Spectrophotometric determination of Mycophenolic Acidand Mirtazapine in pure and Pharmaceutical Formulations

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Three simple and sensitive spectro photometric methods(A-C) have been developed for the estimation of Mycophenolic acid and Mirtazapine.Method A is based on the oxidation of MYCO with excess N-Bromo Succinimide(NBS)and determining the unreacted NBS with an oxidisable dye Celestine blue(CB(λ_{Max} =540nm); Method B is based on the reduction of MYCO with Folin-Clocalteau reagent(λ_{Max} =775nm)and Method C is based on quantitative precipitation of MIRT with Phospo molybdic Acid(PMA) and estimating the PMA (released with acetone from it's adduct) by reducing it with Co (II)-EDTA complex (λMax =840nm) .These methods obey Beer's law limits and give reproducible results.The percentage recoveries are 99.17,99.84,99.11 respectively.

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-enoic acid. Mycophenolate is potent and can be used in place of the older anti-proliferative azathioprine. It is official in Martindale[1],MI[2],CIMS[3] and MIRT is official in BP[4],USP[5],EP[6],MI[2],PDR[7],CIMS[3]. A very few physio-chemical methods appeared in the literature for the determination of MYCO in pharmaceutical formulations (less)&plasma samples. The methods so far reported includes TLC, spectrophotometric (UV and visible), Tandem mass spectrometry etc., the analytically important functional groups of MYCO,MIRT were not properly exploited designing suitable spectrophotometric methods for the determination of MYCO.Mirtazapine is chemically known as 1,2,3,4,10,14b-hexahydro-2-methylpyrazino [2,1-a] pyrido [2,3-c] benzazepine.

In the present paper,We describe three visible spectrophotometric methods based on the colour development rection with the released precipitant in the filtrate from the precipitate in the filtratewith chromogenic reagent Co(II) – EDTA.

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 wereprepared freshly. Aqueous solutions of NBS solution(Loba, 0.01%,5.618 x 10^{-4}M),CB solution (Chroma, 0.02%,5.49 x 10^{-4}M),Hydrochloric acid,FC reagent,Loba, (2N),Na_2CO_3 solution(10%;8.75x10^{-1}M)PMA((Loba, 0.3%,8.71 x 10^{-3}M),CobaltnitrateSolution(Qualigens:3.0%w/v,1.03x10^{-1}M)EDTAsolution (Qualigens:4.0%w/v,1.07x10^{-1}M) were prepared in triple distilled water.

Standard drug solutions:

A 1mg/ml solution was prepared by dissolving 100mg of pure MYCO,MIRT in 100ml of water and further diluted to50μg/ml for A,100μg/ml for method B, 200μg/ml for the method C respectively.

Recommended procedures:

Method A: To each25ml of graduated test tubes containing standard MYCO solution (0.5 – 3.0ml, 50μg/ml), 1.25ml of 5M HCl and 2.5ml of NBS were added and tsolution was diluted to to 20ml with distilled water. After 10 min, 5 ml of CB solution was added, mixed thoroughly. The absorbances were measured after 5 min at 540nm against distilled water. The blank (omitting drug) and dye (omitting drug and oxidant) solutions were prepared in a similar manner and their absorbances were measured against distilled water. The decrease in absorbance corresponding to consumed oxidant,which reflects 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). Calibration graph was prepared by plotting the decrease in absorbance of Dye against the amount of the drug. The amount of drug was calculated from its Beer-Lambert plot.

Method B: Aliquots of standard MYCO solution (0.5 – 6ml, 100 μg/ml), were transferred in to a series of 25ml calibrated tubes.Then 2.0ml of 2N F-C solution was added and after 5 min 7.0ml of 10%Na_2CO_3 were added and kept aside for 30 min. The volume was made upto the mark with distilled water. The absorbance was measured at 770nm against a similar reagent blank. The amount of drug was computed from Beer-Lambert plot

Method C: Aliquots of the standard MIRT solution (0.5-2.5ml, 200 μg/ml) were delivered into a series of centrifuge tubes containing 0.25ml of conc. HCl and the volume of each tube was adjusted to 3.0ml with distilled water,then 1.5 ml of phospomolybdic acid was added and centrifuged for 5 min. The precipitate was collected through filtration, followed by washing with distilled water until it is free from reagent. The precipitate in each tube was dissolved in 5ml of acetone and transferred into a 25ml graduated tube. 1 ml each of cobalt nitrate and EDTA solutions were successively added and the test tubes were heated for 10 min at 60-70^{0}C.The test tubes were cooled and the solution in each tube was made up to the mark with distilled water. The absorbance was measured after 10 min at 840 nm against a similar reagent blank. The amount of drug was calculated from Beer-Lambert plot

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's limits,molar absorptivity, and sandell's sensitivityare given in Table-1.Regression analysis using the method of lest

Squares was made to evaluate the slope(b),intercept(a),and correlation Co-effecient (r) for each systemare presented in Table-1.

The accuracy of the methods was ascertained by comparing the results obtained for pharmaceutical formulationsby the proposed methods and reference method(UV, developed in the laboratory using drugsolutions ,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 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 NBS/CB	Method-B FC	Method-c PMA/Co(II)-EDTA
1	λ_{max}(nm)	540	775	*840*
2	Beer's Law limits(µg/ml)	1-6	5-30	*4-20*
3	Molar absorptivity(l $mol^{-1}cm^{-1}$)	$4.45x10^4$	$4.10x10^4$	*$1.0x10^4$*
4	Correlation coefficient (r)	o.9999	0.9988	*0.9996*
5	Sandell's sensitivity ($\mu g/cm^2$/0.001absorbance unit)	0.0359	0.0195	*$0.040x10^{-3}$*
6	Regression equation(y=a+bc) (i)slope (b)	0.1114	0.0205	*$2.465x10^{-2}$*
	(ii) Standard deviation on intercept(S_b)	$5.12x10^{-4}$	1.401x10-4	*$1.34x10^{-4}$*
	(iii)intercept (a)	-0.0057	-0.0045	*$-1.00x10^{-4}$*
	(iv) standard deviation (S_a)	$5.12x10^{-4}$	$2.728x10^4$	*$1.34x10^{-4}$*
	(v)Standard error of estimation(S_e)	0.2332	$2.931x10^{-3}$	*$1.70x10^{-3}$*
7	Optimum photometric range (µg/ml)	2.9-5.9	1.0-29	*5.6-18.2*
8	Relative Standard deviation	0.2718	0.196	*0.477*
9	Detection limit	0.0137	0.3992	*0.2052*
10	% of range of error(confidence limit) (i)0.05 level	0.2852	0.2057	*0.494*
	(ii)0.01 level	0.4473	0.3226	*0.775*

Table-2

ASSAY OF MYCO,MIRT in PHARMACEUTICAL FORMULATIONS

SAMPLE	**LABELLED AMOUNT(mg)**	%Recovery by Proposed methods			%Recovery by Reference Methods		
		A	B	C	A	B	C
Tablets – T_1	200mg	99.17± 0.40 t = 0.47 F = 1.56	99.84 ± 0.14 t = 0.98 F = 3.18	100.01 ± 0.69 t = 0.58 F = 1.02	99.41 ± 0.25	99.51 ± 0.25	99.51± 0.70
Tablets – T_2	200mg	99.18 ± 0.27 t =1.21 F = 1.07	99.12 ± 0.29 t = 1.02 F = 2.10	99.52 ± 0.54 t = 0.99 F = 1.65	99.66 ± 0.26	99.86 ± 0.26	99.93 ± 0.77
Tablets – T_3	200mg	100.13 ± 0.52 t = 0.18 F = 1.12	99.55 ± 0.55 t = 1.94 F = 1.49	99.61± 0.45 t = 0.51 F = 3.82	99.46 ± 0.49	99.26 ± 0.49	99.48 ± 0.88
Tablets – T_4	200mg	99.21± 0.27 t = 0.26 F = 1.98	99.01 ± 0.32 t = 0.22 F = 2.44	99.16 ± 0.38 t = 0.86 F = 1.17	99.76 ± 0.38	99.86 ± 0.38	99.19 ± 0.35

*Two different batces of capsules from two different Pharmaceutical companies

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

Colored Species formation

Method A:

Method A IS 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 CB

Scheme For Method A:

step-I.

MYC0 + NBS $\rightarrow$ oxidation productsof MYCO + Succinimide + NBS (un reacted)

Step –II

NBS + CB $\rightarrow$ unreacted CB + Oxidation products of dye (colorless)
(colored) (Disruption of cromophores and auxochromes)

Method B:

The colour formation by FC reagent with MYCO may be explained in the following manner based on the analogy with the reports of earlier workers. The mixed acids in the FC reagent preparation involve the following chemical species.

Scheme for Method B:

$3H_2O$, P_2O_5, $I3WO_3$, $5MoO_3$, $I0H_2O$ and

$3H_2O$, P_2O_5, $I4WO_3$, $4MoO_3$, $I0H_2O$

MYCO probably effects a reaction of 1,2 or 3 oxygen atoms, from tungstate and / or molybdate in FC (phosphomolybdo tungstate), thereby producing one or more of the possible reduced species which have a characteristic intense blue colour.

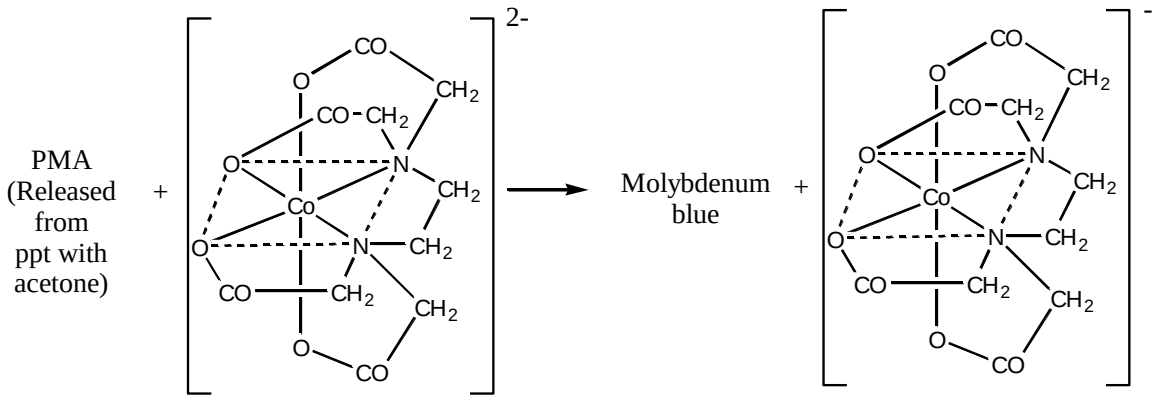
Method C: This method involves two steps .The first step is the quantitative precipitation of MIRT with PMA. Second step is the reduction of PMA (released from the adduct by Co(II)-EDTA complex to generate molybdenum blue. The proposed sequences of reactions are given in the following scheme.

Step I :

MIRT + PMA → MIRT-PMA + PMA

(Precipitate) (Unreacted)

Step II:



CONCLUSION: The proposed methods are 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 methods have good sensitivity and λ_{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 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,MIRT in bulk samples and pharmaceutical formulations depending upon the needs of the specific situation.

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