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A UV Spectrophotometric Method for the Determination of Methotrexate in Pharmaceutical Dosage Form

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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 Zeroorder derivative at 303nm. Method B deals with area under curve measurement (AUC method), which involves the calculation of integrated value of absorbance with respect to wavelength between 300-306nm. 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 Methotrexatein routine analytical work.

Keywords: Methotrexate, Zero order derivatives, Area under Curve method (AUC), UV Spectrophotometer.

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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 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.

MATERIALS AND METHOD

Materials

Methotrexatewas 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 Methotrexatewas weighed and transferred to 50 ml volumetric flask; 10 ml of 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=0showed a sharp peak at 303nm (Figure 1). The absorbance difference at n=0 (dA/dλ)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/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 (100µg/ml) of Methotrexatewere prepared and scanned in the spectrum mode from the wavelength range 300nm to 306nm (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.[2]

Method Validation

Precision

Precision of the method was determined by repeating the assay 3 times for six replicate dilutions of the same concentrations after every two hours on the same day for intraday precision. Performing the assay of the same sample solution after 24 hours and 48 hours carried out intraday precision. The results are shown in the Table 1.

Linearity

A series of volumetric flasks of 10 ml capacity were arranged. To each of these flasks 0.3, 0.5, 0.7, 0.9, 1.0, and 1.5ml of the drug stock solution ware added. The volume was made up with distilled water. The absorbance was measured at 300nm to 306nm against the reagent blank. A linear graph of absorbance V/s concentration was obtained. The concentration range over which the drugs to obeyed Beers- Lamberts law was found to be 3 to 15 µg/ml for methotrexate. The standard calibration table and curve for methotrexate are given in Figure 3 and 4.

Recovery

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 calculated as shown in table 2. All the methods were validated for linearity, accuracy and specificity.

Analysis of tablet formulation

For the estimation of Methotrexatein 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 sonification for 45 min, finally the volume was made up to the mark with distilled water, this was then filtered through Whattman 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].

RESULT AND DISCUSSION

Both the methods A and B for the estimation of methotrexate in tablet dosage form were found to be simple accurate and reproducible. Beer- Lamberts law was obeyed in the concentration range of 3-15 µg/ml. in method A and method B. The validation of the proposed method was further confirmed by recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of methotrexate showed no interference from the common excipients. The value of standard deviation was satisfactory and the recovery studies were close to 100%. Hence, all the two methods can be employed for routine analysis of the drugs in quality control R & D laboratories.

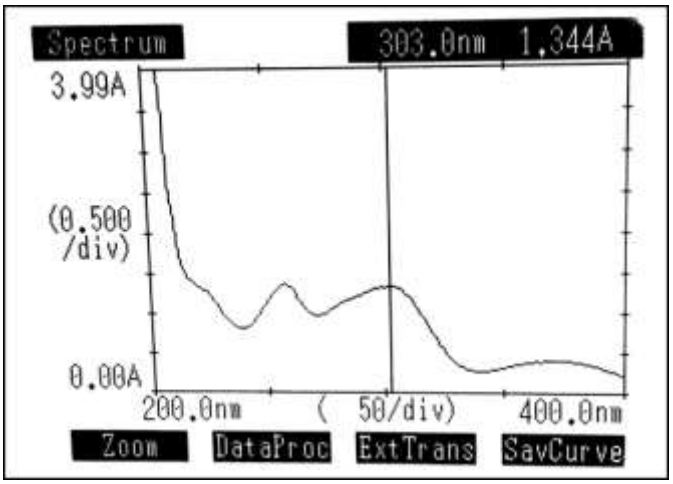

Figure 1: Spectrum by Zero order derivative Method (Methotrexate).

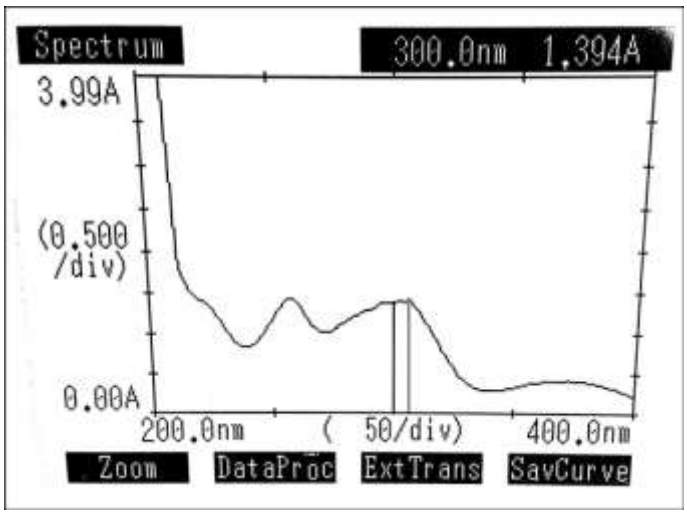

Figure 2: Spectrum by AUC Method (Methotrexate).

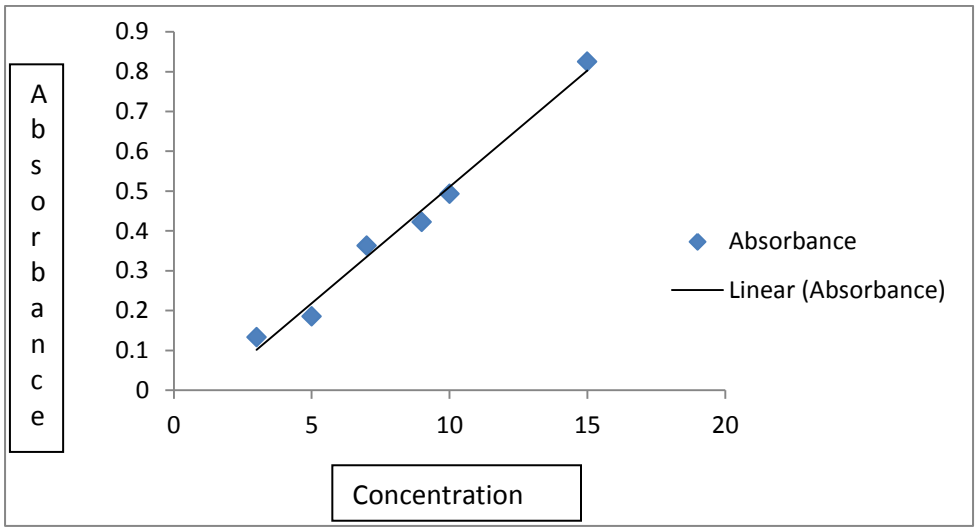

Figure 3: Calibration curves of Method 'A' (Methotrexate).

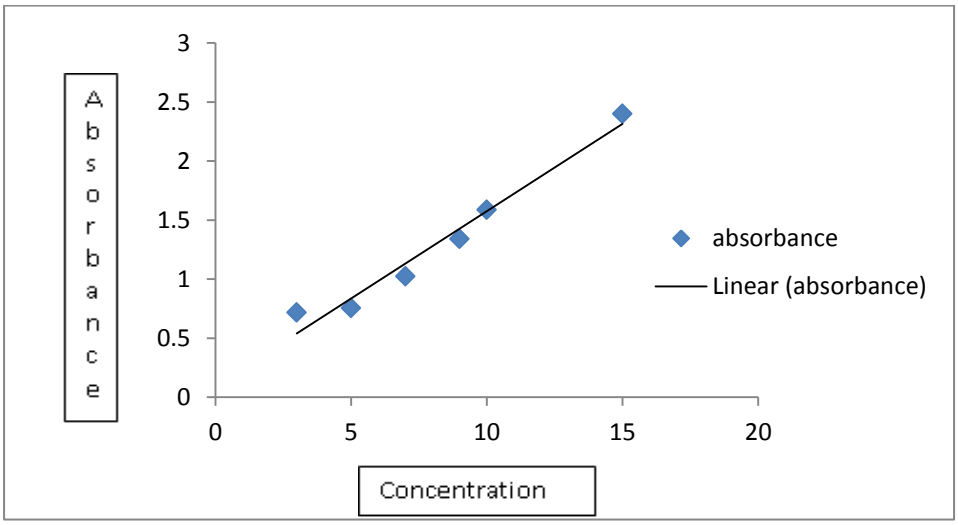

Figure 4: Calibration curves of Method 'B' (Methotrexate)

Table 1: Statistical validation for precision.

Sr. No.	Component	Mean		S.D.		R.S.D.		S.E.	
		A	B	A	B	A	B	A	B
1	**Intra-day**	99.31	98.79	0.2691	0.565	0.2709	0.5721	0.1102	0.2318
2	**Inter-day**	99.02	98.80	0.4766	0.550	0.4813	0.5566	0.1953	0.2254

Table 2: 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	96.5	96.16	0.4163	1.0503	0.4313	1.0922	0.240	0.6064
02		100	97.7	98.16	0.3511	1.0503	0.3593	1.0699	0.2027	0.6036
03	T1	120	96.0	94.0	0.9643	0.300	1.004	0.3184	0.5567	1.1732

When *n=3 at each level of recovery

Table 3: 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	2.48	99.2	0.8461	0.0085	0.3454
B	T1	2.5	2.41	96.4	1.256	0.0130	0.5131

When *n=6 at each level of recovery

Table 4: Optical characteristics and Parameters

Parameters	Method A	Method B
Wavelength(nm) (λ Max)	303	300-306
Beer's – Lambert's range (µg/ml)	3-15	3-15
Coefficient of correlation (r^2)	0.9776	0.882
Regression equation : Y = mx + c		
a – Slope (m)	0.058	0.0165
b – Intercept (c)	0.074	0.132
LOD	12.84	11.51
LOQ	42.82	38.38
Molar absorptivity	0.04710	0.2272

CONCLUSION

Method was developed and validated as per ICH guidelines for estimation of Methotrexate. Both the methods were applied for estimation of this compound in the marketed formulation. The method has been evaluated for the linearity, accuracy, precision and Robustness in order to ascertain the suitability of the method. It has been proved that the developed method was linear in the concentration range of 3 to 15 µg/ml. High percentage recovery showed that method was free from interference of excipients used in the formulations. The result of the study indicates that the proposed UV spectrophotometric method of analysis can be used in quality control departments with respect to routine analysis for the assay of the tablets containing Methotrexate.

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