Available Online at

http://www.ijcpa.in

April-June 2017

DOI : http://dx.doi.org/10.21276/ijcpa

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

Research Article | Volume-4 | Issue-3 | Article ID: 1294

STUDY OF THE EFFECT OF ANHYDROUS SOLVENT ON METHOTREXATE BY USING UV-SPECTROPHOTOMETER

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Received: 10 April 2017 / Revised: 20 April 2017 / Accepted: 28 May 2017 / Available online : 4 June 2017

ABSTRACT

Three simple, precise and economical methods for UV have been developed for determination of Methotrexate in bulk formulation. Method A involves measurement of UV absorbance in Zero order derivative and Method B involves first order derivative are at 302and264nm respectively. Method C deals with Area Under Curve measurement (AUC method), which involves the calculation of integrated value of absorbance with wavelength range between 297-305nm. The drug follows Beer-Lambert's law in the concentration range of 10-50µg/ml in all three methods. Results of analysis were validated statistically and found to be satisfactory. Thus proposed method scan be successfully applied for estimation of Methotrexate in routine analytical work.

Keywords – *Methotrexate, Zero Order derivative, First order derivative, Area Under Curve method (AUC), UV-spectrophotometer.*

1. INTRODUCTION

Methotrexate (MTX) is L-Glutamic acid, N-*(4{(2,4-Diaminopteridin-6-yl)methyl-methyl-amino}benzoyl) amino pentanedioic acid. It is drug used to cure cancer against cancerous cells in body.* It is used for the treatment of lymphoma, lung, breast, trophoblastic neoplasms and leukemia, Methotrexate also affect rheumatoid arthritis by two different mechanisms. For cancer, Drug competitively inhibits dihydrofolate reductase enzyme(DHFR), that inhibits tetrahydrofolate synthesis[1-3]. The Methotrexate drug is official in IP[4], BP[5] and USP[6].

Literature survey reveals that methods like RP-HPLC[7,8] and different Spectrophotometric have been reported for estimation of the Methotrexate in pharmaceutical dosage forms and biological fluids. Official method includes UV Spectrophotometric method for determination of the drug from the tablets dosage form [9].The method were validated according to ICH guidelines [10,11].

2. MATERIALS AND METHODS

2.1 Materials

Methotrexatewas obtained as sample fromAribindo pharmaceuticals.Sodium Carbonate (Anhydrous) and distilled water are used as a solvent in the study.

2.2 Instrument

A Shimadzu UV-1700 UV/VIS spectrophotometer was used with 1cm matched quartz cells wereused for spectral measurements.

2.3 Stock solution

Accurately weigh about 5 mg of Methotrexate was weighed and transferred to 50 mlvolumetric flask 10 ml of Sodium carbonate solution (0.1 N) was added to dissolvecompletely with vigorous shearing/shaking.Then the volumewas made up with distilled water up to the mark to give the drug stock solution of concentration100µg/ml.

2.4 Method A: Zero order derivative

The Zero order derivative spectra at n=0showed a sharp peak at 302 nm(Figure 1).The absobance difference at n=0 (dA/dλ)was calculated by the inbuilt software of the instrument which wasdirectly proportional to the concentration of the standard solution.The standard drug solutions werescanned in the Zero order derivative spectra.A calibration curve was plotted taking the absorbancedifference (dA/dλ) against the concentration of Methotrexate. The coefficient of correlation (r2), slope and intercept values of this method are given in table 1.

2.5 Method B: First order derivative

The First order derivative spectra at n=1showed a sharp peak at 264nm(Figure 2).The absobance difference at n=1 (dA/dλ) were calculated by the software in instrument which is directly proportional to the concentration of the standard solution .The standard drug solution were scanned in the Firstorder derivative spectra.A calibration curve was plotted taking 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.

2.6 Method C: AUC (Area under curve)

The AUC (area under curve) involves the calculation of integrated value of absorbance with respect to between theselected wavelength range λ1 to λ2. The Area bound by curve and the horizontal axis calculated by area calculation process. This wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve (AUC) and concentration.Suitable dilutions of standard stock solution (100µg/ml) of Methotrexatewere prepared and scanned in the spectrum mode from the wavelength range 400nm to 200nm (Figure 3) and the calibration curve was plotted as AUC against concentration ofMethotrexate. 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.

2.7 Analysis of Tablet Formulation

For the estimation of Methotrexatein tablet formulation, tablets were weighed and ground into a fine powder. Tablet powder equivalent to 2.5mgof Methotrexateweighed and transferred to 25 mlvolumetric flask and dissolve in 10 ml of Sodium Carbonate Solution. 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 100µg/ml. Various dilutions of the tablet solution were prepared and analyzed for six times and concentration was calculated by using calibration curve for the three methods.Both the methods were validated according to ICH guidelines.All the methods were validated according to ICH guidelines.

2.8 Method of Validation

2.8.1 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 Interdayandintraday precision. The results are shown in the Table **3.**

2.8.2 Linearity

A series of volumetric flasks of 10 ml capacity were arranged. To each of these flasks 1, 2, 3, 4, and 5 ml of the drug stock solution ware added. The volume was made up with distilled water. The absorbance was measured at 302 nm in method A,264 nm in method B and 297-305 nm in method C 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 10-50 µg/ml for Methotrexate.The standardCalibration table and curve for methotrexate are given in Figure 3 and 4.

2.8.3 Recovery study

Recovery studies were carried out at three different levels i.e. 80%, 100%, 120% by adding the pure drug (8, 10 and 12mg respectively) to previously analyzed tablet powdered sample (2.5 mg) as per ICH guidelines and percentage recovery was calculated as shown in Table **4.**All the methods were validated for linearity, accuracy and specificity.

3. RESULTS AND DISCUSSION

Both the methods A, Band C for the estimation of Methotrexatein tablet form were found to be simple, precise, accurate, rapid and reproducible. Beer-Lambert's law was obeyed in the concentration range of 10-50 µg/ml in methodsA method B and method C.The validation of the proposed method was furtherconfirmed by recovery study data clearly indicate the reproducibility and accuracy of the methods. Evaluated by Interday and intraday study and all three methods were developed and validated as per ICH guidelines for estimation of Methotrexate. 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.

Table 1: Optical characteristics and parameters

Sr.No.	Parameters	Method A	Method B	Method C
1	Wavelength(nm) (λ Max)	302	264	297-305
2	Beer's – Lambert's range (µg/ml)	10-50	10-50	10-50
3	Coefficient of correlation (r^2)	0.9920	0.9990	0.9940
4	Regression equation	Y =0.021x + 0.065	Y=0.001x+0.000	Y=0.002x+ 0.002
5	a – Slope (m)	0.021	0.001	0.002
6	b – Intercept (c)	0.065	0.000	0.002
7	LOD	0.66	12.87	4.29
8	LOQ	0.60	11.70	3.9

Table 2: Assay of the tablet

Sr. No.	Method	Conc. (µg/ml)	Amount found (mg)*	% Mean	S.D.	R.S.D. %	S.E.
1	A	10	9.977	99.77	0.436	4.381	0.178
2	B		8.268	82.68	0.416	5.033	0.170
3	C		9.276	92.76	0.390	4.203	0.201

When*n=6 at each level of recovery

Table 3: Statistical validation for Precision

Sr. No.	Component	Mean			S.D.			R.S.D.			S.E.		
		A	B	C	A	B	C	A	B	C	A	B	C
1	Intra-day	99.31	98.89	98.79	0.47	0.49	0.56	0.48	0.49	0.57	0.22	0.26	0.32
2	Inter-day	99.03	98.58	98.80	0.23	0.31	0.54	0.25	0.29	0.55	0.10	0.23	0.25

Table 4: Recovery studies

Sr. No.	Tablet Sample	Level of recovery %	Mean*			S.D.*			C.O.V			S.E.*		
			A	B	C	A	B	C	A	B	C	A	B	C
01	T1	80	100.12	99.73	99.55	0.9388	0.438	0.316	0.5420	0.440	0.323	0.881	0.488	0.310
02		100	99.10	99.52	99.89	0.3098	0.340	0.434	0.0960	0.345	0.430	0.219	0.136	0.123
03		120	99.30	99.13	98.99	0.4354	0.256	0.289	0.1896	0.265	0.270	0.251	0.437	0.189

When *n=3 at each level of recovery, T1: Folitrex (5mg)

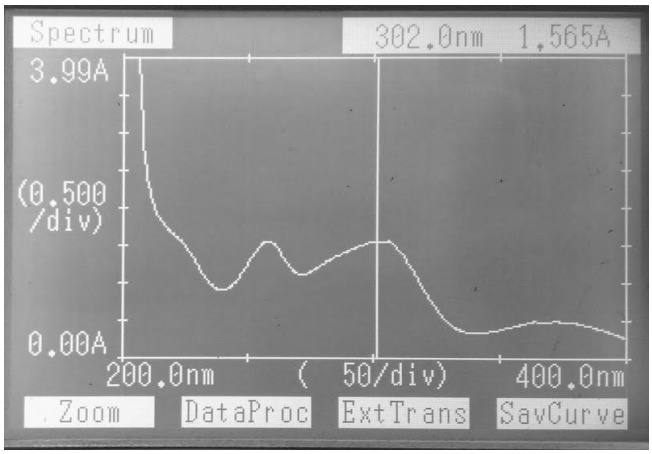


Figure 1: Spectrum by zero order derivative method

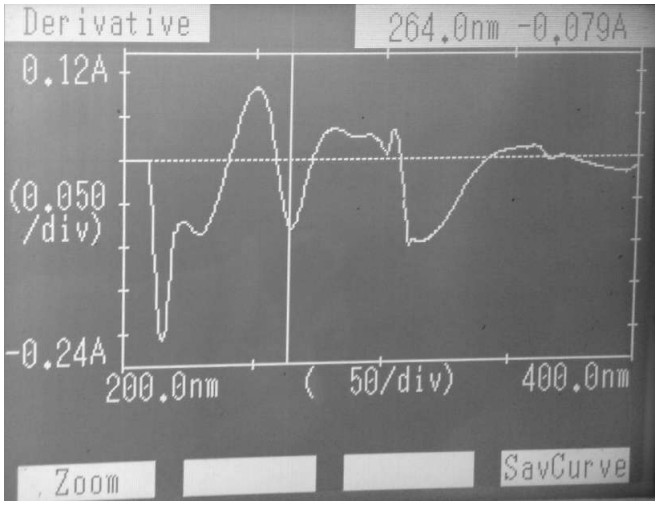


Figure 2: Spectrum by first order derivative method

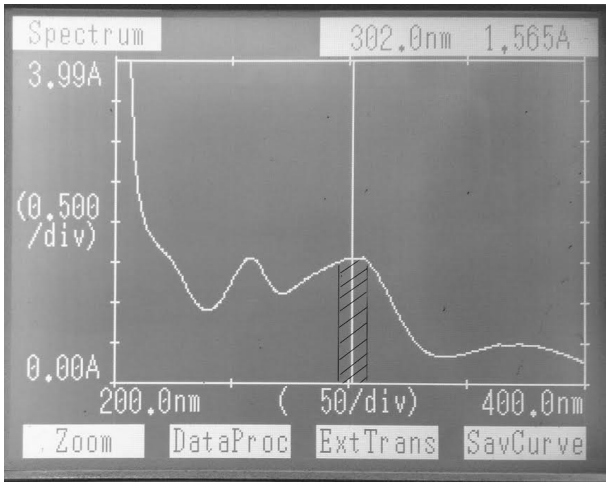
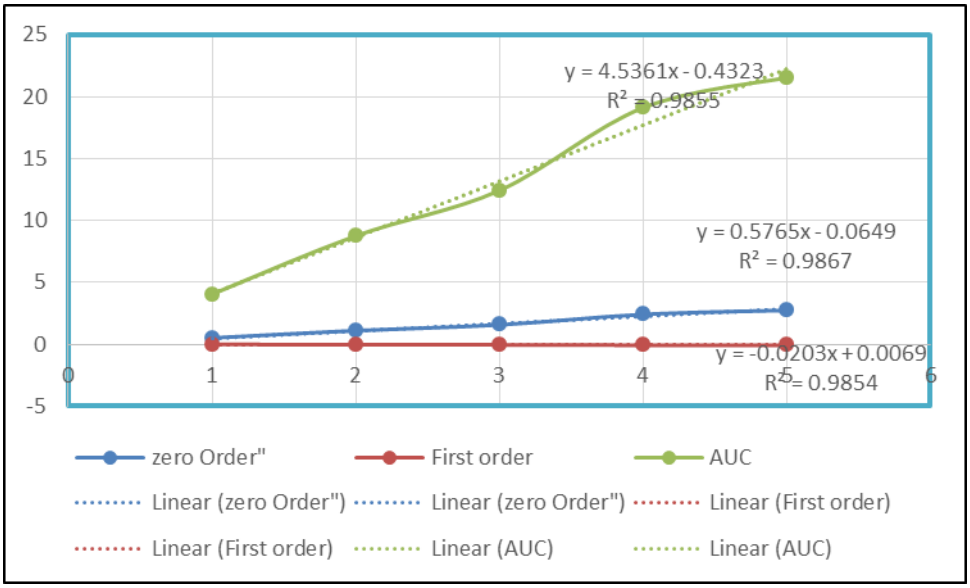
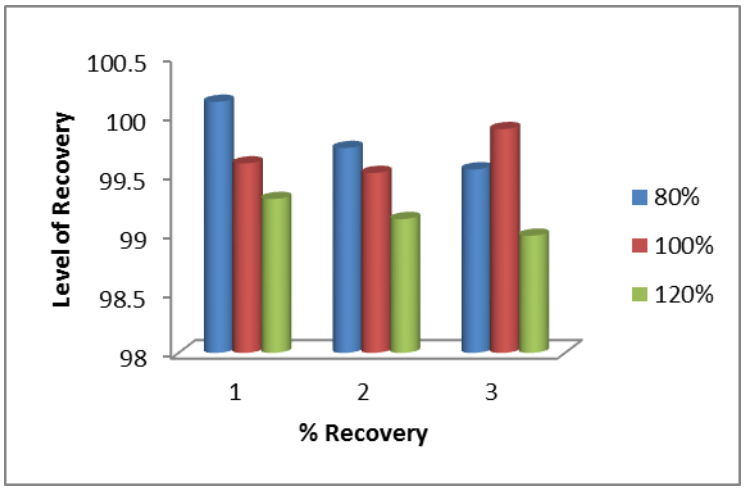


Figure 3: Spectrum by AUC method



Graph 1: optical parameters of method A, B and C



Graph 2: Recovery study by method A, B and C.

4. 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 Methodhas been evaluated for the linearity, accuracy, precision and Robustness in order Ascertain the suitability of the method. It has been proved that the developed method was linear inthe concentration range of 3 to 15 µg/ml. The result of the study indicates that theproposed 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.

5. ACKNOWLEDGEMENTS

The Authors thanks to Chairman and the Principal of Dr. D. Y. Patil College of Pharmacy Akurdi,Pune , for providing facilities to carry out this research investigation.

6. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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