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Determination of mycophenolate mofetil in bulk and pharmaceutical formulations by UV derivative spectrophotometric method

T.Vijaya Bhaskara Reddy[1], N.Sowjanya Reddy[1], G.Ramu[1,2], S.Ravi Prakash Reddy[3], C.Rambabu[1]*

[1]Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Andhra Pradesh, (INDIA)

[2]Department of Chemistry, Sir C.R.Reddy College, Eluru, Andhra Pradesh, (INDIA)

[3]International Pharmaceutical Generics LTD, (CANADA)

E-mail: rbchintala@gmail.com

ABSTRACT

Simple and rapid UV absorption spectrophotometric (Method-A) and UV second order derivative spectrophotometric (Method-B) methods have been developed for the determination of Mycophenolate Mofetil (MMF) in pure and its pharmaceutical formulations. The absorption spectrum, first and second order derivative spectra of working standard solution of concentration 20µg/mL of MMF in methanol was recorded against methanol as a reagent blank and wavelength of maximum absorbance was found to be 249.0nm. From the first derivative spectrum it was found that a valley at 257.4nm showed maximum amplitude. Second derivative spectrum has the maximum amplitude in negative valley at 249.0nm hence the second derivative method was validated by measuring amplititudes at 249.0nm. Standard deviation and percent of relative standard deviation of six replicates were calculated and found to be within the limits. The mean percent of recovery were evaluated at 50%, 100% and 150% concentration levels and found to be within the range 99.1-99.6 percent. The developed methods were found to be linear within the range of concentrations 10-30µg/mL. Slope, intercept and correlation coefficient for the developed method were calculated and found to be satisfactory. The methods have been proved robust and rugged. The developed methods were found to be precise, accurate and stable, therefore readily adapted for routine quality control of MMF by ordinary laboratories. The developed methods were effective for quantitative determination of MMF in bulk and pharmaceutical preparations without any interference of other constitute in tablets of different brand names.

KEYWORDS

Mycophenolate mofetil;
Derivative spectrophotometry;
Amplitude;
Pharmaceutical preparations;
Assay.

INTRODUCTION

Mycophenolate Mofetil (MMF), an immunosuppressant is extensively used in transplant medicine. It is a reversible inhibitor of inosine monophosphate dehydrogenase http://en.wikipedia.org/wiki/Mycophenolate_mofetil - cite_note-Fulton-1 in purine biosynthesis which is necessary for the growth of T cells and B cells. MMF has an empirical formula of $C_{23}H_{31}NO_7$, molecular weight of 433.5grams/mole and the chemical name is 2-morpholinoethyl (E)-6-(1,3- dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-

isobenzofuranyl)-4-methyl-4- hexenoate. MMF is a white to off-white crystalline powder which is soluble in water and is available as CellCept of 500 mg. The chemical structure of the drug is given in Figure 1.

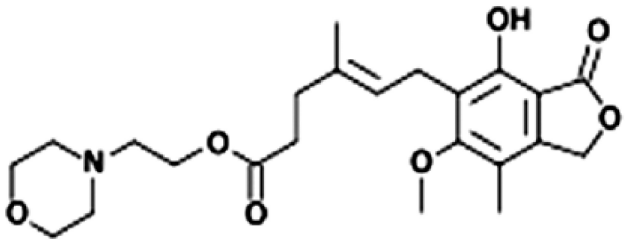

Figure 1 : Chemical structure of mycophenolate mofetil (MMF)

An extensive literature survey was carried out and found some high-performance liquid chromatographic methods for the determination of mycophenolate mofetil in human plasma[1], determination of mycophenolate mofetil and its degradation product mycophenolic acid in dosage form[2], capsules[3], in bulk and tablet dosage form[4-6]. Two liquid chromatography-mass tandem spectrometric methods were developed for the estimation of mycophenolate mofetil in human skin extracts[7] and in bulk and pharmaceutical formulation[8], One HPTLC method[9] for the determination of mycophenolate mofetil in bulk and pharmaceutical formulation, UPLC technique[10] for estimation the Mycophenolate in dosage form, assay in pharmaceutical formulations by electrochemical oxidation of mycophenolate mofetil[11] and two spectrophotometric methods[12,13] for the estimation of mycophenolate mofetil were also reported. Since UV spectrophotometric methods were relatively sensitive when compared with other methods and yet no author has attempted the UV- derivative spectrophotometry as a quantitative method for the determination of the same, so the author has chosen this technique to estimate the MMF in pure and formulations and succeeded. The proposed method was found to be simple, sensitive, economic and time saving, and the developed method was successfully applied for the analysis of formulation with good recovery.

EXPERIMENTAL

Instrumentation

An UV-Visible spectrophotometer (UV-3000) with 1cm matched quartz cells was used for the spectral and absorbance measurements. Semi micro balance (CPA225D) was used for weighing purpose.

MATERIALS AND METHODS

About 10mg MMF was accurately weighed and transferred into a 100 mL volumetric flask, added about 70 mL of methanol, sonicated to dissolve it completely and made volume up to the mark with the same solvent. Further 2 mL of MMF stock solution was pipetted into a 10mL volumetric flask and diluted up to the mark with methanol, and then a series of solutions were prepared by transferring 10mL-30mL of diluted solution (20µg/mL) into 10mL flasks and diluted.

Method development

Assay of MMF was calculated by the proposed methods after a detailed study of the effects of various parameters involved. The absorption spectrum of a solution of 20 µg/mL of MMF was recorded by scanning the absorbance values in the range of wavelength 200-400nm and then first and second derivative spectra were also obtained from the spectrophotometer. From the absorption spectrum (Figure 2) it was found that the wavelength of maximum absorbance was 249.0nm. The first derivative spectrum D^1 (Figure 3) crossed the zero point at a wavelength of 249.0nm and producing positive valley at a wavelength of 240.0nm and negative valley at 257.4nm on either side of the zero crossing point of the spectrum. The valley at 257.4nm showed maximum amplitude than the first valley. Therefore validation of the method was carried out by measuring the amplitudes at this wavelength. The second derivative spectrum D^2 (Figure 4) crossed the x-axis at 243.5nm, 257.4nm and 274.0nm leaving negative valley at 249.0nm and a positive valley at 263.2nm. The maximum amplitude was observed at negative valley; therefore the method was validated by measuring amplititudes at 249.0nm.

Method validation

Linearity

Different aliquots (10mL-30mL; 20µg/mL) of working standard solution of MMF were taken in 10mL calibrated tubes, diluted up to the mark with methanol and

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then kept aside for 10min. The zero order, first order and second order derivative spectra for each of the concentration were recorded over the wavelength range 200-400nm against a reagent blank under similar conditions (Figure 5- Figure 7).

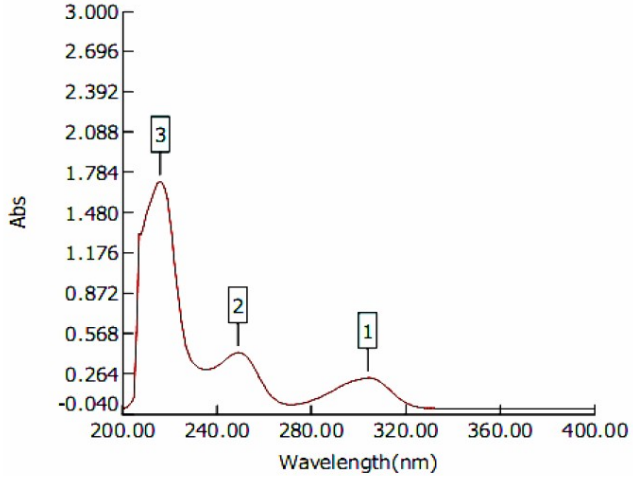

Figure 2 : Absorption spectrum of MMF (20 µg/mL)

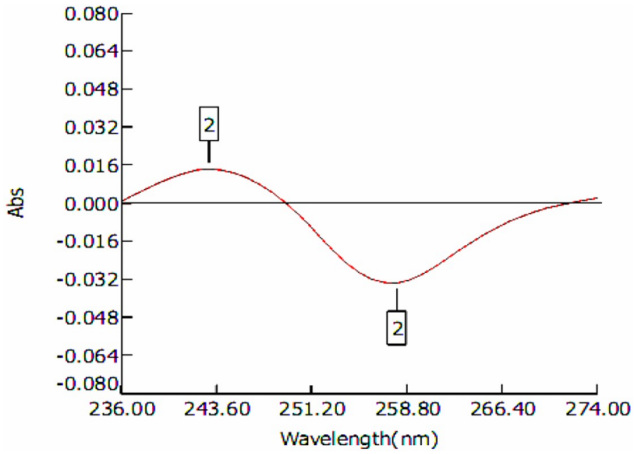

Figure 3 : First derivative spectrum of MMF (20 µg/mL)

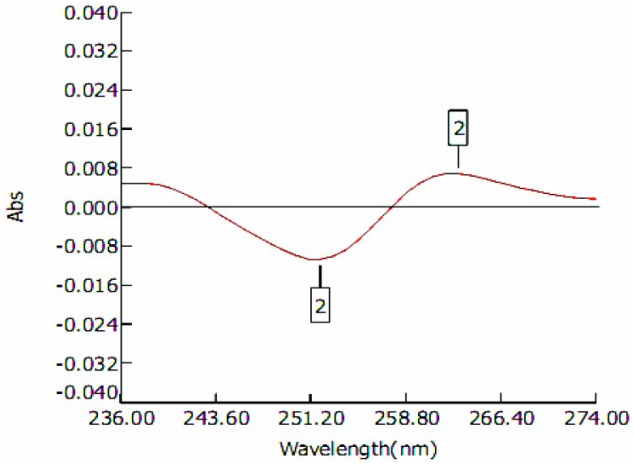

Figure 4 : Second derivative spectrum of MMF (20 µg/mL)

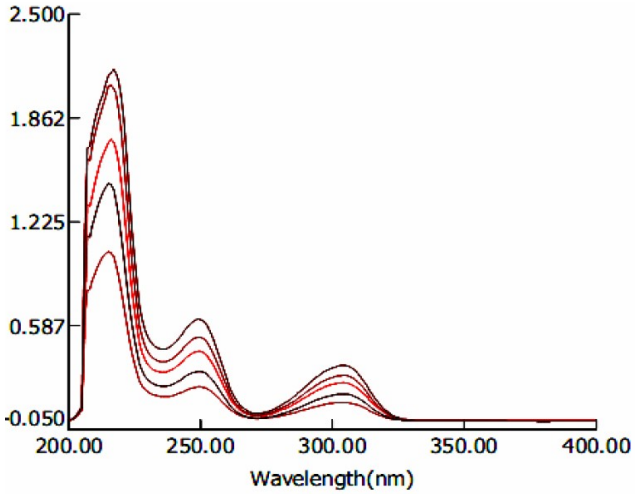

Figure 5 : Absorption spectra of MMF (10-30µg/mL)

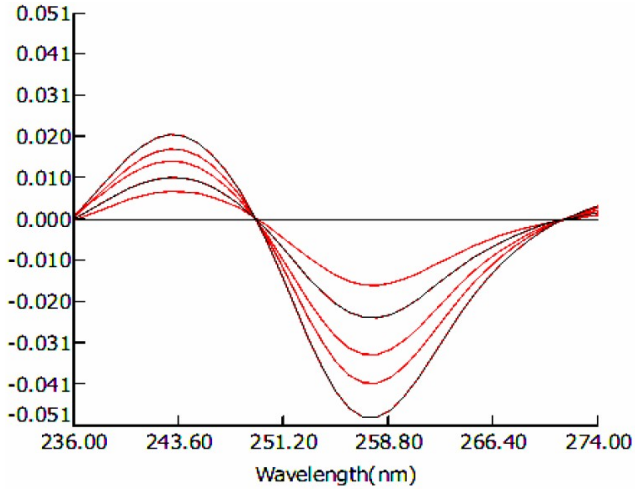

Figure 6 : First order derivative spectra of MMF (10-30 µg/mL)

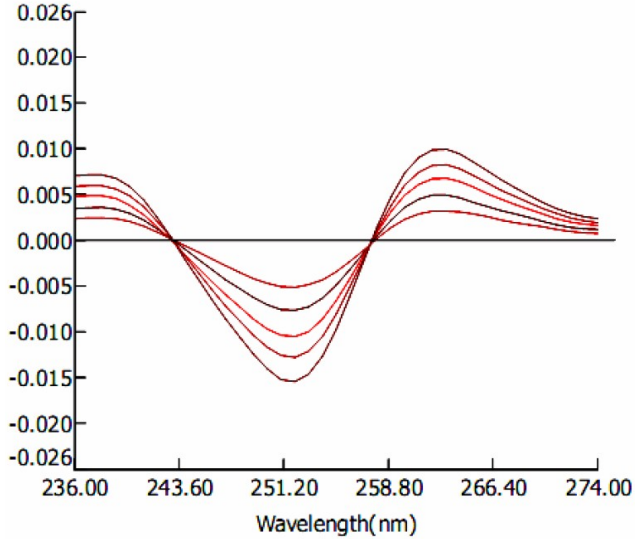

Figure 7 : Second order derivative spectra of MMF (10-30 µg/mL)

Precision

Precision (repeatability) of each proposed method was calculated from the absorbance values or maximum amplitudes of five replicates of a fixed amount of MMF in total solution in D^0, D^1 and D^2 respectively. The standard deviation and percent relative standard deviation were calculated for the proposed methods and presented in TABLE 1.

TABLE 1 : Precision for the developed method

S.No	Concentration µg/mL	Zero Order	First Order	Second Order
Average*		0.4214	0.0321	0.0105
Standard Deviation*	20.0	0.0023	2.39E-4	8.0E-5
%RSD*		0.5463	0.7442	0.7962

***Statistical analysis applied on five replicates of measurements**

Intermediate precision

To evaluate intermediate precision (reproducibility) measurements were performed on different days under the same experimental conditions. In the present study intermediate precision of each proposed method was ascertained from the absorbance values and amplitudes obtained for five replicates of a fixed amount of MMF in total solution on two different days. The standard deviation and percent relative standard deviation were calculated in each case and presented in TABLE 2.

TABLE 2 : Study of intermediate precision of the proposed method

Statistical parameter	Zero order	First order	Second order
Average*	0.4200	0.0320	0.0105
Standard Deviation*	0.0023	2.59E-4	1.0E-5
%RSD*	0.5583	0.8083	0.3763

***Statistical analysis applied on five replicates of measurements**

Accuracy

Accuracy, concordance between the measured value and the true or most probable value was determined at three different amounts (50%, 100%, and 150%) of MMF within the Beer's law limits were taken, measurements were made thrice in each concentration. Standard deviation and percent of relative standard deviation were calculated for three replicate measurements at three concentrations. The results were recorded in TABLE 3(a)- TABLE 3(c).

TABLE 3(a) : Accuracy of the developed method (Zero derivative)

% Concentration	Amount Added	Amount Found	% Recovery	Mean Recovery
50%	10.0	5.00	100.0%	
100%	20.0	9.93	99.3%	99.1%
150%	30.0	14.7	98.0%	

TABLE 3(b) : Accuracy of the developed method (First derivative)

% Concentration	Amount Added	Amount Found	% Recovery	Mean Recovery
50%	10.0	4.99	99.8%	
100%	20.0	9.98	99.8%	99.6%
150%	30.0	14.90	99.3%	

TABLE 3(c) : Accuracy of the developed method (Second derivative)

% Concentration	Amount Added	Amount Found	% Recovery	Mean Recovery
50%	10.0	4.99	99.8%	
100%	20.0	9.98	99.8%	99.2%
150%	30.0	14.70	98.0%	

Calibration plot

In zero order (D^0), a linear straight line was drawn by taking absorbance values on y-axis and concentration on x-axis (Figure 8). In case of derivative method, maximum D^1 and D^2 amplitudes were plotted against concentration of the drug (Figure 9- Figure 10). Linear least squares regression analysis was applied in three cases and slope intercept and correlation coefficient parameters were calculated and were presented in TABLE 4.

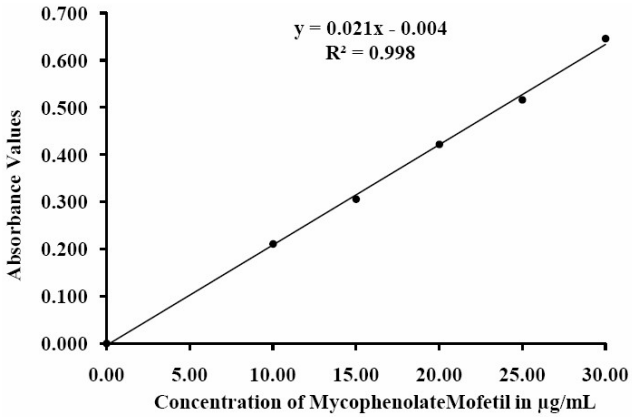

Figure 8 : Linearity plot of absorbance against concentration of MMF

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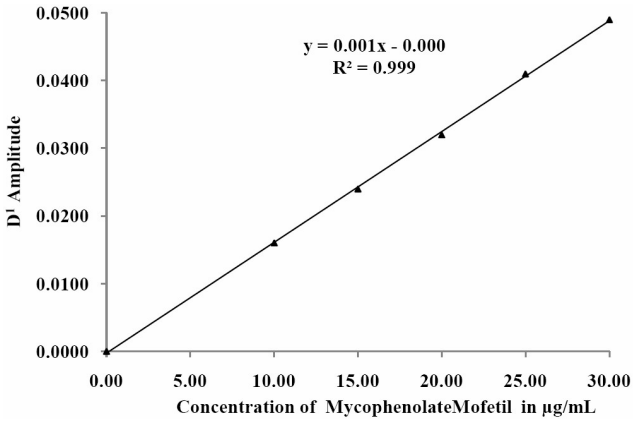

Figure 9 : Calibration plot of first derivative amplititudes against concentration of MMF

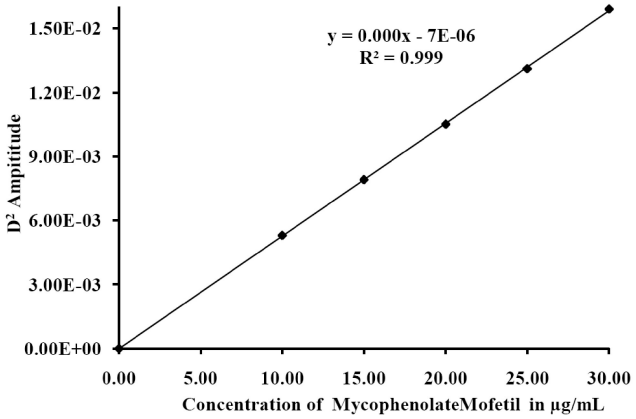

Figure 10 : A linear straight line drawn between second derivative amplititudes and concentration of MMF

TABLE 4 : Linearity of the proposed method

S.No.	Concentration µg/mL	Absorbance	D^1 Amplitude*	D^2 Amplitude*
1	10.0	0.211	0.0160	0.00530
2	15.0	0.306	0.0240	0.00790
3	20.0	0.422	0.0320	0.01050
4	25.0	0.516	0.0410	0.01310
5	30.0	0.646	0.0490	0.01590
Slope		0.0213	0.0016	0.0005
Intercept		0.0042	0.0003	7.0E.-06
Correlation Coefficient		0.9996	0.9997	0.9999

***D^1 and D^2 are first order and second order derivative spectra**

Limit of detection (LOD) and limit of quantization (LOQ)

The LOD and LOQ of the proposed method were calculated by using standard deviation of the intercept (σ) and slope (s) of the calibration curve. These were calculated by using the formulae LOD=3σ/s and LOD=10σ/s and are presented in TABLE 5.

TABLE 5 : LOD and LOQ of MMF

Parameter	Zero Derivative	First Derivative	Second Derivative
LOD	0.170	0.0111	0.0101
LOQ	0.510	0.0471	0.0241

Robustness

Robustness of a method is a study of the effect of small variation of the experimental conditions on reproducibility of the measurements. In the present investigation a study of robustness was carried out by making a small change in wavelength (±2) of measurements. The results of robustness of the D^0, D^1 and D^2 spectroscopy were represented in TABLE 6.

TABLE 6 : Robustness of the proposed method

Wavelength	Absorbance (Zero)	Amplitude (First)	Amplitude (second)
247	0.411	0.0311	0.0104
249	0.422	0.0320	0.0105
251	0.416	0.0292	0.0106

Assay of pharmaceutical formulations

Sample solution

The average weight of five tablets of MMF was accurately calculated and these tablets were grinded well into a uniform powder. Test solution of 20µg/mL was prepared by taking an amount of the tablet powder equivalent to10 mg of MMF. Three different concentration solutions at 50%, 100% and 150% of target concentration were also prepared in similar manner. Cell Cept tablets of 500mg were analyzed by the validated method by measuring absorbance and amplititude of working standard solution and sample solution. The amount of drug present was evaluated in terms of percent of recovery of six replicates and the results were presented in TABLE 7.

RESULTS AND DISCUSSION

The absorption spectrum, first and second derivative spectra of a solution of 20 µg/mL of MMF were recorded in the range of wavelength 200-400nm. The wavelength of maximum absorbance was found to be 249.0nm. In the first derivative spectrum, the valley at 257.4nm showed maximum amplitude therefore validation of the method was carried out by measuring the

TABLE 7 : Assay of pharmaceutical formulations

Spectra	Labeled Amount	Amount Found*	SD	% Recovery	% RSD
D^0	500 mg	498.97	1.384	99.79	1.3869
D^1	500 mg	501.57	0.975	100.31	0.9719
D^2	500 mg	499.89	0.491	99.98	0.4911

Average of six determinations, SD=standard deviation, RSD=relative standard deviation

amplitudes at this wavelength. The maximum amplitude was observed in negative valley at 249.0nm in second derivative spectrum and hence the method was validated by measuring amplititudes at 249.0nm. The developed methods were found to be linear within the range of concentrations 10-30µg/mL. Slope, intercept and correlation coefficient for the developed method were found to be 0.0213, 0.0042 and 0.9996; 0.0016, 0.0003 and 0.9997; and 0.0005, 7.0E-6 and 0.9999 for zero order, first and second derivative methods respectively. Standard deviation and percent of relative standard deviation (%RSD) values for zero order, first and second derivative methods were found to be 0.0023&0.5463, 2.39E-4&0.7442 and 8.0E-5 & 0.7962 respectively. The method has been proved robust at ±2nm wavelength variation. The mean percent of recovery and percent of relative standard deviation were evaluated at 50%, 100% and 150% concentration levels. The mean percent of recovery and percent of relative standard deviation were found to be 99.1% (0.5583), 99.6% (0.8083), 99.2% (0.3763). Low % RSD values and high % recovery values support for high accuracy of the methods.

CONCLUSION

The developed methods were effective for quantitative determination of MMF in bulk and pharmaceutical preparations without any interference of other constitute in the formulation. Tablets of different brand names were analyzed by the proposed methods and assay of the drug was calculated. The derivative spectrophotometric methods developed by the author were simple sensitive, selective, reproducible, and stable. The developed methods could be readily adapted to routine quality control of MMF by ordinary laboratories.

REFERENCES

[1] Irene Tsina, Martin Kaloostian, Rosy Lee, Thomas Tarnowski, Belinda Wong; Journal of Chromatography B: Biomedical Sciences and Applications, **681(2)**, 347-353 **(1996)**.

[2] A.Protic, Lj.Živanovíc, M.Zečević, B.Jocić; Journal of Chromatographic Science, **47(2)**, 149-155 **(2009)**.

[3] L.Yang, D.He; **28(7)**, 1177-1179 **(2008)**.

[4] Surekha, M.Lakshmi, Gandla, Kumaraswamy, G.L.Ashwini; **5(3)**, 1771-1773 **(2012)**.

[5] T.Vijaya Bhaskara Reddy, G.Ramu, M.Sravan Kumar, C.Rambabu; Asian Journal of Chemistry, **25(9)**, 4788-4790 **(2013)**.

[6] A.L.Rao, P.V.Srinivas, J.V.L.N.S.Rao; **2(3)**, 266-269 **(2010)**.

[7] T.Vijaya Bhaskara Reddy, N.Sowjanya Reddy, G.Ramu, S.V.Venkatrao Sure, C.Rambabu; Journal of Pharmacy Research, **7(7)**, 640-646 **(2013)**.

[8] M.Plätzer, K.Jahn, J.Wohlrab, R.H.Neubert; J.Chromatogr.B Biomed.Sci.Appl., **755**, 355-9 **(2001)**.

[9] S.Kathirvel, K.Rajendra Prasad, K.Madhu Babu; Pharmaceutical Methods, **3(2)**, 90-93 **(2012)**.

[10] Kunithala Vasanth Kumar, Chinthakindi Kiran Kumar, Vemula Sateesh Kumar et al.; Asian Journal of Pharmaceutical & Clinical Research, **5(3)**, 233-237 **(2012)**.

[11] S.N.Prashanth, K.C.Ramesh, J.Seetharamappa; International Journal of Electrochemistry, http://dx.doi.org/10.4061/2011/193041, Volume Article ID 193041, 7 **(2011)**.

[12] S.Verma, H.Gupta, O.Alam, P.Mullick, N.Siddiqui, S.A.Khan; Journal of Applied Spectroscopy, **76(6)**, 876-882 **(2009)**.

[13] K.B.Vinay, H.D.Revanasiddappa, M.S.Raghu, Sameer A.M.Abdulrahman, N.Rajendraprasad; Journal of Analytical Methods in Chemistry, Article ID 875942, **2012**, 8 **(2012)**.