WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 11, 1042-1047. Research Article ISSN 2277– 7105

UV METHOD FOR DETERMINATIONIN OF METHOTREXATE TABLET DOSAGE FORM

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Article Received on
27 Aug 2015,

Revised on 20 Sep 2015,
Accepted on 14 Oct 2015

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ABSTRACT

Two simple, precise and economical UV methods have been developed for estimation of Methotrexat in bulk formulation. Method A involves measurement of UV absorbance in Zero order derivative at 374nm. Method B deals with area under curve measurement (AUC method), which involves the calculation of integrated value of absorbance with respect to wavelength between 372-376nm. The drug follows Beer-Lambert's law in the concentration range of3-10ug/ml in both the methods. Results of analysis were validated statistically and were found to be satisfactory. Thus proposed methods can be successfully applied for estimation of Methotrexatin routine analytical work.[1,2,3]

KEYWORDS: Methotrexat, Zero order derivative, Area Under Curve method (AUC), UV Spectrophotometer.

INTRODUCTION

Methotrexate is described chemically L-Glutamic acid, N-{4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamine]benzoyl}-, Folex: Methotrexate; Mexate.[1]It is a class of anticancer drug. It is abbreviated MTX and as amethopterin is antimetabolite and antifolate drug.[1-3]the drug is official in Indian pharmacopoeia,[4] ,USP,[5]and BP.[6] Literature survey reveals that there are few UV Spectroscopic methods.[7-11] and one HPLC,[12] method is reported for the determination of methotrexate in plasma and urine of humans, rats and dogs. So an attempt was made to develop two simple, accurate, rapid and precise spectrophotometric methods for the determination of Methotrexate in tablet and formulation.

EXPERIMENTAL

Materials

Methotrexatwas obtained as gift sample from Matrix Ltd. NaOH are used as a solvent in the study.

Instrument

A shimadzu UV-1700 UV/VIS Spectrophotometer was used with 1cm matched quartz cells were used for spectral measurements.

Stock solution

Accurately about 5 mg of Methotrexatwas weighed and transferred to 50 ml volumetric flask; 50 ml of NaOH was added to dissolve the drug completely with vigorous shaking. Then the volume was made up with the NaOH up to the mark to give the drug stock solution of concentration 50µg/ml.

Method A

The Zero order derivative spectra at n = 0showed a sharp peak at 374 nm (Figure 1). The absorbance difference at n=0 (dA/dλ)was calucalted by the inbuilt software of the instrument which was directly proportional to the concentration of the standard solution.The standard drug solutions were scanned in the Zero order derivative spectra.A calibration curve was plotted taking the absorbance difference (dA/dλ) against the concentration of Methotrexate. The coefficient of correlation (r^2), slope and intercept values of this method are given in table 1.[2]

Method B

The AUC (area under curve) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. This wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. Suitable dilutions of standard stock solution (50µg/ml) of Methotrexatwere prepared and scanned in the spectrum mode from the wavelength range 372nm to 376nm (figure 2) and the calibration curve was plotted as AUC against concentration of Methotrexat. The method was checked by analyzing the samples with known concentration. As the results obtained were satisfactory low, the method was applied for pharmaceutical formulations.

Analysis of tablet formulation

For the estimation of Methotrexatin tablet formulation by the two methods, ten tablets were weighed and ground into a fine powder. Tablet powder equivalent to 2.5 mg of Methotrexat weighed and transferred to 25 ml volumetric flask and dissolve in 25 ml of NaOH. It was kept for ultra sonification for 45 min, finally the volume was made up to the mark with NaOH, this was then filtered through Whatman filter paper to get tablet stock solution of concentration to 100 µg/ml. Various dilutions of the tablet solution were prepared and analyzed for six times and concentration was calculated by using calibration curve for the two methods. Both the methods were validated according to ICH guidelines,[13] Recovery studies were carried out atthree different levels i.e. 80%, 100% and 120% by adding the pure drug (4, 6 and 8 mg respectively) to previously analyzed tablet powdered sample (2.5mg) as per ICH guidelines.[14] and percentage recovery was calulated as shown in table 2. All the methods were validated for linearity, accuracy and specificity.

RESULT AND DISCUSSION

The methods A & B for the estimation of Methotrexatin tablet form were found to be simple, precise, accurate, rapid & reproducible. Beer- Lambert's law was obeyed in the concentration range of 3-10 µg/ml in both the methods. The values of standard deviation were satisfactory low and the recovery studies were close to 100%.The derivative spectroscopic method applied has the advantage that it locates the hidden peaks in the normal spectrum when the spectrum is not sharp and it also eliminates the interference caused by the excipients present in the formulation. The AUC method has advantage that it is applicable to be drug which shows the broad spectra without a sharp peak. Hence the two methods can be employed for routine analysis of the drugs in quality control, R&D laboratories.

Table 1: Optical characteristics and parameters

Parameters	Method A	Method B
Wavelength(nm)	374	372-376
Beer's – Lambert's range (µg/ml)	3-10	3-10
Coefficient of correlation (r^2)	0.9451	0.9964
Regression equation : Y = mx + c		
a – Slope (m)	0.020	0.104
b – Intercept (c)	0.026	0.281
LOD	5.576	0.1143
LOQ	16.899	0.8289
Molar absorptivity	0.015517	…

Table 2: Assay of the Tablet.

Method	Tablet Formulation	Label claim(mg)	Amount found (mg)*	% mean	S.D.	R.S.D.	S.E.
A	T1	2.5	2.3	92	0.7528	0.818	0.3072
B	T1	2.5	2.4	96	1.0954	1.1410	0.4471

Table 3 Recovery Studies

Sr. No.	Tablet Sample	Level of recovery %	Mean*		S.D.*		R.S.D.*		S.E.*	
			A	B	A	B	A	B	A	B
01		80	98.57	99.52	1.1503	0.7016	1.1669	0.7049	0.6641	0.4050
02	T1	100	99.59	100.04	0.7743	0.0871	0.7774	0.0870	0.4470	0.0502
03		120	100.28	100.21	0.5519	0.2211	0.5503	0.2206	0.3177	0.1276

When *n=3 at each level of recovery

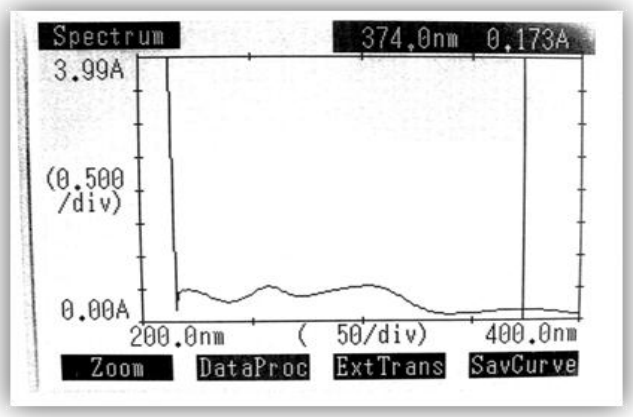

Fig. 1: Spectrum by Zero order derivative method.

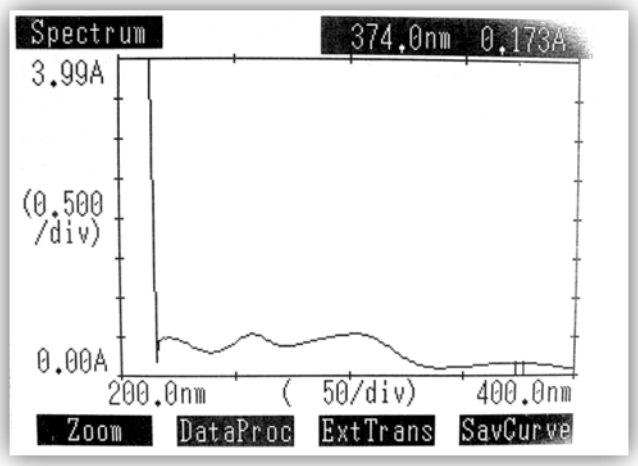

Fig.2: Spectrum by AUC method.

CONCLUSION

An accurate and precise zero order derivative and AUC method have been developed and evaluated for the analysis of methotrexate using (0.1N) NaOH as solvent. The percentage recovery and obtained concentrations of active ingredient where within the acceptable limits. These methods can be used for the estimation of methotrexate in bulk and formulation for quality control studies.

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