THE PHARMA RESEARCH

A Journal of Pharmacy Research

Original Article ISSN- 0975-8216

Impact- 0.529 Refer website

NBS-METOL AS CHROMOGENIC REAGENT FOR THE DETERMINATION OF MYCOPHENOLICACID

CH.V.R.MURTHY[1],ACHARYULU,M.L.N[2*], ,B.V.SRINIVAS[3], T.S.REDDY[4] C.S.RAMA LAKSHMI[5]

Affiliation:

[1]Department of Chemical Engineering, AU College of Engineering (A), Andhra University, Visakhapatnam-530003 Andhra Pradesh (India)

[2] Department of AS&H, VITAM College of Engineering, Mindivanipalem , Anandapuram Mandal,Visakhapatnam, A.P , India.

[3]Department of Organic chemistry, Foods, Drugs and Water, A.U, Visakapatnam-530003

[4]DLR College (Degree & P.G COURSES), Gollalamamidada, E.G.Dtt, A.P-533344.

[5]C.S.R. Lakshmi, Asst. Professor, Dayanand Sagar College of Pharmacy, Kumaraswamy Layout, Bangalore

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

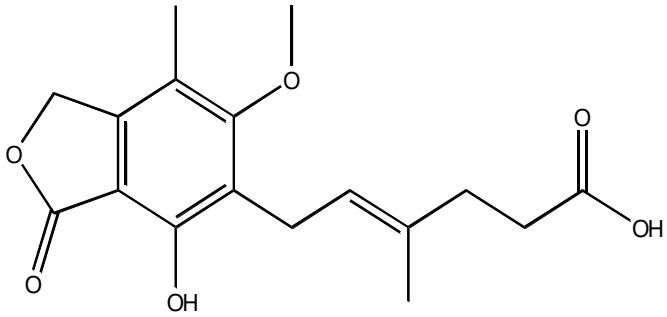

Fig.1. CHEMICAL STRUCTURE OF MYCO

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 x 10^{-3}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 x 10^{-3}M) Prepared by dissolving 300 mg of p-N-methyl Aminophenol

sulphate in 100 ml of distilled water.SA solution(Sd–fine,1.16x10^{-2}M,0.2%,) Prepared by dissolving 200 mg of Sulphanilamide in 100 ml of distilled water.Acetic acid (qualigens,8.75× 10^{-1} M),5 ml of glacial acetic acid was diluted to l00 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 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.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 undertakenn 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 coured 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	λ_{max}(nm)	520
2	Beer's Law limits(µg/ml)	1-16
3	Molarabsorptivity(l $mol^{-1}cm^{-1}$)	$2.94x10^{4}$
4	Correlation coefficient (r)	0.9999
5	Sandell's sensitivity (µg/cm^2/0.001absorbance unit)	$1.73x10^{-3}$
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 (µg/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.

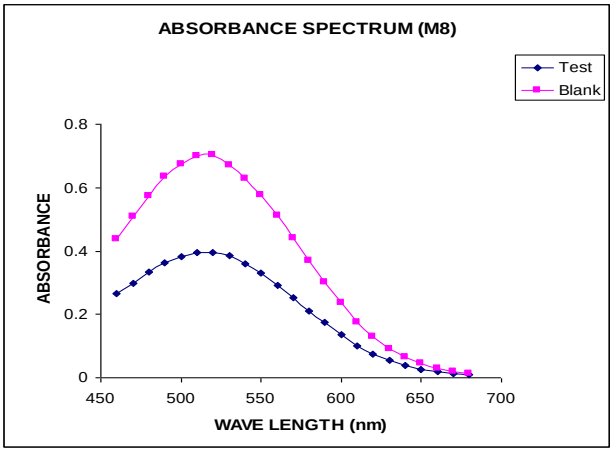

Fig.2Absorption Spectrum of NBS/METOL System

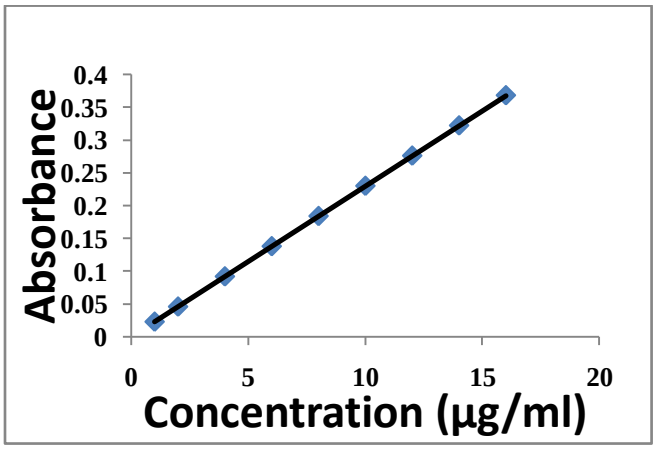

Fig.3 Beer"s plot of NBS/METOL System

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 being determined either by PMAP –SA, the unreacted NBS develops colour when treated with PMAP – SA, the *in situ* formed PMBQMI from PMAP involves in (charge – transfer colour complex) formation.(**Fig.4**)

Step 1:

MYCO + NBS $\rightarrow$ Oxidation products of MYCO + Un reacted NBS.

Step2:

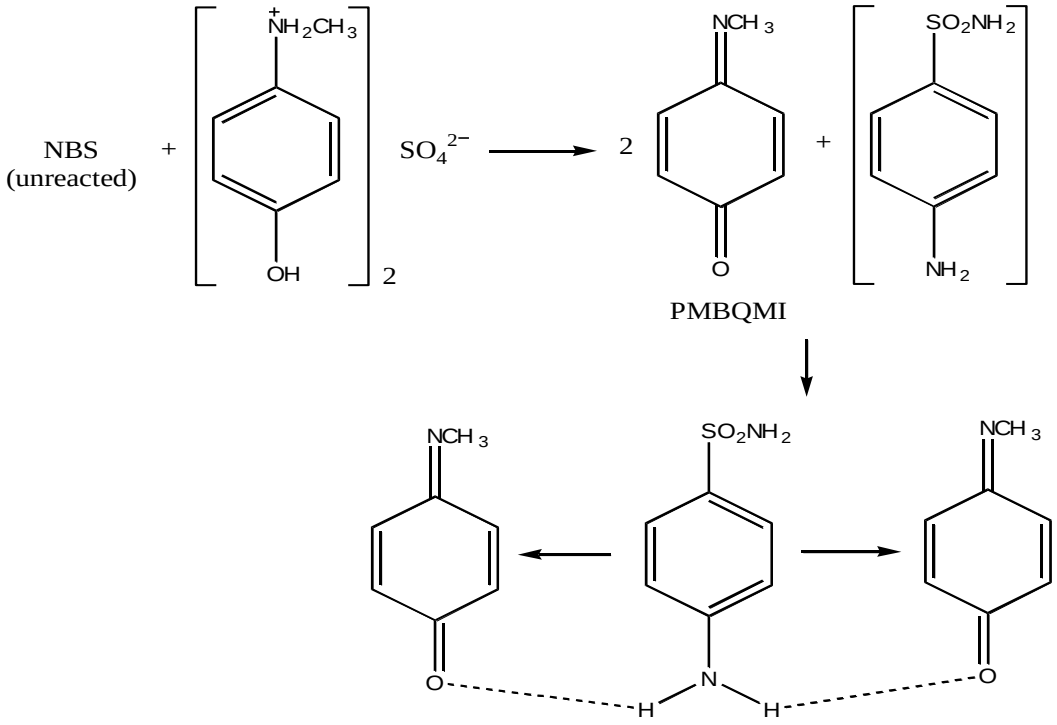

Fig.4 Probable colored complexes of NBS/METAOL with PMAP/SA and subsequent reactions

CONCLUSION

The proposed method is superior in one way or another in terms of simplicity, (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.

Acknowledgements

The author* express his deep sense of gratitude to UGC, SAP, PHASE-III for its assistance.

REFERENCES

1. Martinadale: The complete drug reference' (Extra Pharmacopoeia) 30th Edn. 1993.
2. The Merck Index, 13th Edn. Merck & Co Inc, New York, 1996.
3. CIMS-97 April2007.
4. Mehdi Ahadi Barzoki, Mohammadreza Rouini, Kheirolla Gholami, Mahboob Lessan-Pezeshki, Saeed Rezaee, Determination of Mycophenolic Acid in human Plasma by HPLC, DARU 2005;13,3.
5. Sorensen, Louise Marie; Mogensen, Jesper; Nielsen, Kristian Fog, Simultaneous determination of ochratoxin A, mycophenolic acid and fumonisin B in meat products, Analytical & Bioanalytical Chemistry; 2010,398;3;1535.
6. K. B. Vinay, H.D.Revanasiddappa, M.S.Raghu,Sameer. A. M. Abdulrahman, and N. Rajendra prasad, Spectrophotometric Determination of Mycophenolate Mofetil as Its Charge-Transfer Complexes with Two π – Acceptor**s,**Journal of Analytical Methods in Chemistry,20, 2; 1;1
7. A. Narendra, D. Deepika, and M. Mathrusri Annapurna, Validated SpectrophotometMathrusrids for the Determination of Mycophenolate: An Anti-Neoplastic Agent in Bulk and Pharmaceutical Dosage Forms, Journal of Chemistry,Volume 2013, Article ID 523193,4.
8. Frank E. Ga, ner, Harold J. Wesselman, GLC of mycophenolic acid and related compounds, Journal of Pharmaceutical Sciences, 1970;59;8,1157–1159,
9. Stern, S. S, Tallan, M. N, Jan iles, K. K. M, Ritter, J. K, dupes, R. E, Smith, P.C., Drug Metab. dispos, Drug Metabolism and Disposition: the Biological Fate of Chemicals, 2007;35,3, 449-454.
10. Jacobson P, Green K, Rogosheke J, Brunstein C, Ebeling B, Defor J, Mcglave P, Weisdorf D, Therapeutic monitoring of mycophenolate mofetil, J. Clin Pharmacol, 2007,2,1;184-191.
11. Kuhn, J; Gotting, C, Kleesiek, K., Sample cleanup-free determination of Mycophenolic Acid and its glucuronide in serum and plasma using the novel technology of ultra-performance liquid chromatography electrospray ionization

tandem mass spectrometry, *Talanta*, 2010;80,5,15,1894-98.

12. Tripodi, U.P., Leucangioli, S.E., Barbara, C.L., Roriguez, R.G., Carrducci, C.C., Electrochemical Oxidation of an Immunosuppressant, Mycophenolate Mofetil, and Its Assay in Pharmaceutical Formulations, Chromatographia, 2001, 93-98.
13. Sparidans, R.W., Hoetelmans, R.M., Beijne, J.H., J, Liquid chromatographic assay for simultaneous determination of abacavir and mycophenolic acid in human plasma using dual spectrophotometric detection, Chromatogr Biomed Sci Appl.2001, 750;1,155.
14. Pastor, A., LoRusso, A., PiMonte., Fiorella., Manucci., Cilizna., Federici., Giordioi, Rapid determination of mycophenolic acid in plasma by reversed-phase high-performance liquid chromatography, Journal of Chromatography, 2002,776,2;251-254.
15. Berka,A,Vuteran,J,Zyka,J,Newer Redox Titrants,Tr.By Weiss,H, Pergamon Press, Oxford, 1965-53.
16. Tiwari,R.D, Pande, U.E, J.Int.Chem.Soc.,1974,51,112.
17. Schneider,P.O, Thibert,R.J,Watton,R.J,Mikro Chim.Acta,1972, 925.
18. Upadhyaya, K.N, J.Anal.Chem.1978, 202,412.
19. Sharma,J.P, Shukla,V.K.S, Dudey, A.K, Analyst,1976,101,867.
20. 20.K. Basaviah, B. C. Somasekhar, Titrimetric and Spectrophotometric Determination of Metaprolol tartrate in Pharmaceuticals Using N-Bromosuccinimide,, E-Journal of Chemistry, 2007;4,1,117-127, 21.
21. Ibrahim A Darwish, Samiha A. Hussein, Ashraf M Mahmoud, Ahmed I. Hassan, N-Bromosuccinimide/fluorescein system for spectrophotometric determination of H2-receptor antagonists in their dosage form international Journal of Research in Pharmaceutical Scienceptor010,1;2:86
22. Ibrahim A Darwish, Samiha A Hussein, Ashraf M Mahmoud, Ahmed I Hassan, A sensitive spectrophotometric method for the determination of H2-receptor antagonists by means of N-bromosuccinimide and p-aminophenol, Acta Pharmaceutica,2008;58,1:87-97.
23. N. A. Alarfaj, S. A. Altamimi, L. Z. Almarshdy, Spectrophotometric Determination of MefenamicAcid in Pharmaceutical Preparations, Asian Journal of Chemistry, 2009,21,1; 217-216.
24. KN Prashanth, K Basavaiah*, Simple and Rapid Spectrophotometric Determination of Propranolol Hydrochloride as Base Form

in Pharmaceutical Formulation through Charge Transfer Complexation , Chemical Sciences Journal, 2012; CSJ-71

25. Hassan Askal, Ibrahim Refaat, Ibrahim Darwish, Mostafa Marzouq, Evaluation of N-bromosuccinimide as a new analytical reagent for the spectrophotometric determination of fluoroquinolone antibiotics, Chem Pharm Bull, 2007;55;11:1551-6.
26. C.S. P Sastry, V. A. N. Sarma, U. V Prasad, C. S. R Lakshmi,N-Bromosuccinimide as an Analytical Reagent For The Spectrophotometric Determination of Benzimidazole Anthelmintics**,** international journal of ph, rmaceutical sceiences,1997,59,4:161-164.
27. Hassan Askal et al Evaluation of N-bromosuccinimide as a new analytical reagent for the spectrophotometric determination of sciences, nolone antibiotics, Chem. Pharm. Bull. 2007;55;1551-6.
28. Sastry C.S.P, Sastry B.S, The Eastern Pharmacist, 1986,29,67.
29. Sastry, C.S.P, Lingeswara Rao, J.S.V.M, The Eastern Pharmacist*,* 1995,3,57.