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NBS-METOL AS CHROMOGENIC REAGENT FOR THE DETERMINATION OF MYCOPHENOLICACID

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ABSTRACT

A simple and sensitive spectro-photometric method is developed for the estimation of Mycophenolic acid. It is based on the oxidation of MYCO with N-Bromo Succinimide followed by color product formation between unreacted NBS and P-N-methyl aminophenol sulfate (PMAP) -SA. The absorption maxima was found to be at 520nm. The method obeys Beer's law within the limits (1-15µg/ml) and gives reproducible results. The percentage recoveries in the formulations found to be 99.26 to 99.86 respectively.

Key Words: Mycophenolic acid (MYCO), N-Bromo Succinimide (NBS), PMAP, Sulphanilic Acid (SA).

INTRODUCTION

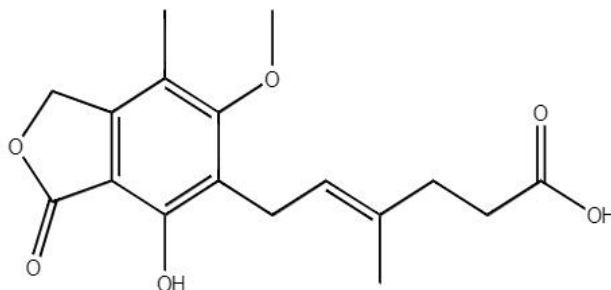


**Fig.1. CHEMICAL
STRUCTURE OF MYCO**

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 TLC [4], TLC-HPLC [5], spectro photometric (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 NBS [15-27] as the chromogenic reagent is very much proved versatile and simplest reagent because of its well stability and the stability of colored complexes it formed with a series of reagents was been well utilized by good number of researchers. It is clear from the literature that usage of NBS/METOL [28-29] combination with PMAP/SA for the determination 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.

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. NBS solution ($5.61 \times 10^{-3} \text{M}$) was Prepared by dissolving 100mg of N-Bromo Succinimide (0.1%,) in 100ml of distilled water and) standardized Iodometrically. PMAP solution (Loba, 0.3%, $8.71 \times 10^{-3} \text{M}$) Prepared by dissolving 300 mg of p-N-methyl Aminophenol

sulphate in 100 ml of distilled water. SA solution (Sd-fine, $1.16 \times 10^{-2} \text{M}$, 0.2%,) Prepared by dissolving 200 mg of Sulphanilamide in 100 ml of distilled water. Acetic acid (qualigens, $8.75 \times 10^{-1} \text{M}$), 5 ml of glacial acetic acid was diluted 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 50 µg/ml-400 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 stepwise with distilled water to get working standard solutions and analysed under procedures described for bulk samples.

Procedure:

Aliquots of standard MYCO solution (0.5–4.0 ml, 100 µg/ml), were transferred into a series of 25ml calibrated tubes. Then 1.0ml of Acetic acid and 2.0 ml of NBS solutions were added. The volume was brought to 10ml with distilled water and kept aside for 15 minutes at room

temperature. Then 1.5ml of PMAP solution was added. After two minutes, 2.0 ml of SA solution was added and the volume was made up to the mark with distilled water. The absorbance was measured at 520 nm (**Fig.2**). A blank experiment was also carried out omitting the drug. The decrease in the absorbance and in turn the drug concentration was obtained by subtracting the absorbance of the test solution from the blank. The amount of MYCO was computed from its Beer's 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 in Table-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. Research work carried out using PMAP as one of the chromogenic reagents Sastry's laboratory over the past years lead to the moulding of a very simple molecule like P-N-methyl aminophenol sulfate (PMAP or Metol) as a versatile chromogenic reagent, capable of reacting with different functional groups under different conditions enabling the estimation of many pharmacodynamic and chemotherapeutic agents. PMAP is a bifunctional substrate, when treated with an oxidizing agent undergoes oxidation with two electron transfer to yield the very unstable and highly reactive P-N-methyl benzoquinone monoimine (PMBQMI). The extent of oxidation of PMAP and the stability of

the quinoneimine formed depends upon the experimental conditions (pH, concentration of the oxidizing agent, and its redox potential). The selection of an appropriate oxidant in the colorimetric estimation of different compounds with PMAP depends upon its reactivity towards the compound and PMAP and also on the behaviour of its reduced form. In developing the method, a systematic study of the effects of various relevant parameters in the concerned was 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.

TABLE:1 OPTICAL CHARACTERISTICS, PRECISION, ACCURACY OF THE METHODS PROPOSED IN THE DETERMINATION OF MYCO

S.No	OPTICAL CHARACTERISTICS	NBS/PMAP-SA
1	$\lambda_{\max}(\text{nm})$	520
2	Beer's Law limits($\mu\text{g}/\text{ml}$)	1-16
3	Molar absorptivity($\text{l mol}^{-1}\text{cm}^{-1}$)	2.94×10^4
4	Correlation coefficient (r)	0.9999
5	Sandell's sensitivity ($\mu\text{g}/\text{cm}^2/0.001$ absorbance unit)	1.73×10^{-3}
6	Regression equation($y=a+bc$) (i)slope (b)	0.0230
	(ii) Standard deviation on intercept(S_b)	0.00869
	(iii) Intercept (a)	0.01060
	(iv) Standard deviation (SA)	0.0827
	(v) Standard error of estimation (S_e)	0.0303
7	Optimum photometric range ($\mu\text{g}/\text{ml}$)	5.8-15.8
8	Relative Standard Deviation *	0.3379
9	Detection limit	0.1424
10	% of range of error(confidence limit) (i)0.05 level	0.3546
	(ii)0.01 level	0.5838

Table-2 DETERMINATION OF MYCO IN PHARMACEUTICAL FORMULATIONS

SAMPLE	LABELLED AMOUNT(mg)	AMOUNT FOUND	
		PROPOSED METHOD	REFERENCE METHOD
Tablets – T ₁	200mg	100.13± 0.51 t = 1.28 F = 2.54	99.51 ± 0.25
Tablets – T ₂	200mg	99.63 ± 0.50 t = 0.62 F = 3.69	99.86 ± 0.26
Tablets – T ₃	200mg	99.78 ± 0.50 t = 0.41 F = 1.04	99.26 ± 0.49
Tablets – T ₄	200mg	99.81 ± 0.35 t = 0.50 F = 1.17	99.86 ± 0.38

*Tablets from four different pharmaceutical companies.

**Average ± standard deviation of six determinations, 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.

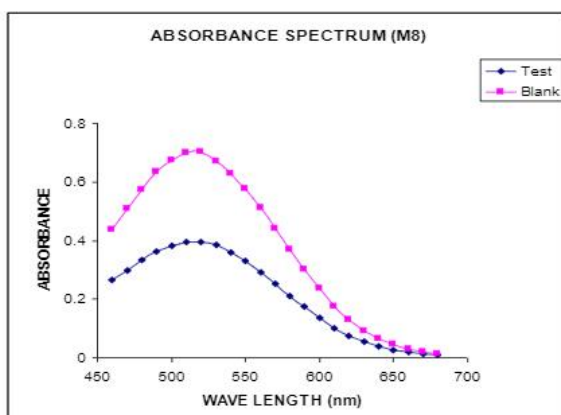


Fig.2 Absorption Spectrum of NBS/METOL System

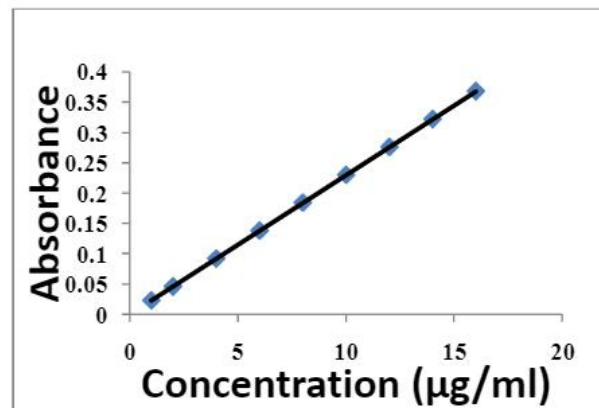
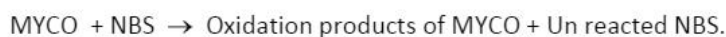


Fig.3 Beer's plot of NBS/METOL System

Colored Complexes:

These methods are based on the oxidation of MYCO by NBS to form oxidation products (probably mixtures, but reproducible under proposed experimental conditions, excess NBS

Step 1:



Step2:

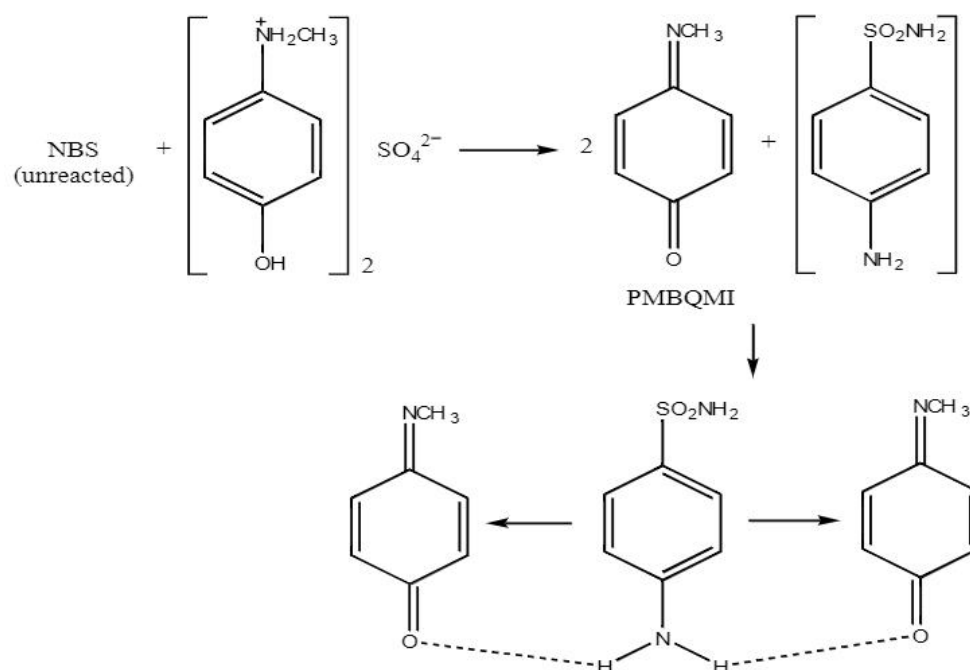


Fig.4 Probable colored complexes of NBS/METAOL with PMAP/SA and subsequent reactions

CONCLUSION

The proposed method is superior in one way or another in terms of simplicity, (λ_{max} , ϵ_{max} , stability of colored 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} . Statistical 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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