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Spectrophotometric Determination of Mycophenolic Acid in Bulk and Dosage Forms Using 1,10 Ortho Phenanthroline

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ABSTRACT

A simple and sensitive spectrophotometric method is developed for the estimation of Mycophenolic acid(MYCO) based on the formation of the coloured complex due to the involvement of Fe(II) formed through the oxidation of MYCO with Fe(III) and O-phenanthroline.The absorption maxima is found to be at 510nm.The method obey Beer's law with in the limits (2-7.5µg/ml) and give reproducible results.The percentage recoveries in the formulations found to be 99.26 to 99.86 respectively.

Key Words: Mycophenolic acid, Beer"s Law; 1,10,Ortho Phenanthroline(PHEN)

INTRODUCTION

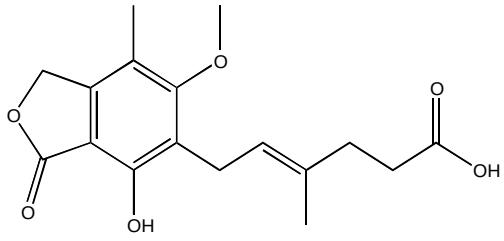


Fig.1. Chemical structure of myco

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 immunmosuppresant drug and can be used in place of the older anti-proliferative azathioprine. 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 TLC[4],TLC-HPLC[5], spectrophotometric (UV and visible)[6,7], GLC[8,9,10], UPLC[11], Electro chemical[12],RPLC[13,14]. The analytically important functional groups of MYCO were not properly exploited designing suitable spectrophotometric methods for the determination of the

selected drug.The applications of [Fe(III)/PHEN System],[15-18] as the chromogenic reagent is well observed in good number of methods that were developed to determine the assay of drugs. Ferric salts play a prominent role in the colorimetric determination of organic compounds.Many phenols, hydroxamic acid ester and more complicate compounds containing phenolic hydroxyl in their molecule react with Ferric salt in an aqueous, water-alcohol or chloroform solution to give intense colouration due to the formation of complex.Acting as an oxidant, a Ferric salt converts into a Ferrous salt. It can easily be detected by the usual reagents for divalent Iron, Potassium Ferricyanide,O-phenanthroline, bipyridyl ortriazine.O-Phenanthroline forms a complex of low absorption value with Fe(III) which functions as a better oxidant than Fe (III) itself. The reduction product is tris complex of Fe(II), well known as Ferroin. Based on its complexing tendency and oxidising properties, Ferric salt was suggested in the estimation of several drugs.In the present investigation the drug was treated with excess Ferric salt under specified experimental conditions. Acting as an oxidant, Ferric salt converts to Ferrous salt which corresponds to the drug concentration. It was estimated by the usual reagent for divalent Iron, Ortho phenanthroline or hexacyanoFerrate(III).Fe (III) along with Potassium Ferri cyanide or O-phenanthroline has been used as an analytical reagent for the estimation of MYCO.Also it is clear from the literature that usage of [Fe(III)/Phen System] for the determination of assay of the selected drug by the author was not attempted. Therefore in this paper, the author has made a valid attempt to develop a sensitive and reproducible method for the assay of the MYCO.

MATERIALS AND METHODS

Instumentation:
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. Fe(III) solution (Wilson Labs; 0.054%,3.32 x 10^{-3}M),Prepared by dissolving 54 mg of anhydrous Ferric Chloride in 100 ml of distilled water.PHEN solution(Merck,0.2%,1.10x10^{-2}M)Prepared by dissolving 200 mg of O-phenanthroline in 100 ml of distilled water with warming. Ortho Phosphoric acid solution (Qualigens,2.0 x 10^{-2}M) Prepared by diluting 1.27 ml of O-phosphoric acid to 100 ml with distilled water.

Preparation of Standard drug solution:
A 1mg/ml solution was prepared by dissolving 100mg of pure MYCO in 100ml of water and further diluted to get 25 µg/ml-150 µg/ml.

Pharmaceutical formulations:
The tablet powder equivalent to 100 mg of MYCO was extracted with 3x25 ml of chloroform and filtered. The combined filtrate was evaporated to dryness and the residue was dissolved in 100ml 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 procedure described for bulk samples.

Procedure:
Into a series of 25ml calibrated tubes, aliquots of standard MYCO solution (0.5 –3.0ml, 50µg/ml) were transferred and then solutions of Fe(III) (1.5ml) and Phen(2.0ml) was added successively.The total volume in each flask was brought to 10.0 ml with distilled water and heated for 30 min in a boiling water bath.After cooling to room temperature,2.0ml of O-phosphoric acid was added, the volume in each flask was made up to the mark with distilled water.The absorbance of the coloured complex solution was measured after 5 min at 510 nm(**Fig.2**) against a reagent blank prepared simillarly. The content of the drug was calculated from it's Beer'plot (**Fig.3**)

RESULTS AND DISCUSSION

The optimum conditions for this method were established by varying one parameter at a time and keeping the others fixed and observing the effect produced on the absorbance of the coloured species. Beer's law limits, molar extinction coefficient, Sandell's sensitivity and regression characteristics of the method are presented inTable-1. The relative standard deviation and % range of error are also given in Table-1. Recovery studies were carried out by addition of known standard drug solution to pre analyzed sample solution. Results of recovery studies were presented in Table-2.The interference studies in the determination of MYCO in pharmaceutical formulations

revealed that the normally existing excipients and additives like hydroxyl propyl cellulose, lactose, carboxy methyl cellulose were found not to interference even when present in excess.

TABLE:1 Optical characteristics, precision, accuracy of the methods proposed in the determination of myco

S.No	OPTICAL CHARACTERISTICS	Fe(III)/Phen
1	λ_{max}(nm)	510
2	Beer's Law limits(µg/ml)	2-7.5
3	Molarabsorptivity(l $mol^{-1}cm^{-1}$)	$3.0x10^{4}$
4	Correlation coefficient (r)	0.9999
5	Sandell's sensitivity (µg/cm^2/0.001absorbance unit)	$2.0x10^{-3}$
6	Regression equation(y=a+bc) (i)slope (b)	0.0502
	(ii) Standard deviation on intercept(S_b)	$2.25x10^{-4}$
	(iii)intercept (a)	0.0002
	(iv) standard deviation (Sa)	$9.705x10^{-4}$
	(v)Standard error of estimation(S_e)	$1.21x10^{-4}$
7	Optimum photometric range (µg/ml)	1.99-5
8	Relative Standard deviation *	0.4969
9	Detection limit	0.0833
10	% of range of error(confidence limit) (i)0.05 level	0.5216
	(ii)0.01 level	0.8586

Table-2.Determinationof myco in pharmaceutical formulations

SAMPLE	LABELLED AMOUNT(mg)	AMOUNT FOUND	
		PROPOSED METHOD	REFERENCE METHOD
Tablets – T_1	200mg	99.97 ± 0.49 t = 1.10 F = 1.27	99.51 ± 0.25
Tablets – T_2	200mg	99.63 ± 0.20 t = 1.70 F = 2.67	99.86 ± 0.26
Tablets – T_3	200mg	99.50± 0.62 t = 0.59 F = 1.18	99.26 ± 0.49
Tablets – T_4	200mg	99.96 ± 0.26 t = 1.23 F = 1.74	99.86 ± 0.38

**Tablets from four different pharmaceutical companies.; **Average ± standard deviation of six determin ations, the t-and F-test values refer to comparison of the proposed method with the reference method.; Theoretical values at 95 % confidence limit,F = 5.05, t = 2.57.*

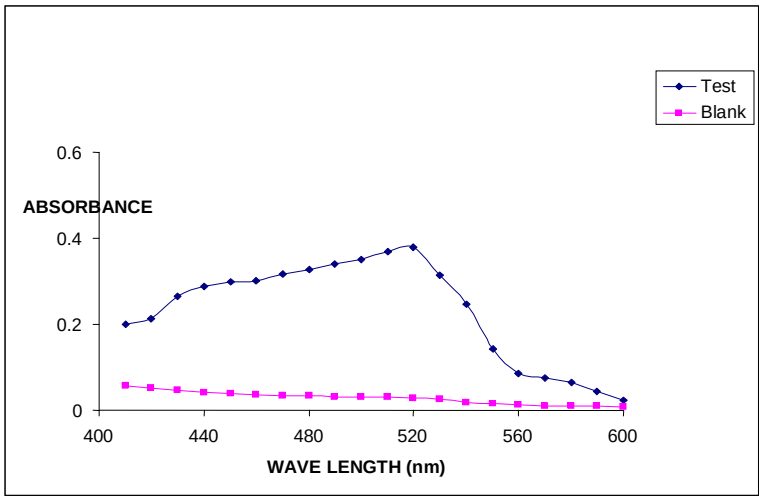


Fig.2 Absorption Spectrum of Fe(III)/Phen SYSTEM

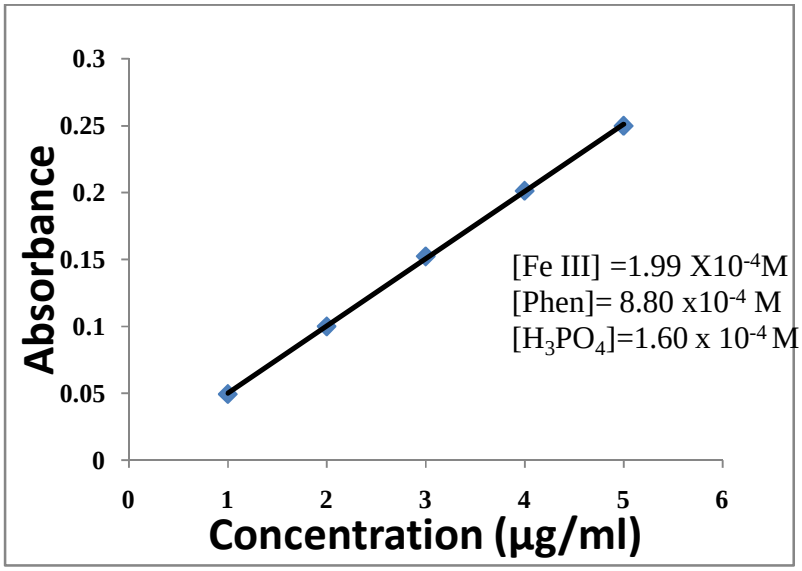
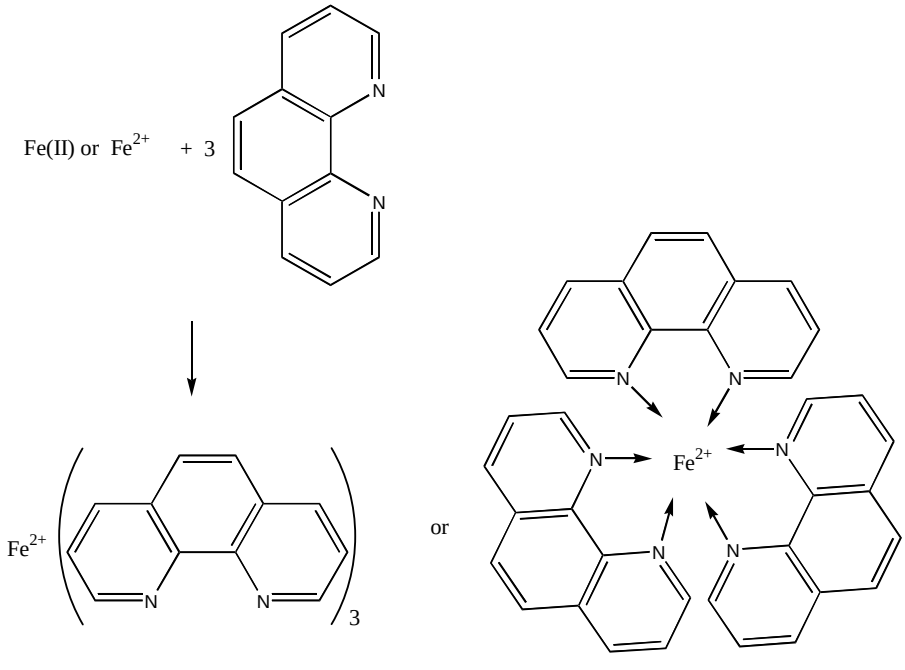


Fig. 3 Beer"s plot of Fe(III)/Phen SYSTEM

Colored Complexes:

In this method,the formation of the coloured complex is due to the involvement of Fe(II) (formed through the oxidation of MYCO with Fe(III) and O-phenanthroline.The structure of the complex can be regarded as shown below(**Fig.4**).

MYCO+Fe(III) → Oxidation products of MYCO + Fe(II) + Fe(III)
(un reacted)



Ferroin complex

Fig.4 Probable colored complex of Fe(III)/PHEN

CONCLUSION

The proposed method is superior in one way or other in terms of simplicity, λ_{max}, ϵ_{max} ,stability of coloured species over very few visible spectrophotometric 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 reveal that the proposed method is suitable for the 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.

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