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Archives of Applied Science Research, 2014, 6 (1):109-114
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Sensitive visible spectrophotometric methods for the determination of anagrelide in pure and pharmaceutical dosage forms

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ABSTRACT

Two simple and sensitive visible spectrophotometric methods (A and B) have been developed for the determination of anagrelide in pure and pharmaceutical dosage forms. Method A is based on the oxidation of 3-methyl benzothiazololinone-2-hydrazone (MBTH) by ferric chloride followed by its coupling with anagrelide in acidic medium forming a dark green colored chromogen with an absorption maximum at 618 nm. Method B is based on the reaction of anagrelide with Folin-Ciocateu(FC) reagent under alkaline conditions forms an intensive blue colored chromogen with an absorption maximum at 685 nm. Beer's law is obeyed in the concentration range of 5-30 µg/ml for method A and 10-70 µg/ml for method B. These methods were successfully applied to pharmaceutical dosage forms and no interference was observed from common pharmaceutical excipients and additives.

Key words: Anagrelide, MBTH, FC reagent, Visible Spectrophotometry.

INTRODUCTION

Anagrelide[1-4](AGD) is chemically known as 6,7-Dichloro-1,5-dihydroimidazo[2,1-b]quinazolin-2(3H)-one monohydrochloride used for the treatment of essential thrombocytosis and chronic myeloid leukemia. It works by inhibiting the maturation of megakaryocytes into platelets. Anagrelide hydrochloride was approved by the FDA in 1997 for the treatment of patients with thrombocythemia, secondary to myeloproliferative disorders, to reduce the elevated platelet count and the risk of thrombosis and to ameliorate associated symptoms including thrombo-hemorrhagic events. The chemical structure of the drug is shown in Fig 1.

The literature survey revealed that only few methods have been reported for the determination of anagrelide in plasma by GC-MS[5],LC-MS[6]and bulk drugs by HPLC[7]and no visible specrophotometric methods have been reported. The analytical application of MBTH and FC indicated that they have not been earlier reported as reagents for the spectrophotometric determination of anagrelide in pharmaceutical dosage forms or biological fluids. Hence ,the authors have made an attempt and succeed in the development of these methods for the spectrophotometric determination of anagrelide in pharmaceutical dosage forms.

MATERIALS AND METHODS

Analytical grade anagrelide was obtained from local pharmaceutical laboratory and its pharmaceutical dosage forms were obtained from commercial sources. A Tech-comp model UV-2301 UV-Visible spectrophotometer (Hitachi software) with 1 cm matched quartz cells was used for all spectral measurements.

Reagents:
MBTH (0.2%): 0.2 g of MBTH was dissolved in 100 ml of distilled water.
$FeCl_3$ (0.5%): 0.5 g of $FeCl_3$ was dissolved in 100 ml of 0.5 N HCl.
FC Reagent (10%): 10 ml of FC reagent with 100 ml of distilled water.

Na_2CO_3 (15%): 15 g of Na_2CO_3 was dissolved in 100 ml of distilled water.

Preparation of standard drug solution:
Accurately weighed 100 mg of anagrelide was dissolved in 100 ml of distilled water to give a concentration of 1 mg/ml. This stock solution was further diluted with water to obtain the working standard concentration of 100µg/ml for method A and 200µg/ml for method B.

Assay Procedure:
Effect of reaction time and stability of coloured product:
The colored product was developed rapidly after the addition of the reagents and attained maximum intensity after about 10 min at room temperature (methods A and B). The absorbance values were measured at time intervals of 5 min to study the stability of the colored product. The developed color was found to be stable for a period of more than 1.5 h in both the methods under the described experimental conditions.

Method validation:
Linearity:
A linear correlation was observed between the absorbance at respective wavelengths and concentrations of AGD in the ranges as given in Table 1. The regression analysis using method of least squares was made for the slope, intercept, and correlation coefficient, obtained from different concentrations, and the results are summarized in Table 1. The optical characteristics such as absorption maxima, Beer's law limit, molar absorptivity, and Sandell's sensitivity, the limits of detection and quantitation calculated as per the current ICH guidelines are compiled in Table 1.

Sensitivity:
The limit of detection (LOD) and limit of quantitation (LOQ), for the proposed methods, were evaluated as per ICH guidelines using the following formula:

$$LOD = 3\,S_a / Slope \quad ; \quad LOQ = 10\,S_a / Slope$$

where S_a is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte, The high values of molar absorptivity and low values of Sandell's sensitivity and LOD in both the methods indicate the high sensitivity of the proposed methods (Table 1).

Intraday and Inter day accuracy and precision:
Further the accuracy and precision (intra-day) of the proposed methods were evaluated by replicate analysis of calibration standards at three different concentration levels in the same day. Accuracy and precision of inter day were measured by performing the calibration standards at three cited concentrations on five consecutive days. The results show that these methods have reasonable precision (Table 1).

Application to the tablet Analysis:
The developed methods (A and B) were applied to the determination of AGD in tablet solutions at three different concentrations. The results were compared with those of the reported method .Statistical analysis of the results using the Student's-t and F-tests revealed no significant difference between the reported method and the official method at the 95% confidence level with respect to accuracy and precision (Table 2)

Recovery:
The accuracy and reliability of the methods developed were further ascertained by recovery experiments performed on synthetic mixtures of AGD with several excipients such as talc (20 mg), dextrose (10 mg), sodium alginate (10 mg), starch (15 mg), acacia (20 mg), and magnesium stearate (10 mg) by the proposed methods and recoveries obtained by each method, were in the range 98.1 – 101.9% .

The accuracy and precision of the proposed methods were further ascertained by performing recovery studies by standard addition technique. Preanalyzed tablet powder was spiked with pure drug at three different concentrations, and the total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug added was quantitative and revealed that co formulated substances did not interfere in the determination. The results of recovery study are compiled in Table 3.

Recommended Procedure:

Method A:

Aliquots of standard drug solution ranging from 0.5-3.0 ml (100µg/ml) were transferred to a series of 10 ml volumetric flasks. To each flask, 1.0 ml of MBTH and 1.5 ml of ferric chloride were added and allowed to stand for 10 min. Finally the volume was made up to mark with distilled water and the absorbance was measured at 618 nm against a reagent blank. The colored species was stable for 2.3 h and the calibration curve was prepared to calculate the amount of drug.

Method B:

Varying aliquots of standard drug solution ranging from 0.5-3.5 ml (200µg/ml) were taken into a series of 10 ml volumetric flasks. To each flask,1.5 ml of FC reagent and 1.0 ml of sodium carbonate were added and allowed to stand for 10 min. The volume was made up to mark with distilled water and mixed well. The absorbance of the colored compound was measured at 685 nm against a reagent blank . The colored species was stable for 1.5 h and the amount of drug in the sample was computed from its calibration curve.

up to 100 ml and appropriate aliquots of the drug solution were treated as described above.

RESULTS AND DISCUSSION

In method A, MBTH was oxidized by ferric chloride in acidic medium followed by its coupling with the drug to form dark colored complex having absorption maximum 618 nm. On oxidation MBTH loses two electrons and one proton forming an electrophilic intermediate, which is the active coupling agent. This intermediate undergoes electrophilic substitution with the drug to form the colored product.

In method B, the mixed acids in FC reagent involve the following chemical species:

3 H_2O. P_2O_5. 13 WO_3. 5 MoO_3. 10 H_2O and

3 H_2O. P_2O_5. 14 WO_3. 4 MoO_3. 10 H_2O

Anagrelide effects the reduction of 1, 2 or 3 oxygen atoms from the tungstate and/or molybdate, there by producing one or more of several possible reduced species, which shows the intense blue color having absorption maximum 685 nm.

The optical characteristics such as absorption spectra (Fig.1&2), Beer's law limit (Fig.3&4), molar absorptivity, Sandell's sensitivity and percent relative standard deviation were calculated and the results are summarized in table 1. Regression characteristics like slope, intercept, and correlation coefficient were calculated and shown in Table 1. Commercial formulations of anagrelide were successfully analyzed by the proposed methods and the results are presented in table 2. For an additional demonstration of accuracy, recovery experiments were performed by adding fixed amount of the drug to the pre-analysed formulation at three different concentration levels. These results are summarized in table 3. The ingredients usually present in formulations did not interfere with the proposed methods.

Table 1 Optical, regression characteristics and precision of proposed methods

Parameter		Method A	Method B
λ max, nm		618	685
Beer's law limits (µg/ml)		5-30	10-70
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)		5.5366×10^3	3.5148×10^3
Sandell's sensitivity (µg/cm^2/0.001 abs.unit)		0.0463	0.0729
Slope		0.035679	0.021202
Intercept		0.016679	0.037417
Correlation coefficient		0.9989	0.9991
% RSD*	Intra day	0.88	0.64
	Inter day	0.98	1.10
Limit of detection (µg/ml) (LOD)		0.75	0.10
Limit of quantification (µg/ml)(LOQ)		2.50	3.50

** Average of six determinations*

Table 2 Assay of Anagrelide in pharmaceutical dosage forms

Sample	Labeled amount, mg/capsule	Amount obtained, mg		% Assay	
		Method A	Method B	Method A	Method B
1	1.0	0.9846 F=1.44 t=1.54	0.9763 F=1.74 t=1.23	98.46	97.63
2	0.5	0.4794 F=1.78 t=0.74	0.4901 F=2.45 t=1.14	95.88	98.03

The t and F test values refer to comparision of the proposed methods with the reference method. Theoretical values of 95% confidence limit, F=5.05 , t=2.57

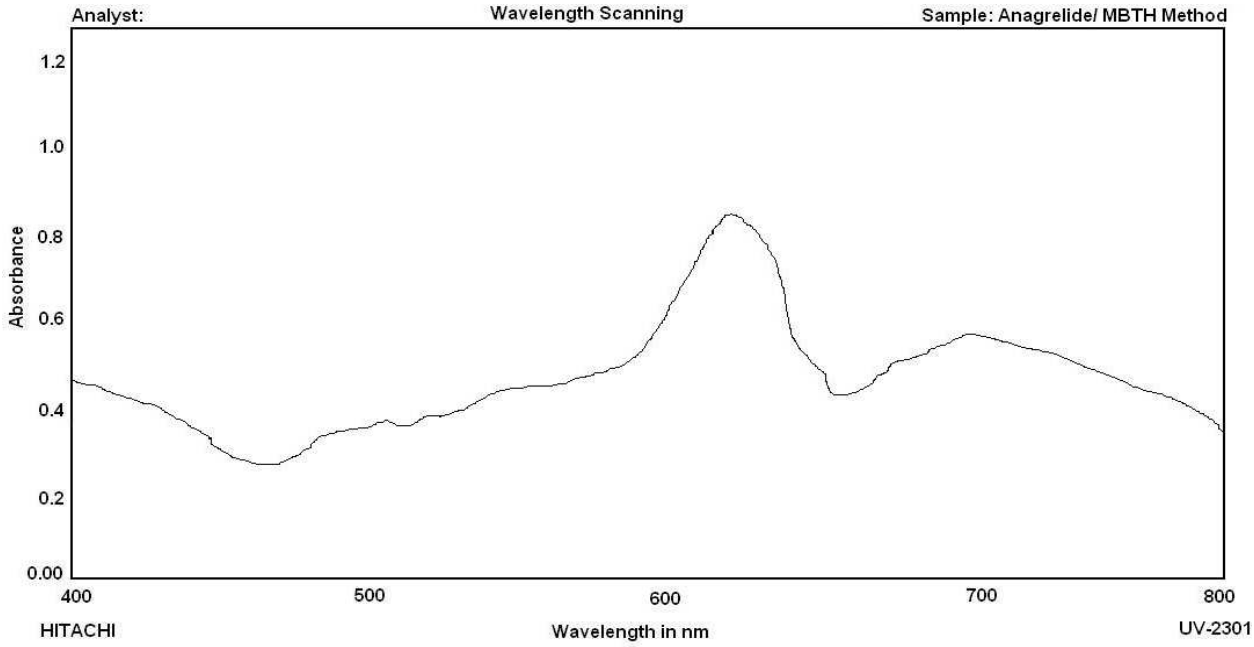

Fig.1 Absorption spectrum of Anagrelide with MBTH

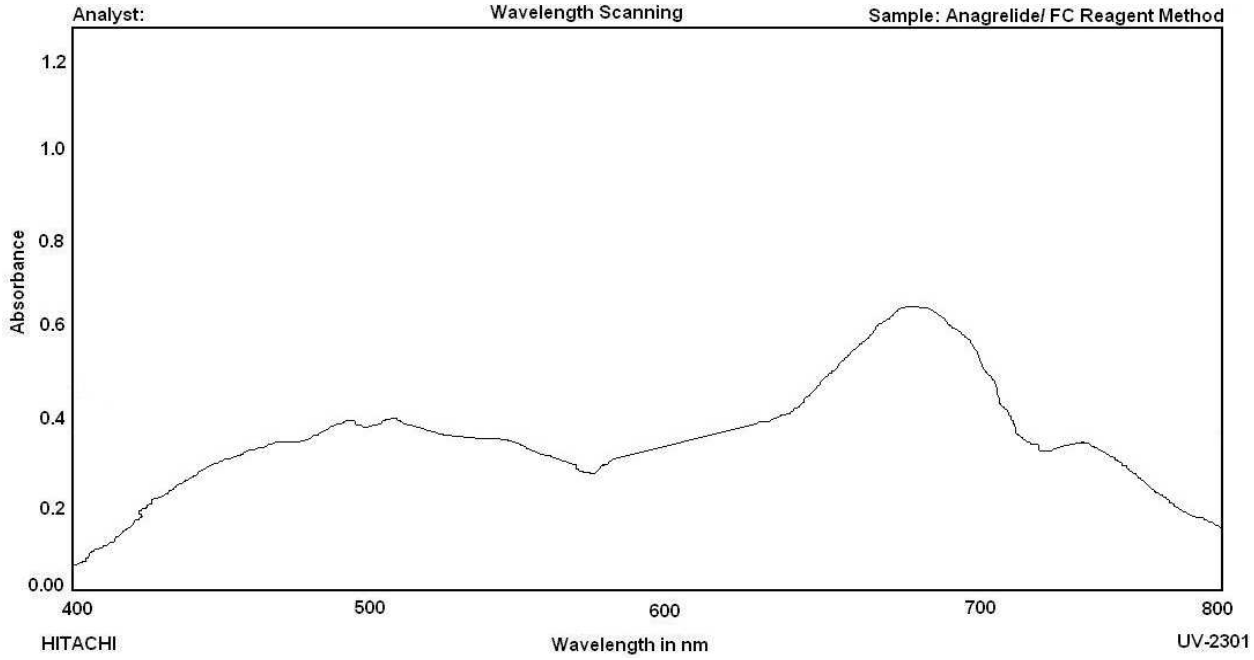

Fig.2 Absorption spectrum of Anagrelide with MBTH

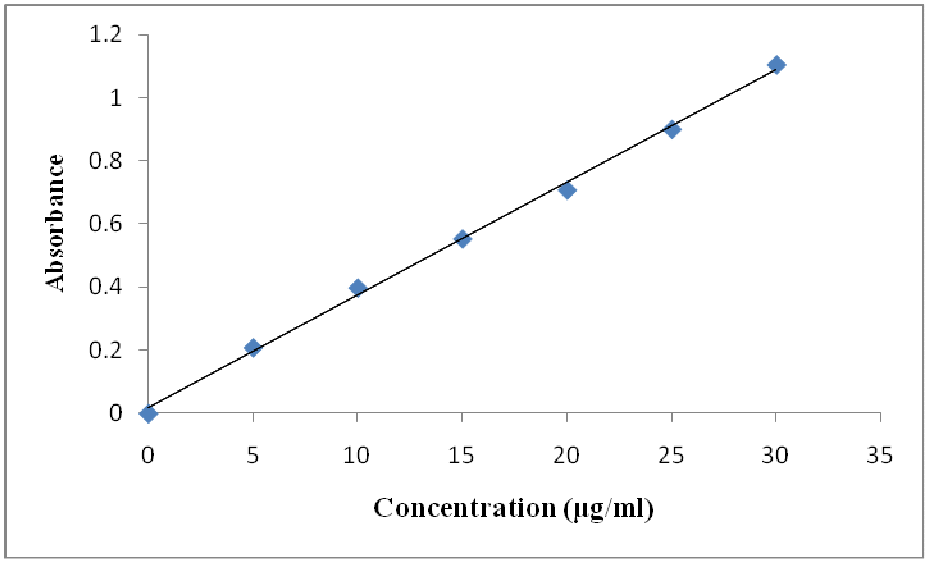

Fig.3 Calibration curve of Anagrelide with MBTH

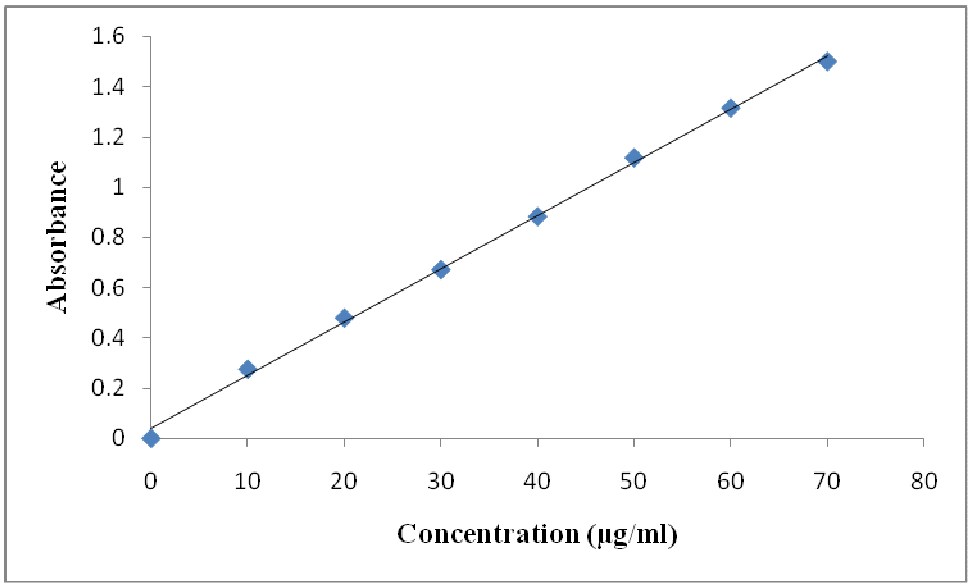

Fig.4 Calibration curve of Anagrelide with FC

Table 3 Recovery studies

Spiked level	Method A				Method B			
	Pre-analyzed amount, (µg/ml)	Amount added, (µg/ml)	Amount obtained, (µg/ml)	% Recovery	Pre-analyzed amount, (µg/ml)	Amount added, (µg/ml)	Amount obtained, (µg/ml)	% Recovery
50%	10	5	14.71	98.07	20	10	29.66	98.87
	10	5	14.86	99.07	20	10	29.47	98.23
	10	5	14.82	98.80	20	10	29.51	98.37
100%	10	10	19.71	98.55	20	20	39.33	98.32
	10	10	20.24	101.20	20	20	40.26	100.65
	10	10	19.84	99.20	20	20	39.47	98.67
150%	10	15	25.47	101.88	20	30	50.89	101.78
	10	15	25.22	100.88	20	30	50.22	100.44
	10	15	24.89	99.56	20	30	50.56	101.12

CONCLUSION

It could be concluded that the developed methods for the determination of anagrelide are simple, sensitive, accurate and precise and can be satisfactorily applied to the analysis of anagrelide in bulk and pharmaceutical dosage forms. The advantage of the methods is that they do not require any tedious extraction or heating procedures. Furthermore,

no expensive or toxic reagents or organic solvents are required. The methods developed are rapid and do not involve any stringent experimental conditions, which influence the sensitivity and reliability of the methods. The methods are unaffected by slight variations in the experimental conditions such as reagent concentrations, and temperature.

Acknowledgements
The authors are thankful to department of chemistry, Acharya Nagarjuna University, Guntur for providing all facilities for carrying the work.

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