

SPECTROPHOTOMETRIC DETERMINATION OF METHOTREXATE IN TABLET DOSAGE FORM**Samrat Suryawanshi*, Pratiksha Shinde, Nita Thamke, Mukesh Mohite.**

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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 258nm. Method B deals with area under curve measurement (AUC method), which involves the calculation of integrated value of absorbance with respect to wavelength between 252-264nm. The drug follows Beer-Lambert's law in the concentration range of 3-15µg/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 routine analytical work.

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

Methotrexate is described chemically L-Glutamic acid, N-{4-[[[2,4-Diamino-6-pteridiny]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 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**

Methotrexates obtained as gift sample from Matrix Ltd. NaOH & distilled Water 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 Methotrexate 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 distilled water up to the mark to give the drug stock solution of concentration 100µg/ml.

Method A

The zero order derivative spectra at $n = 0$ showed a sharp peak at 258nm (Figure 1). The absorbance difference at $n = 0$ ($dA/d\lambda$) was calculated 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 λ_1 and λ_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 200nm to 400nm (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 of NaOH. It was kept for ultra-signification for 45 min, finally the volume was made up to the mark with distilled water, 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 at three 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 calculated as shown in table 2. All the methods were validated for linearity, accuracy and specificity.

RESULT AND DISCUSSION

The methods A and B for the estimation of Methotrexate tablet form were found to be

Table 1: Optical and parameters.

S. No.	Parameters	Method A	Method B
1	Wavelength(nm) (λ Max)	258	252-264
2	Beer's – Lambert's range (µg/ml)	3-15	3-15
3	Coefficient of correlation (r^2)	0.913	0.987
4	Regression equation : $Y = mx + c$		
5	a – Slope (m)	0.064	0.598
6	b – Intercept (c)	0.150	0.150
7	LOD	108.04	11.51
8	LOQ	360.15	38.38
9	Molar absorptivity	0.0574	0.5748

characteristics

Table 2: Assay of the Tablet

Sr. No.	Method	Tablet Formulation	Label claim(mg)	Amount found (mg)*	% mean	S.D.	R.S.D.	S.E.
1	A	T1	2.5	2.38	93.55	0.8998	0.961	0.3673
2	B	T1	2.5	2.30	95.56	1.5705	1.64	0.6144

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	91.53	94.43	0.4509	0.6110	0.4926	0.6470	0.2603	0.3527
02		100	92.6	95.06	0.5567	0.8523	0.6011	0.8965	0.3214	0.4920
03		120	91.73	95.46	0.7767	0.5507	0.8467	0.5768	0.4484	0.3179

When *n=3 at each level of recovery

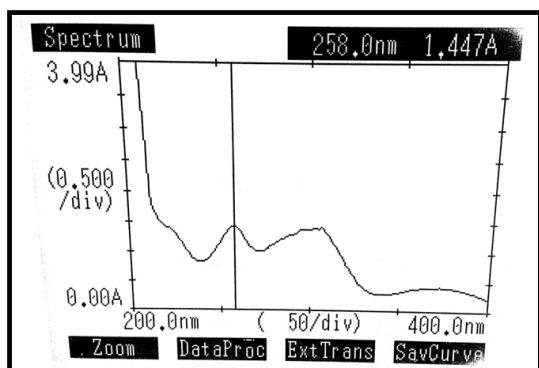


Fig. 1: Spectrum by First order derivative method.

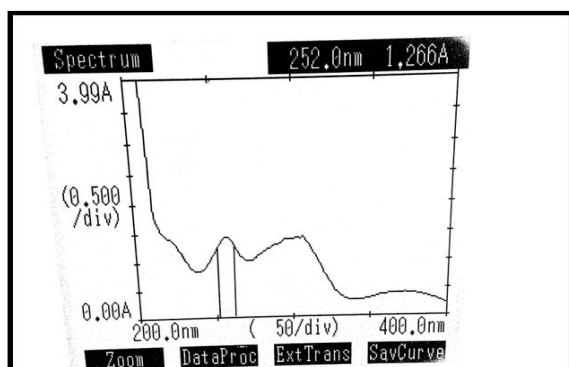


Fig.2: Spectrum by AUC method.

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 were within the acceptable limits.

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