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Oxidimetric determination of mycophenolic acid in pure and pharmaceutical formulations

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ABSTRACT

Three simple and sensitive spectro photometric methods (A-C) have been developed for the estimation of Mycophenolic acid. Methods A&B are based on oxidation of the drug with oxidant (Potassium permanganate, Mno_4^-,Ferric chloride, Fe (III))followed by the estimation of un reacted oxidant with Fast green FCF (FGFCF). Method C involves the addition of excess CAT of a known concentration in the presence of 0.25MHCl and determining the un reacted CAT by measuring the absorbance of the Dye Gallocyanine. The absorption maxima were found to be at ($\lambda_{Max\ 620nm\ for\ A,740\ nm\ for\ B\ and\ 540\ nm\ for\ C}$). These methods obey Beer's law limits (1-10µg/ml (A),2-10µg/ml (B) and 3-12µg/ml (C)and give reproducible results. The percentage recoveries are99.02, 99.98 and 99.80 respectively.

KEYWORDS

Mycophenolic acid (MYCO);
GalloCyanine (GC);
Chloramine-T (CAT);
Fast green FCF (FGFCF);
Potassium permanganate.

INTRODUCTION

Mycophenolic acid (MYCO) is chemically known as (E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methylhex-4-enoicacid (Figure1). It is official in Martindale[1],MI[2],CIMS[3]. The active functional groups present in MYCO are Aliphatic carboxylic acid, Double bond, Active methylene, Para cresol. A very few physio-chemical methods appeared in the literature for the determination of MYCO in pharmaceutical formulations (less)&more for the plasma samples. The methods[4-25] so far reported includes TLC, spectrophotometric (UVand visible), Tandem mass spectrometry etc., the analytically important functional groups of MYCO were not properly exploited designing suitable spectrophotometric methods for the determination of the selected drugs.

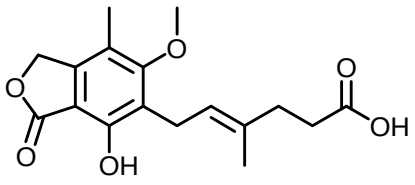

Figure 1 : Chemical structure of mycophenolic acid

In the present paper, We describe three visible spectrophotometric methods based on reaction of the bio active compound with Permanganate (Method-A), the un reacted permanganate was determined by FGFCF[26-37]. The method (B) based on the oxidation of MYCO by excess ferric salt (Fe (III)or Fe^{3+}) to form sulphone

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derivative and reduced form Fe (III) (i.e. Fe (II) or Fe^{2+}) which subsequently reacts with potassium Ferricyanide to give ferrous Ferri cyanide. In the method (C),CAT undergoes hydrolysis in aqueous acid medium to give sodium hypochlorite followed by Hypochlorous acid. This reacts with MYCO to form the relevant oxidant products, probably a mixture which appears to be reproducible under the specified experimental conditions. The remaining Hypochlorous acid may be responsible for the bleaching of the colour GC through destruction of the extended chromophoric system, by excess of CAT and the un reacted CAT oxidizes the Oxazine Dye, Gallocyanine to a colorless form thereby causing a decrease in the absorbance of GC[40-42]. A few visible methods[43-46] developed for the estimation of some drugs which suffer from one or other disadvantage such as low sensitivity, lack of selectivity and simplicity.

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. Aqueous solutions of $KMnO_4$ Solution (BDH, 0.0316%, 2.0 x10^{-3}M),FG FCF Solution (Chroma, 0.1%,1.236 x 10^{-4}M)for method-A, Potassium Ferricyanide (BDH,0.1%,3.02x10^{-3}M),Fe (III) solution (Wilson: abs,0.054%, 3.32 x10^{-3}M),HCl solution (IM) for method-B, CAT solution (Loba,0.02%,7.10 x 10^{-4}M),GC solution chroma, 0.01%,2.97 x 10^{-4}M), HCl solution (IM)for Method-C respectively in tripple distilled water.

Standard drug solutions

A 1mg/ml solution was prepared by dissolving 100mg of pure MYCO in 100ml of water and further diluted to of300µg/ml (for A),100µg/ml (for B),100µg/ml (for C) respectively.

Recommended procedures

(a) Method A

Into a series of 25ml tubes containing aliquots of standard MYCO solution (0.25 – 3.0ml, 300 µg/ml),0.5ml of $KMnO_4$ solution was added and the total volume in each tube was brought to 5 ml with distilled water and kept aside for 10min at laboratory temperature. Then 4.0ml each of FG FCF solution and Sodium Sulfate solution were added successively and kept aside for 5 min. The volume was made upto the mark with distilled water. The absorbance was measured at 620nm (Figure 2) against distilled water. The decrease in absorbance corresponding to consumed permanganate and in turn the drug concentration was obtained by subtracting the decrease in absorbance of the test solution (dye – test) from that of the blank solution (dye – blank). The amount of MYCO was deduced from the Beer's plot (Figure 5).

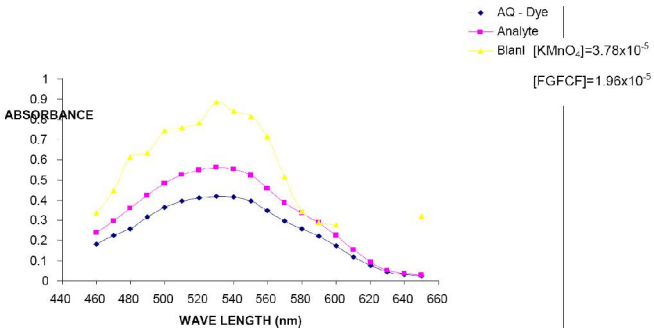

Figure 2 : Absorption spectrum of myco with MnO_4^-/FGFCF

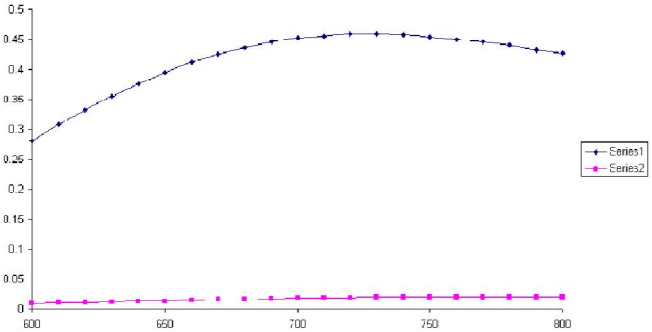

Figure 3 : Absorption spectrum of myco with Fe (II)/ Fe(III) System

(b) Method B

In to a series of 25ml calibrated tubes, aliquots of standard MYCO solution (0.5 – 2.0ml, 100µg/ml) were transferred and 1 ml of 3.32 x 10^{-3}M $FeCl_3$ solution was added. The tubes were stoppered immediately and shaken well for 5 min. Then 0.5ml of 3.02 x 10^{-3}M Potassium Ferri cyanide solution was added into each tube and was closed with lids immediately. After 5 min,

one ml of IN HCl was added and final volume was made up to 25ml with distilled water. The absorbance of the solution in each tube was measured immediately at 740 nm (Figure 3) against the similar reagent blank. The amount of drug was calculated from the Beer's plot (Figure 6).

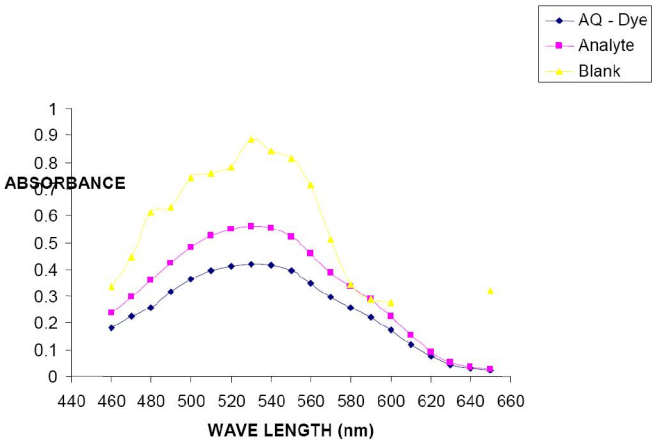

Figure 4 : Absorption spectrum of myco with GC/CAT System

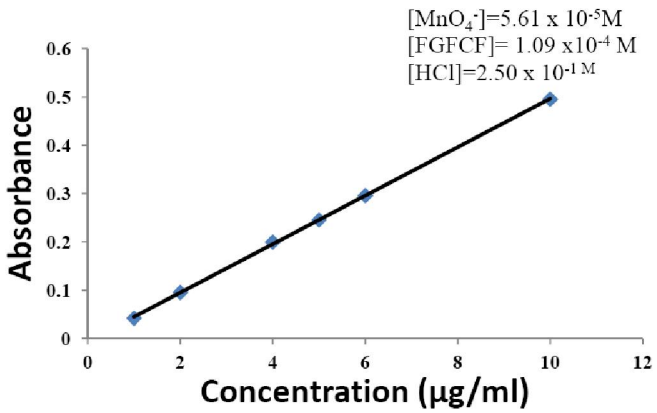

Figure 5 : Beer"s plot of MYCO with MnO_4^-/FGFCF System

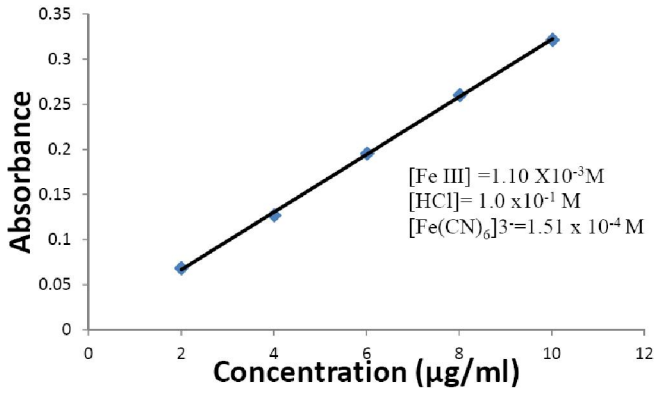

Figure 6 : Beer"s plot of MYCO with Fe(II)/Fe(III) System

(c) Method C

To each of 25ml graduated tubes containing standard MYCO solution (0.5- 3.0 ml, 100g/ml), 1.25ml of HCl and 2.0ml of 0.02% CAT were added and the solution was diluted to 20ml with distilled water. After 10 min, 5 ml of GC solution was added, mixed thoroughly and the absorbances were measured after 5 min at 540 nm (Figure 4) against reagent blank. The blank experiment was carried out in similar manner. The decrease in absorbance corresponding to consumed CAT, which in turn to the drug quantity was obtained by subtracting the absorbance of the blank solution from that of the test solution. The calibration graph was drawn by plotting the decrease in the absorbance of the dye (GC), against amount of drug. The amount of drug in any sample was computed from Beer's plot (Figure 7).

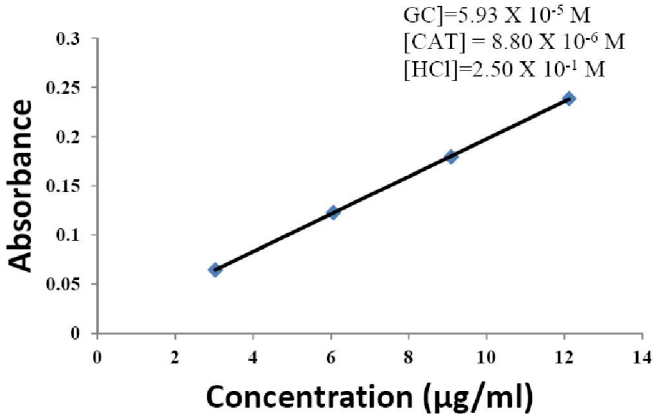

Figure 7 : Beer"s plot of MYCO with GC/CAT System

RESULTS AND DISCUSSION

In developing these methods, 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 are given in TABLE 1. 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 methods was 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. Re-

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coveries were determined by adding standard drug to the pre analysed pharmaceutical formulations. The ingredient s usually present in pharmaceutical formulations did not interfere in the proposed methods.

TABLE 1 : Optical and regression charecteristics,precision and accuracy of proposed methods

S.No	OPTICAL CHARACTERISTICS	Method-A	Method-B	Method-c
1	λ_{max}(nm)	620	730	530
2	Beer's Law limits(μg/ml)	1-12	2-16	3-12
3	Molar absorptivity(l $mol^{-1}cm^{-1}$)	$4.71x10^4$	$4.08x10^4$	$1.91x10^4$
4	Correlation coefficient (r)	0.9999	0.9998	0.9999
5	Sandell's sensitivity (μg/cm^2/0.001absorbance unit)	0.0815	$1.56x10^{-3}$	$7.8x10^{-3}$
	Regression equation(y=a+bc) (i)slope (b)	0.0507	0.03148	0.0191
	(ii) Standard deviation on intercept(S_b)	$6.02x10^{-4}$	0.0128	$1.185x10^{-4}$
6	(iii)intercept (a)	-0.0069	0.00470	0.006319
	(iv) standard deviation (S_a)	$2.346x10^{-3}$	0.1290	0.1120
	(v)Standard error of estimation(S_e)	$5.5x10^{-3}$	0.1668	$8.031x10^{-4}$
7	Optimum photometric range (μg/ml)	3.2-13	6-16	6.02-12
8	Relative Standard deviation	0.3394	0.3013	0.4291
9	Detection limit	0.138	0.2344	0.1545
10	% of range of error(confidence limit) (i)0.05 level	0.3562	0.3163	0.4503
	(ii)0.01 level	0.5864	0.5206	0.7413

TABLE 2 : Assay of myco in pharmaceutical formulations

SAMPLE	LABELLED AMOUNT (mg)	% Recovery by Proposed methods			% Recovery by Reference Method
		A	B	C	
Tablets –T_1	200mg	99.02 ± 0.32 t = 0.98 F = 1.63	99.98 ± 0.98 t = 0.48 F = 1.51	99.80±0.93	99.41 ± 0.25
Tablets –T_2	200mg	100.92 ± 0.37 t = 0.32 F = 3.42	99.89 ± 0.39 t = 0.98 F = 1.57	99.52±0.76	99.66 ± 0.26
Tablets –T_3	200mg	99.01 ± 0.47 t = 0.27 F = 1.39	100.37 ± 0.16 t = 1.95 F = 3.18	100.11±0.69	99.46 ± 0.49
Tablets –T_4	200mg	99.95 ± 0.34 t = 0.40 F = 2.16	100.30 ± 0.27 t = 0.26 F = 1.98	99.91±0.15	99.76 ± 0.38

***Two different batces of capsules from two different pharmaceutical companie; +Average ±Standard deviation of six determinations, the t-and f-tests values refer to the comparison of the proposed method with the reference method**

Colored species formation

(a) Method A

In this method, CAT undergoes hydrolysis in aqueous acid medium to give sodium hypochlorite followed by Hypochlorous acid. This reacts with MYCO to form the relevant oxidant products, probably a mixture which appears to be reproducible under the specified experimental conditions. The remaining Hypochlorous acid may be responsible for the bleaching of the colour GC through destruction of the extended chromophoric system

Colored complex of method A

Step -I

Myco + $KmnO_4$ → Mixtures of carboxylic acids + $KMnO_4$ + Mn (II)

(unreacted)

Step – II

$KMnO_4$ + FG FCF → unreacted FG FCF + Mixture of compounds with

(unreacted) (colored) rupture of conjugate system (reproducible but Not stoichiometric as several alternate pathways possile)

This method is based on the oxidation of MYCO by excess ferric salt (Fe (III) or Fe^{3+}) to form Sulphone derivative and reduced form Fe (III) (i.e. Fe (II) or Fe^{2+}) which subsequently reacts with potassium Ferri cyanide to give Ferrous Ferri cyanide

Colored complex of method B

MYCO + Fe (III) → Sulphone derivative + Fe (II) 3 Fe (II) + 2Fe $(CN)_6^{3-}$ → $Fe_3[Fe(CN)_6]_2$

Method C

In this method, CAT undergoes hydrolysis in aqueous acid medium to give sodium hypochlorite followed by Hypochlorous acid. This reacts with MYCO to form the relevant oxidant products, probably a mixture which appears to be reproducible under the specified experimental conditions. The remaining Hypochlorous acid may be responsible for the bleaching of the colour GC through destruction of the extended chromophoric system

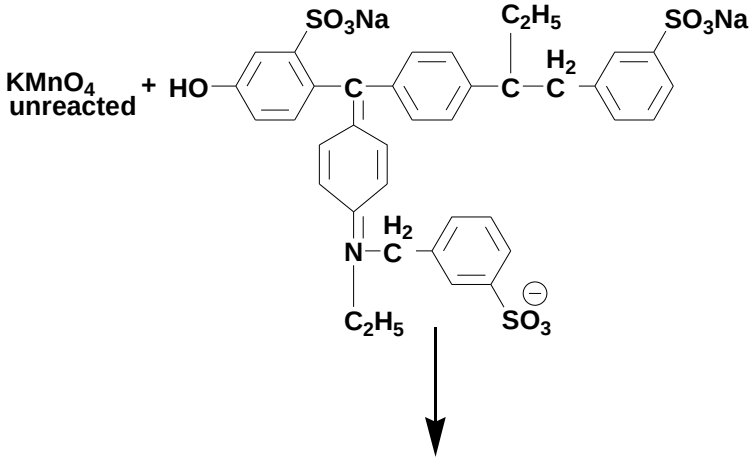

Figure 8 : Colored Complex of MYCO with MnO_4^-/FGFCF

Colored complex of method C

MYCO + CAT → Oxidation products of MYCO + un reacted CAT

Un reacted CAT +GC → Oxidation products of GC + GC un reacted

(colour less) (coloured)

CONCLUSION

The proposed methods are superior in one way or other (simplicity, λ_{max}, ° $_{max}$, 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 methods have good sensitivity and $_{max}$. Stastical analysis of the results (TABLE 1) shows that the proposed procedures have good precision and accuracy. Results of the analysis of pharmaceutical formulations (TABLE 2) reveal that the proposed methods are suitable for their analysis with virtually no interference of the usual additives. All the proposed methods are 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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