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Research Article

DEVELOPMENT OF UV – VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE ANALYSIS OF METHOTREXATE IN TABLET FORMULATIONS

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ABSTRACT

Two simple, precise and economical UV methods have been developed for estimation of Methotrexate in bulk formulation. Method A involves measurement of UV absorbance in zero order derivative at 259nm. Method B deals with area under curve measurement (AUC method), which involves the calculation of integrated value of absorbance with respect to wavelength between 256-262nm. The drug follows Beer-Lambert's law in the concentration range of 3-10µg/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 Methotrexate in routine analytical work.

Keywords: Methotrexate, Zero order derivative, Area Under Curve method (AUC).

INTRODUCTION

Methotrexate is described chemically L-Glutamic acid, N-{4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamine]benzoyl}-,
Folex: Methotrexate; Mexate. 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 surveyreveals 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 formulation.

EXPERIMENTAL

MATERIALS

Methotrexate was obtained as gift sample from Matrix Ltd. Methanol AR grade and 0.1N NaOH were 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 Methotrexate was weighed and transferred to 50 ml volumetric flask; 10 ml of 0.1N NaOH was added to dissolve the drug completely with vigorous shaking. Then the volume was made up with the (0.1N) NaOH up to the mark to give the drug stock solution of concentration 100µg/mL.

Method A

The zero order derivative spectra at n = 0showed a sharp peak at 259 nm (Figure 1). The absorbance difference at n=0 $(dA/d\lambda)$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 $(dA/d\lambda)$ against the concentration of Methotrexate. The coefficient of correlation (r^2), slope and intercept values of this method are given in table 1.

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 $\lambda 1$ and $\lambda 2$. 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 (100µg/ml) of Methotrexate were prepared and scanned in the spectrum mode from the wavelength range 400nm to 200nm (figure 2) and the calibration curve was plotted as AUC against concentration of Methotrexate. 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 Methotrexate in tablet formulation by the two methods, ten tablets were weighed and ground into a fine powder. Tablet powder equivalent to 2.5 mg of Methotrexate weighed and transferred to 25 ml volumetric flask and dissolve in 10 ml 0.1N NaOH. It was kept for ultra sonification for 45 min, finally the volume was made up to the mark with 0.1N 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 8mg respectively) to previously analyzed tablet powdered sample (2.5mg) as per ICH guidelines[14] and percentage recovery was calculated 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 Methotrexate in tablet form were found to be simple, precise, accurate, rapid & reproducible. Beer- Lambert's law was obeyed in the concentration range of 3-10µg/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.

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. The proposed method could be applied for routine analysis in quality control laboratories.

Table 1: Optical characteristics and parameters

Parameter	Method A (Zero order derivative)	Method B (Area under curve)
Wavelenght (nm)	259	256-262
Beer's – Lambert's rang (µg/ml)	3-10	3-10
Coefficient of correlation (r^2)	0.9994	0.99
Regression equation : (Y*)	Y = 0.051x+0.004	Y = 0.428x+0.020
a-Slope (m)	0.051	0.428
b – Intercept (c)	0.004	0.020
Limit of detection(mcg/ml)	0.4779	9.3228
Limit of quantitation (mcg/ml)	28.401	28.251
Molar absorptivity	0.0508	0.4257

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	2.4	92.16	0.7527	0.8167	0.3072
B	T1	2.5	2.3	94.83	0.8944	0.9431	0.3651

When *n=6 at each level of recovery

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	T1	80	99.23	98.81	0.9613	1.0458	0.9687	1.0583	0.5550	0.6037
02		100	98.45	98.62	0.5923	0.5553	0.6016	0.5630	0.3419	0.3206
03		120	98.85	98.89	0.5907	1.0000	0.5975	1.0112	0.3410	0.5773

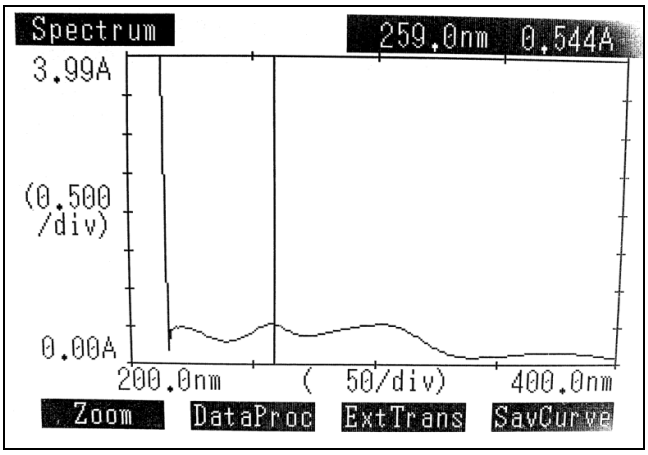

Fig. 1: Spectrum by Zero order derivative method

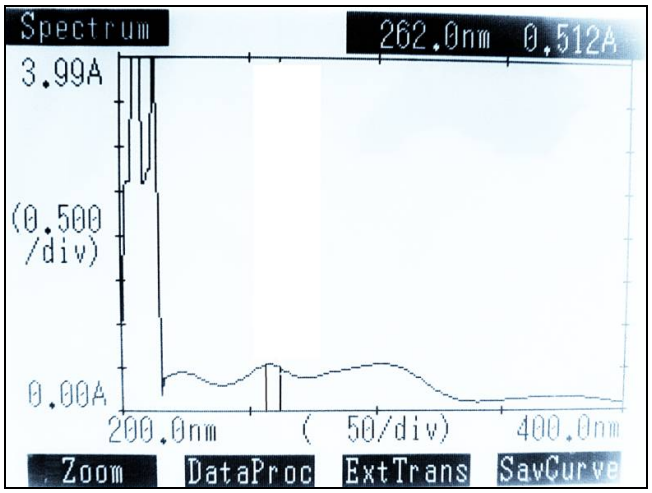

Fig. 2: Spectrum by AUC method

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