INNOVARE JOURNAL OF SCIENCES

Vol 1, Issue 2 , 2013

ISSN-2321-5496

Research Article

ANALYTICAL APPLICATIONS OF SAFRANIN O

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Received: 31 July 2013, Revised and Accepted: 19 August 2013

ABSTRACT

A simple and sensitive spectrophotometric method has been developed for the estimation of Mycophenolic acid. The method is based on the formation of Ion-Association complex with MYCO formed an ion -association complex with basic dye, SafraninO. The cationic form of the dye SAFO involves in the formation of neutral coloured ion-association complexe with negative charge (acid groups in the drug) which is extractable into chloroform and behaves as a single unit being held together by electrostatic attraction. The absorption maxima were found to be at $\lambda_{Max\ 520\ nm.}$ The method obeys Beer's law within the limits 10-40µg/ml and gives reproducible results. Molar absorptivity value is obtained as$8.424x10^4$ L mol^{-1} cm^{-1} and recovery was found to be 99.17 ± 0.92 to 99.62 ± 0.27. Interferences of the other ingredients and excipients were not observed. The proposed method can be used for the determination of MYCO both in pure and pharmaceutical formulations.

Keywords: Mycophenolicacid (MYCO), SafraninO (SAFO), Ion- association Complex

INTRODUCTION

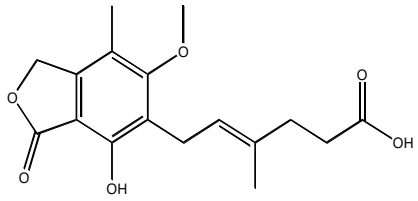

Fig.1: CHEMICAL STRUCTURE OF MYCO

Mycophenolic acid(MYCO)[1-3] is chemically known as(E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoic acid(Fig.1). Mycophenolate is potent and can be used in place of the older anti-proliferative azathioprine. Pharmaceutical chemistry is a science that makes use of the general laws of chemistry to study drugs *i.e.* their preparation, chemical nature, composition, structure, influence on an organism and studies the physical and chemical properties of drugs, the methods of quality control and conditions of their storage. A very few physio-chemical methods appeared in the literature for the determination of MYCO in pharmaceutical formulations (less) and more for the plasma samples. The methods so far reported includes LC[4],TLC[5],HPLC[6-8],spectro photometric(UVandvisible)[9-11].The analytically important functional groups of MYCO were not properly exploited designing suitable spectrophotometric methods for the determination of the selected drug.The presence of hydrophilic substituents such as – OH or – COOH often prevents extraction of the complex into the organic phase. According to the same principle, basic dyes[12]can be used for the assay of acidic drugs. In the present paper, We describe one visible spectrophotometric method based on the Ion-Association Complex [13-16] with the Dye MB, with MYCO for its assay. Good number of methods was reported in the literature using SAFO [17-23] as chromogenic reagent for the assay of drugs other than the drug selected by the author.

EXPERIMENTAL

A UV – 1601, and SHIMADZU digital spectrophotometer with 1cm matched quartz cells were used for the spectral and absorbance measurements. A SYSTRONICS digital pH meter 361 was used for pH measurements. All the chemicals and reagents were of analytical grade and the solutions were prepared freshly, Buffer solution (pH 9.8),SAF-O Solution (Fluka, 0.01%,2.857 x 10^{-4}M) were prepared in triple distilled water and Chloroform is used as it is.

Standard drug solution

A 1mg/ml solution was prepared by dissolving 100mg of pure MYCO in 100ml of water and further diluted to of 80µg/ml.

Recommended procedure

Aliquots of analyte MYCO (0.5-2.5ml, 20µg/ml) is taken in a series of separation flasks and then 1ml of buffer (pH = 9.8) and 5ml of SAF O were added and the volume is made up to 10ml. Then 10ml of chloroform is added and shaken well for 5 minutes. The organic layer is separated and the absorbances were measured each at 520nm (Fig.2). The concentration of drug is calculated from its Beer's plot. (Fig.3).

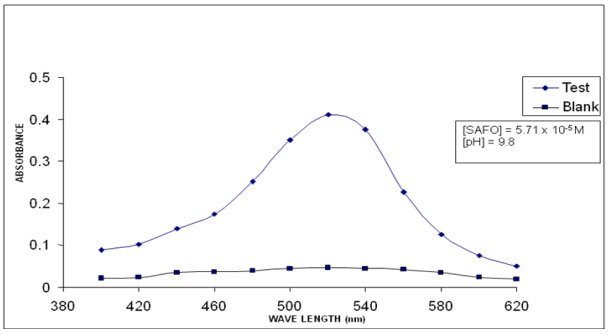

Fig.2:Absorption Spectrum of Myco With SAFO

For pharmaceutical formulations

The tablet powder equivalent to 100mg of MYCO was extracted with 3x25 ml of chloroform and filtered. The combined filtrate was evaporated to dryness and the residue was dissolved in100ml of distilled water to achieve a concentration of 1mg/ml stock solution. The solution was further diluted step wise with distilled water to get working standard solutions and analysed under procedures described for bulk samples.

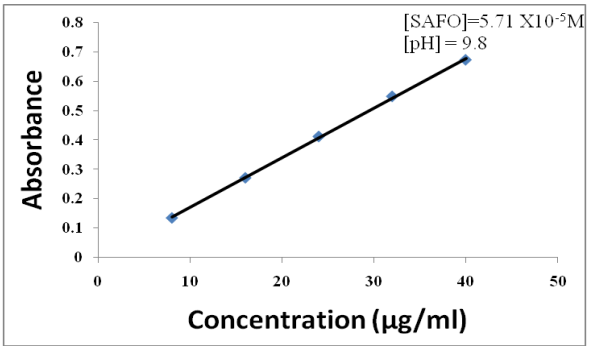

Fig.3: Beer's plot of Myco With SAFO

RESULTS AND DISCUSSION

In developing the method, a systematic study of the effects of various relevant parameters in the concerned were undertaken by varying one parameter at a time and controlling all other parameters to get maximum colour development, minimum blank colour, reproducibility and reasonable period of stability of final coloured species formed. The conditions so obtained were incorporated in the recommended procedures. The optical characteristics such as Beer'slimits, molar absorptivity, and sandell's sensitivity, regression analysis using the method of least squares was made to evaluate the slope(b),intercept(a),and correlation Co-efficient (r) for each system are presented in Table-1.The accuracy of the method is ascertained by comparing the results obtained for pharmaceutical formulations by the proposed methods and reference method by UV, developed in the laboratory using drug solutions , Stastically by the t-and f-tests and the results are summarized Table-2. Recoveries were determined by adding standard drug to the pre analysed pharmaceutical formulations. The ingredients usually present in pharmaceutical formulations did not interfere in the proposed method.

Table1: Optical And Regression Charecteristics, Precision And Accuracy Of Proposed Method

S.No	OPTICAL CHARACTERISTICS	SAFO
1	λ_{max}(nm)	520
2	Beer's Law limits(µg/ml)	Oct-40
3	Molar absorptivity(l $mol^{-1}cm^{-1}$)	$8.424x10^4$
4	Correlation coefficient (r)	0.9996
5	Sandell'ssensitivity (µg/cm^2/0.001absorbance unit)	0.0233
6	Regression equation(y=a+bc) (i)slope (b)	0.0169
	(ii) Standard deviation on intercept(S_b)	$2.169x10^{-4}$
	(iii)intercept (a)	0.00382
	(iv) standard deviation (S_a)	$5.75x10^{-3}$
	(v)Standard error of estimation(S_e)	$5.489x10^{-3}$
7	Optimum photometric range (µg/ml)	15.9-39.8
8	Relative Standard deviation	1.7191
9	Detection limit	1.021
10	% of range of error(confidence limit) (i)0.05 level	1.8044
	(ii)0.01 level	2.9702

Colored Species formation

MYCO being an acid it forms an ion association complex with a basic dye MB, which is extractable into chloroform from aqueous phase. The cationic form of the dye MB , involves in the formation of neutral coloured ion- association complex with negative charge acid group in the drug(Fig.4) which are extractable into chloroform behave as a single unit being held together by electrostatic attraction. It is supported by slope ratio method which was obtained as 1 : 1 .

Table2: Assay Of Myco In Pharmaceutical Formulations

Sample	Labelled Amount(mg)	%Recovery by Proposed method	%Recovery by Reference Method
Tablets – T_1	200mg	99.25 ± 0.62 t = 1.86 F = 3.75	99.41 ± 0.25
Tablets – T_2	200mg	99.22 ± 0.95 t = 1.13 F = 2.99	99.66 ± 0.26
Tablets – T_3	200mg	99.16 ± 0.38 t = 1.23 F = 1.66	99.46 ± 0.49
Tablets – T_4	200mg	99.62 ± 0.27 t = 1.10 F = 1.98	99.76 ± 0.38

*Two different batches of capsules from two different Pharmaceutical companies

+Average ±Standard deviation of six determinations, the t-and f-tests values refer to the comparison of the proposed method with the reference method.

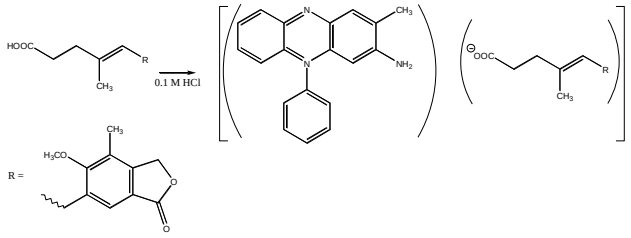

Fig.4: Colored complex of Myco With MB

CONCLUSION

The proposed method is superior in one way or other (simplicity, λ max, ϵ_{max},stability of coloured species) over very few visible spectro photometric methods reported so far. It can be seen from the results presented above, that the proposed method has good sensitivity and λ_{max}. Stastical analysis of the results (Table-1) shows that the proposed procedure has good precision and accuracy. Results of the analysis of pharmaceutical formulations (Table-2) reveal that the proposed method is suitable for their analysis with virtually no interference of the usual additives. The proposed method is simple, sensitive, and reliable and can be used for routine determination of MYCO in bulk samples and pharmaceutical formulations depending upon the needs of the specific situation.

ACKNOWLEDGEMENTS

The author expresses his deep sense of gratitude to UGC, SAP, Phase-III, for its assistance.

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