugopal .et al has developed a new RP-HPLC method for anagrelide in pure and pharmaceutical formulations by using C18 column, Methanol: ACN: Water (80:15:5) as mobile phase and the retention time was found to be 4.48min. S.Ramanjaneyulu .et al has reported a HPLC method for the determination of Anagrelide in pharmaceutical preparations by using C18 column, ACN: Water (40:60) as mobile phase and the retention time was found to be 2.349min. (1) Zhu Z .et al has reported a LC-MS method for the determination of Anagrelide in Human plasma by using Intersil ODS column. Literature survey (5-9) revealed that few analytical methods available for determination of anagrelide by RP-HPLC, LC –MS. But there is no method for determination of anagrelide by UV spectroscopy. Hence we planned to develop a simple and effective for determination of anagrelide in bulk and pharmaceutical dosage forms.

MATERIALS AND METHODS

Chemicals and Instrument: Anagrelide gift sample was provided by Spectrum research solutions, Hyderabad, Anagrelide (agrylin) capsules bought from nearest local pharmacy store. DMSO, NaOH were purchased from Merck Chemicals pvt.ltd, Hyderabad. PG Instruments, T60 UV Spectrophotometer with 1 cm quartz cell is used for analysis. Software is UV Win 5.

Standard Stock Solution Preparation: 10mg of standard anagrelide was accurately weighed and transferred to100 ml volumetric flask and dissolved in 20 ml DMSO and make upto100 ml with 0.1 N NaOH to obtain concentration of 100µg/ml.this

solution is used as standard solution. For calibration curve, dilutions were made from this solution.

Preparation of sample solution: Weigh 10 tablets and powder them. Take accurately weighed powder equivalent to 10 mg in a 100 volumetric flask. Add 20 ml DMSO to dissolve it. Shake well and sonicate, made up to volume with o.1NaOH, then filter the solution through whatmann filter paper. Use the filtrate for further dilutions.

Measurement of Absorbance: The absorbance of the solution containing anagrelide at 4µg/ml was determined in the UV range 200-400nm using appropriate blank. The λ max was found to be 300 nm. Serial dilutions of 2,3,4,5,6,7,8,9,10 were prepared from stock solution and absorbance was measured at 300nm against blank (Figure 2).

RESULTS AND DISCUSSION

Linearity: 2-10µg/ml concentrations were prepared and absorbance was tested for 3 replicates of each sample at 300nm. The calibration curve was plotted between absorbance and concentration and it was found to be linear with mentioned concentrations. The equation was found to be 0.084x - 0.006 and regression coefficient was found to be 0.999(Figure 3).

Precision:

System precision: It is evaluated by testing 4µg/ml solution in 6 replicates. Average, standard deviation and RSD were found to be 0.3375, 0.0015 and 0.00444.

Intraday precision: From stock solution, 4µg/ml was prepared and test 6 replicates. Average, standard deviation and RSD was found to be 0.3395, 0.001384 and 0.004078 respectively.

Interday precision: It is evaluated by two different analysts, two different instruments within same day. The average, standard deviation and RSD were found to be 0.3403, 0.004274 and 0.0125 respectively(Table 1).

Accuracy: Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts to tablet. The recovery was performed at three levels 50,100,150% of anagrelide concentration 4µg/ml. Three samples were prepared at each recovery level and analyzed. The recovery value was found to be 101.97% (Table 2).

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 detection limit (LOD) and quantitation limit (LOQ) may be expressed as: LOD = 3.3[SD/S], LOQ = 10[SD/S] ;Where,SD = Standard deviation of the calibration curve, S = Slope of the calibration curve, The LOD was found to be 0.0419g/ml and LOQ was found to be 0.127g/ml. The values of LOD and LOQ were shown in the Table.

Robustness: The robustness of the method was determined by deliberate changes in the method like changes in the solvent temperature. The robustness of the method shows that there were no marked changes in the values obtained, which demonstrate that the developed method is robust (Table 3).

Solution Stability: Stability of the solution was tested by keeping the solution for 24hrs, and checks the absorbance of the solution. The absorbance of the solution was found to be 0.335. No significant change was observed in the absorbance of the solution, so that the solution was stable up to 24hrs.

Assay: The assay was done by preparing the 4µg/ml solution from the sample stock solution and made the 6 replicates of the solutions. Measured the absorbance of the solution at 300nm and label claim was found to be 0.49 mg.

CONCLUSION

The developed method was found to be simple, sensitive, accurate, precise and reproducible and can be used for routine quality control analysis of Anagrelide in bulk and pharmaceutical formulation.

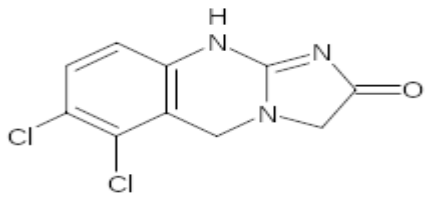

Figure 1: Structure of Anagrelide

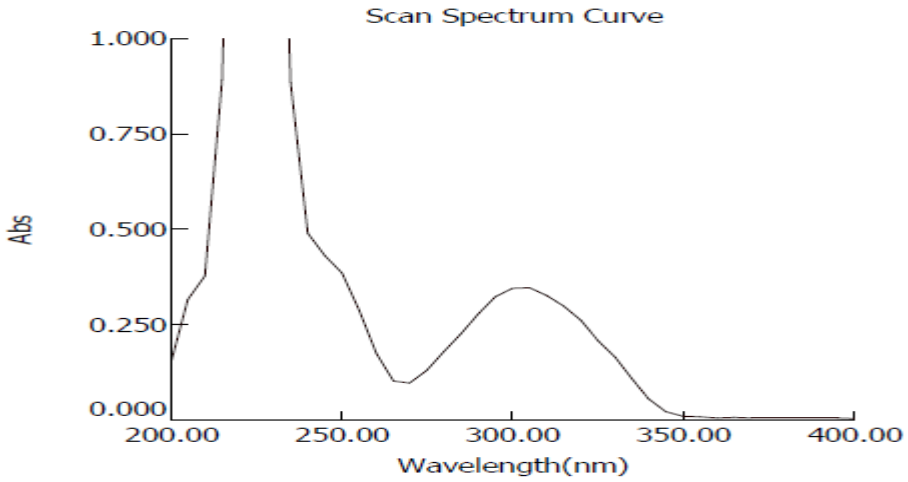

Figure 2: Spectra of Anagrelide

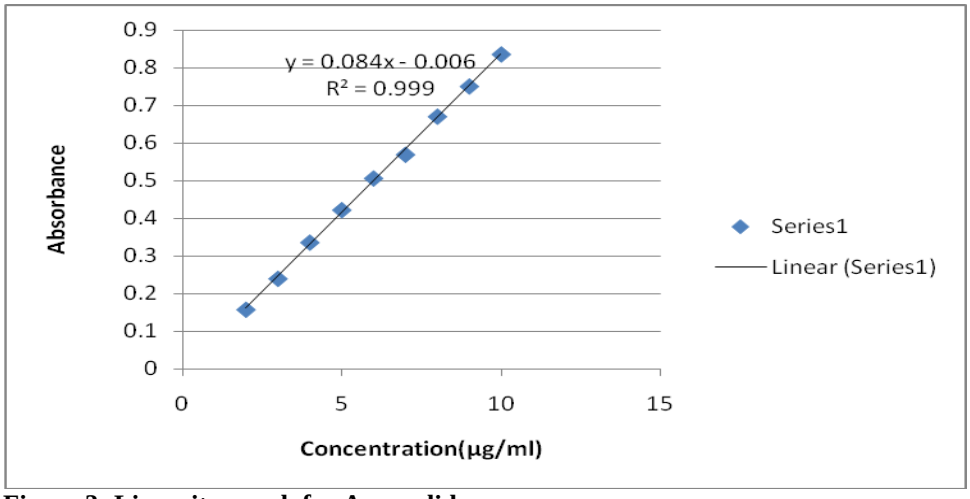

Figure 3: Linearity graph for Anagrelide

Table 1: Intraday and Interday precision

S.NO.	INTRA DAY	INTER DAY
1	0.338	0.347
2	0.34	0.335
3	0.339	0.342
4	0.34	0.342
5	0.338	0.339
6	0.342	0.337
AVG	0.3395	0.340333
SD	0.001384437	0.004274
RSD	0.004077871	0.012558

Table 2: Accuracy data

	spiked pm	std ppm	recovered	% recovery
50%	2	4	2.142857	107.1429
	2	4	2.142857	107.1429
	2	4	2.166667	108.3333
100%	4	4	3.988095	99.70238
	4	4	3.988095	99.70238
	4	4	4.011905	100.2976
150%	6	4	5.892857	98.21429
	6	4	5.892857	98.21429
	6	4	5.940476	99.00794

Table 3: Robustness data

S.No.	At 30°C	At 18°C
1.	0.337	0.335
2.	0.338	0.336
3.	0.34	0.338
4.	0.338	0.334
5.	0.337	0.338
6.	0.335	0.334
AVG	0.3375	0.335
SD	0.0015	0.001675
RSD	0.004444	0.004988

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